Abstract Book – NFN Fall Symposium 2017

Part of:

Abstracts NFN fall meeting 2017
Dietary sodium restriction versus diuretics for salt-sensitive hypertension in chronic kidney disease

D.M. Bovée¹, A.H.J. Danser¹, R. Zietse¹, E.J. Hoorn¹

¹Erasmus MC, Rotterdam, The Netherlands

Objective: Fluid overload and salt-sensitive hypertension are hallmarks of advanced chronic kidney disease (CKD) and associated with worse outcomes. Dietary sodium (Na⁺) restriction is an accepted intervention, but long-term adherence remains a challenge. Distal diuretics could provide an alternative approach, but they are considered less effective in advanced CKD because of reduced tubular secretion. Here, we compared both approaches head-to-head.

Methods: Twenty-six patients with CKD stage 3 or 4 and hypertension were included in this single-center, open-label, randomized cross-over trial (baseline eGFR 39 ± 13 ml/min/1.73 m²). Renin-angiotensin inhibitors and diuretics were discontinued 2 weeks prior to interventions and during study period. Subsequently, we compared dietary Na⁺ restriction (60 mmol/day) versus amiloride/hydrochlorothiazide (5/50 mg once daily). Both interventions lasted for two weeks and were separated by a 2-week wash-out period. The primary endpoint was 24h systolic blood pressure (SBP).

Results: Urinary Na⁺ excretion was successfully lowered with dietary Na⁺ restriction (160 ± 66 to 64 ± 37 mmol/day, p < 0.01), and remained similar with diuretics (154 ± 47 to 153 ± 63 mmol/day, p = 0.95). Dietary Na⁺ restriction lowered 24-hour SBP (134 ± 12 to 129 ± 14 mmHg, p < 0.05), while diuretics had a greater effect (138 ± 12 to 124 ± 13 mmHg, p < 0.01 for within and between interventions). Both maneuvers significantly lowered indices of fluid overload, including body weight (-1.6 ± 1.1 kg with dietary Na⁺ restriction and -1.9 ± 1.5 kg with diuretics), NT-pro-BNP (median -10 and -7 pmol/L), and overhydration as assessed by bioimpedance (-0.6 ± 0.6 and -1.3 ± 0.7 L). Both interventions also lowered eGFR (-2 ± 4 and -5 ± 5 ml/min/1.73 m², p<0.05 for both) and showed a trend towardsalbuminuria reduction (median -5 mg/day and -20 mg/day). The reduction in overhydration and eGFR was greater with diuretics than with dietary Na⁺ restriction (p < 0.05).

Conclusion: Distal diuretics and dietary Na⁺ restriction effectively lower blood pressure in CKD 3 and 4 in the absence of renin-angiotensin inhibitors. Both interventions also lower indices of fluid overload. Diuretics produce greater effects than dietary Na⁺ restriction. These beneficial effects may outweigh the (hemodynamic) reduction in eGFR.
Magnesium prevents vascular calcification by inhibition of hydroxyapatite crystal formation

Anique D. ter Braake,¹ Paul T. Tinnemans,² Catherine M. Shanahan,³ Joost G. J. Hoenderop,¹ and Jeroen H. F. de Baaij¹,⁴

¹Department of Physiology, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Nijmegen, The Netherlands,²Institute for Molecules and Materials, Radboud University, Nijmegen, The Netherlands,³BHF Centre of Research Excellence, Cardiovascular Division, James Black Centre, King’s College, London, United Kingdom ⁴Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, United Kingdom

Objective: Magnesium (Mg²⁺) has been shown to effectively prevent vascular calcification in multiple experimental calcification models. Vascular calcification is common in chronic kidney disease and contributes to increased mortality in these patients. Mg²⁺ has been hypothesized to prevent the upregulation of osteoblastic gene expression that drives calcification. However, extracellular effects of Mg²⁺ on calcium (Ca²⁺)-phosphate (Pi) crystal formation have been largely neglected. This study aimed to investigate the effects of Mg²⁺ on both intracellular changes associated with vascular calcification as well as effects on crystal formation in the extracellular space.

Methods: Bovine vascular smooth muscle cells (bVSMC) were calcified using β-glycerophosphate (BGP). Osteoblastic transdifferentiation was assessed by transcriptional analysis, cellular alkaline phosphatase (ALP) activity and development of apoptosis. X-ray powder diffraction, scanning electron microscopy and energy dispersive spectroscopy on crystals isolated from cell culture supernatants were used to map extracellular effects of Mg²⁺ on crystal formation and crystal composition.

Results: Mg²⁺ effectively prevented BGP-induced calcification in bVSMC. BGP did not cause changes in mRNA expression of the osteogenic genes bone morphogenetic protein 2, Runt related transcription factor 2 or ALP. Moreover, ALP activity was stable and apoptosis was only detected after calcification independent of Mg²⁺. In addition, blocking of the Mg²⁺ channel transient receptor potential melastatin 7 using 2-Aminoethoxydiphenyl borate did not abrogate the protective effects of Mg²⁺, indicating that intracellular Mg²⁺ is not involved in BGP-induced calcification of bVSMCs. Extracellular Mg²⁺ prevented the formation of hydroxyapatite crystals, which formed extensively after BGP treatment. Further analysis of the composition of the hydroxyapatite crystals showed that Mg²⁺ supplementation resulted in reduced Ca²⁺ and Pi fractions of 68% and 41%, respectively, without increasing the fraction of Mg²⁺.

Conclusion: This study demonstrates that Mg²⁺ prevents bVSMC mineralization through inhibition of Ca²⁺-apatite formation in the extracellular space, independent of VSMC transdifferentiation. These results emphasize the need for randomized-controlled clinical trials assessing the effects of Mg²⁺ supplementation on vascular calcification.
O3.
G2/M cycle arrest in human transplanted kidneys correlates to the development of fibrosis, functional decline and graft loss

T.T. Pieters¹, T. Vanhove³, T.Q. Nguyen², M.C. Verhaar¹, D. Kuypers³, R. Goldschmeding², M.B. Rookmaaker¹

¹Departments of Nephrology and Hypertension, and ²Pathology, University Medical Center Utrecht, The Netherlands, ³Department of Nephrology, University Hospitals of Leuven, Belgium

Objective: Kidney transplantation (KTx) is the only curative treatment of end-stage renal failure. Although short-term graft survival has vastly improved in the last decades, long-term graft loss due to interstitial fibrosis and tubular atrophy (IF/TA) remains a major problem, especially in patients who suffer from delayed graft function (DGF) due to ischemia-reperfusion-related acute tubular injury (ATI) after KTx. Insight into the pathophysiology of kidney fibrosis is crucial for the development of new therapeutic approaches. Maladaptive repair, characterized by G2/M arrested tubular epithelial cells, has emerged as a key mediator of IF/TA after ATI in animal experiments. However, little is known about the role of maladaptive repair and G2/M cell cycle arrest in human kidney fibrosis. In this retrospective study, we studied the relation between G2/M cell cycle arrest in tubular cells in biopsies and histological and functional outcome in KTx recipients.

Methods: We included 64 patients, of which 32 presented with DGF and 32 with early graft function. Surveillance biopsies of these patients taken at 3 months after KTx were evaluated for G2/M cell cycle arrest. In the 32 patients with DGF, ATI was studied in the indication biopsies taken at approximately 7 days after KTx. G2/M cell cycle arrest was assessed by staining for the G2/M marker phosphorylated Histone H3 (pHH3) and the proliferation marker Ki67. ATI was quantified morphologically in biopsies that were free of any other pathological changes (e.g. acute rejection) except ATI. Renal outcome was evaluated by histological severity of fibrosis 2 years after KTx (Remuzzi score), kidney function decline in 2 years (eGFR, CKD-epi), and 10-year death-censored graft survival.

Results: At 3 months after KTx, patients who suffered from DGF had more G2/M arrested cells as compared to those with early graft function (median 1.17 cells/1mm² [range 0.00-3.79] vs median 0.59 cells/1mm² [range 0.00-1.83]; p<0.05). Furthermore, the amount of arrested cells at 3 months after KTx correlated to the extent of histological damage during ATI (rₑ=−0.78 p<0.0005). The amount of G2/M arrested cells also correlated to histological severity of fibrosis 2 years after transplantation (rₑ=0.52, p<0.01) and functional decline over 2 years (rₑ=−0.44, p<0.01). Finally, the amount of G2/M arrested cells were associated with a higher risk of 10-year death-censored graft survival (univariate cox regression, HR=3.98, p<0.01).

Conclusion: Our data suggest that ATI after KTx induces cell cycle arrest in regenerating tubules that subsequently leads to progressive IF/TA, functional decline and eventually graft loss. Surprisingly, arrested tubular cells were also present in biopsies of patients with early graft function, which might be attributed to the chronic stress (e.g. medication) that kidney grafts are exposed to during KTx. To our knowledge, this is the first report on the relation between G2/M arrested cells and renal outcome in patients. Our data imply that therapeutic targeting of cell cycle arrested kidney cells may provide a promising tool to decrease IF/TA and improve long-term survival of transplanted kidneys.
Objective: Older patients approaching end-stage renal disease face the decision whether or not to start dialysis. Conservative care is argued to be a reasonable alternative as dialysis is not always associated with a survival benefit, as shown in our previous survival analysis. To truly foster decision-making, we analyzed more patient-relevant outcomes and treatment costs in an extended cohort.

Methods: We conducted an observational single-center cohort study in 366 patients aged ≥70 years with stage 4/5 chronic kidney disease, who chose either dialysis (n=240) or conservative care (n=126) after careful counselling. Using a value-based health care approach (value = outcomes/cost): survival; health-related quality of life, assessed with KDQOL-SF questionnaire; and treatment burden were evaluated, together with treatment costs.

Results: The overall survival benefit of patients choosing dialysis compared with patients choosing conservative care diminished or disappeared in patients aged ≥80 years or with severe comorbidity. There were no differences between patients managed conservatively (n=23) and patients started on dialysis (n=34) on physical and mental health summary scores (all P >0.1). Patients choosing conservative care had 352.7 hospital free days per year versus 282.7 in patients choosing dialysis, measured from date of treatment decision (incidence rate ratio: 1.15, 95% confidence interval 1.09 to 1.21, P <0.001). Annual treatment costs were significantly lower in patients choosing conservative care (cost ratio: 0.43, 95% confidence interval 0.28 to 0.67, P <0.001).

Conclusion: Conservative care is a reasonable treatment option in older patients with advanced chronic kidney disease, particularly those with the highest ages or severe comorbidity. By achieving similar outcomes at lower treatment burden and lower treatment costs, value could be created for both society and older renal patients choosing conservative care.
O5.
Glomerular Enhancer of Zeste Homolog-2 (EZH2) histone methyltransferase reduces glomerular endothelial glycocalyx during diabetic nephropathy by regulating hyaluronan synthesis

Marloes Sol¹, Jiedong Qiu², Johan van der Vlag³, Jacob van den Born⁴, Benito A. Yard², Jan-Luuk Hillebrands¹, Jan A.A.M. Kamps¹, Guido Krenning¹

¹ Dept. of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, The Netherlands, ² Dept. of Nephrology, Endocrinology and Rheumatology, Medical Faculty Mannheim, University of Heidelberg, Germany, ³ Dept. of Nephrology, Radboud University Medical Center Nijmegen, The Netherlands, ⁴ Dept. Internal Medicine, Div. Nephrology, University Medical Center Groningen, University of Groningen, The Netherlands

Objective: Diabetic nephropathy (DN) is the leading cause of end-stage renal failure worldwide. The glomerular endothelial glycocalyx is the first barrier that prevents leakage of circulating proteins. Injury to the glycocalyx evokes proteinuria and kidney failure. The polycomb group methyltransferase Enhancer of Zeste Homolog 2 (EZH2) inhibits expression of its target genes through methylation of lysine 27 on histone 3 (H3K27Me3). We recently performed a target screen for genes involved in glycocalyx turnover, which indicated that EZH2 inhibits glycocalyx synthesis in glomerular endothelial cells. We hypothesized that EZH2 activity is increased in the glomerular endothelium during DN thereby reducing glycocalyx synthesis.

Methods: H3K27me3 was analyzed in glomerular endothelial cells by immunofluorescence in BTBR<sup>ob/ob</sup> mice, a mouse model for DN. Glycocalyx in these mice was measured by the binding of fluorescently-labeled wheat germ agglutinin. In glomerular endothelial cells, EZH2 was silenced by RNAi. Gene expression was assessed by Quantitative Real-time PCR.

Results: H3K27me3 in glomerular endothelial cells was increased 1.5-fold compared to nondiabetic mice (p=0.026). Albumin-creatinine ratios of BTBR<sup>ob/ob</sup> mice correlated with the increase in H3K27me3 (p=0.044; r²=0.674). A 2-fold loss of glomerular glycocalyx was observed in BTBR<sup>ob/ob</sup> mice (p=0.002). Silencing of EZH2 in glomerular endothelial cells led to a decrease in H3K27me3 and an 8-fold increase in the hyaluronan synthesizing enzyme HAS1 (p<0.001). ENCODE database analysis revealed a binding site for EZH2 in the HAS1 gene, suggesting that HAS1 is a direct target of EZH2. Interestingly, the hyaluronan degrading enzymes, HYAL1 (p=0.002), HYAL2 (p=0.015), and HYAL3 (p=0.014) were all decreased upon knockdown of EZH2.

Conclusion: Our data suggests that EZH2-mediated epigenetic changes reduce endothelial glycocalyx via reduction of hyaluronan in DN.
**O6. A novel endothelial cell based complement dependent cytotoxicity test in kidney transplantation**

R.G.M. Lammerts; J. van den Born; W.A. Dam; B.G. Hepkema; BJ Kroesen; M.R. Daha; M.A.J. Seelen; S.P. Berger

*Department of Nephrology, UMCG*

**Objective:** Relevant alloantibodies in kidney transplantation comprise anti-HLA antibodies, blood group antibodies (ABO incompatible) and anti-endothelial cell antibodies (AECA) and can initiate antibody-mediated rejection (ABMR). ABMR is characterized by complement activation products, such as C4d, on the endothelium of the microvasculature in the kidneys at the time of rejection. However, endothelium directed cytotoxicity of the various antibodies and the role of complement (regulation) are not fully elucidated.

**Methods:** In this study, we set up a flow cytometry method and an endothelial complement dependent cytotoxicity test (EC-CDC) to evaluate the involvement of various transplant related antibodies in the process of complement mediated endothelial damage. We used primary endothelial cells (EC) cultured from donor kidney perfusate after machine perfusion, circulating human EC progenitors or conditionally immortalized human glomerular EC. Antibody binding and complement activation was evaluated by FACS analysis and compared to classical donor lymphocyte CDC (L-CDC) and EC-CDC.

**Results:** Firstly, ABO incompatible serum caused complement mediated cell cytotoxicity in the EC-CDC. Secondly, sera containing high titer HLA-DSA that tested negative in the negative L-CDC, caused complement dependent cytotoxicity in the EC-CDC. This correlated with increased IgG binding and activation of C3 by flow cytometry. Serum, suspected to contain AECA, caused abundant cell death in the EC-CDC whereas no cytotoxicity was seen in the L-CDC.

**Conclusion:** We successfully developed an EC-CDC and flow cytometry method to assess complement dependent endothelial cell damage and thereby show DSA mediated cytotoxicity and cytotoxicity of AECA that were undetectable by the classical L-CDC method, confirming the potential value of endothelial cell based cross-matching in transplantation.
O7.

Angiotensin-neprilysin inhibition causes a blood-pressure independent renoprotective effect in diabetic hypertensive rats

Estrellita Uijl12, Lodi C.W. Roksnoer12, Marian C. van Groningen3, Robert Zietse2, Jaap A. Joles4, Ewout J. Hoorn2 and A.H. Jan Danser1

1Division of Pharmacology and Vascular Medicine, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands, 2Division of Nephrology and Transplantation, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands, 3Department of Pathology, Erasmus MC, Rotterdam, The Netherlands, 4Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands

Objective: We recently showed that dual blockade with an Angiotensin Receptor/Neprilysin Inhibitor (ARNI) reduces proteinuria and glomerulosclerosis more strongly than single AR blockade in rats with diabetes and hypertension. Here, we investigated whether this renoprotective effect is due to better blood pressure regulation, improved renal hemodynamics and/or suppression of renal inflammation.

Methods: TGR(mREN2)27 rats (a model of angiotensin II-dependent hypertension) were made diabetic with streptozotocin for 12 weeks, and treated with placebo (n=10), valsartan (n=7) or sacubitril/valsartan (ARNI; n=8) for the final three weeks. Renal histological scores were analyzed by a blinded renal pathologist.

Results: Sacubitril/valsartan and valsartan monotherapy lowered telemetric blood pressure similarly, with a change from baseline mean arterial pressure of -50 ± 4 mm Hg and -43 ± 4 mm Hg, respectively (P=0.3). Both valsartan and sacubitril/valsartan lowered albuminuria. However, only sacubitril/valsartan additionally reduced cardiac hypertrophy, and renal histological scores for chronic renal ischemia and globally sclerotic glomeruli. As expected, urinary atrial natriuretic peptide (ANP) concentration was high in vehicle-treated controls (1.5 ± 1.1 ng/h), most likely due to uncontrolled hypertension. Of interest, valsartan lowered urinary ANP (0.5 ± 0.2 ng/h) whereas sacubitril/valsartan caused higher levels (1.6 ± 0.9 ng/h), despite similar blood pressure reduction. Sacubitril/valsartan, but not valsartan alone, preserved eRPF (10.7 ± 1.0 mL/min vs. 6.5 ± 0.9 mL/min; P=0.04 vs control), and a similar trend was observed for GFR (2.1 ± 0.4 mL/min vs. 1.4 ± 0.2 mL/min; P=0.1 vs control). No treatment affected filtration fraction or infiltration of immune cells in the kidney.

Conclusion: Angiotensin-neprilysin inhibition causes a blood-pressure independent renoprotective effect. This renoprotective effect may be due to a direct effect of ANP on the glomerular filtration barrier rather than through renal hemodynamics, as filtration fraction remained unchanged.
O8. Metabolite-sensing in human proximal tubule epithelial cells stimulates indoxyl sulfate secretion

Jitske Jansen, Katja Jansen, Amr Othman, Ruben Poesen, Björn Meijers, Majorie B. van Duursen, Rosalinde Masereeuw

\[1\] Div. Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, the Netherlands. \[2\] Department of Microbiology and Immunology, Division of Nephrology, University Hospitals Leuven, Leuven, Belgium. \[3\] Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Objective: Recently, we established 3D bioengineered kidney tubules capable of active metabolites (e.g. uremic toxins) secretion through the concerted action of essential renal transporters. We hypothesize that renal cells can sense elevated metabolite levels and can stimulate their renal excretion. In this study, we evaluated our hypothesis in healthy volunteers and examined the metabolite-sensing renal excretion pathway in 2D cultures and 3D bioengineered kidney tubules.

Methods: Healthy volunteers were subjected to high protein diet for 14 days. Urinary indoxyl sulfate concentrations were measured using LC-MS/MS and RNA was extracted from urinary renal epithelial cells prior and post high protein diet.

Results: Urinary indoxyl sulfate secretion was significantly enhanced (p<0.01) and organic anion transporter-1 (OAT1) gene expression tends towards an increase, though not significant. Proximal tubule epithelial cells exposed to indoxyl sulfate (100 µM) for 24h, showed an increased gene expression of OAT1 (p<0.001) compared to control. An Aryl hydrocarbon receptor (Ahr) reporter assay showed that indoxyl sulfate is an Ahr ligand (p<0.001) and stimulated nuclear translocation of the aryl hydrocarbon receptor nuclear translocator protein (ARNT; p<0.001). Enhanced transport was inhibited by an Ahr antagonist CH-223191 (p<0.001) as well as an EGF receptor ligand cetuximab (p<0.001). Indoxyl sulfate exposure in the presence of cetuximab counteracts ARNT translocation (p<0.001). Bioengineered 3D kidney tubules exposed to indoxyl sulfate for 24h were perfused using a microfluidics system and results tends towards an increased secretory clearance of indoxyl sulfate (123 ± 18% of control).

Conclusion: The Ahr-ARNT complex and concomitant EGFr signaling plays a pivotal role in the metabolite-sensing regulatory secretion pathway in renal epithelial cells. This mechanistic insight provides opportunities for the development of novel therapeutic avenues to preserve kidney function in patients.
O9. 
*In vitro* models to detect circulating permeability factors in patients with recurrent FSGS

den Braanker DJW¹, Deegens JKJ¹, Maas R¹, Schreuder MF², Wetzels JFM¹, van der Vlag J¹, Nijenhuis T¹

¹Departments of Nephrology and, ²Pediatric Nephrology, Radboudumc, Nijmegen, the Netherlands

**Objective:** Patients with recurrent focal segmental glomerulosclerosis (FSGS) suffer from heavy proteinuria after kidney transplantation, often within 24 hours. Immediate start of plasma exchange therapy can reduce proteinuria. It is suggested that a circulating plasma permeability factor (CPF) causes the glomerular injury in patients with (recurrent) FSGS. Many candidate CPF have been proposed. However, none has been validated, because results could not be reproduced by other groups, or because only one experimental model was applied. Before CPF can be identified, reproducible methods to detect the presence of CPF in plasma of patients with recurrent FSGS are required. We aim to establish experimental models to detect CPF.

**Methods:** Cell viability of differentiated conditionally immortalized human podocytes (hPod) and mouse glomerular endothelial cells (mGEnC) was assessed using Cell Counting Kit-8. Trans endothelial electrical resistance (TEER) and albumin passage across a mGEnC monolayer were measured. In all assays, cells were exposed to 10% plasma for 24 hours. Plasma of twelve patients with steroid-resistant FSGS was used, in which known mutations linked to glomerular disease were excluded, and one patient with a laminin mutation. In nine patients FSGS recurred after kidney transplantation, four patients were not transplanted. Patient plasma was compared to pooled plasma of five healthy donors.

**Results:** Plasma of five patients significantly decreased hPod viability, compared with pooled healthy donor plasma. Plasma of five patients significantly reduced mGEnC viability. Plasma of four patients did not decrease viability, but clearly affected hPod or mGEnC morphology. Plasma of two patients (of which one with laminin mutation) did not affect viability or morphology. Plasma of three patients induced detachment of mGEnC and significantly decreased TEER and increased albumin passage.

**Conclusion:** Our results showed that CPF in plasma of patients with steroid-resistant FSGS can be detected using our *in vitro* hPod and mGEnC cell viability models. mGEnC TEER and albumin passage models were less sensitive. Plasma CPF activity was demonstrated to specifically act on hPod, mGEnC, or both. Thus, while responses to CPF-containing plasma could be measured, we did not yet find one or more models that uniformly detect the presence of CPF. Further research is needed to explain why some plasmas affect hPod and some mGEnC.
**O10.**

**Heparan sulfate biosynthesis enzymes differentially determine complement factor H/factor H-related protein binding to glomerular endothelial cells**

Markus A. Loeven¹, Simon C. Satchell², Toin H. van Kuppevelt³, Johan van der Vlag¹

¹Department of Nephrology, ³Department of Biochemistry, Radboudumc, Nijmegen, NL. ²Academic Renal Unit, University of Bristol, Southmead Hospital, Bristol, UK

**Objective:** Unregulated complement activation is characteristic of the renal diseases atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G). Complement factor H (FH) regulates activation on host tissues by binding to host cell glycans, particularly heparan sulfates (HS). FH-related proteins (CFHRs) further modulate complement regulation by competing with FH for cell surface ligands. HS is synthesized by a complex biomachinery and extensively modified by acetylation, sulfation and epimerization, which determines protein binding. Disturbed renal HS biosynthesis could affect binding of FH/CFHRs, resulting in aHUS/C3G. Therefore, we investigated the role of HS biosynthesis enzymes in FH/CFHR binding to conditionally immortalized human glomerular endothelial cells (ciGEnCs).

**Methods:** HS biosynthesis enzyme expression (EXT1, NDST1, NDST2, GLCE, HS2ST and HS6ST1) was silenced in ciGEnCs using siRNA. Knockdown was evaluated by quantitative PCR and functional effects on HS domain expression and binding of FH, CFHR1, CFHR2 and CFHR5 was determined using ELISA/flow cytometry. Relevant HS modifications were further investigated by competing with selectively desulfated heparin for immobilized ciGEnC HS (HS_Glx).

**Results:** FH, CFHR1 and CFHR5 bound to ciGEnCs, while CFHR2 did not. Depositing C3b on ciGEnCs significantly increased binding of FH/CFHRs and competition with all tested CFHRs during alternative pathway activation led to complement dysregulation on ciGEnCs. HS biosynthesis enzyme knockdown differentially reduced binding of HS-specific antibodies AO4B08 and EW3D10. Silencing NDST2, GLCE, HS2ST and HS6ST1 expression significantly reduced binding of FH, whereas CFHR1 binding appeared to be mediated by NDST1, NDST2 and GLCE. Binding of CFHR5 to ciGEnCs was relatively unaffected by RNA interference. CFHR1 and CFHR5 blocked the interaction between FH and HS_Glx. While FH binding depended on N-, 2-O- and 6-O-sulfation, CFHR1 and CFHR5 binding depended primarily on N-sulfation and was generally more sensitive to competition with selectively desulfated heparins.

**Conclusion:** In conclusion, pathogenic changes in HS biosynthesis, particularly a reduction in HS2ST and HS6ST1 activity, could shift the balance of FH/CFHR binding to glomerular endothelium, resulting in complement activation and aHUS/C3G. Competition with 2-O-/6-O-desulfated heparins however might be able to restore complement control on glomerular endothelial cells, by selectively reducing CFHR binding to glomerular HS while retaining FH-mediated complement inhibition.
O11. 
Primary coenzyme Q10 deficiency and renal disease: case series and review of literature

Anne M. Schijvens, MD1, Charlotte M.H.H.T. Bootsma-Robroeks, MD PhD1, H.J. (Eric) Steenbergen, MD PhD2, E.A.M. (Marlies) Cornelissen, MD PhD1, Nicole C.A.J. van de Kar, MD PhD3, Michiel F. Schreuder, MD PhD1

1Departments of Pediatric Nephrology, Amalia Children’s Hospital, and 2Pathology, Radboud university medical center, Nijmegen, the Netherlands

Objective: Mitochondrial cytopathies include a group of diseases with impaired oxidative phosphorylation, characterized by multi organ involvement, large variability in both phenotype and genotype and a progressive course of disease. Renal symptoms caused by mitochondrial cytopathies are rarely isolated. Nonetheless, in patients with a primary CoQ10 deficiency, renal dysfunction might be the only manifestation at presentation. Early diagnosis of CoQ10 deficiency enables treatment in a pre-symptomatic stage and might prevent or postpone its consequences. Unfortunately, no clear phenotype – genotype correlation is known, as a result of which diagnosis is often delayed and irreversible damage has already taken place. In this report, we describe the clinical course of three patients with a primary Q10 deficiency to underline the importance of early diagnosis. In addition, we provide a review of current literature and give recommendations to enhance early diagnosis.

Methods: We identified three patients with variable renal phenotypes who were ultimately diagnosed with a primary Q10 deficiency. The first patient is an 18 year old girl with mild cognitive impairment, and diagnosed with chronic kidney failure of unknown origin at the age of 14. The second and third patient both presented with a steroid resistant nephrotic syndrome at 24 and 19 months of age, respectively.

Results: In the presented patients and in literature, there is a large clinical and genetic heterogeneity of coenzyme Q10 deficiency. Patient 1 rapidly progressed to end stage renal disease after which genetic analysis revealed a mutation in the ADCK4 gene. The second patient suffered from a multidrug resistant nephrotic syndrome. A bilateral nephrectomy was required. Afterwards, genetic analysis showed a combined heterozygous COQ2 mutation. The third patient was diagnosed with steroid resistant nephrotic syndrome and ultimately went into remission with additional immunosuppressive medication. As lactate levels were elevated at the time of diagnosis, the suspicion of a mitochondrial cytopathy was raised. Genetic analysis revealed a homozygous pathogenic mutation in the COQ2 gene. Based on the genetic results, in all three patients, mitochondrial CoQ10 levels in leukocytes were measured and were found to be significantly decreased. Oral coenzyme Q10 supplementation was initiated. Two out of three patients already suffered from neurological and/or renal damage.

Conclusion: Early diagnosis of primary coenzyme Q10 deficiency is crucial as oral supplementation of CoQ10 may limit disease progression and improve clinical symptoms. Q10 deficiency should be explored in children with steroid resistant nephrotic syndrome and chronic kidney failure with unknown etiology.
O12.
Bone marrow derived myeloid cells contribute to tissue capsule formation in a rat model of chronic kidney disease for generation of in vivo tissue engineered blood vessels

T. Bezhaeva¹, W. Geelhoed¹, D. Wang², H. Yuan², F.R. Damanik³, AJ van Zonneveld¹, P.H.A. Quax⁴, L. Moroni³, Song Li² and J.I. Rotmans¹
¹Departments of Internal Medicine and Einthoven Laboratory for Experimental Vascular Medicine, and ⁴Vascular Surgery Leiden University Medical Center, The Netherlands ²Department of Bioengineering, University of California, Los Angeles, USA ³Dept. of Regenerative Medicine, Maastricht University, The Netherlands

Objective: Tissue engineered blood vessels (TEBVs) could offer a suitable alternative for arteriovenous conduits, circumventing the limitations of synthetic grafts and avoiding the need for maturation of fistulas. Recently, we developed a novel method to generate TEBVs by utilizing the foreign body response directed to a subcutaneously implanted polymeric rod, which culminates in the formation of a fibrocellular tissue capsule (TC). Upon extrusion of the polymer rod, the remaining TC is grafted into the vasculature whereupon it differentiates towards a blood vessel. Up to now, the origin of cells in this TC remains unknown. In the present study we aimed to elucidate on whether cells are derived from the bone marrow (BM) precursors, or local proliferation and transdifferentiation of resident cells contribute towards TC formation in the setting of chronic kidney disease (CKD).

Methods: 26 Sprague Dawley (SD) rats were randomly divided into 2 experimental groups: healthy rats (n=10) and rats with CKD (n=16). Both groups received BM transplantation from transgenic SD-EGFP rats ubiquitously expressing GFP whereupon the CKD group underwent 5/6 nephrectomy. Renal function was monitored and the efficacy of the BM transplant was assessed by FACS analysis. Next, polymeric rods were subcutaneously implanted and left in place for 1 and 3 weeks. TCs were harvested and their cellular composition as well as gene expression profile was analysed.

Results: CKD was established and persistent during the whole period of TC formation as confirmed by elevation in serum creatinine (WT 0.48±0.11 vs. CKD 1.1±0.34) and BUN levels (WT 18.6±5.4 vs. CKD 40.25±14.21). The percentage of GFP+ cells in peripheral blood 3 weeks after BM transplantation was comparable between the groups (WT 73.82±6.39 vs. CKD 74.83±5.88). Cellular organization of TC gradually changed in time from a disorganized highly nucleated structure at 1 week towards circumferentially aligned tissue at 3 weeks in both WT and CKD groups (Fig.1A). TC harvested after 1 week were GFP+/CD68+, confirming contribution of BM derived cells in TC formation. Interestingly, the most inner layer of the TC at 3 weeks was composed of αSMA+/CD68+ cells (Fig.1B).

Conclusion: Early cellular response upon rods implantation was characterized by a high number of GFP+ BM derived cells detected in TC after 1 week post implantation. Further transdifferentiation of CD68+ macrophages towards αSMA+ myofibroblasts underline a crucial role of inflammatory cells as precursors of myofibroblasts in matured TC. Initial observation did not show any difference in TC thickness between CKD and WT groups, making our in vivo approach of autologous vascular tissue engineering a relevant strategy for future clinical use in hemodialysis patients.
Objective: Chronic kidney disease (CKD) is a condition marked by progressive loss of kidney function, which can eventually lead to end stage renal disease. Genome wide association studies (GWAS) identified many genetic risk factors for CKD. However, linking these common variants to genes that are causal for CKD etiology remains challenging. In our study we aimed to assess the effect of CKD-associated genetic variation on transcriptional activity of DNA regulatory elements such as enhancers and repressors, followed by identification of genes regulated by these elements to complement classic GWAS annotation which links candidate genes primarily based on genetic variation in or near protein coding regions.

Methods: In a proof of principle approach, regulatory regions in linkage disequilibrium with CKD-associated variant rs11959928 were cloned from 20 individual donors in the self-transcribing active regulatory region (STARR) reporter plasmid. The produced library of STARR plasmids, which contain a minimal promoter followed by an incorporated candidate enhancer sequence, was transformed in primary renal endothelium and epithelium. The activity of each enhancer in these primary renal cells, was reflected by its ability to induce promoter activity leading to RNA transcription of the enhancers sequence, allowing parallel assessment of all genomic variation in the enhancer. Additionally, 36 CKD-associated susceptibility regions that co-localize with DNA regulatory elements were used as bait in circular chromosome conformation capture sequencing (4C-seq) in primary renal endothelium and epithelium to identify target genes of regulatory elements that are potentially compromised in their function by CKD-associated common variants.

Results: Five common variants in a regulatory element in linkage with the CKD-associated SNP rs11959928 strongly affected allele specific activity in both examined cell types. Four of these variants had a reference allele frequency of 45.2-74.8% in the library input, yet virtually only the reference alleles were transcribed in both cell types. The fifth allele had a wild-type penetrance of 31.8-36.5% in the library input, but its frequency was strongly reduced in the transcribed RNA, illustrating that disease-associated SNPs may not only affects gene coding sequences, but might also affect transcriptional regulation. Our 4C-seq analyses revealed interactions of regulatory regions within CKD-associated susceptibility regions with the transcriptional start sites of 304 target genes (p<1E-08), identifying these genes as putative target genes of dysfunctional transcription regulatory elements.

Conclusion: Our data not only functionally demonstrates the potential effect of common small variants on transcriptional regulation, we also annotated multiple new genes to previously reported CKD–associated SNPs and provided first time evidence for interaction between these loci and their putative target genes. This current pipeline provides a novel technique for hypothesis generation and complements classic CKD GWAS interpretation.
O14. Diagnostic exome sequencing in 254 dutch patients with familial and sporadic kidney disease

Ilse M. Rood¹, Marlies E.A. Cornelissen², Jeroen K.J. Deegens¹, Tom Nijenhuis¹, Rutger J. Maas¹, Erik-Jan Kamsteeg³, Nicole van de Kar², Linda Koster², Jeroen Schoots³, Michiel F. Schreuder², Jack F.M. Wetzels¹, Ernie M.H.F. Bongers³*, Dorien Lugtenberg³†

Departments of ¹Nephrology, ²Pediatrics, and ³Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

Objective: Diagnostic exome sequencing has proven to be an effective strategy for identifying mutations in heterogeneous disorders. We implemented diagnostic exome sequencing, using filtered data for known renal disease genes, to investigate the genetic diagnostic yield of clinically and genetically heterogeneous renal diseases by using one test in routine diagnostics.

Methods: In a clinically heterogeneous cohort of 254 unrelated Dutch patients with suspected hereditary renal disorders, diagnostic renal disease gene panel exome sequencing (up to 226 genes) was performed as a first step. Exome sequencing data was analyzed for (likely) pathogenic variants and copy number variations in known renal disease genes using a bioinformatic applied filter. If no causative mutations were identified, patients were offered whole exome analysis (beyond the renal disease gene panel) in a second step.

Results: In the renal disease gene panel analysis, (likely) pathogenic mutations were identified in 89 out of 254 patients (35%). In total, 33 novel variants in 20 distinct genes were detected. The diagnostic yield in patients with glomerular disease, cystic renal disease, electrolyte disorders, and renal insufficiency of unknown cause was 39%, 35%, 25% and 63%, respectively. Whole exome analysis discovered a novel renal disease gene (CLDN10) in one patient and revealed variants in 12 candidate genes in 12 patients. Exome sequencing facilitated re-analysis of the complete cohort for the recently published gene for nephrotic syndrome, MAGI2, but it did not demonstrate causative mutations.

Conclusion: We conclude that a two-step procedure of exome sequencing analysis is a powerful diagnostic strategy for identifying causative mutations of hereditary renal diseases in a heterogeneous patient cohort using one test, including the systematically detection of copy number variations. This approach enables an easy update of the renal disease gene panel, easy re-analysis of exome data and novel gene discovery providing emerging evidence for its application as a primary genetic test in the diagnostic work up of patients suspect for a hereditary renal disease.
P1. Sodium-sensitive blood pressure response in type 1 diabetes is accompanied by impeded skin lymphangiogenesis

E.F.E. Wenstedt\textsuperscript{1}, MD; N.M.G. Rorije\textsuperscript{1}, MD; R.H.G. Olde Engberink\textsuperscript{1}, MD; B.J.H. van den Born\textsuperscript{2}, MD PhD; J. Aten\textsuperscript{3}, PhD; L. Vogt\textsuperscript{1}, MD PhD

\textsuperscript{1} Department of Internal Medicine, Division of Nephrology, Academic Medical Center, University of Amsterdam, the Netherlands; \textsuperscript{2} Department of Internal Medicine, Division of Vascular Medicine, Academic Medical Center, University of Amsterdam, the Netherlands; \textsuperscript{3} Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands

**Objective:** New insights in sodium homeostasis revealed that sodium can be non-osmotically stored within the skin. In animal models, high sodium diet (HSD) increased skin sodium content and attracted skin macrophages, inducing an increment of the lymphatic capillaries in both amount and size. Disruption of this system was associated with sodium-sensitive hypertension. This study investigates the effects of HSD on skin lymphatic and blood capillaries as well as blood pressure (BP) in type 1 diabetic patients (DM1), who are known to be prone for sodium-sensitive hypertension.

**Methods:** We performed a randomized crossover study in males with DM1 and healthy controls. All subjects pursued an 8-day low sodium diet (LSD: <50 mmol Na+/day) and HSD (>200 mmol Na+/day). Diet order was randomized and time in-between diets was 1-2 weeks. After each diet, BP measurements and skin biopsies were obtained. Macrophages, vascular endothelium and lymphatic endothelium were identified through immunohistochemistry, using antibodies for CD68, CD31 and D2-40, respectively. Analysis was performed using ImageJ Software (National Institutes of Health, USA). Macrophage and capillary density were defined as the number of macrophages or capillaries per megapixel. Lymphatic cross sectional surface area was determined by the average inner surface area of the lymphatic capillaries as a percentage of the histological slice surface area.

**Results:** This study included 8 patients with DM1 and 12 controls (mean age (SD) 28 (6) vs. 23 (4)) who were similar regarding BMI (23 vs. 22, p=0.49) and eGFR (121 vs. 120, p=0.83). In DM1 patients, mean arterial pressure was higher after HSD as compared to LSD (mean (SD) 85(5) vs. 80(3) mmHg, p=0.03) whereas in controls no differences were observed (78(5) vs. 78(5) mmHg, p=0.66). HSD increased lymphatic cross sectional surface area in controls (p=0.01) but not in DM1 patients (p=0.25). Less CD68+ macrophages were present in DM1 patients compared to controls (LSD: p<0.001, HSD: p<0.001). In both groups, there was a strong association between lymphatic capillary density and macrophage density (DM1 r=0.57 p=0.02; controls r=0.71 p=0.02).

**Conclusion:** The sodium-sensitive BP increase in DM1 patients is accompanied by impeded skin lymphangiogenesis and reduced skin macrophage content. Lymphangiogenesis may help to prevent sodium-sensitive hypertension.
P2.  
*NPH1* gene deletions cause ESRD in 0.9% of adult-onset cases

Snoek R1*, van Setten J2, Keating BJ3, van der Zwaag A1, Knoers NVAM1, de Borst MH4*, van Eerde AM1*

1 Department of Genetics and Center for Molecular Medicine, University Medical Center Utrecht, The Netherlands, 2 Department of Cardiology, University Medical Center Utrecht, The Netherlands, 3 Department of Surgery, Penn Transplant Institute, Philadelphia, PA, USA, 4 Department of Nephrology, University Medical Center Groningen, The Netherlands (*on behalf of the TransplantLines-Genetics consortium)

**Objective:** Nephronophthisis (NPH) is the most prevalent (15%) genetic cause for end-stage renal disease (ESRD) in children and 16% is caused by mutations in the autosomal recessive *NPH1* gene. Little is known about the prevalence of these in adult-onset ESRD. With data generated to perform genome-wide association studies in adult-onset ESRD patients, we aim to determine the prevalence of homozygous *NPH1* full gene deletions.

**Methods:** Renal transplant recipients were genotyped using the Affymetrix Axiom Tx GWAS Array, designed for the iGeneTRAIN consortium, containing ~350,000 evenly spaced probes to cover the whole genome. Autosomal copy number variants (e.g. deletions) were determined based on median log2 ratios. All findings were independently validated. In this abstract we report on n=1272, from the TransplantLines-Genetics cohort. At the time of the conference we will have expanded this to n≈4000 and other than Caucasian ethnicities, as we are currently analyzing data from various cohorts participating in the iGeneTRAIN consortium. Cases are included in the analysis when they had adult-onset ESRD, defined as start of renal replacement therapy (RRT) at age ≥ 18 years.

**Results:** 1250 cases met the age criteria, of whom 11 (0.9%) showed a homozygous deletion of the *NPH1* gene. Median age at start of RRT was 35 years (range 18-42), with eight cases aged ≥30. Notably only three out of 11 cases (27%) were diagnosed as having NPH. The other cases (8/11, 73%) were noted as chronic kidney disease with unknown etiology (n=5), glomerulonephritis (n=1), sporadic primary reflux nephropathy (n=1) and autosomal dominant polycystic kidney disease (n=1).

**Conclusion:** NPH is a classical pediatric kidney disease. However, we show that homozygous *NPH1* full gene deletions alone cause 0.9% of all adult-onset ESRD, with the majority of *NPH1* cases being aged ≥30 years. Considering that other types of mutations in *NPH1* were not analyzed, and the other 19 known NPH genes were not even investigated, NPH is a relatively frequent cause of adult-onset ESRD. As only 27% of *NPH1* cases was registered as NPH, these results warrant wider application of genetic testing in adult-onset ESRD.
P3. 
Long-term outcomes of arteriovenous fistulas and grafts in a large European cohort

Bram M Voorzaat, MD1, Koen EA van der Bogt, MD2, Friedo W Dekker, PhD3, Joris I Rotmans, MD, PhD1

Departments of Internal Medicine (1), Surgery (2) and Clinical Epidemiology (3), Leiden University Medical Center

Objective: For hemodialysis (HD), arteriovenous fistulas (AVFs) are the preferred type of vascular access (VA). Most data on VA durability originate from North America. As practice patterns and patient characteristics differ between Europe and the US, we evaluated outcomes of radiocephalic AVFs, upper arm AVFs and AVGs in a large retrospective cohort of Dutch HD patients.

Methods: This Dutch Vascular Access Study cohort consists of 1,656 VAs in 1,221 patients from 8 hospitals. To obtain independent observations, only the first successfully matured VA of each patient was included. Primary patency started at VA creation and ended at the first intervention or abandonment. Functional patency started at the first cannulation and ended at abandonment. Patency was censored at death or transplant. Patency outcomes are presented as median VA survival and analysed using Kaplan-Meier analysis. Hazard ratios for patency loss were calculated using Cox regression analysis using RCAVF as the reference. Procedure rates are presented per year of functional patency.

Results: 863 VAs (420 RCAVF, 341 upper arm AVF, 102 AVG) were analysed. The median primary patency for RCAVF was 13.8 ± 1.8 months, for upper arm AVFs 26.6 ± 4.5 months and for AVGs 11.4 ± 1.8 months. The hazard ratio for loss of primary patency was higher for AVGs than RCAVF (HR 1.52, 95% confidence interval: 1.18 – 1.96), and lower for upper arm AVFs (HR 0.74, 0.60 – 0.90). The median of death-censored functional patency was not met during the study follow-up (fig. 1). At 48 months 82% of RCAVF, 79% of upper arm AVFs and 73% of AVGs were still functionally patent (death-censored). The number of procedures was lowest for RCAVF (0.8 ± 2.1/year) versus upper arm AVFs (1.4 ± 8.7/year) and AVGs (2.5 ± 5.8/year).

Conclusions: In the Dutch Vascular Access Study cohort, long-term functional patency was comparable between the 3 groups of arteriovenous access configurations. However, the number of procedures required to maintain AVG patency is 3-fold higher compared to RCAVF.

Funding: unrestricted research grant by Proteon Therapeutics

[Figure 1] – Death-censored primary and functional patency
Abstracts NFN fall meeting 2017

P4. Dyslipidemia in nephrotic rats associates with abnormalities in hepatic lipoprotein receptors

Pragyi Shrestha1, Daphne Dekker2, Wendy Dam1, Bart van de Sluis2, Jacob van den Born1

1Dept Nephrology and 2Dept Pediatrics, section Molecular Genetics, Univ. Med. Center Groningen, The Netherlands

Objective: Liver is the primary organ for clearance of triglyceride-rich remnant lipoproteins (TRL) via LDL receptor (LDLR), Lipoprotein Receptor-related Protein (LRP-1) and by the heparan sulfate side chains of syndecan-1. Hepatic LDLR degradation might occur by Proprotein convertase subtilisin/kexin type 9 (PCSK9). Elevated plasma TRL, probably as a result of abnormalities in hepatic clearance, could be a major cause of dyslipidemia, cardiovascular diseases and mortality in chronic kidney disease (CKD). We therefore aim to investigate the expression of hepatic lipoprotein receptors and PCSK9 in a dyslipidemic proteinuria rat model.

Methods: Eight male Wistar rats received 1.8 mg adriamycin/kg BM i.v. in order to induce nephrotic kidney disease. Six control rats were injected with saline. General parameters including kidney function, proteinuria, serum triglycerides and cholesterol were monitored weekly. Animals were sacrificed 12 weeks after disease induction and tissues and plasma were collected. Liver tissues were evaluated for the expression of LDLR, LRP-1, syndecan-1, heparan sulfate and PCSK9 by immunofluorescence staining, western blotting and qRT-PCR. Plasma PCSK9 was measured by ELISA. Statistical difference and correlations were tested by Mann Whitney test, Dunnett’s multiple comparison test and Spearman Rank correlation.

Results: Rats treated with adriamycin showed increased proteinuria, decreased creatinine clearance and increased serum triglycerides and cholesterol (all p<0.001) compared to control group. Although no differences were found in the protein and mRNA expression of hepatic LDLR, LRP-1 and syndecan-1, we found that the expression of liver PCSK9 was increased in adriamycin treated rats compared to the control group (p<0.001, immunofluorescence), but not in mRNA and total protein levels (qRT-PCR and Western blot). Besides, hepatic heparan sulfate expression (evaluated by FGF2 binding) was significantly reduced in livers of adriamycin rats compared to control group (p<0.02) despite unchanged expression levels of heparan sulfate synthesizing and degrading enzymes. Increased serum triglycerides and cholesterol were correlated with increased hepatic PCSK9 (r=0.83, p=0.0004 / r=0.80, p=0.001 respectively) and serum PCSK9 (r=0.83, p=0.0001 / r=0.82, p=0.0001 respectively). Serum cholesterol was inversely correlated with hepatic heparan sulfate staining (p<0.03). Similarly, urine protein concentration and plasma creatinine were also correlated with increased serum PCSK9 (r=0.63’ p=0.022/ r=0.70, p=0008).

Conclusion: These data show that dyslipidemia in adriamycin nephrotic rats is associated with abnormalities in PCSK9 and syndecan-1 (via its heparan sulfate side chains). These data suggest loss of TRL clearance capacity under nephrotic conditions.
P5. The limits of cold ischemia time in kidney transplantation of deceased donors

Hessel Peters-Sengers¹, Julia H.E. Houtzager², Mirza M. Idu³, Martin B.A. Heemskerk³, L.W. Ernest van Heurn², Jaap J. Homan van der Heide¹, Stefan P. Berger⁴, Jesper Kers⁵, Thomas M. van Gulik², Frederike J. Bemelman¹

¹Department of Nephrology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, ²Department of Surgery, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands, ³Dutch Transplant Foundation, Leiden, the Netherlands, ⁴Department of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands, ⁵Department of Pathology, Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands

Objective: Cold Ischemic Time (CIT) is a well-known risk factor among the renal transplant community, however its precise limits to define high-risk donor kidneys for transplantation are not yet clear. There is evidence that limits of CIT are lower for donation after circulatory-death (DCD) compared to donation after brain-death (DBD) kidneys. The aim of this study was to compare precise limits for CIT in DCD and DBD donor kidneys with graft failure and mortality as outcome, and relate these limits of both donor types to donor age.

Methods: We used the prospective Dutch Organ Transplantation Registry (NOTR) to include recipients (n=2153) aged 18 years or older, of a first kidney from a DBD donor (n=1266, 58.8%) or a DCD category III donor (n=887, 41.2%) between January 1, 2005 and January 1, 2012. Donor kidneys are preserved with static cold storage. Kidneys allocated to overseas patients and donors aged ten years or younger were excluded. CIT was non-linearly modelled with splines. Associations and interactions between CIT, donor type, donor age and five-year (death-censored) graft survival and mortality were evaluated.

Results: Median CIT was 16.2 hours (IQR 12.8 – 20.0), ranging from 3.4 hours to a maximum of 44.7 hours. With ten hours of cold ischemia time as reference, adjusted risk of five-year graft failure was significantly higher at 14 hours for DCD kidneys (hazard ratio (HR), 1.88, 95%CI 1.01-3.50, p=0.046), and this risk was significantly higher at a later stage of 17 hours for DBD kidneys (HR 1.57, 95%CI 1.01-2.43, p=0.042). At a cold ischemia time of 22 hours or higher, adjusted five-year graft failure risk was significantly higher for circulatory-death donor kidneys compared with brain-death donor kidneys (HR for 22 hours: 1.45, 95%CI 1.01-2.49, p=0.043). At a cold ischemia time of 19 hours transplanting a kidney from a 60 year-old circulatory death donor kidney, increased the risk of graft failure compared to kidney from a 60 year-old brain-death donor (adjusted HR 1.33, 95%CI 1.00-1.78, p=0.045). The additional insult of increased cold ischemia in kidneys from circulatory-death donors was also found for death-censored graft failure, but did not show significant impact on the outcome of mortality.

Conclusion: Our study shows that warm ischemically damaged donor kidneys in the DCD procedure are more vulnerable to CIT in static cold storage. If CIT exceeds 22 hours, the risk of graft failure is significantly higher for DCD kidneys compared to DBD kidneys. A lower limit of 19 hours applies for older DCD donor kidneys. Future studies can implicate whether there may be a different limit for CIT when using machine perfusion.
P6.
Effect of SGLT2 inhibition on fibroblast growth factor 23 and 25(OH) vitamin D in the IMPROVE trial

M.A. de Jong¹, S.I. Petrykiv², G.D. Laverman³, D. de Zeeuw², Stephan J.L. Bakker¹, H.J.L. Heerspink² M.H. de Borst¹

Div. of¹ Nephrology and ²Pharmacology, UMCG, Groningen, the Netherlands, ³Dept of Nephrology, ZGT, Almelo and Hengelo, the Netherlands

Objective: Sodium glucose co-transporter 2 (SGLT2) inhibitors like dapagliflozin are novel drugs for the treatment of diabetes mellitus, which also promise to slow the progression of kidney disease. Previous studies found that SGLT2 inhibition increases serum phosphate and PTH. However, the effects on fibroblast growth factor 23 (FGF23) and vitamin D are less well studied.

Methods: This is a post-hoc analysis of a double-blind, randomized, cross-over trial, enrolling patients on stable RAAS blockade, albumin:creatinine ratio between 100 and 3500 mg/g, eGFR ≥ 45 ml/min/1.73m² and HbA1c ≥ 55 and <100 mmol/mol. Patients were treated with dapagliflozin 10 mg/d (DAPA) or placebo during 2 consecutive periods of 6 weeks each, with a 6-week wash-out in between. Plasma C-terminal FGF23 was measured with ELISA (Immutopics Inc), 25(OH) vitamin D with LC-MS/MS. Data are shown as mean (95%CI). Endpoints were assessed with linear mixed models.

Results: Thirty-three patients (age 61±9 yrs; 24% female; median 24h UAE 470 mg/24hr) completed the study. Baseline characteristics and results are shown in table 1. DAPA increased serum phosphate, PTH and FGF23 compared to both baseline and placebo. Serum calcium and 25(OH)D did not change (p=0.9, p=0.8). DAPA reduced eGFR, but change in eGFR and change in bone and mineral parameters were not correlated (all P>0.5). All effects of DAPA were reversed 6 weeks after discontinuation.

Table 1: baseline values and mean change (95%CI) from baseline during Dapa and Placebo study periods. * denotes P < 0.01 vs baseline. † denotes P< 0.01 vs placebo.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Δ Placebo</th>
<th>Δ DAPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate (mg/dL)</td>
<td>3.37 (3.19;3.59)</td>
<td>0.03 (-0.12;0.19)</td>
<td>0.25 (0.09;0.40)*†</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>48.0 (40.5;55.4)</td>
<td>1.9 (-2.2;6.1)</td>
<td>10.4 (5.7;14.1)*†</td>
</tr>
<tr>
<td>FGF23 (RU/mL)</td>
<td>124.3 (100.6;153.6)</td>
<td>4.9% (-5.3;16.2%)</td>
<td>24.9% (12.3;38.8%)*†</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9.42 (9.26;9.58)</td>
<td>0.06 (-0.04;0.20)</td>
<td>0.06 (-0.04;0.19)</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>19.29 (16.21;22.37)</td>
<td>-1.4 (-3.2;0.4)</td>
<td>-1.3 (-3.1;0.6)</td>
</tr>
<tr>
<td>eGFR (ml/min*1.73m²)</td>
<td>69.7 (63.5;76.4)</td>
<td>0.9 (-1.6;3.4)</td>
<td>-4.4 (-7.0;-1.9)*†</td>
</tr>
</tbody>
</table>

Conclusion: Dapagliflozin treatment induced a significant rise in serum phosphate, PTH and FGF23 levels, independent of concomitant effects on eGFR. Serum calcium and 25(OH)D levels remained unchanged. In light of the high prevalence of bone and mineral disorders in this population, future studies should assess the clinical significance of these alterations.
P7. Dietary protein and kidney function decline in older post-myocardial infarction patients: the Alpha Omega Cohort study

Kevin Esmeijer1,2, Johanna M. Geleijnse3, Johan W. de Fijter1, Daan Kromhout3,4, Ellen K. Hoogeveen1,2,5

1. Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands
2. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands
3. Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands
4. Department of Epidemiology, University Medical Center Groningen, Groningen, The Netherlands
5. Department of Nephrology, Jeroen Bosch Hospital, Den Bosch, the Netherlands

Objective: Chronic kidney disease is an independent risk factor for cardiovascular morbidity and mortality. Post-myocardial infarction (MI) patients have an accelerated decline of kidney function. Restriction of protein intake is one of the potentially modifiable factors to slow down kidney function decline, but little is known about the role of specific types of protein. The aim of this study was to examine dietary protein intake from different sources in relation to kidney function decline in stable older post-MI patients.

Methods: We measured serum cystatin C (cysC) and creatinine (cr) at baseline and after 41 months, in 2,426 Dutch post-MI patients of the Alpha Omega Cohort (79% men, aged 60-80, median time since MI 4 years). Data on dietary intake was collected using a 203-item food frequency questionnaire. We examined the association of protein intake (total, animal and vegetable) per g/kg ideal body weight with annual cystatin C and combined creatinine-cystatin C based glomerular filtration rate (eGFR_{cysC} or eGFR_{cr-cysC}) by multivariable linear regression.

Results: Of all patients, 19% had diabetes, 56% had a blood pressure of ≥140/90 mmHg, and 23% were obese. At baseline, mean (SD) eGFR_{cysC} was 81.5 (19.5) mL/min/1.73m², mean annual eGFR_{cysC} decline was -1.30 mL/min/1.73m². The mean (SD) daily intake of total, animal and vegetable protein intake was 1.05 (0.28), 0.65 (0.23), and 0.41 (0.11) g/kg ideal body weight. After multivariable adjustment, every incremental intake (g/kg ideal body weight) of total protein was associated with an additional annual eGFR_{cysC} decline (95% CI) of -1.59 (-2.60 to -0.59) mL/min/1.73m²; for animal protein it was -1.68 (-2.73 to -0.62) and for vegetable protein it was -1.03 (-3.43 to 1.36). Analyses of specific sources of animal protein showed that each incremental 50 g/day (about one portion) intake of red meat was associated with an additional annual eGFR_{cysC} decline of -0.31 (-0.57 to -0.05) mL/min/1.73m². Intake of white or processed meat, and dairy was not associated with eGFR_{cysC} decline (all p>0.5). Results were comparable when eGFR_{cr-cysC} was used as an outcome.

Conclusion: In contrast to dietary vegetable protein, intake of animal protein, especially red meat, was associated with accelerated kidney function decline in post-MI patients.
P8. 
Comparison of estimated versus measured GFR decline in the association with ESRD and mortality

Marieke HC van Rijn, Karen Leffondre Marie Metzger, Martin Flamant, Jean-Philippe Haymann, Benedicte Stengel, Jan AJG van den Brand

Department of Nephrology, Radboudumc, Nijmegen, The Netherlands (on behalf of the NephroTest study group)

Objective: We compared the association of eGFR and mGFR decline with risk for ESRD and mortality in patients with CKD.

Methods: We included 1734 adult patients with CKD stage 1 to 4 who had a total of 4790 simultaneous eGFR and mGFR measurements, over a median 3.3-year follow-up (IQR: 2.0-5.4). mGFR was measured with 51Cr-EDTA renal clearance and CKD-EPI eGFR was based on IDMS-traceable creatinine. We used shared parameter joint models to estimate the association between current value and slope of eGFR or mGFR and ESRD or death, adjusted for baseline age, gender, and albumin to creatinine ratio (ACR).

Results: Patients (mean age 59±15 yrs, 31% women) had a median of 2.0 (IQR 1.0-4.0) visits, a mean mGFR of 43.5 ml/min/1.73m², and a median ACR of 8.0 mg/mmol (IQR: 1.5-46.2). eGFR and mGFR decline was comparable, 1.87 (2.02-1.73) versus 1.88 (2.04-1.73) mL/min/1.73m2/year, respectively. HRs for death were similar for both current value and slope of mGFR or eGFR. In contrast, HRs for ESRD were lower when using current value and slope of mGFR than eGFR.

Conclusion: This study shows that the association of GFR slope with mortality is similar whether using eGFR or mGFR, but not that with ESRD. The hazard ratio of ESRD is lower with mGFR than eGFR. Therefore, mGFR decline may be considered as an alternative endpoint for ESRD rather than eGFR in clinical trials.

<table>
<thead>
<tr>
<th></th>
<th>HR for death (95% CI)</th>
<th>HR for ESRD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mGFR</td>
<td>CKD-EPI eGFR</td>
</tr>
<tr>
<td>Current GFR (ml/min/1.73m²)</td>
<td>0.98 (0.97 – 0.99)</td>
<td>0.98 (0.97 – 0.99)</td>
</tr>
<tr>
<td>Current GFR slope (ml/min/1.73m² per year)</td>
<td>0.89 (0.78 – 1.02)</td>
<td>0.89 (0.79 – 0.99)</td>
</tr>
<tr>
<td>2log ACR (mg/mmol)</td>
<td>1.04 (0.99 – 1.09)</td>
<td>1.05 (1.00 – 1.09)</td>
</tr>
</tbody>
</table>

ACR: Urinary albumin to creatinine ratio
P9. Clinical consequences of different albumin measurement methods in patients with membranous nephropathy

Anne-Els van de Logt¹, Coralien Vink¹, Sanna Rijpma², Miranda van Berkel², Jack Wetzels¹

¹ Dept. Nephrology and ² Clinical Laboratory, Radboudumc, Nijmegen, The Netherlands

Objective: Albumin levels are often used to define disease or predict risk. Cut off values are used to define nephrotic syndrome or advise toward the use of prophylactic anticoagulant therapy in patients with membranous nephropathy (MN). However, differences between albumin assays, although recognized (Bachmann 2016) are often unnoticed. In this study we aimed at quantifying differences between different albumin assays (immunonephelometry (reference method), bromcresol green (BCG) and bromcresol purple (BCP)) in nephrotic patients with MN.

Methods: Plasma samples were collected from nephrotic patients with MN. Samples were stored at -80 °C. Albumin was measured with BCG, BCP and immunonephelometry in plasma in our hospital. A round robin was organized to compare the results of the BCG assay used in three regional hospitals.

Results: We included 23 MN patients (83 % male), mean age was 60 ± 13 years, median serum creatinine level was 107 µmol/l (IQR 83-152) and median protein creatinine ratio 6.3 g/10 mmol (IQR 3.9-8.9). Mean serum albumin in nephelometric assay was 21.1 ± 4.5 g/l. Whereas bias with the BCP was limited (0.3 ± 1.8 g/l), BCG reported higher bias (5.1 ± 1.9 g/l). Three regional hospitals, that used BCG, also reported higher serum albumin values. Variation between centers was large. Bias in hospital 1 and 3 was very high; respectively 6.7 ± 2.1 g/l and 7.7 ± 2.3 g/l. In hospital 2 a bias comparable with our assay (4.8 ± 1.9 g/l) was noticed. Calculated accuracy of prophylactic anticoagulant therapy using a cut-off value of 25 g/l ranged between 53 % and 83 %, indicating that up to 47 % of patients might not receive appropriate therapy.

Conclusion: A large bias and impression was noticed between different albumin assays in patients with MN. The BCG assay overestimates serum albumin values. There were large between-centers differences. Inaccuracy of serum albumin assays will contribute to incorrect treatment decision. Clinicians should be aware of these variations. More attention to calibration and standardization is urgently needed.
P10.
Explaining factors for ethnic differences in estimated GFR in the Netherlands: the HELIUS study

B.J.M.V. Huisman, B. Hafkamp, C. Agyemang, M.B. Snijder, R.J.G Peters, B.J.H van den Born, L. Vogt.

Academic Medical Center, University of Amsterdam

Objective: Ethnic minority groups have a higher prevalence of chronic kidney disease (CKD) as compared to subjects from Dutch origin. Besides albuminuria class, CKD definition is based on estimated glomerular filtration rate (eGFR). eGFR has however never been validated for the various ethnic groups living in the Netherlands and might lead to uncertainty in CKD staging. We investigated to what extent ethnic differences in eGFR among 6 ethnic groups living in Amsterdam, the Netherlands, can be explained by differences in demographics and traditional cardiovascular risk factors.

Methods: Baseline data from the HELIUS study, a multi-ethnic cohort study, were used. Analyses were conducted among 18,534 participants (aged 18-70 years) of Dutch, South-Asian Surinamese, African Surinamese, Ghanaian, Moroccan and Turkish ethnic origin. We used multiple regression analyses to determine ethnic differences in eGFR (CKD-EPI definition), with additional adjustments for age, sex, educational level and traditional cardiovascular and kidney risk factors to study to what extent they explained the ethnic differences in eGFR.

Results: Mean (SE) eGFR was higher in all ethnic minority groups as compared to those of Dutch origin (eGFR 95mL/min/1.73m$^2$), ranging from 1.9±0.41 in subjects from South-Asian Surinamese origin to 15±0.39mL/min/1.73m$^2$ in subjects from Moroccan origin. Adjustment for age, gender, educational level, BMI, diabetes mellitus, hypertension, smoking, non-HDL-cholesterol and albuminuria diminished the ethnic differences in eGFR for most ethnic groups but these differences were still highly significant (Table 1).

Conclusion: Our results show that eGFR is higher among ethnic minority groups as compared to people from Dutch origin. Regarding previously found higher CKD prevalence in ethnic minority groups of HELIUS, these findings prompt for development of ethnicity specific GFR estimations. The currently recommended use of the CKD-EPI-based eGFR, which only distinguishes 2 ethnic groups, might lead to an underestimation of CKD risk and other eGFR-associated outcomes in multi-ethnic populations.

Table 1 Differences in eGFR between ethnic minority groups vs Dutch participants

<table>
<thead>
<tr>
<th>eGFR (ml/min/1.73m$^2$)</th>
<th>Difference (± SE)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1 (crude)</td>
<td>Dutch (reference)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>South-Asian Surinamese</td>
<td>1.9 ± 0.41</td>
</tr>
<tr>
<td></td>
<td>African Surinamese</td>
<td>7.9 ± 0.39</td>
</tr>
<tr>
<td></td>
<td>Ghanaian</td>
<td>9.0 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Turkish</td>
<td>12.5 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>Moroccan</td>
<td>15.0 ± 0.39</td>
</tr>
<tr>
<td>Model 2 (adjusted)</td>
<td>Dutch (reference)</td>
<td></td>
</tr>
<tr>
<td>Age, sex, BMI, Educational level</td>
<td>South-Asian Surinamese</td>
<td>1.0 ± 0.34</td>
</tr>
<tr>
<td>Diabetes mellitus, Hypertension</td>
<td>African Surinamese</td>
<td>8.7 ± 0.32</td>
</tr>
<tr>
<td>Smoking, Non-HDL cholesterol</td>
<td>Ghanaian</td>
<td>7.6 ± 0.38</td>
</tr>
<tr>
<td>Albumin/creatinine ratio</td>
<td>Turkish</td>
<td>7.3 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>Moroccan</td>
<td>9.8 ± 0.33</td>
</tr>
</tbody>
</table>

Abstracts NFN fall meeting 2017
P11.
Genetic research in structural kidney malformations in humans: What’s next?

Anukrati Nigam, Nine V.A.M. Knoers, and Kirsten Y. Renkema

Department of Genetics, Center for Molecular Medicine, UMC Utrecht, Utrecht University, Utrecht, The Netherlands

Objectives: Congenital anomalies of the kidney and urinary tract (CAKUT) consisting of varied structural malformations is the major cause of end-stage renal disease in children, occurring in 3-6 per 1000 live births. Major types of CAKUT involve kidney dysplasia, duplex renal collecting system, and obstructive ureter abnormalities. Up till now, research in CAKUT has been mostly focused on rare monogenic causes, which seem to explain only 10-20% of CAKUT cases. The genetic etiology is largely unknown. Involvement of genetic factors is supported by the occurrence of familial cases and the presence of CAKUT in known genetic multi-organ syndromes. So far, multiple genetic approaches have been directed towards the protein coding parts of the genome. We believe that the understanding of the genetic etiology is more challenging because of genetic and phenotypic heterogeneity and incomplete penetrance of the phenotype. Thus, our aim is to identify all genetic variation in severe CAKUT patients by whole genome sequencing (WGS). By annotating coding as well as non-coding variants and the integration with cross-omics data will lead to the identification of key pathways involved in CAKUT etiology.

Methods: Case-parent trios DNA samples of multicystic dysplastic kidney patients from the AGORA data- and biobank will undergo WGS on the Illumina HiSeq X Ten platform. The WGS data will be processed using an in-house pipeline, involving Burrows-Wheeler Aligner (BWA; Wellcome Trust Sanger Institute) for mapping of sequence reads to the reference human genome and Genome Analysis Tool Kit (GATK; Broad Institute) for germline variant calling. Variant annotation and prioritization will be done based on the minor allele frequency, gene location, coding effect, and \textit{in silico} predictions based on protein domain conservation and amino acid changes. A genome-wide association study (GWAS) will be performed on SNP array data from CAKUT cases and unaffected controls; and analyzed using PLINK. Prioritization and interpretation of genetic markers for CAKUT will be performed using bioinformatics tools like DEPICT (Data-driven Expression-Prioritized Integration for Complex Traits) and FUMA GWAS (Functional Mapping and Annotation of Genome-Wide Association Studies). Further, WGS data will be integrated with GWAS, RNA sequencing, and ChIP-Seq data by using bioinformatic tools including R/bioconductor packages and pathway analysis tools. Appropriate functional characterization experiments will be applied for the identified candidate variants.

Results: WGS data analysis and extensive data integration might lead to the identification of causal variants, molecular dysfunction of a particular gene, regulatory elements, interacting partners, and essential molecular pathways involved in CAKUT etiology.

Conclusion: This study will bring us one step closer towards understanding the complex etiology of kidney diseases using current state of the art methods of Next Generation Sequencing.
Decline of kidney function during the pre-dialysis period in chronic kidney disease patients: a systematic review and meta-analysis

Cynthia J. Janmaat¹, Merel van Diepen¹, Cheyenne C.E. van Hagen¹, Joris I. Rotmans², Friedo W. Dekker¹, Olaf M. Dekkers¹,²

Departments of ¹Clinical Epidemiology and ²Internal Medicine, Leiden University Medical Center, Leiden, the Netherlands

Objective: Substantial heterogeneity exists in reported kidney function decline in pre-dialysis chronic kidney disease (CKD). By design, decline rates can be studied in either CKD 3-5 cohorts or dialysis-based studies. In the former, patients are followed from a certain point in the pre-dialysis phase and not all patients start dialysis therapy, while in the latter patients are selected into the study based on the fact they initiated dialysis at some point. Decline rates obtained from dialysis-based studies could overestimate the true underlying kidney function decline in the pre-dialysis period. We performed a systematic review and meta-analysis, assessing and comparing the rate of kidney function decline during the pre-dialysis period in CKD stage 3-5 patients, as estimated in these two different study types.

Methods: We searched PubMed, EMBASE, Web of Science and Cochrane to identify eligible studies reporting kidney function decline during pre-dialysis in adult CKD patients. Random-effects meta-analysis was performed to obtain weighted annual mean estimated glomerular filtration rate (eGFR) declines (mL/min/1.73m²). With random-effects meta-regression analysis annual eGFR declines were compared between CKD 3-5 cohorts and dialysis-based studies.

Results: We identified 1231 unique publications that were assessed for eligibility; 60 publications were included (43 CKD 3-5 cohorts, 17 dialysis-based studies). Mean follow-up until dialysis initiation ranged between 0.2-8.2 years. Mean baseline eGFR ranged between 10-45 and 6-35 mL/min/1.73m² for CKD 3-5 cohorts and dialysis-based studies, respectively. The meta-analysis yielded a weighted annual mean [95%-confidence interval (95%-CI)] eGFR decline during pre-dialysis of 2.4 (2.2, 2.6) mL/min/1.73m² in CKD 3-5 cohorts compared to 8.5 (6.8, 10.1) in dialysis-based studies. This difference was confirmed with meta-regression (difference 6.0 (95%-CI: 4.8, 7.2) mL/min/1.73m²).

Conclusion: Dialysis-based studies report faster mean annual eGFR decline during pre-dialysis than CKD 3-5 cohorts. Importantly, guidance for clinical decision-making in CKD patients and anticipated treatment choices should be based on eGFR decline data from CKD 3-5 cohorts. Similarly, these cohorts should also be used for power calculations in RCTs with CKD progression during pre-dialysis as outcome.
P13. 
Estimated GFR to predict post-donation renal function in living kidney donors

Marco van Londen, Jessica van der Weijden, Stephan J.L. Bakker, Stefan P. Berger, Gerjan Navis, and Martin H. de Borst

Department of Internal Medicine, Division of Nephrology, University Medical Center Groningen, Groningen, the Netherlands

Objective: We tested the capacity of pre-donation estimated GFR (eGFR) equations to predict post-donation measured GFR (mGFR). We also calculated pre-donation eGFR cut-offs leading to an adequate post-donation mGFR.

Methods: In 873 living donors who donated in our center between 1984 and 2017, we prospectively measured creatinine-based eGFR (CKD-EPI, Cockcroft-Gault, MDRD), 24 hour urinary creatinine clearance and mGFR (continuous iotholamate) before and at 3 months after donation. We used linear regression to test the performance of the different eGFR formulas. We also calculated pre-donation eGFR cut-offs with a 95% specificity for post-donation mGFR thresholds of 40, 50, 60 or 70 mL/min/1.73m² using receiver operating characteristic curves.

Results: Mean donor age was 53±12 years, 48% of donors were male. Pre-donation mGFR was 102±16 mL/min/1.73m² and post-donation mGFR. The pre-donation eGFR_Cockcroft-Gault displayed the strongest association with post-donation mGFR (st. β 0.51, p<0.001). Pre-donation eGFR cut-offs leading to post-donation mGFR are shown below:

<table>
<thead>
<tr>
<th>Required post-donation mGFR (mL/min/1.73m²)</th>
<th>40</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-donation eGFR cut-offs (mL/min/1.73m²)</td>
<td>77</td>
<td>93</td>
<td>99</td>
<td>105</td>
</tr>
<tr>
<td>eGFR_{CKD-EPI}</td>
<td>75%</td>
<td>36%</td>
<td>21%</td>
<td>10%</td>
</tr>
<tr>
<td>% of donors meeting cut-off</td>
<td>62%</td>
<td>25%</td>
<td>14%</td>
<td>10%</td>
</tr>
<tr>
<td>eGFR_{MDRD}</td>
<td>80</td>
<td>94</td>
<td>102</td>
<td>107</td>
</tr>
<tr>
<td>% of donors meeting cut-off</td>
<td>72</td>
<td>97</td>
<td>108</td>
<td>118</td>
</tr>
<tr>
<td>eGFR_{Cockcroft-Gault}</td>
<td>87%</td>
<td>34%</td>
<td>19%</td>
<td>10%</td>
</tr>
<tr>
<td>% of donors meeting cut-off</td>
<td>102</td>
<td>142</td>
<td>164</td>
<td>176</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>73%</td>
<td>25%</td>
<td>12%</td>
<td>8%</td>
</tr>
</tbody>
</table>

Conclusion: We show that the eGFR can be used in a subgroup of donors with a high probability of good post-donation renal function without requiring mGFR measurement. The mGFR remains important in the majority of donors with an eGFR below these cut-offs values.
P14. Pregnancy in renal transplant patients: is azathioprine better than a calcineurin inhibitor?

Dorien Feyaerts¹, Lina Rigodanzo Marins¹, Bram van Cranenbroek¹, Olivier W.H. van der Heijden², Henk W. van Hamersvelt³, Irma Joosten¹, Renate G. van der Molen¹

¹Departments of Laboratory Medicine, ²Obstetrics and Gynaecology, ³Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands

Objective: Pregnancy after renal transplantation (RTX) is associated with an increased risk of complications such as preeclampsia (PE) and preterm birth (PTB). In these patients, azathioprine (AZA) and calcineurin inhibitors (CNI) can be used as immunosuppressive drugs (ISD) during pregnancy. Previous research showed that ISD influence the composition and function of peripheral blood immune cells. Since uterine immune cells play an essential role in embryonic implantation, placentation, and fetal tolerance, we hypothesize that the choice of ISD may determine the degree to which the placental and peripheral immune function are affected, with consequences for maternal and fetal health.

Methods: We performed a retrospective study between 1997 and 2013 of 26 RTX patients (mean age 31 ± 4 years) who carried a total of 40 pregnancies. We investigated the maternal and fetal outcomes according to ISD use. Furthermore, we initiated a prospective flowcytometric study to characterize lymphocytes isolated from peripheral blood (PBMC), term placental tissue (DPMC), and cord blood (CBMC), obtained at the time of delivery from RTX patients and healthy pregnant controls.

Results: In the retrospective study 40% of pregnancies were complicated by PE, 47% by PTB, and 43% by low birth weight. Patients using CNI developed PE earlier as compared to patients using AZA (gestational age at onset respectively 32 wks and 37 wks, p=0.009). Proteinuria developed during pregnancy in most patients irrespective of type of ISD (AZA 0.50 g/L, p=0.008; CNI 0.84 g/L, p=0.002). No statistical differences were found in the course of renal function with respect to immunosuppressive regimens. Patients using CNI showed a higher diastolic blood pressure within 6 months post-delivery (p=0.005). Fetal outcomes were similar, although newborns of patients using CNI tended to have a lower birth weight.

Immunophenotyping showed a decreased percentage of CD4⁺CD25⁺CD127⁻ regulatory T cells (Treg) in PBMC from all RTX patients compared to healthy controls. CBMC from neonates born to RTX patients also contained a lower percentage of Tregs and a lower percentage of B cells as compared to neonates born to healthy controls, irrespective of the type of ISD used during pregnancy. Compared to healthy controls, the placenta of RTX patients contained increased percentages of Tregs in patients using AZA during pregnancy but not in patients using CNI.

Conclusion: The maternal and fetal outcomes in our retrospective study favor the use of AZA above CNI as a preferred immunosuppressive choice for pregnant RTX patients. Our preliminary immune profiling data showed an altered lymphocyte phenotype and suggests a role for Treg in the higher incidence of pregnancy related complications in pregnant RTX recipients.
P15.
Peritubular capillary loss in the first month after kidney transplantation is more pronounced in patients with rejection compared to delayed graft function.

Anke A. Keijbeck¹, Floor M.E.G. Steegh¹, Mariëlle A.C.J. Gelens², Ernst L.W. van Heurn³, Maarten H.L. Christiaans², Carine J. Peutz-Kootstra¹

Department of ¹Pathology, ²Internal Medicine, Maastricht University Medical Centre, Maastricht, The Netherlands. ³Department of Surgery, Academic Medical Centre, Amsterdam, The Netherlands

Objective: Loss of peritubular capillaries (PTC) in patients with chronic transplant dysfunction is associated with worse outcome. We have shown previously that PTC loss occurs in the first three months after transplantation and precedes renal function decline (Steegh et al. JASN 2011). PTC density in the first weeks after transplantation has not yet been studied.

Methods: A cohort of 205 patients, who had a kidney transplantation between August 2003 and December 2009 at the Maastricht University Medical Centre and of whom representative protocol biopsies were taken at transplantation, and 3 and 12 months posttransplant, was analysed. In 102 of these patients an indication biopsy was taken in the first month after transplantation because of delayed graft function (DGF) or rise of creatinine. PTC numbers were studied as described earlier (Steegh et al. JASN 2011).

Results: Recipients who underwent an indication biopsy more often received a DCD graft. Consequently the ischemia times were higher than in recipients who did not have an indication biopsy. Furthermore, patients with an indication biopsy developed more IF/TA 1 year after transplantation (p=0.04). In patients with indication biopsies, a significant loss of PTC density occurs already in the first month after transplantation (Figure 1 panel A) (p<0.01). This PTC loss is more pronounced in patients suffering from rejection than patients with DGF (Figure 1 panel B). However, in the rejection group there is a stabilisation of the PTC loss between 1 and three months, while in the DGF group there is further loss of PTCs (p<0.01).

Conclusion: We found that PTC loss occurs in the first weeks after transplantation. The pattern of PTC loss in the first 3 months after transplantation differs between patients with rejection and DGF. Prevention of microvascular damage during and early after transplantation may be crucial to prevent chronic transplant dysfunction.
P16.
Routine hemodialysis does not result in optimal plasma magnesium concentrations.

Niki H.J. Leenders, MD; Tiny Hoekstra, PhD; Frans J. van Ittersum, MD PhD; Joost G.J. Hoenderop, PhD; Marc G. Vervloet, MD PhD

1Department of Nephrology, VU University Medical Center, Amsterdam, The Netherlands
2Department of Physiology, Radboud University Medical Center, Nijmegen, The Netherlands

Objective: Lower plasma magnesium (Mg) concentrations have been associated with a higher overall and cardiovascular mortality in hemodialysis patients. The optimal level of plasma Mg in hemodialysis patients appears to be above the reference range for the healthy population (typically 0.70-1.00 mmol/L). Plasma Mg is not routinely measured after hemodialysis. Aim of this study was to determine the effect of standard hemodialysis treatment on plasma Mg.

Methods: Plasma Mg was measured in duplicate before (Mg_pre) and after (Mg_post) 6 consecutive dialysis sessions in 34 patients on a regular 3 times weekly hemodialysis schedule with a standard 0.50 mmol/L dialysate magnesium concentration.

Results: Mean Mg_pre was 0.88 mmol/L (SD 0.14), 76% of patients had a mean Mg_pre below 1.00 and the coefficient of intra-individual biological variation was 5.6%. Mean Mg_post was 0.78 (SD 0.05). During dialysis, there was a statistically significant change of Mg: mean intra-dialytic decrease 0.10 mmol/L (95%-CI 0.06-0.13). Analysis with linear mixed models showed that a 0.10 mmol/L higher Mg_pre was associated with a 0.03 mmol/L higher Mg_post (95%-CI 0.024-0.037). The equation derived from this model showed that Mg_post equalled Mg_pre (no intra-dialytic change) at a Mg_pre of 0.74 mmol/L and that there was an intra-dialytic decline of plasma Mg at higher Mg_pre values and an increase of plasma Mg at lower Mg_pre values. If added to the model, baseline factors including gender, age, serum albumin, hemoglobin, venous bicarbonate, height and weight; and dialysis characteristics including vascular access type, dialysis duration, ultrafiltration volume, blood flow and dialysis efficiency did not change this association.

Conclusion: In the majority of the hemodialysis patients Mg_pre is suboptimal. Routine hemodialysis further declines magnesium in the majority of patients. Current dialysate magnesium concentrations may be too low.
Abstracts NFN fall meeting 2017

P17.
Online hemodiafiltration effectively removes free light chain kappa

J. Sträter1, S.A. Nurmohamed1, N. van de Donk2, F.J. van Ittersum1·M.P.C. Grooteman1
1VU University medical center, department of nephrology; 2 VU university medical center; department of hematology

Objective: Acute kidney disease is a common complication in multiple myeloma (MM). There is an ongoing debate whether or not removal of free light chains (FLC) is beneficial in the recovery of renal function. In addition, it is not known which extracorporeal removal technique is most effective in removing FLC. Plasma-exchange (PE) and dialysis with high-cut-off (HCO) membranes are the methods most investigated. FLC kappa exists predominantly as a monomer with a molecular weight of 22.5 kDa and FLC lambda as a dimer with a molecular weight of 45 kDa. As online hemodiafiltration (HDF) with high flux dialyzers is very effective in removal of solutes with a molecular weight below 40 kDa, we hypothesized that online HDF will effectively remove FLC kappa in patients with multiple myeloma.

Methods: In two patients with FLC kappa related acute kidney injury, we applied daily postdilution online HDF with high flux dialyzers (Cordiax FX100 (Fresenius®)) in an attempt to reduce the FLC kappa content. Convection volume per treatment was targeted at 25L. Treatment duration was 4 hours. Vascular access: central venous catheter. Furthermore, anti-myeloma therapy was administered in order to reduce the rate of FLC production. The levels of FLC kappa were measured before and after hemodiafiltration in blood.

Patient A was diagnosed with kappa light chain MM with kappa to lambda FLC ratio of 699 (N 0.37-3.1). Bortezomib and dexamethasone were initiated immediately after diagnosis. He had a rapidly progressive renal failure suggestive of cast-nephropathy, though not histologically proven. FLC kappa levels were measured 12 times before and after dialysis.

Patient B was diagnosed IgG kappa plus kappa light chain plasma cell leukemia and was treated with carfilzomib, lenalidomide and dexamethasone. One week after starting treatment she developed acute anuric kidney failure due to histologically proven cast nephropathy. FLC kappa levels were measured 59 times before and after HDF.

Results: In patient A mean FLC kappa reduction rate of 57% per session was reached (achieved convection volume 23.4 ± 2.8 L/treatment [mean ±SD]). In patient B the mean FLC kappa reduction rate was 45% (achieved convection volume 20.0 ± 3.2 L/treatment). As demonstrated in the figures below, HDF online was very effective in removal of FLC kappa. As also depicted in the figures, the rebound of FLC kappa was high when therapy to reduce production failed. Ultimately both patients had recovery of renal function and could stop dialysis.

Conclusion: HDF online is effective in removing FLC kappa. Compared to data in the literature it may even be at least as effective as dialysis with HCO membranes and PE. It remains questionable to what extent HDF online added to the recovery of renal function in our patients. Large trials are necessary to resolve these issues.
P18. Do hemodialysis and peritoneal dialysis differ regarding their effect on coronary artery calcification?

Thijs T. Jansz\textsuperscript{1}, Franka E. van Reekum\textsuperscript{1}, Akin Özyilmaz\textsuperscript{2,3}, Marianne C. Verhaar\textsuperscript{1}, Brigit C. van Jaarsveld\textsuperscript{1}, on behalf of the NOCTx investigators.

\textsuperscript{1}Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, the Netherlands, \textsuperscript{2}Division of Nephrology, Department of Internal Medicine, University Medical Center Groningen, Groningen, the Netherlands, \textsuperscript{3}Dialysis Center Groningen, Groningen, the Netherlands, \textsuperscript{4}Department of Nephrology, VU University Medical Center, Amsterdam, the Netherlands

Objective: Identifying modifiable factors of vascular calcification in end-stage renal disease is crucial in light of the associated high cardiovascular morbidity and mortality. In this cross-sectional study, we aimed to compare coronary artery calcification and levels of biomarkers associated with vascular calcification in patients treated with hemodialysis and peritoneal dialysis.

Methods: We assessed coronary artery calcification using multi-slice computed tomography in 121 patients treated with hemodialysis (≤ 16 hours / week) and 46 patients treated with peritoneal dialysis, who were included in the NOCTx study (NCT00950573). Biomarker measurements were performed in a subset of 55 hemodialysis and 33 peritoneal dialysis patients using enzyme-linking immuno-assays and multiplex assays.

Results: Patients treated with hemodialysis were somewhat older (53.1 ± 12.2 versus 49.8 ± 15.1 years) and had been on dialysis longer (26 [IQR 12 – 57] versus 14 [IQR 7 – 33] months). In univariate and multivariate analyses, coronary artery calcification in hemodialysis patients (median score 208 [IQR 1 – 809]) was not significantly different from peritoneal dialysis patients (median score 84 [IQR 0 – 1066]). In hemodialysis patients, phosphate levels tended to be higher compared with peritoneal dialysis patients (1.68 ± 0.39 versus 1.59 ± 0.35 mmol/L). Osteoprotegerin was evidently lower in hemodialysis patients (2.95 ± 1.33 versus 3.43 ± 1.81 μg/L, p < 0.01), while fetuin-A and inactive matrix Gla protein (dp-ucMGP) levels did not differ significantly between hemodialysis and peritoneal dialysis patients. Only dp-ucMGP was independently associated with extent of coronary artery calcification. Inflammatory markers C-reactive protein, interleukin-1β and interleukin-6 did not differ significantly between hemodialysis and peritoneal dialysis patients. Probably due to intermittent fluid overload in hemodialysis, NT-proBNP was significantly higher in hemodialysis patients (2217 ± 1817 versus 1045 ± 1372 pmol/l, p = 0.01).

Conclusion: Uremia per se is detrimental for the coronary vasculature, seemingly irrespective of treatment with hemodialysis or peritoneal dialysis. Whether coronary artery calcification and its progression are affected by other renal replacement therapies needs further evaluation. Prospective studies are needed to elucidate the prognostic significance of the differences in levels of biomarkers associated with vascular calcification.
P19.
Anxiety symptoms are independently associated with all-cause mortality in chronic dialysis patients

R.W. Schouten¹,2, G.L. Haverkamp¹,2, E. Nadort¹,2, C.E.H. Siegert, F.W. Dekker³, A. Honig¹,4

¹Department of Nephrology, OLVG, Amsterdam, The Netherlands. ²Department of Psychiatry, OLVG, Amsterdam, The Netherlands. ³Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, the Netherlands. ⁴Hospital Psychiatry, VUmc, Amsterdam, the Netherlands. In cooperation with: Department of Nephrology, VU medical centre, Amsterdam, The Netherlands. Department of Nephrology, MHC hospital, The Hague, The Netherlands. Department of Nephrology, Haga hospital, The Hague, The Netherlands.

Objective: Depressive and Anxiety symptoms are common in patients with end stage renal disease receiving dialysis therapy. These symptoms have a large impact on the quality of life and are known to be associated with several adverse clinical outcomes, such as mortality. The association of depressive symptoms with mortality has been studied extensively in dialysis patients, in contrast to anxiety symptoms. The aim of this study is to examine the association between anxiety symptoms and mortality in a large dialysis cohort in the Netherlands.

Methods: 701 chronic dialysis patients were included in a prospective cohort study in 10 dialysis centres in the Netherlands from 2012 till January 2017. Both prevalent (n=437) and incident (n=264) patients on haemodialysis and peritoneal dialysis were included. Patients completed self-reports on depressive symptoms (Beck Depression Inventory), anxiety symptoms (Beck Anxiety Inventory), Quality of Life, Religion and Acculturation every 6 months. At the same moment biochemical parameters were measured. Patients were followed up with all-cause mortality as the primary end point. Depressive and Anxiety symptoms were analysed using a cut-off for depression and anxiety (BDI/BAI ≥16) and as a continuous variable. A cox proportional hazard model was used with several confounders including age, primary renal disease, co-morbidity and ethnicity to adjust the hazard ratio.

Results: The median follow-up time for the cohort was 1139 days (IQR 1120-1261). There were 172 deaths (26%), 142 transplantations (21%) and 33 patients were lost to follow-up (5%), 354 patients (50%) are still in the cohort in January 2017. 31% of the patients had depressive symptoms (cut-off BDI≥16) and 28% had anxiety symptoms (cut-off BAI≥16). Depressive symptoms at baseline showed an adjusted hazard ratio (HR) for mortality of 1.4 (CI 1.0-1.9, p=0.06). Anxiety symptoms showed an adjusted HR for mortality of 1.6 (1.2-2.6, p=0.02). Linear and logistic regression models show both anxiety and depressive symptoms are associated with hospitalisation rate. Multivariate adjustment included demographic, social, dialysis-related and clinical variables, such as the Charlson Comorbidity Score. When both depression and anxiety are present the adjusted HR for mortality is 1.7 (1.1-2.5, p=0.02), this is the case in 12% of the patients.

Conclusion: Anxiety symptoms are independently associated with adverse outcomes, such as hospitalisation rate and all-cause mortality. Furthermore, in our cohort anxiety symptoms show a higher hazard ratio for mortality compared to depressive symptoms. We recommend future studies to include both depressive and anxiety symptoms when assessing mental health in dialysis patients. Further studies are needed to investigate whether treating anxiety and depressive symptoms is effective in both quality of life improvement and improving clinical adverse outcomes.
P20.
Ethnic differences in depressive symptoms and its associated clinical outcomes

R.W. Schouten1,2, G.L. Haverkamp1,2, E.Nadort1,2, C.E.H. Siegert2, F.W. Dekker3, A. Honig1,4

1Department of Nephrology, OLVG, Amsterdam, The Netherlands. 2Department of Psychiatry, OLVG, Amsterdam, The Netherlands. 3Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, the Netherlands. 4Hospital Psychiatry, VUmc, Amsterdam, the Netherlands. In cooperation with: Department of Nephrology, VU medical center, Amsterdam, The Netherlands. Department of Nephrology, MHC hospital, The Hague, The Netherlands. Department of Nephrology, Haga hospital, The Hague, The Netherlands.

Objective: Depressive symptoms are common in patients receiving dialysis therapy for end-stage renal disease. Previous studies have concluded depressive symptoms are independently associated with hospitalisation rate and mortality. However several studies suggest there are ethnic differences in these associations. The aim of this study is to examine the ethnic differences in depressive symptoms at its association with hospitalisations and all-cause mortality.

Methods: 701 chronic dialysis patients were included in a multi-ethnic cohort study from 10 dialysis centres in the Netherlands from 2012 till August 2016. Both prevalent (n404) and incident (n297) patients on haemodialysis and peritoneal dialysis were included. Patients completed self-reports on depressive symptoms (Beck Depression Inventory), Anxiety (Beck Anxiety Inventory), Quality of Life, Religion and Acculturation every 6 months. At the same moment biochemical parameters were measured. Patients were followed up with all-cause mortality as the primary end point. Depressive and Anxiety symptoms were analysed using a cut-off for depression and anxiety (BDI/BAI ≥16) and as a continuous variable. A cox proportional hazard model was used with several confounders including age, primary renal disease, co-morbidity and ethnicity to adjust the hazard ratio. Logistic regression models were used to investigate association between hospitalisation and depressive symptoms.

Results: The median follow-up time for the cohort was 1139 days (IQR 1120-1261). There were 172 deaths (26%), 142 transplantations (21%) and 33 patients were lost to follow-up (5%), 354 patients (50%) are still in the cohort. 31% of the patients had depressive symptoms (cut-off BDI≥16) and 28% had anxiety symptoms (cut-off BAI≥16). Immigrant patients showed a higher prevalence of depressive symptoms compared to natives (40% vs 21% respectively). The effect of depressive symptoms on survival differs between ethnic groups. Natives have a hazard ratio on survival of 1.6 (p<0.05) vs 1.2 of immigrants (not significant). Further analysis is undergoing, investigating possible differences in biochemical mechanisms between ethnic groups.

Conclusion: Immigrant dialysis patients show a better survival compared to native dialysis patients, despite the higher prevalence of depression. Natives show a significant association between depressive symptoms and adverse outcomes, such as hospitalisation and survival. These associations are not present in immigrant dialysis patients. These differences might be explained by a difference in different coping mechanisms, different biochemical pathways or a different validity of the self-reports among ethnic groups. We recommend future studies to take ethnic differences into account when investigating mental health and associated adverse outcomes in dialysis patients.
P21.
Cognitive e-health therapy for depressive symptoms in dialysis patients: protocol for a randomized controlled trial ‘DIVERS-II’

R.W. Schouten\textsuperscript{1,2}, E.Nadort\textsuperscript{1,2}, C.E.H. Siegert\textsuperscript{2}, F.W. Dekker\textsuperscript{3}, P. van Oppen\textsuperscript{4}, A. Honig\textsuperscript{1,5}
\textsuperscript{1}Department of Nephrology, OLVG, Amsterdam, The Netherlands. \textsuperscript{2}Department of Psychiatry, OLVG, Amsterdam, The Netherlands. \textsuperscript{3}Department of Clinical Epidemiology, Leiden University Medical Centre, Leiden, the Netherlands. \textsuperscript{4}Department of Clinical Psychology, GGZ-Ingeest, VUmc, Amsterdam Public Health, Amsterdam, the Netherlands. \textsuperscript{5}Hospital Psychiatry, VUmc, Amsterdam, the Netherlands.


Objective: Depressive symptoms in patients receiving dialysis therapy are highly prevalent, underdiagnosed and undertreated. Randomized trials investigating the clinical and cost-effectiveness of psychotherapy with sufficient power are lacking. This study aims to investigate the clinical and cost-effectiveness of a tailor-made cognitive e-health therapy for depressive symptoms in dialysis patients.

Methods: This abstract describes a protocol for a multicentre, cluster randomized trial with two parallel arms. Usual care is being compared with a tailormade self-help cognitive based therapy (CBT) for depressive symptoms. We aim to include a total of 206 patients (103 per arm). The sample size calculation has been corrected for the design effect for the cluster-randomization. A cluster randomisation method will be used to reduce contamination, with a total of 40 clusters using the morning and afternoon shifts of the 10 dialysis centres. The self-help intervention is based on a frequently used problem solving therapy and has been tailor made for dialysis patients. The intervention consists of 5 modules with explanatory text, videos, exercises and weekly feedback of trained psychologists. Treatment as usual (TAU) is defined as the routine care patients receive in routine specialized mental healthcare, consisting of face-to-face CBT and antidepressant medications. We will not interfere with TAU, however we will carefully monitor treatment utilization through patients record and self-reported healthcare utilization. The primary outcome is lowering of depressive symptoms using the Beck Depression Inventory-II (BDI). Patients who score a BDI of 13 or higher will be randomized. Patients scoring a BDI of 12 or lower will be monitored in a parallel follow-up cohort. The main analysis will assess differences in BDI-score pre- and post-treatment between the 2 arms. An intention-to-treat (ITT) analysis will be used. Multilevel analysis accounts for baseline BDI, dialysis centres and clusters. Secondary aims consist of examining the cost-effectiveness in QALY’s using the SF-12 and EQ-5D. Biochemical changes in cortisol, inflammation and tryptophan before and after treatment will be examined to investigate possible biochemical pathways involved in depression in dialysis patients. We aim to start inclusion in December 2017. This project has been funded by ZonMw, OLVG and Stichting Zabawas.

Results: Not applicable.

Conclusion: Not applicable.
P22.
Subtypes of depressive symptoms and their association with mortality in dialysis patients

Robbert W. Schouten\textsuperscript{1,2}, Victor J. Harmse\textsuperscript{2}, Carl E.H. Siegert\textsuperscript{1}, Friedo W. Dekker\textsuperscript{3}, Wouter van Ballegooijen\textsuperscript{4}, Adriaan Honig\textsuperscript{2,5}

\textsuperscript{1}Department of Nephrology, OLVG hospital, Amsterdam, The Netherlands. \textsuperscript{2}Department of Psychiatry, OLVG hospital, Amsterdam, The Netherlands. \textsuperscript{3}Department of Clinical Epidemiology, Leiden, The Netherlands. \textsuperscript{4}Department of Clinical Psychology, GGZ Ingeest/VUmc/Amsterdam Public Health, Amsterdam, The Netherlands. \textsuperscript{5}Hospital Psychiatry, VUmc, Amsterdam, the Netherlands

Objective: Depression is a heterogeneous condition and common in dialysis patients. Unraveling specific subtypes of depression may help to improve screening and treatment. We aim to confirm the somatic-affective and cognitive-affective subtypes in dialysis patients and assess the relation of these subtypes with clinical outcomes, such as hospitalizations and mortality.

Methods: In this prospective study we examined subtypes of depression in dialysis patients with confirmatory factor analysis. We analyzed data of 684 prevalent and incident chronic dialysis patients from 10 dialysis centers in the Netherlands. Furthermore we examined the association between different subtypes of depressive symptoms and mortality, hospitalization and Quality of Life (QoL) using cox proportional hazards analysis and multivariate linear regression models with adjustment for socio-demographic, dialysis related and clinical characteristics.

Results: In total 684 dialysis patients were included, consisting of 431 prevalent and 242 incident patients. We distinguished somatic-affective and cognitive-affective dimensions of depression in this cohort. Somatic dimension scores were associated with all-cause mortality, increased hospitalization and a reduced QoL. Cognitive dimension scores were only associated with a reduced QoL. The association between the somatic subtype and clinical outcomes remained significant after adjustment for demographic factors, dialysis related and somatic comorbidity.

Conclusion: Compared to the cognitive dimension, somatic dimension were stronger associated with all-cause mortality, increased hospitalization and a reduced QoL. These findings show that the somatic subtype of depression needs specific clinical attention in screening and treatment of depressive symptoms in dialysis patients. Future research should take these symptoms dimensions into account when investigating depressive symptoms in dialysis patients, especially in the association with clinical outcomes.
P23.
The impact of symptoms on health related quality of life in elderly pre-dialysis patients; effect and importance in the EQUAL study

Pauline WM Voskamp¹, Merel v Diepen¹, Marie Evans², Fergus J Caskey³, Maurizio Postorino⁴, Maciej Szymczak⁵, Moniek vd Luijtgaarden⁶, Christoph Wanner⁷, Kitty J Jager⁶, Friedo W Dekker¹, on behalf of the EQUAL Study Investigators

¹Dept of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands, ²Dept of Clinical Sciences Intervention and Technology, Karolinska University Hospital Huddinge, Stockholm, Sweden, ³UK Renal Registry, Southmead Hospital, Bristol, UK, ⁴Clinical Epidemiology and Pathophysiology of Renal Diseases and Hypertension, Azienda Ospedaliera Bianchi Melacrino Morelli, Reggio Calabria, Italy, ⁵Dept of Nephrology and Transplantation Medicine, Wroclaw Medical University, Wroclaw, Poland, ⁶ERA-EDTA registry, Dept of Medical Informatics, Academic Medical Center, Amsterdam, The Netherlands, ⁷Division of Nephrology, University Hospital of Würzburg, Würzburg, Germany

Objective: Quality of life (QoL) is an important outcome in chronic kidney disease (CKD). Among the multiple determinants for QoL, patients feel symptoms are of high importance. However, the effect of symptoms on QoL is unknown. The aims of this study were to investigate the impact of symptoms on quality of life in pre-dialysis patients, assessed by both the effect of symptoms and their importance in addition to kidney function and other clinical variables.

Methods: We used participants of the EQUAL Study, a European prospective follow-up study in late stage 4/5 chronic kidney disease patients aged ≥65 years, who were included between March 2012 and December 2015. Baseline and six months follow-up data were used. Patients scored their symptoms with the Dialysis Symptom Index, and QoL with the RAND-36 item Health Survey (RAND-36). The RAND-36 results in a physical component summary score (PCS) and a mental component summary score (MCS). We used linear regression to estimate the relation between symptoms and QoL at baseline and over time, and to calculate the impact of symptoms on QoL expressed as explained variance. Adjustments were made for confounders. Missing data were imputed.

Results: 1079 (73%) patients filled in a baseline patient questionnaire (median age 75, 66% male, 98% Caucasian). Mean symptom number at baseline was 13 (SD 7). The most prevalent symptoms were fatigue and decreased interest in sex. At baseline the adjusted association (95% CI) between symptom number and MCS was -0.80 (-0.90 to -0.70), with PCS this was -0.52 (-0.63 to -0.40). The adjusted association between symptom number and MCS over time was -0.40 (-0.57 to -0.23), for PCS this was -0.16 (-0.26 to -0.05). Univariable explained variance of symptom number was 0.23 for MCS and 0.13 for PCS.

Conclusion: In pre-dialysis patients symptoms have a substantial impact on QoL: they negatively affect QoL and explain a large part of the variance in QoL.
P24.
Investigating the impact of home dialysis on quality of life, clinical outcomes, and costs: rationale and design of the DOMESTICO project

AA Bonenkamp¹, A van Eck van der Sluijs², JAJ Bart³, MH Hemmelder⁴, GA de Wit⁵, A Özyilmaz⁶, FM van der Sande⁷, FTJ Boereboom⁸, AJ Luik⁹, CWH de Fijter¹⁰, FJ van Ittersum¹, FW Dekker¹¹, MC Verhaar², AC Abrahams² en BC van Jaarsveld¹

¹VU medical center, Amsterdam ²UMCU, Utrecht ³Dutch Association of Kidney Patients (NVN), Bussum ⁴Medical Center Leeuwarden ⁵Julius Center for Health Sciences and Primary Care, Utrecht ⁶Dialysis Centre Groningen ⁷MUMC, Maastricht ⁸Dianet Dialysis Center, Utrecht ⁹VieCuri, Venlo ¹⁰Onze Lieve Vrouwe Gasthuis, Amsterdam ¹¹LUMC, Leiden

Objective: The percentage of patients treated with a form of home dialysis (either peritoneal dialysis or home haemodialysis) is steadily decreasing in the Netherlands, from 33% in 2002 to 18% in 2016. Previous studies show at least, comparable survival for home dialysis patients compared to in-centre haemodialysis patients. However, in the light of patient-centered care the focus of attention is turning away from mortality to patient reported outcomes. Quality of life, an important patient reported outcome measure, is suggested to improve with home dialysis. However, these data are obtained from small, outdated and cross-sectional studies and are thus limited. In addition, the dialysis population as a whole is ageing importantly, which makes findings in these studies less applicable to current patients. To identify which patients might benefit from home dialysis, new actual data are needed. Therefore, a large prospective follow-up study with adequate correction for confounders is necessary to determine the quality of life of home dialysis patients compared to patients treated with conventional in-centre HD.

Methods: Dutch nOcturnal and hoME dialysis Study To Improve Clinical Outcomes (DOMESTICO) is a multicenter, prospective, observational cohort study that will start including incident dialysis patient from December 2017. We aim to include 800 home dialysis patients and a comparison group of at least 800 in-centre haemodialysis patients. All dialysis patients >18 years are eligible for this study. The primary outcome is Quality of Life, measured every 6 months by Patient Reported Outcome Measures (SF-12, Disease Specific Index and Eq5D-5L). Secondary outcomes include clinical outcomes (hospitalisation, technique failure, phosphate and anaemia control, nutritional status, mortality) and cost-effectiveness. Randomization in this study is not feasible, due to patients’ strong preferences for treatment modality. Therefore, extensive adjustment for confounding factors will be applied.

Results: First results will be expected mid-2019.

Conclusion: Modality choice should be individualized with the aim to optimize the patient’s quality of life. DOMESTICO will investigate the effect of home dialysis on Quality of life in relation to clinical outcomes and costs in comparison to conventional in-center HD. This should help to identify which patients benefit the most from home-based dialysis.

Melissa Uij1,4, Chi M. Hau2, Mohamed Ahdi3, Victor Gerdes3, Sandrine Florquin1, Rienk Nieuwland2, Joris J.T.H. Roelofs1

1Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands, 2Laboratory of experimental clinical chemistry, Academic Medical Center, the Netherlands, 3Department of internal medicine, Slotervaart Hospital, Amsterdam, the Netherlands

Objective: Diabetic nephropathy (DN) is a major complication of diabetes and is characterized by a proinflammatory and procoagulant state. Microvesicles (MV) are small cell-derived vesicles that are secreted under physiological conditions, including inflammation and coagulation. MV numbers, size, cellular origin, composition and function can alter during disease. Evidence shows that MV can actively regulate cellular processes influencing disease progression. In this study, we characterized plasma MV in type 2 diabetes mellitus (T2DM) patients with albuminuria.

Methods: We divided 103 T2DM patients from the outpatient clinic for diabetes of the MC Slotervaart in 3 groups based on 24h urinary albumin levels; normoalbuminuria (<30mg/day), microalbuminuria (30-300mg/day), and macroalbuminuria (>300mg/day). Patients >65 years and smokers were not included in this study. MV from citrated plasma were stained with the live-cell labeling dye Calcein violet AM (CV), and simultaneously labeled for cell surface molecules CD14 (monocytes), CD235a (erythrocytes), CD3 (t-cells), CD45 (leukocytes), CD61 (platelets), CD62p (activated platelets), CD34 (endothelial cells), CD62e (activated endothelial cells), CD66b (granulocytes) or IgG1 control antibody. The number of double positive MV were determined using flow cytometry (A60-Micro, Apogee). This study was approved by a medical ethics committee and informed consent was obtained from all patients.

Results: The total number of CV-positive MV, erythrocyte-derived MV, and leukocyte-derived MV were significantly higher in both micro- and macroalbuminuric patients, compared with the diabetic controls. In addition, the number platelet-derived MV and granulocyte-derived MV were only increased in patients with macroalbuminuria compared with the diabetic controls. Activated endothelium-derived MV were significantly higher in microalbuminuria patient compared with the diabetes controls, and these patients show a trend towards higher monocyte-derived MV.

Conclusion: Our data show that MV from T2DM patients with micro- and macroalbuminuria display different profiles of cellular origin. DN patients have increased numbers of activated endothelial cell-, leukocyte-, and platelet-derived MV, which possibly reflects the procoagulant and proinflammatory state that accompanies DN. More research will be needed to further investigate the role of these MV in the progression of DN.
P26. 
Mobilization of nonosmotically stored sodium after water loading in healthy individuals

Rosa D. Wouda, Shosha Dekker, Joelle Reijm, Rik H. Olde Engberink, Liffert Vogt

Division of Nephrology, Academic Medical Center, Amsterdam, Netherlands

Objective: Recently it was discovered that significant amounts of sodium (Na⁺) can be stored without concurrent water retention. These observations indicate the presence of a third compartment for Na⁺ distribution. The role of this compartment under hypotonic conditions is not known. In this study we investigated whether Na⁺ can be released from its nonosmotic stores after a hypotonic fluid load.

Methods: Twelve healthy male subjects had a water loading test (WL; 20 ml water/kg in 20 min). During a 240 min follow-up, we compared the observed plasma [Na⁺], fluid and cation excretion with values predicted by the Barsoum-Levine and Nguyen-Kurtz formula. These formulas are used for guidance of fluid therapy during dysnatremia and do not account for nonosmotic Na⁺ stores.

Results: 30 min after WL plasma [Na⁺] was decreased with -3.2±0.5 mmol/L (mean (SE)), after which plasma [Na⁺] increased gradually. The observed maximal decrease in plasma [Na⁺] after WL was significantly overestimated by the Barsoum-Levine (-4.4±0.3 mmol/L) and Nguyen-Kurtz formula (-5.2±0.4 mmol/L)(p<0.05). In addition, 120 min after WL the Barsoum-Levine and Nguyen-Kurtz formula overestimated urine volume, while cation excretion was significantly underestimated with a cation gap of 54±18 mmol and 60±19 mmol, respectively (p<0.05). At 240 min, this gap was 29±18 mmol and 38±17 mmol, respectively (p=NS).

Conclusion: These data demonstrate that healthy individuals are able to mobilize osmotically inactivated Na⁺ after a hypotonic fluid load. Further research is needed to expand knowledge on the Na⁺ buffer and assess its impact on therapy of dysnatremia.
Ciliary phenotyping in urine-derived patient cells to determine the pathogenicity of novel variants in ciliopathy genes

Machteld M. Oud, Dorien Lugtenberg, Helger G. Yntema, Lisenka Vissers, Ronald Roepman, Ernie M. Bongers

Dept. of Human Genetics, Radboud University Medical Centre, Nijmegen, The Netherlands; Radboud Institute for Molecular Life Sciences, Nijmegen, The Netherlands

Objective: Ciliopathies are rare hereditary disorders caused by dysfunction of the cilium, a small signaling organelle present on nearly every human cell. Ciliopathies display extensive clinical and phenotypic heterogeneity, which complicates accurate diagnosis of patients. Studies of urine-derived renal epithelial cells (URECs) of ciliopathy patients vs controls allow for functional interpretation of variants of unknown significance detected by next-generation sequencing techniques. The aim of this study was to determine the pathogenicity of two novel IFT140 variants in a patient with visual impairment, vertical nystagmus, progressive hearing impairment, short stature, and mild skeletal abnormalities.

Methods: Mutations in IFT140, encoding an intraflagellar transport complex-A (IFT-A) protein, are associated with skeletal ciliopathies. Cilium length and ciliogenesis frequency was evaluated by immunofluorescence (IF) cytochemistry using markers of the ciliary axoneme. Dysfunction of the retrograde IFT-A complex can be visualized by IF staining showing an accumulation of IFT88 at the ciliary tip.

Results: The ciliary phenotype in URECs from the patient showed normal ciliogenesis, cilium length, and ciliary localization of IFT140, however, they showed abnormal retrograde IFT. About 40% of the cilia from the patient’s URECs showed an accumulation of IFT88 in the tip, whereas this was not seen in URECs from the healthy mother or controls, and only in 10% of the healthy father’s URECs.

Conclusion: URECs can be used to study the pathogenicity of variants in ciliopathy genes like IFT140. The novel mutations found in IFT140 cause abnormal intraflagellar transport, which supports the clinical diagnosis of short-rib thoracic dysplasia 9. Functional studies in urine-derived patient cells prove to be an attractive non-invasive procedure to facilitate accurate diagnosis of ciliopathy patients.
P28. Quantification of urinary extracellular vesicles

Charles J. Blijdorp¹, Thomas Hartjes¹, Martin E. Van royen¹, Robert Zietse¹, Ewout J. Hoorn¹

¹Department of Nephrology, Erasmus Medical Center, Rotterdam, Netherlands

Objective: Urinary extracellular vesicles (uEVs) have emerged as a powerful non-invasive tool to study renal epithelial transport in humans. However, the optimal method to quantify and normalize uEVs remains unclear, especially for spot urines. Our objective therefore was to determine (1) whether novel high-throughput methods to quantify uEVs could replace current standards and (2) whether it is justified to use urine creatinine concentration to normalize spot urines.

Methods: Four healthy subjects were subjected to overnight thirsting (10 pm-noon) followed by water loading (20 ml/kg in 30 min). Spot urines were collected during thirsting (T1-2) and after water loading (WL1-4, noon-7 pm). Subsequently, 4 uEV quantification techniques were compared: (1) nanoparticle tracking analysis (NTA), (2) uEV isolation by ultracentrifugation followed by immunoblotting of CD9, CD63, CD81, ALIX, and TSG101, (3) a timeresolved fluorescence immunoassay (TRFIA) that captures CD9+ uEVs, and (4) EVQuant, a novel technique which counts individual fluorescently labeled EVs after immobilization in a matrix. A Bland-Altman analysis was used to compare methods using NTA as reference.

Results: As expected, urine osmolality was near-maximal during thirsting, decreased after water loading and then increased again (Figure). The results of the 4 uEV quantification methods showed similar dynamics as urine osmolality suggesting that uEV number changes in proportion to urinary concentration (Figure). Of interest, EVQuant identified 2.4 ± 0.6 times more uEVs than NTA. Using NTA as reference, the Bland-Altman analysis showed that EVQuant had the lowest bias (% difference 6 ± 27) followed by TRFIA (10 ± 21). Of the uEV-markers, CD9 agreed best with NTA (-12 ± 34). uEV number correlated strongly with urine creatinine (Figure) and osmolality (r² for both 0.9, P<0.0001).

Conclusion: uEV number is proportional to urinary concentration and both urine creatinine and osmolality can be used to normalize spot urines for uEV number. EVQuant is a promising alternative to NTA and appears more sensitive for uEV detection. These uEV quantification methods be used to analyze if changes in a uEV protein of interest are the result of more protein per uEV or the excretion of more uEVs containing this protein.
P29.
Automatic segmentation of histopathological slides from renal allograft biopsies using artificial intelligence

Meyke Hermsen\textsuperscript{1}, Thomas de Bel\textsuperscript{1}, Milly van de Warenburg\textsuperscript{1}, Jimmy Knuiman\textsuperscript{1}, Eric Steenbergen\textsuperscript{1}, Geert Litjens\textsuperscript{1}, Bart Smeets\textsuperscript{1}, Luuk Hilbrands\textsuperscript{2}, Jeroen van der Laak\textsuperscript{1}

Departments of\textsuperscript{1}Pathology and\textsuperscript{2}Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands

Objective: Histopathological analysis of renal biopsies depends on the identification and assessment of specific histological structures. Both in research and routine diagnostics, this analysis can be time-consuming and suffer from observer variability. Recently, it has been shown that the combination of high resolution whole slide imaging (WSI) and artificial intelligence yields powerful new avenues for tissue section analysis. This study aims to develop an algorithm based on a specific type of artificial intelligence (a convolutional neural network; CNN) to fully automatically segment structures in cortical fragments of renal allograft biopsies. Automated segmentation of renal tissue allows an unbiased, reproducible computation of morphological characteristics of important structures. This in turn can be used as support in diagnostic quantitative measure-based decisions as for instance is employed in the Banff-classification.

Methods: The neural network was trained using a set of Periodic acid-Schiff (PAS) stained slides (n=26) of renal allograft biopsies. WSIs were produced using a 3DHISTECH Pannoramic 250 Flash II digital slide scanner with a 20x objective lens. We used a U-net architecture CNN, which has been proven to be specifically useful in biomedical image segmentation tasks. Training was based on exhaustive annotations in one to two randomly selected rectangular areas per WSI (size approximately 3000 x 4000 pixels; comparable to one 200x microscopic field of view). A total of nine classes were annotated: Glomeruli, Sclerotic glomeruli, Proximal tubuli, Distal tubuli, Atrophic tubuli, Undefined tubuli, Arteries, Capsule and Interstitium. All annotations were revised by a pathology resident (JK), under consultation of an experienced nephropathologist (ES). Our CNN was evaluated using the Dice coefficient for each individual class. This coefficient expresses the quality of the segmentation on a scale ranging from 0-1, taking into account both recall and precision. Because of the limited amount of annotations for certain classes, cross-validation was applied.

Results: We found the following Dice coefficients for the different histological segments: Glomeruli: 0.89, Sclerotic glomeruli: 0.43, Proximal tubuli: 0.88, Distal tubuli: 0.77, Atrophic tubuli: 0.32, Undefined tubuli: 0.11, Arteries: 0.71, Capsule: 0.47 and Interstitium: 0.85.

Conclusion: This study shows that segmentation of WSIs of PAS-stained renal allograft biopsies using a CNN is feasible. Segmentation of several important classes (Glomeruli, Interstitium, Arteries, Proximal-, and Distal tubuli) was highly accurate. CNNs learn from being exposed to many example images. The most probable reason for the lower performance for the other classes are the relatively low number of annotated regions for these classes, combined with a high level of variability inherently present in these tissue structures. To our knowledge, this is the first time artificial intelligence is being deployed in a nine-class segmentation task in the field of kidney transplant histopathology. Results of this study show the promising potential of CNNs in obtaining quantitative, spatial and morphometric information from renal tissue in an objective, reproducible, high-scale fashion supporting diagnostic decisions based on quantitative measures.
P30.
Convertase-stabilizing factors in patients with complement-mediated renal diseases

M.A. Michels¹, N.C. van de Kar¹, M. Okroj², A.M. Blom³, S.A. van Kraaij⁴, E.B. Volokhina¹,⁴*, L.P. van den Heuvel¹,⁴,⁵*

¹Department of Pediatric Nephrology, Amalia Children Hospital, Radboud university medical center, Nijmegen, the Netherlands. ²Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG, Medical University of Gdańsk, Poland. ³Medical Protein Chemistry, Department of Translational Medicine, Lund University, Malmö, Sweden. ⁴Department of Laboratory Medicine, Radboud university medical center, Nijmegen, the Netherlands. ⁵Department of Pediatrics, University Hospitals Leuven, Leuven, Belgium

*Contributed equally

Objective: The autoantibody C3 nephritic factor (C3NeF) plays a pathogenic role in C3 glomerulopathy (C3G) by stabilizing the key enzymatic complex of complement activation, the C3 convertase. However, the reliability of currently used assays to detect C3NeF is limited. Recently, we developed a method to measure convertase stability in the physiological milieu of whole serum. We now optimized the method for simple detection of convertase-stabilizing factors such as C3NeF in large patient cohorts.

Methods: Convertase stability was measured in a hemolytic assay using the C5-blocking agent eculizumab to separate the alternative pathway into two steps: formation of C3/C5 convertases by test sera in a time-variable first step and formation of lytic membrane attack complexes in a standardized second step for readout. Samples of 15 controls and 27 patients with C3G were analyzed. In addition, convertase stability was assessed in a family with complement Factor B (FB) mutation (p.Lys323Glu) and atypical hemolytic uremic syndrome (aHUS), a complement-mediated disease not associated with C3NeF.

Results: Healthy controls were tested to define the normal convertase activity profile: maximal convertase activity was reached after 10-15 min of incubation and after 30 min the activity of all controls had returned to background levels. When serum or a purified Ig fraction containing C3NeF was added to control serum, convertase stability was increased at t=30 min (P<0.001). Thus, detectable convertase activity at t=30 min or later was chosen as a marker for presence of convertase-stabilizing factors such as C3NeF. In our cohort, 16 out of 27 (59%) patients showed increased convertase stability. Interestingly, prolonged convertase activity was also detected in an aHUS family and segregated with the FB mutation in both affected and non-affected family members.

Conclusion: We present optimization of a simple, reliable, and cost- and time-effective assay for detecting convertase-stabilizing factors (C3NeF and some mutations) in patients with various complement-mediated renal diseases. This study may give insight in disease pathogenesis and treatment strategies in these patients.
P31.
Dietary sodium-induced changes in the microcirculatory system of the skin are related to blood pressure response in healthy males

E.F.E. Wenstedt¹, MD; R.H.G. Olde Engberink¹, MD; N.M.G. Rorije¹, MD; B.J.H. van den Born², MD PhD; J. Aten³, PhD; L. Vogt³, MD PhD

¹ Department of Internal Medicine, Division of Nephrology, Academic Medical Center, University of Amsterdam, the Netherlands, ² Department of Internal Medicine, Division of Vascular Medicine, Academic Medical Center, University of Amsterdam, the Netherlands ³ Department of Pathology, Academic Medical Center, University of Amsterdam, the Netherlands

Objective: Studies in animal models indicate that not only the kidney but also the skin microcirculation may play a pivotal role in sodium-sensitive hypertension. In response to high sodium diet (HSD), skin lymphatic capillaries increase in both amount and size, which is shown to be mediated by macrophages. We investigated changes in the lymphatic and blood skin microcirculation of healthy males after a low and high sodium diet in relation to blood pressure (BP) changes.

Methods: We performed a randomized crossover study in healthy males. All subjects pursued an 8-day low sodium diet (LSD: <50 mmol Na+/day) and HSD (>200 mmol Na+/day). Diet order was randomized and time in-between diets was 1-2 weeks. After each diet, BP measurements and skin biopsies were obtained. Macrophages, vascular endothelium and lymphatic endothelium were identified through immunohistochemistry, using antibodies for CD68, CD31 and D2-40, respectively. Analysis was performed using ImageJ Software (National Institutes of Health, USA). Macrophage and capillary density were defined as the number of macrophages or capillaries per megapixel. Lymphatic cross sectional surface area was determined by the average inner surface area of the lymphatic capillaries as a percentage of the histological slice surface area.

Results: Twelve subjects with a mean (SD) age of 22 (4) years were included. Overall, there was no BP increase after HSD vs. LSD (mean arterial pressure (SD): 78 (5) vs. 78 (5), p=0.66). HSD increased lymphatic cross sectional surface area (p=0.01). No differences in macrophage, lymphatic or blood capillary density were observed between the diets. Macrophage density correlated with both lymphatic capillary density (r=0.71 p=0.02) and blood capillary density (r=0.43 p=0.04). There was a correlation between lymphatic and blood capillary density after LSD (r=0.76, p=0.01) but not after HSD (r=0.16, p=0.62). Changes in mean arterial pressure (HSD-LSD) correlated with changes in blood capillary density (r=0.74, p=0.01) and changes in macrophage density (r=0.68, p=0.02) but not with lymphatic capillary density (r=0.06, p=0.87) or lymphatic cross sectional surface area (r=0.20, p=0.56).

Conclusion: High sodium diet is associated with skin lymphangiogenesis and a loss of correlation between the lymphatic and blood microcirculation. This phenomenon, reflecting increased lymph flow, might oppose a sodium-induced BP rise. By contrast, because both blood capillary and macrophage changes within the skin correlate with BP changes following sodium intervention, these parameters might be causally linked to sodium-sensitivity and hypertension development.
P32.
Relaxin receptor deficiency impairs outward remodeling in arteriovenous fistulas for haemodialysis

T. Bezhaeva¹, M.R. de Vries², W. Geelhoed¹, E.P. van der Veer¹, S. Versteeg³, C.M.A. van Alem¹, N. Eijkelkamp³, AJ van Zonneveld¹, P.H.A. Quax² and J.I. Rotmans¹
¹Departments of Internal Medicine and Einthoven Laboratory for Experimental Vascular Medicine, ²Vascular Surgery, Leiden University Medical Center, The Netherlands; ³Laboratory for Translational Immunity, University Medical Center Utrecht, The Netherlands

Objective: A proper functioning vascular access site is a lifeline for a lifetime for patients required hemodialysis. The pathophysiology of arteriovenous fistula (AVF) non-maturation failure is incompletely understood but impaired outward remodeling and intimal hyperplasia are both considered to contribute. In the setting of AVF non-maturation, this adverse vascular response results from a complex interplay between vascular smooth muscle cells (VSMC) extracellular matrix (ECM) components and inflammatory cells. Relaxin (RLN2) is a hormone exhibiting its action on the peripheral vasculature via interaction with its receptor (RXFP1), resulting in vasodilatation, increase in passive compliance, decrease in fibrosis and inflammation. In the present study, we evaluated the role of RXFP1⁻/⁻ on AVF maturation in a murine model of AVF failure and cell specific effects of RXFP1⁻/⁻ on VSMCs and macrophages in vitro.

Methods: AVFs were created in an end-to-side manner between the jugular vein and the carotid artery in mice (n=10 per group). AVFs were harvested at day 14 and processed for morphometric and immunohistochemical analysis. Blood pressure was measured with CODA noninvasive system. All in vitro experiments were performed on primary cells isolated from RXFP1⁻/⁻ and wild type (WT) mice. The capacity of VSMCs to invade a collagen matrix was assessed by haptotaxis assay.

Results: At 14 days after AVF surgery, RXFP1⁻/⁻ mice showed 22% smaller venous circumference at the venous outflow tract which coincides with a 30% increase in elastin content (Fig.1a). We observed a 84% increase in the amount of infiltrating CD45⁺ leukocytes in RXFP1⁻/⁻ mice, from which pro-inflammatory Mac3⁺/CCR2⁺ macrophages were increased by 74%, anti-inflammatory Mac3⁺/CD206⁺ subset by 43% and GR1⁺ neutrophils by 70%, when compared to WT mice. In vitro, VSMCs from RXFP1⁻/⁻ mice exhibited a synthetic VSMCs phenotype, characterized by up regulation in type 1 collagen, fibronectin, TGFβ and PDGF mRNA levels. Venous VSMCs derived from RXFP1⁻/⁻ mice showed an 80% increase in cell migration within a type I collagen matrix, when compared to VSMC obtained from WT mice. Finally, the expression of RXFP1 and RLN2 was confirmed in αSMA⁺ cells within the neointima of human AVFs, confirming the relevance of these observations for hemodialysis patients (Fig.1b).

Conclusion: Deletion of RXFP1 results in a marked decrease in venous outward remodeling in AVF. The latter might relate to a shift towards synthetic/proliferative VSMCs phenotype, ECM expansion and up regulation of inflammatory response in the venous outflow tract of AVF, making relaxin-relaxin receptor axis a potential target to improve AVF maturation.
Targeted C4 inhibition by affinity purified immunoglobulins

Elena Volokhina1,2, Rianne van Sloten1, Nicole van de Kar1, Marloes Michels1, Thea van der Velden1, Marcin Okrój3, Anna Blom4, Lambertus van den Heuvel1,2,5

1Department of Pediatric Nephrology, Radboud university medical center, Nijmegen, The Netherlands; 2Department of Laboratory Medicine, Radboud university medical center, Nijmegen, The Netherlands; 3Department of Medical Biotechnology, Intercollegiate Faculty of Biotechnology UG-MUG, Medical University of Gdańsk, Poland; 4Medical Protein Chemistry, Department of Translational Medicine, Lund University, Malmö, Sweden; 5Department of Pediatrics, University Hospitals Leuven, Belgium

Objective: Immunoglobulins (Igs) can activate complement when bound to their antigens. Moreover, they may inhibit complement activation and in clinical practice intravenous Igs are widely used to treat immunodeficiencies as well as inflammatory conditions. Complement inhibitory properties of Igs are poorly understood, which limits their use for targeted complement modulation in renal disease. In this study we describe immunoglobulin preparations with specific complement inhibiting properties.

Methods: Igs from healthy donors were purified using Protein L or Protein A/G affinity chromatography. Classical (CP) and alternative pathway (AP) activation was assessed using hemolytic assays. Activation of C1q, C4b, C3b and C5b-9 in CP was assessed by ELISA. Purified fractions were analyzed by SDS-PAGE and Coomassie staining.

Results: Igs purified from serum using Protein L, but not Protein A/G inhibited CP in normal human serum (NHS) to below 10% of normal activity when added at 13 mg/mL. Igs from lepirudin, citrate and heparin plasma showed similar inhibiting results, while those isolated from EDTA plasma had no effect. None of the fractions had effect on AP. CP ELISA revealed normal deposition of C1q, and strongly decreased deposition of C4b, C3b and C5b-9. All fractions showed same band pattern in SDS-PAGE. All purified Igs retained ability to activate CP when heat-aggregated.

Conclusion: Thus, Igs purified using Protein L chromatography block complement in NHS at the stage of C4. These findings have important therapeutic potential for the inhibition of classical complement pathway in the future in such conditions as antibody-mediated renal graft rejection, lupus nephritis and antiphospholipid syndrome.
Renal oxygenation during chronic nitric oxide synthase inhibition as recorded by telemetry

Tonja W. Emans$^{1,2}$, Jaap A. Joles$^2$, Ben J. Janssen$^3$ and C.T.P. (Paul) Krediet$^1$

$^1$ Internal Medicine-Nephrology, AMC-UvA, Netherlands; $^2$ Nephrology & Hypertension, UMC Utrecht, Netherlands; $^3$ Pharmacology and Toxicology, University Maastricht, Netherlands

Objective: Renal hypoxia has been advanced as a crucial factor in the vicious circle of disease progression leading to kidney failure. Nitric oxide (NO) is involved in renal vascular regulation. NO synthase (NOS)-inhibition leads to hypertension while decreasing renal blood flow and thus oxygen delivery. Furthermore, NO inhibits mitochondrial oxygen consumption. Therefore, we hypothesized that NOS inhibition would induce renal hypoxia. We report telemetrically monitored mean arterial pressure and oxygen pressure ($pO_2$) in renal cortex and medulla in conscious rats during chronic NOS inhibition.

Methods: Oxygen sensitive electrodes were implanted in either renal cortex (n=6) or medulla (n=7) in healthy rats. After recovery and stabilization, baseline $pO_2$ was recorded for one week. Then, to inhibit NOS, L-NNA (40mg/kg/day) was administered via drinking water for two weeks. A separate group of rats (n=8), instrumented with blood pressure recording telemeters, followed the same protocol. Terminal glomerular filtration rate (GFR), renal blood flow (RBF), renal oxygen extraction and natriuresis were assessed under isoflurane anesthesia in all L-NNA rats (n=21) and in untreated controls (n=6).

Results: NOS inhibition rapidly induced hypertension (164 ± 6 vs. 108 ± 3 mmHg, p<0.001) and progressive proteinuria (82 ± 13 vs. 17 ± 2 mg/day, p<0.01). After an initial dip, cortical oxygenation returned to baseline. In contrast, medullary oxygenation decreased progressively (up to -23 ± 8% vs. baseline; p<0.05). Terminal GFR (1374 ± 74 vs. 2098 ± 122 µl/min) and RBF (5014 ± 336 vs. 9966 ± 905 µl/min) were reduced vs. control (both p<0.01). Terminal sodium reabsorption efficiency ($T_{Na}/QO_2$ also decreased (13.0 ± 0.8 vs. 22.8 ± 1.7 µmol/µmol, p<0.01).

Conclusion: Chronic NOS inhibition induced temporal changes in renal $pO_2$. Cortical $pO_2$ was not persistently altered, despite reduced RBF and therefore oxygen supply. In contrast, medullary $pO_2$ decreased progressively. Chronic NO deficiency leads to decreased renal perfusion and reabsorption efficiency (possibly of mitochondria) resulting in progressive medullary hypoxia. This suggests that juxtamedullary nephrons are particularly sensitive to chronic NO depletion.

Supported by: Netherlands Organisation for Health Research (ZonMW, 40007039712461)
P35. HMGA1-driven long non-coding RNAs mediate endothelial-to-mesenchymal transition in kidney fibrosis

R. Bijkerk1, A. Lafzi2, W. Stam1, J.M.G.J. Duijs1, A. Koudijs1, E. Lievers1, T.J. Rabelink1, H. Kazan2 and A.J. van Zonneveld1

1Department of Internal Medicine (Nephrology) and the Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands.
2Department of Computer Engineering, Antalya International University, Antalya, Turkey

Objective: Chronic kidney disease associates with the development of interstitial fibrosis characterized by a loss of the microvasculature and myofibroblast formation. Endothelial cells (ECs) are important for maintaining a healthy microvasculature while ECs also provide a potential source for myofibroblasts through endothelial-to-mesenchymal transition (EndoMT). Here, we aimed to identify a role for long non-coding RNAs (lncRNAs), novel central post-transcriptional regulators, in ECs in the development of kidney fibrosis.

Methods: We used VE-cadherin-ERT2;tdTomato mice to label and trace endothelial cells. We applied both the ischemia-reperfusion injury (IRI) and unilateral urethral obstruction (UUO) models followed by FACS sorting of the tomato-positive cells from healthy and diseased kidneys. Subsequently, we isolated RNA from these cells and profiled for lncRNAs, as well as gene expression, using comprehensive genome-wide transcript arrays.

Results: Upon kidney injury, we observed substantial co-localization of VE-cadherin-derived tomato positive signal with α-SMA staining, indicating that a significant portion (~15-20%) of myofibroblasts originated from ECs. We confirmed that ECs acquired a myofibroblast phenotype by using qPCR on FACS sorted tomato-positive cells showing reduced expression of EC markers CD31 and VE-cadherin while myofibroblast markers α-SMA and col1α1 increased. In UUO and IRI, we found 586 and 416 lncRNAs to be differentially expressed (>2-fold, p<0.05) in the VE-cadherin-derived tomato-positive cells, respectively. Using bioinformatics analyses to determine transcription factor motif-enrichment amongst differentially expressed lncRNAs we found strong enrichment for HMGA1 binding sites. Using ChIP-seq, we validated binding of HMGA1 to the promoter of the lncRNA MALAT1, one of the differentially expressed and conserved lncRNAs. We subsequently demonstrated in an in vitro model for EndoMT that blocking MALAT1 with gapmers enhanced TGF-β induced EndoMT. Lastly, we found circulating MALAT1 levels to be increased in CKD patients compared to healthy controls.

Conclusion: We demonstrated that HMGA1-induced lncRNAs mediate EndoMT which may provide novel strategies to counteract the development of kidney fibrosis.
Abstracts NFN fall meeting 2017

P36.
Evaluation of tumorigenic potential of conditionally immortalized proximal tubule epithelial cells for bioartificial kidney application

Milos Mihajlovic¹, Sam Hariri¹, Miriam J. Oost¹, Koen G.C. Westphal¹, Manoe J. Janssen¹, Luuk Hilbrands² and Rosalinde Masereeuw¹

¹Div Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht, the Netherlands; ²Department of Nephrology, Radboudumc, Nijmegen, the Netherlands

Objective: Novel renal replacement treatments, such as a bioartificial kidney (BAK) devices, are needed to improve current hemodialysis. However, when developing a BAK, availability of functional cells and their safety are frequently encountered problems. The aim of this study was the evaluation of the tumorigenic potential of readily available conditionally immortalized human proximal tubule epithelial cells (ciPTEC) for use in BAK.

Methods: A urine derived ciPTEC line was characterized for loss of contact-inhibition by focus formation assay and proliferation, as well as anchorage-independent growth, over a period of 4 weeks. In addition, the expression of temperature-sensitive SV40 large T antigen, used for conditional immortalization of cells, and its effect on cell cycle distribution was addressed. Cells were examined for apoptosis-resistance in function of SV40 large T antigen, as well as for invading ability in extracellular matrix-based 3D environment. Next, targeted locus amplification (TLA) technology was employed to determine genomic integration of transgenes, the eventual functional effects and on chromosomal stability. Finally, subcutaneous injection of ciPTEC in athymic nude rats (Hsd:RH-Foxn1nmu) aimed to provide more insight into their tumorigenic potential.

Results: Focus formation assay showed that ciPTEC grew in stable monolayers maintaining contact-inhibition after 4 weeks of culture (<1% foci compared to >50% by HeLa cells; p<0.001). Cell cycle analysis confirmed this as >85% of cells were present in G0/G1. Anchorage-independent growth of ciPTEC indicated that the cells do require anchorage for efficient growth (less than 1 colony per field, compared to 15 colonies for HeLa cells; p<0.001). SV40 large T antigen expression was significantly downregulated after culturing cells at non-permissive temperature for 7 days. Moreover, cell viability analysis suggested that cells are not apoptosis-resistant and that exposure to the MDM2 inhibitor nutlin-3a, induced apoptosis in cells at non-permissive temperature, by stabilizing p53 levels after downregulation of SV40 large T antigen expression. Only 7% of cells were able to migrate through the extracellular matrix. Chromosome analysis of the late-passage cells showed a mosaic tetraploid chromosome complement, indicating the presence of chromosomal instability after long culture periods. TLA analysis showed that the transgene integration had occurred in the intronic regions of six distinct endogenous genes. Most notably, BCL2L1 and EEA1, which play important roles in apoptosis and endocytosis respectively, were affected. However, neither process was found to be negatively altered in ciPTEC. Finally, preliminary results from athymic nude rats suggest that ciPTEC do not possess tumorigenic effect in vivo.

Conclusion: Both in vitro and in vivo results indicate that ciPTEC do not show typical cancer cell-like behavior and do not have tumorigenic potential, suggesting their safe use in an extracorporeal BAK device.
P37.
In Two-kidney One-clip Hypertensive Sheep Cardiac and Contralateral Renal Sympathetic Nerve Activity are Differentially Controlled

Tycho R. Tromp1,2, Jaap A. Joles2, Rohit Ramchandra1

1The University of Auckland, Auckland, New Zealand, 2University Medical Center Utrecht, Utrecht, Netherlands

Objective: Hypertension is often initiated and maintained by elevated sympathetic tone. We investigated changes in directly recorded sympathetic nerve activity (SNA) to the heart and nonclipped kidney in two-kidney oneclip (2K-1C) hypertensive sheep.

Methods: Adult ewes either underwent unilateral renal artery clipping (n=12) or sham surgery (n=15). Two weeks later, the carotid artery was cannulated and electrodes were placed in the (nonclipped) renal and/or cardiac nerve. Blood pressure (BP), heart rate (HR) and baseline and baroreflex control of SNA were recorded in the conscious sheep one week later.

Results: Unilateral renal artery clipping induced hypertension (systolic blood pressure 130±3 vs 111±4 mmHg in shams, p<0.001) after 21±5 days, and shifted the heart rate baroreflex curve rightwards (BP50 120±13 vs 104±16 mmHg, p<0.01). HR was unchanged. The renal SNA (RSNA) baroreflex curve was also shifted rightwards (BP50 93±3 vs 78±2, p<0.01) and showed increased gain (p<0.05). In the hypertensive group, cardiac SNA (CSNA) burst incidence (bursts/100 beats) was increased (39±14 vs 25±9 in normotensives, p<0.05), whereas RSNA burst incidence was decreased (69±20 vs 93±8 in normotensives, p<0.01).

Conclusion: In this ovine model of 2K-1C renovascular hypertension we show that cardiac and contralateral renal sympathetic nerve activity were differentially controlled three weeks after clipping: baseline CSNA was increased whilst RNSA to the nonclipped kidney was decreased. We speculate that the observed contralateral RSNA decrease is a homeostatic response to increased blood pressure and the sodium avid state of the clipped kidney.

Figure 1 - Differential control of contralateral renal and cardiac sympathetic nerve activity (SNA). Baseline contralateral renal SNA was decreased whereas cardiac SNA was increased (A). Baroreflex control of contralateral renal (B) and cardiac SNA (C) was differentially regulated.
A proteome comparison between fetal and mature renal extracellular matrix

Laura Louzao-Martinez MSc\textsuperscript{1,2}, Christian van Dijk MSc\textsuperscript{1}, Niek Bekker\textsuperscript{1}, Caroline Cheng PhD\textsuperscript{1,3}

\textsuperscript{1}Department of Nephrology and Hypertension, University Medical Center Utrecht, The Netherlands; \textsuperscript{2}Netherlands Heart Institute; \textsuperscript{3}Experimental Cardiology, Department of Cardiology, Erasmus University Medical Center, The Netherlands

Objective: The extracellular matrix (ECM) is a collection of molecules deposited by the surrounding cells, making it a tissue-specific 3D structure in which cells are embedded. The ECM not only gives structural support to the tissue, it also contains biochemical cues that can influence many biological processes, including cell migration, proliferation and differentiation, both during kidney development and repair processes. Characterization of the composition of the renal ECM, especially the differences between the developing fetal and the more static mature ECM, will give important insights into ECM structure and function, that can be used in the renal tissue engineering field for implementation in biomaterials.

Methods: We analysed healthy kidney samples from both adult and fetal human donors. The ECM was enriched in these samples by a process involving decellularization. Cellular components, including nucleic acid, were successfully removed using SDS and Triton-X as detergents, without disrupting the structure and morphology of the ECM. To characterize the differences between the fetal and mature renal ECM proteome, we used SDS-PAGE and liquid chromatography-mass spectrometry analyses. Protein verification was conducted using immunohistochemistry. Furthermore, key ECM components found with our proteomics screen were functionally tested using a methodology that permits the reliable anchorage of native cell-secreted ECM to glass coverslips. By using siRNA mediated knock-down technologies, we investigated the ability of the modified ECM to support the adhesion of several renal cells \textit{in vitro}.

Results: We identified 94 fetal and 76 mature renal ECM proteins, from which the majority could be classified as structural proteins (60 and 51, respectively). These core proteins included collagens, glycoproteins and proteoglycans. The remaining ECM proteins could be classified as ECM affiliated proteins, including ECM regulators and secreted factors. Relative protein quantification showed a dominance of collagens and glycoproteins in both the fetal and mature ECM: 62\% of the fetal and 64\% of the mature selection consisted of collagens and 33\% and 28\%, respectively, of the total signal consisted of glycoproteins. 23 of the identified proteins were significantly enhanced in the fetal renal ECM compared to the mature ECM. Only 6 ECM proteins were found the be significantly enriched in the mature renal ECM. Of these 23 fetal proteins, elastin microfibril interfacer 1 (EMILIN1) and fibrillin-1 were found to be one of the most highly expressed glycoproteins in the fetal renal ECM. We demonstrated using captured native cell-secreted ECM depleted form EMILIN1 or fibrillin-1, that EMILIN1 is needed for proper adhesion of Human Renal Proximal Tubular Epithelial Cells (HRPTECs). EMILIN1 knockdown within the captured ECM significantly reduced the paxillin area, a focal adhesion protein, of adhered HRPTECs.

Conclusion: Collectively, we created a catalogue of fetal and mature renal ECM proteins and identified specific differences in ECM composition between fetal and mature kidneys. Key proteins that are more enriched in the fetal renal ECM could be promising candidates for implementation in renal tissue engineering.