Abstract Book

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Oral Abstract Presentations

O1

Effect of somatostatin analogues on the vasopressin pathway in patients with Autosomal Dominant Polycystic Kidney Disease

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Objective
The vasopressin V2 receptor antagonist tolvaptan slows the rates of total kidney volume growth and GFR decline in ADPKD, but its effect is limited and aquaretic side effects hamper wide spread clinical use. Therefore somatostatin analogues are of interest, which also interfere with cyst formation. Interestingly, several studies have suggested that somatostatin is involved in renal water handling, indicating that there may be an interaction between the somatostatin and vasopressin pathways. We therefore investigated if the somatostatin analogue lanreotide has an effect on vasopressin levels and renal water handling in patients with ADPKD.

Methods
Patients were included who participated in the DIPAK-1 study, a randomized, open-label controlled trial to test the efficacy and safety of the somatostatin analogue lanreotide in later stage ADPKD. Patients were invited for a baseline visit, and randomized to receive either lanreotide or standard care in a 1:1 ratio. Blood and 24 hour urine samples were collected at baseline and after 12 weeks. Free water clearance (FWC) was calculated as 24 hour urine volume-((Urine osmolality*24 hour urine volume)/plasma osmolality) and fractional free water clearance (FFWC) as (FWC/eGFR)*100%.

Results
Overall, 305 ADPKD patients were included, 53% female, 48±7 years of age and eGFR 50±11 ml/min/1.73m^2. Overall, we observed no differences in change in plasma copeptin, 24 hour volume, FWC and FFWC between patients receiving lanreotide or standard care at week 12. In patients with eGFR >50 mL/min/1.73m^2 there was a relative decrease in FWC and FFWC in patients receiving lanreotide compared to standard care (-0.04 ± 0.74 vs. 0.28 ± 0.75 L/24hr, p=0.01 and -0.16 ± 0.24 vs. 0.48 ± 1.34 %, p=0.005, resp.). There was no significant difference in change in 24 hour urine volume and plasma copeptin (-0.05 ± 0.73 vs. 0.15 ± 0.71 L/24hr, p=0.10 and -2.86 ± 15.5 vs. -0.32 ± 3.8 pmol/L, p=0.12, resp.).

Conclusion
Although the somatostatin analogue lanreotide did not affect vasopressin levels or renal water handling in the overall group of patients, it slightly lowered free water clearance in patients with relatively preserved renal function. This effect was independent of vasopressin, as copeptin levels did not increase in this patient group.

O2

Modulation of *YAP* level prevent cyst formation in 3D cyst assay but not in *Pkd1* mutant mouse model

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Objective

The transcriptional co-activator YAP (Yes-associated protein) and its ortholog TAZ (transcriptional co-activator with PDZ-binding motif) are final effector molecules of the Hippo signaling pathway, involved in controlling organ size restriction. We previously showed altered Hippo signaling in Autosomal Dominant Polycystic Kidney Disease (ADPKD), a frequent genetic cause of renal failure. We observed strong nuclear accumulation of YAP in dilated tubules and cysts of several *Pkd1*-mutant mouse models and in human cystic tissues, which was accompanied by up-regulation of the YAP transcriptional targets. Interestingly, YAP and TAZ are transcriptional co-activators for a variety of transcription factors, many of them implicated in ADPKD. Therefore we hypothesize that modulating nuclear localization of YAP/TAZ may slow down renal cystic disease.

Methods

We developed mIMCD3 cells *YAP* knock-out (KO), *Pkd1* KO or *YAP*/*Pkd1* double KO, and MDCK cells *TAZ* KO using CRISPR/Cas9 system. We tested the cystic capacity of the different genotypes using 3D cysts assay. For the *in vivo* experiments, we used a kidney-specific tamoxifen inducible *Pkd1* deletion mouse model and we induced gene deletion at postnatal day 18 leading to cyst formation in all the nephron segments. Two weeks after gene inactivation we administered antisense oligonucleotides targeting *YAP* (YAP ASO). We used scrambled ASO as control. Mice were sacrificed 8 weeks after *Pkd1* inactivation.

Results

Pkd1 KO mIMCD3 cells show increased ability to form cyst in 3D cultures compared to wt. When also *YAP* was KO together with *Pkd1* we observed significant reduction in cyst formation while cyst formation was completely abolished in *YAP* single KO cells. Interestingly, we could not achieve homozygous *TAZ* KO in mIMCD3 suggesting that YAP and TAZ might have specific and unique roles in the different nephron segments. This is supported by the different pattern of expression of YAP and TAZ in kidneys, where YAP and TAZ can be found more enriched in different and distinct nephron segments. For this reason, we knocked-out *TAZ* in MDCK cells. *TAZ* KO MDCK cells were able to form cyst in 3D cultures suggesting that YAP but not TAZ might be involved in cyst formation. To test this hypothesis, we used ASO targeting *YAP* in *Pkd1* mutant mice. We validated the reduction of YAP, both at gene and protein level, while TAZ levels remained unchanged. Nevertheless we did not see any change in cyst progression in YAP ASO treated mice compared to scrambled ASO group. Also, the expression of known YAP and TAZ targets, such as *Cyr61, AmotL2* and *Wtip*, were not changed after *YAP* knock-down (KD) with ASO.

Conclusion

From this study we can conclude that modulation of YAP level using ASOs do not slow down cystic disease. Although YAP and TAZ have a distinct pattern of expression and show some differences in cystic potential in 3D assay, they also have largely overlapping transcription targets which are not altered by *YAP* KD. This suggests that TAZ might be overtaking YAP functions during cyst formation.

O3
Identification of ARL15 as a novel determinant of the renal handling of magnesium

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Objective: The renal handling of magnesium (Mg^{2+}) primarily dictates body Mg^{2+} homeostasis. Thus, disturbed transport of Mg^{2+} in the kidney leads to abnormal Mg^{2+} levels in serum, that relates to cardiovascular, metabolic and neurological disorders. Yet, many of the molecular determinants of the renal handling of Mg^{2+} remain unknown to date. In this study, we aimed to disclose new molecular players in renal Mg^{2+} handling.

Methods: We conducted the first meta-analysis of genome-wide association studies (GWAS) of urinary magnesium (uMg) combining seven European-based cohorts (general population and genetic isolates), with a total of 9,099 individuals. Subsequently, the identified loci were characterized in relevant cellular and animal models for renal Mg^{2+} transport.

Results: Two genome-wide significant loci associated with urinary Mg^{2+}-to-creatinine ratio were identified: rs3824347 located on chromosome 9, near the *TRPM6* gene, coding for a Mg^{2+} channel; and rs35929 located on chromosome 5, a variant of the *ARL15* gene known for its association with metabolic phenotypes, but without a prior physiologic link to Mg^{2+} homeostasis. In the zebrafish model, gene expression of the highly conserved *ARL15* ortholog, *arl15b*, was regulated by dietary Mg^{2+} in kidney and *arl15b*-knockdown resulted in renal Mg^{2+} wasting. Consistently, this phenotype was rescued by human ARL15 expression. Immunohistochemistry studies in specific nephron segments revealed that ARL15 localizes in the thick ascending limb of Henle's loop; and in the distal convoluted tubule, where TRPM6 is enriched. In human embryonic kidney 293 cells, ARL15 regulated TRPM6-mediated currents.

Conclusion: In conclusion, this combined observational and experimental approach uncovered a new molecular determinant of the renal handling of Mg^{2+}. Therefore, *ARL15* mutations should be considered in patients with inherited hypomagnesemia.

O4

Amino acid loss during hemodialysis in end-stage renal disease patients

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Objective: Poor nutritional status is frequently observed in chronic hemodialysis (HD) patients and associated with adverse clinical outcomes and increased mortality. Loss of amino acids during HD may contribute to protein malnutrition in these patients. It remains to be established whether dietary protein intake is sufficient to compensate for the amount of amino acids lost in the dialysate during HD.

Methods: Ten anuric chronic HD patients, undergoing dialysis three times per week, were selected to participate in this study. Spent dialysate was collected continuously and plasma samples were obtained directly before and after HD. Amino acid profiles in spent dialysate, pre-HD, and post-HD plasma were measured through ultra-performance liquid chromatography to determine amino acid concentrations and, as such, net loss of all amino acids. In addition, food intake during HD was closely monitored. A paired-samples *t*-test was conducted to compare pre-HD and post-HD plasma amino acid concentrations. Correlations between amino acid concentrations in pre-HD plasma and spent dialysate were assessed through determining Spearman's Rank Correlation Coefficients.

Results: Throughout a HD session 12.0±0.7 g amino acids were lost in the dialysate, of which 8.3±0.5 g non-essential amino acids, 3.7±0.3 g essential amino acids, and 1.6±0.2 g branched-chain amino acids. As a consequence, plasma total amino acid and essential amino acid concentrations declined significantly from 2879±47 and 795±17 μmol/L to 2273±50 and 655±15 μmol/L, respectively ($P<0.05$). Amino acid profiles of pre-HD plasma and spent dialysate were similar. Moreover, amino acid concentrations in pre-HD plasma and spent dialysate were strongly correlated ($\rho=0.92$, $P<0.001$). Dietary protein and energy intake during a single HD session averaged 20.1±2.9 g and 572±50 kcal, respectively.

Conclusion: During a single HD session 8 – 15 g amino acids are lost in the dialysate. Habitual dietary intake during HD is insufficient to compensate for this loss, resulting in a significant decline of plasma amino acid concentrations. The observed amino acid deficit can contribute substantially to protein malnutrition in end-stage renal disease patients.

O5

TREM-1 limits the maladaptive repair following renal ischemia/reperfusion by preventing senescence and preserving mitochondrial function of tubular cells.

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Objective: Long-term sequelae of acute kidney injury (AKI) include the incomplete recovery of renal function, resulting in chronic kidney disease (CKD). Triggering receptor expressed on myeloid cells-1 (TREM-1) is a pattern recognition receptor which amplifies the inflammatory response elicited by Toll like receptors and most importantly, it is expressed by macrophages and epithelial cells. These two cell types are well-known to play a pivotal role in the adaptive repair post-AKI. Therefore, we investigated the role of TREM-1 in renal regeneration by using a preclinical model of AKI.

Methods: WT and TREM1/3 KO mice were subjected to renal ischemia/reperfusion (IR) and sacrificed 1, 5 and 10 days later. By means of several techniques we determined parameters of renal function, inflammation and repair in both mice strains. Additionally, through *ex-vivo* experiments we have extensively studied the metabolic profile, mitochondrial activity and proliferative capacity of WT and TREM1/3 KO tubular epithelial cells (TECs).

Results: WT and TREM1/3 KO mice displayed no major differences during the acute phase of injury, however, they showed significantly increased mortality in the recovery phase. This detrimental effect was associated with a maladaptive repair response, characterized by persistent tubular damage and inflammation, fibrosis and tubular senescence. *In vitro* we found that hypoxia induces an upregulation of TREM-1 in TECs. Interestingly, at steady-state TREM1/3 KO TECs displayed an altered mitochondrial homeostasis and cellular metabolism, which was associated with a G2/M arrest, most-likely due to an excessive ROS production. Indeed, when WT and TREM1/3 KO TECs were exposed to an extra ROS-generating trigger, such as *in vitro*-simulated IR, TREM1/3 KO TECs manifested a senescent phenotype with enhanced secretory phenotype, resulting in decreased wound healing capacities.

Conclusions: Taken together these results unraveled a novel mechanism under the control of/regulated by the innate immune receptor TREM-1 that takes place in senescent TECs which comprises of a disruption of mitochondria homeostasis, redox state and a decline in cell metabolic activities. This suggests that novel intervention strategies targeting TREM-1 may steer tubular metabolism, improve tubular regeneration and prevent the risk of AKI-CKD progression.

O6

Liposomal delivery improves the efficacy of prednisolone to reduce renal inflammation in a mouse model of acute renal allograft rejection

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Objective: Despite reduced incidence of acute cellular rejection, first line treatment with high doses of glucocorticoids is not always effective. The treatment is also associated with onset of severe side effects and long term graft survival and patient health are affected. In this study a mouse model of acute renal allograft rejection was used to investigate whether liposomal encapsulation of prednisolone facilitates local delivery to the allograft and enhances its local therapeutic effect.

Methods: Male BalbC^{HanZtm} mice received a kidney transplant from male C57BL/6J^{HanZtm} donors (n=10 per group). Recipients were injected daily with 5 mg/kg cyclosporine A and received either 10 mg/kg prednisolone (P), or liposomal prednisolone (LP) intravenously on day 0, 3, and 6, or no additional treatment (NA). Functional MRI was performed on day 6 and organs were harvested on day 7 for further analysis with FACS, qPCR and histology.

Results: Staining of liposomes reveals accumulation in the allograft, and diffuse presence throughout the interstitium (Fig 1a). After LP treatment, the expression of the glucocorticoid receptor responsive gene *Fkbp5* is upregulated in the allograft, (fig 1b). FACS analysis of renal allografts shows a reduced number of CD45+, CD3+, and F4/80+ cells after LP treatment (fig 1c) and MRI analysis revealed better allograft perfusion than untreated mice (fig 1d).

Conclusion: Liposomal delivery results in higher local bioavailability of prednisolone, reduced cellular infiltrate in the transplanted kidney, and increased perfusion. Future clinical studies should reveal if treatment with liposomal prednisolone results in improved efficacy and reduced side effects in patients with acute renal allograft rejection.

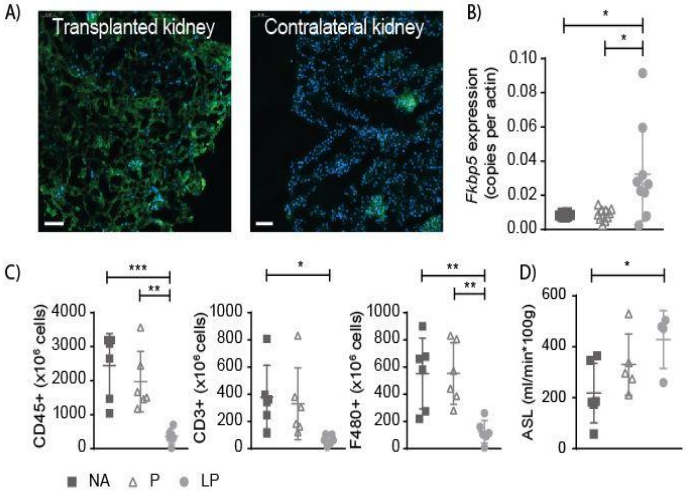

Figure 1 A) Representative images of the whole organ are shown in a 200x magnification with a scale bar of 50 µm. An anti-PEG antibody was used to visualize liposomes (green). B) The mRNA expression of the glucocorticoid receptor responsive gene *Fkbp5* in the allograft is plotted as copies per actin. C) The cellular infiltrate in the allograft was quantified with FACS. The influx of CD45+, CD3+, and F4/80+ cells after treatment is shown. D) Treatment effect of NA, P, and LP on perfusion (ASL) is presented. Results from individual mice are shown with a group mean±SD.

O7

Salt-sensitive hypertension in type 1 diabetes mellitus is accompanied by an incapacity to reduce systemic vascular resistance – a randomized experimental cross-over study

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Objective

Diabetes mellitus type 1 (DM1) is suggested to be associated with salt sensitivity. However, strong evidence to support this is scarce, and the mechanisms behind it are not fully known. For a long time, the volume-regulating function of the kidney was thought to be the only important factor in salt-sensitive hypertension. Due to insufficient sodium excretion by the kidney, sodium excess was considered to increase extracellular fluid volume (ECFV) and cardiac output (CO), resulting in a blood pressure (BP) rise. However, recent publications put forward that systemic vascular resistance (SVR) might be central in BP regulation. We hypothesized that in salt-sensitive hypertension in DM1, vasodysfunction rather than increases in ECFV and CO plays a role.

Methods

We performed a dietary intervention study in 8 DM1 patients and 12 age-matched healthy controls (HC), with normal BP, body mass index, and kidney function. All subjects adhered to an 8-day low salt diet (LSD) and an 8-day high salt diet (HSD), in randomized order. After each diet, hemodynamic measurements included BP (Omron) and CO (Nexfin™), and SVR was calculated accordingly. To assess volume-dependent changes, ECFV, interstitial fluid volume (IFV) and plasma volume (PV) were calculated by the use of iohexol and ^{125}I-albumin distribution. Due to heterogeneity in BP responses, HC were divided in two subgroups: those with versus those without a BP increase after HSD.

Results

After HSD, patients with DM1 showed a BP increase (mean arterial pressure HSD vs LSD (mean (SD)): 85 (5) mmHg vs. 80 (3) mmHg; P=0.03) while BP in HC did not rise (78 (5) mmHg vs. 78 (5) mmHg; P=0.85). The difference in BP response was not accompanied by differences in urinary sodium excretion between the DM1 patients and HC nor by differences in CO, ECFV or ICF. HC without a BP increase after HSD (n=5) showed a SVR reduction after HSD, while this reduction was not present in the HC with a BP increase after HSD (n=7) or in the DM1 patients.

Conclusion

DM1 is associated with increased salt sensitivity compared to HC, coinciding with incapacity for vasodilation.

O8

Trained autoimmunity as a driver in the pathogenesis of Systemic Lupus Erythematosus

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Introduction: Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by autoantibodies against chromatin. Elevated levels of circulatory chromatin are detected in SLE patients, which may be a result of aberrancies in apoptosis or neutrophil extracellular trap (NET) formation or insufficient clearance of apoptotic material or NETs. Recently, we showed that SLE-derived PBMCs appeared far more sensitive to apoptotic microparticles (MPs) than those from controls, for which there is no clear explanation yet. In fact, recently the concept of trained immunity was described, meaning that innate immune cells can develop an unspecific memory after first exposure to disease or pathogen associated molecular patterns (DAMPs/PAMPs) which results in a stronger response after subsequent exposures to the same or different stimuli. Therefore, we hypothesized that sources of nuclear antigens in SLE, including MPs and NETs, can train PBMCs, thereby induce trained autoimmunity.

Methods: Adherent monocytes from SLE patients or healthy donors were let to rest for five days and then the cells were stimulated for 24 hours with different TLR agonists (TLR2 (Pam3CSK4), TLR4 (LPS:B5), TLR7 (R848), TLR9 (ODN2006)). After the stimulation, IL-6 and TNF-α levels were measured by ELISA. Adherent monocytes from healthy volunteers were trained with 10% plasma from healthy volunteers or SLE patients for 24 hours. After five days of resting period, the cells were restimulated with different TLR agonists and IL-6, TNF-α levels were measured by ELISA. Adherent monocytes from healthy volunteers were trained 24 hours with different stimuli (untrained, heat killed *C. albicans*, MPs, NETs). After five days of resting, cells were stimulated with TLR agonists for 24 hours and IL-6 and TNF-α levels were measured by ELISA.

Results: Monocytes from SLE patients tend to produce higher levels of proinflammatory cytokines (IL-6 and TNF-α) when stimulated with different TLR agonists after five days of resting. In line with this, plasma samples from those patients were capable to train healthy monocytes as measured as elevated levels of IL-6 and TNF-α production after second stimulation. *In* vitro-produced NETs induced monocyte training dose dependently, which was based on secretion of higher levels of IL-6 and TNF-α in response to second stimulation after the resting period. Training by NETs was comparable to that induced by *C. albicans*, which is positive control for innate immune training. MPs, on the other hand, induced training at a lesser extent.

Conclusion: SLE monocytes are possibly trained *in vivo* to give higher proinflammatory response upon TLR stimulation. This training is induced by factors present in the circulation of SLE patients as SLE plasma can train healthy monocytes for a more proinflammatory phenotype. Innate immune cells can be trained by MPs and NETs, factors highly present in the circulation of SLE patients. All in all, trained autoimmunity play an important role in the pathogenesis of SLE and lupus nephritis.

O9

Caloric Restriction Improves Established Proteinuria in Adriamycin-Induced Nephropathy

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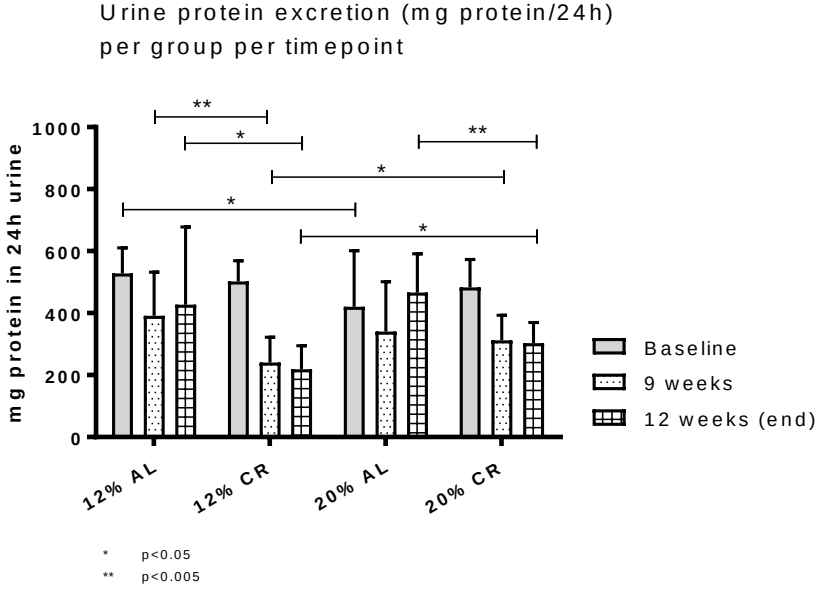
Introduction: Reduction of proteinuria is an important strategy to prevent loss of kidney function. Preclinical studies suggest that caloric restriction, induced at young age, protects against age-related proteinuria. None of these studies investigated the effect of caloric restriction in established renal disease. We hypothesized that caloric restriction in established proteinuria reduces urinary protein excretion.

Materials and methods: Male Wistar rats (n= 56; age 12± 2 week) were intravenously injected with 2.1mg/kg Adriamycin. At 6 weeks after injection, baseline urinary protein excretion, was measured. At 7 weeks after injection rats were randomly assigned to 4 groups: An ad libitum (AL) and a caloric restriction (CR) group (60% of AL food intake) fed with a 12% protein diet (12%AL, 12%CR) and an AL and a CR group fed with a 20% protein diet (20%AL, 20%CR). All groups were treated for 12 wks. urinary protein excretion was measured at week 9 and 12 and mean arterial blood pressure at the end of the study.

Results: Baseline urinary protein excretion was similar in all groups ($p>0.20$) with a median value of 495 and an interquartile range of 127mg/24h. After 12 weeks of diet, all animals exposed to caloric restriction had a 20.3% lower urinary protein excretion ($p=0.003$) compared to ad libitum fed animals. At week 9 this effect was only seen in the 12%CR group. Blood pressure in animals exposed to caloric restriction was -21.2% lower ($p<0.0001$) compared to ad libitum fed animals.

Conclusions: Caloric restriction lowers urinary protein excretion and blood pressure in rats with established proteinuria. Protein restriction was effective if applied in combination with caloric restriction. These results may guide future intervention studies in patients with proteinuric and obesity-related nephropathy.



O10

Precurved non-tunnelled catheters for haemodialysis are comparable in terms of infections and malfunction as compared to tunnelled catheters - a retrospective cohort study

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Objective The main limitations of central venous catheters (CVCs) for haemodialysis access are infections and catheter malfunction. Our objective was to assess if precurved non-tunnelled CVCs (NTCVCs) are comparable to tunnelled CVCs (TCVCs) in terms of infection and catheter malfunction and to assess whether precurved NTCVCs are superior to straight CVCs.

Methods. In this retrospective, observational cohort study, adult patients in whom a CVC for haemodialysis was inserted between 2012-2016 were included. The primary endpoint was a combined endpoint consisting of the first occurrence of either an infection or catheter malfunction. The secondary endpoint was a combined endpoint of the removal of the CVC due to either an infection or catheter malfunction. Using multivariable analysis, cause-specific hazard ratios for endpoints were calculated for TCVC versus precurved NTCVC, TCVC versus NTCVC and precurved versus straight NTCVC.

Results. A total of 1603 patients were included. No difference in reaching the primary endpoint was seen between TCVCs, compared to precurved NTCVCs (HR 0.91, 95% CI 0.70-1.19, *P*=0.48). TCVCs were removed less often, compared to precurved NTCVCs (HR 0.65, 95% CI 0.46-0.93, *P*=0.02). A trend was seen for less infections and catheter malfunctions when precurved jugular NTCVCs were compared to straight NTCVCs (HR 0.60, 95% CI 0.24–1.50, *P*=0.28), and were removed less often (HR 0.41, 95% CI 0.18–0.93, *P*=0.03).

Conclusions. TCVC and precurved NTCVCs showed no difference in reaching the combined endpoint of catheter-related infections and catheter malfunction. TCVCs get removed less often because of infection/malfunction than precurved NTCVCs.

O11

Dietary magnesium prevents vascular calcification but induces osteomalacia in klotho deficient mice

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Objective: Klotho is a key modulator of the phosphate (Pi) and calcium (Ca^{2+}) balance, is protective against vascular calcification, and is diminished in chronic kidney disease (CKD). Klotho knock-out ($^{-/-}$) mice are therefore an important model for CKD-induced calcification. In CKD, serum magnesium (Mg^{2+}) inversely correlates with incidence and severity of vascular calcification. This study aims to determine the effects of Mg^{2+} on development of aortic calcification, and its effects on bone in Klotho$^{-/-}$ mice.

Methods: Klotho$^{-/-}$ and Klotho$^{+/+}$ mice were fed a normal (0.05% w/w) or high (0.48% w/w) Mg^{2+} diet from birth for 8 weeks. Aortic calcification was detected by Von Kossa staining and RNA-sequencing was performed. Serum electrolyte and hormone concentrations were determined. MicroCT and bone histology was used to study bone architecture and metabolism.

Results: Aortic calcification was present in Klotho$^{-/-}$ mice on a normal Mg^{2+} diet but not in Klotho$^{+/+}$ mice. High Mg^{2+} diet prevented aortic calcification in Klotho$^{-/-}$ mice. Mg^{2+} reduced aortic gene expression of *Runx2* and matrix gla protein, demonstrating the preventive effect of Mg^{2+} on pro-osteogenic signaling. Potential novel mechanisms by which Mg^{2+} prevented calcification were studied by RNA sequencing. Pathways mediating inflammation and extracellular matrix remodeling were enhanced in Klotho$^{-/-}$ mice and reversed by high Mg^{2+}. In addition, high Mg^{2+} diet decreased femoral mineral-bone density by 20% and increased osteoid area, indicating a state of osteomalacia. High Mg^{2+} did not change known mineralization modulators parathyroid hormone, 1.25-dihydroxyvitamin D and Ca^{2+} in serum. Interestingly, Mg^{2+} prevented calcification despite increasing fibroblast growth factor-23 and Pi concentration in Klotho$^{-/-}$ mice. In Saos-2 cells, magnesium dose-dependently inhibited mineralization without affecting osteoblastic matrix production, alkaline phosphatase activity and gene expression.

Conclusion: Mg^{2+} prevents vascular calcification potentially through modification of inflammatory and extracellular matrix remodeling pathways. However, Mg^{2+} affects bone mineralization as well. These results indicate that magnesium treatment is promising to halt vascular calcification, but the bone mineralization defects should be taken into account, and require further study.

O12

Cellular origin and microRNA content of plasma extracellular vesicles in diabetic nephropathy

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Objective: Diabetic nephropathy (DN) is a major complication of diabetes and is responsible for 45% of all end-stage renal disease cases. Extracellular vesicles (EVs) are small cell-derived vesicles that are involved in intercellular communication. MV characteristics, including cellular origin, number, composition and function, are altered in type 2 diabetes (T2DM). EV and microRNA (miRNA) in EV influence vascular disease progression by modulating inflammation and by promoting thrombus formation. In this study we aimed to characterize the cellular origin and miRNA of EVs in plasma samples of T2DM patients during various stages of DN.

Methods: 96 T2DM patients were divided in three groups based on 24 h urinary albumin levels; normoalbuminuria (<30mg/day), microalbuminuria (30-300mg/day), and macroalbuminuria (>300mg/day). EV were measured in plasma and were detected with the viability dye Calcein Violet and cell specific markers by flow cytometry. EV miRNA content was investigated using microRNA profiling qPCR array (752 miRNAs). Seven differentially expressed miRNAs were validated by qPCR.

Results: In microalbuminuria patients, total numbers of EV, and EV from endothelial cells, leukocytes and erythrocytes were significantly elevated. In macroalbuminuria patients, total EV numbers and EV derived from platelets, leukocytes, granulocytes and erythrocytes were elevated compared to diabetic controls. Profiling of the miRNAs in the EV revealed seven differentially expressed miRNAs. Validation of these miRNAs showed significantly increased EV expression of miR-99a-5p in macroalbuminuria patients compared to normo- and microalbuminuria patients. Elevated levels of miR-99a-5p in EV may contribute to renal inflammation and fibrosis, as miR-99a-5p targets fibroblast growth factor receptor 3 and mammalian target of rapamycin.

Conclusion: EV from T2DM patients with micro- and macroalbuminuria display pro-inflammatory and pro-coagulant profiles. Further research is needed to explore the role of EV and EV mediated miR-99a-5p delivery in diabetic renal injury.

Poster session A

“Pitched Posters from Poster Highlights session”

P1

A flow cytometry-based assay for alternative and terminal complement pathway activity in the glomerular endothelial microenvironment

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Objective

Deregulation of the alternative pathway of complement activation in the glomerular endothelial microenvironment is characteristic of the renal diseases atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G). Ultimately, terminal pathway activation damages glomerular endothelial cells and leads to loss of renal function. The plasma protein complement factor H regulates alternative pathway activation on host tissues by recognizing host cell glycans such as heparan sulfate or sialoglycans. Factor H-related proteins (FHRs) further modulate complement regulation by competing with factor H for these cell surface glycan ligands. Additionally, host cells are protected by membrane-associated complement regulators. Atypical HUS and C3G have been associated with mutations in members of the factor H protein family and membrane-associated inhibitors, indicating that both contribute to complement regulation in the glomerulus. When evaluating deregulation in the glomerular endothelial microenvironment using serum from patients with complement-mediated nephropathies, we observed that alternative pathway activation is difficult to achieve on glomerular endothelial cells *in vitro*. We therefore sensitized glomerular endothelial cells to alternative and terminal pathway activation by inhibiting cell surface regulators, enabling quantification of complement deregulation in the glomerular endothelial microenvironment.

Methods

Alternative and terminal pathway regulator expression in conditionally immortalized human glomerular endothelial cells (ciGEnCs) was evaluated using real-time PCR and flow cytometry. Detected regulators were inhibited using monoclonal antibodies and ciGEnCs were incubated with human serum. C3 and C9 deposition on the cell surface was evaluated using flow cytometry as measures for alternative and terminal pathway activity, respectively.

Results

ciGEnCs expressed the alternative pathway regulators membrane cofactor protein (CD46) and decay-accelerating factor (CD55), but not complement receptor 1 (CD35), as well as the terminal pathway regulator protectin (CD59). Inhibiting CD46, CD55 and CD59 using monoclonal antibodies resulted in significantly increased C3 and C9 deposition. Adding factor H-specific antiserum, depleting factor H from human serum or supplementing serum with recombinant FHRs increased alternative and terminal pathway activity on the cell surface. Incubation with aHUS patient sera revealed significant deregulation on ciGEnC membranes compared to serum from healthy controls.

Conclusion

In conclusion, ciGEnCs were successfully sensitized to alternative and terminal pathway activation by inhibiting membrane-associated complement regulators, enabling the detection of complement deregulation in aHUS patient sera. Sera from additional aHUS patients as well as patients with C3G are currently being screened.

P2

Plasma Malondialdehyde and Risk of New-Onset Diabetes After Transplantation in Renal Transplant Recipients

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Objective

New-onset diabetes after transplantation (NODAT) is a frequent complication following renal transplantation, adversely affecting patient and graft survival. Oxidative stress has been associated with diabetes mellitus; however, data regarding NODAT are limited. We prospectively determined the association between the oxidative stress biomarker malondialdehyde (MDA) and NODAT in renal transplant recipients (RTR).

Research design and methods

Nondiabetic RTR with a functioning graft for ≥1 year were eligible. Total plasma MDA concentration was measured by high-performance liquid chromatography. Multivariable Cox proportional hazards regression analyses were performed to determine the prospective association of plasma MDA concentration with the development of NODAT.

Results

We included 516 RTR (age 51±13 years, 57% men). Plasma MDA concentration was 2.54 [1.92-3.66] µmol/L. During a median follow-up of 5.3 [4.6–5.9] years, 56 (11%) RTR developed NODAT. In crude Cox regression analyses, plasma MDA concentration were inversely associated with the risk of NODAT (hazard ratio (HR) 0.76 [95% CI 0.59-0.95]; P=0.02). This association remained independent of adjustment for potential confounders, including plasma glucose concentrations and smoking status. The effect of plasma MDA on NODAT was modified by the intakes of vitamin E ($P_{interaction}$=0.06), alpha lipoic acid (ALA) ($P_{interaction}$=0.02) and linoleic acid (LA) ($P_{interaction}$=0.02), with strongest inverse associations of plasma MDA with NODAT among patients with high vitamin E intake, (HR 0.58 [95% CI 0.38-0.89]; P=0.02), high ALA intake (HR 0.61 [95% CI 0.43-0.88]; P=0.008) and high LA intake (HR 0.63 [95% CI 0.44-0.90]; P=0.01).

Conclusions

Plasma MDA concentrations are inversely and independently associated with the risk of NODAT in RTR. Oxidative stress may play an under recognized role in the post-transplant glucose homeostasis.

P3

Parietal epithelial cells maintain the epithelial cell continuum forming Bowman's space in glomerulosclerosis

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Objective: The Bowman's space of a glomerulus is formed by the continuum of glomerular epithelial cells, i.e. the podocytes on the glomerular tuft and the parietal epithelial cells on Bowman's capsule, which show a sharp transition at the vascular pole of the glomerulus. In focal segmental glomerulosclerosis (FSGS), a hallmark of progressive glomerular disease, glomeruli show segmental scarring. It has been proposed that these scar lesions interrupt the epithelial continuum of the Bowman's space (BS) leading to misdirected filtration of pre-urine into the tubulo-interstitial space surrounding the nephron, which in turn lead to tubulo-interstitial fibrosis and inflammation. We studied the transition between PECs and podocytes in sclerotic glomeruli to investigate if PECs form new connections with podocytes and maintain the cell continuum of the BS.

Methods: We used kidney tissue from 40-50 weeks old Munich Wistar Frömter (MWF) rats (n=5). MWF rats are spontaneously hypertensive and develop glomerulosclerosis at 35 weeks after birth. Immunofluorescent stainings for PEC, PEC matrix and podocyte markers were performed to study the spatial localization of PECs in respect to the podocytes. In addition, connections between PECs and podocytes were studied at ultrastructural level using electron microscopy. Furthermore, we performed 3D reconstruction of registered whole slide images of serial sections double stained for podocytes and PECs. We used this information to examine the alteration of the BS and its connection to the proximal tubule.

Results: Analysis of the PEC marker and PEC matrix staining revealed the presence of ingrown PECs between the sclerotic and healthy glomerular segments. At the end of these ingrowths, PECs were positioned directly adjacent to the podocytes which, in healthy glomeruli, is only seen at the vascular pole. This observation was verified at ultrastructural level. The gained results lead to the assumption that PECs might have a role in restoring the epithelial continuum, thereby creating a barrier between sclerotic and healthy glomerular parts which results in a change of shape of the BS. 3D reconstructions of the BS of sclerotic glomeruli revealed that in the vast majority of examined sclerotic glomeruli the BS, surrounding the healthy segments of the glomeruli, are still connected to each other and to the proximal tubule.

Conclusion: Parietal epithelial cells might play a role in maintaining or restoring the epithelial cell continuum forming BS, by creating new connections to podocytes. This action results in the formation of a barrier between healthy and affected glomerular segments. We observed that the altered BS in sclerotic glomeruli was still connected to the proximal tubule, indicating that the morphologically normal segments of the glomerulus can maintain their filtration activity and thus function. To prove that PECs form real new cell-cell contacts with podocytes, future experiments are needed that visualize junctions between these two cell types. In addition, our findings need to be verified in human FSGS lesions.

P4

Pharmacologically stimulating the NOS-NO-sGC pathway to prevent podocyte injury

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Objective: Nitric Oxide (NO) production was shown to play a cardinal role in the kidney, e.g regulating renal and glomerular hemodynamics, as well as tubular transport processes. Little is known, however, about the specific effects of local NO synthesis on podocytes, and thereby the role of NO in maintaining the integrity of the glomerular filtration barrier. We hypothesize that NO production by glomerular endothelial cells (GEnCs) might act as a protective paracrine factor in the glomerulus and prevent podocyte injury. NO is known to stimulate soluble guanylyl cyclase (sGC), producing cGMP, which (as we have previously shown) inhibits deleterious podocyte signaling processes, e.g. by reducing expression and activity of the Ca^{2+}–permeable Transient Receptor Potential Channel 6 (TRPC6). Importantly, several pharmacological sGC activators/stimulators were recently introduced and are currently prescribed for non-renal disorders. We therefore aim to characterize glomerular NOS-NO-sGC signaling and to investigate the potential of repurposing sGC activators to prevent podocyte injury.

Methods: *In vitro* experiments were performed using conditionally immortalized mouse and human glomerular endothelial cells (mGEnCs and ciGEnCs, respectively), as well as mouse and human podocytes (MPC5 and hPOD, respectively). mGEnCs were cultured for 7 days both in static conditions and under a flow of 5dyn/cm^2 to mimic the physiological glomerular blood flow. NO production was visualized using the NO-sensitive dye DAF-FM diacetate. Podocyte injury was induced by incubation with 0.25µg/mL adriamycin for 24hrs and the effect of co-exposure to the NO donor *S*-Nitroso-*N*-acetyl-DL-penicillamin (SNAP, 200µM) or the sGC activator Riociguat (20µM) was evaluated.

Results: Expression of all three forms of nitric oxide synthases (NOS; i.e nNOS, iNOS and eNOS) was observed in mGEnCs, whereas ciGEnCs lacked nNOS. Interestingly, MPC5 and hPOD also expressed the iNOS and eNOS isoforms. In addition, NO production could be confirmed in all cell types. When mGEnCs were cultured under flow, the expression of eNOS and iNOS was elevated two- and six-fold, respectively, and NO production was increased ~three-fold. sGC subunit expression was confirmed in podocytes. Upon exposure of podocytes to SNAP and Riociguat, expression of the podocyte injury marker desmin was decreased. Importantly, Riociguat prevented the adriamycin-induced TRPC6 overexpression in human podocytes.

Conclusion: GEnCs, but also podocytes, express multiple isoforms of NO producing enzymes and are able to produce NO *in vitro*. Flow stimulates the expression of these enzymes, and thereby NO production, in GEnCs. In addition, NO prevents podocyte injury and might therefore preserve the integrity of the glomerular filtration barrier in an autocrine or paracrine manner. Because several market-approved drugs stimulate the NOS-NO-sGC-cGMP TRPC6-inhibiting pathway, further characterization of this pathway might disclose new therapeutic strategies for proteinuric glomerular disease.

P5

A unique renal transplantation program comes of age: 5 year follow-up of the Transatlantic Airlift in the Dutch Antilles

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Objectives: The prevalence of end-stage renal failure in the Dutch Caribbean is one of the highest in the world with an estimated prevalence of 145 per 100 000 residents. To respond to the high incidence of renal failure, the Academic Medical Center in Amsterdam together with the Eurotransplant Foundation began a unique transatlantic collaboration with two hospitals in Aruba: the St. Elisabeth Hospital in 1998, and the Dr. Horacio E. Oduber Hospital in 2003. We aimed to investigate the outcome of this transatlantic program with particular interest in the impact of high cold ischemia time (CIT) in recipients of deceased donors.

Methods: We studied transplantations from April 2007 to May 2018. Patients were followed up for a maximum of 5 years. All patients received induction therapy with basiliximab with triple regimens maintenance therapy. Only living donors and donation after brain-death donors (DBD) were accepted. CIT was categorized into 4 groups, defined as the time from cold flushing until the graft is implanted into the recipient. All DBD donor kidneys were preserved by means of machine perfusion. P-value of <0.05 was considered statistically significant.

Results: During the studied period, 88 patients received a DBD and 31 patients received living donor kidney. Donor age was 53.3 (SD 13.5) and 52.2 (SD 13.7) years for living and DBD donor kidneys, respectively. Mean age of recipients of living donor kidneys was 49.0 years (SD 15.6), and 53.1 years (SD 12.8) for DBD. Original recipient disease included hypertension (31.9%), diabetes (15.1%), glomerulonephritis (4.2%), and cystic kidney disease (5.0%). Mean CIT was 2.9 hours (SD 0.6) for living, and 27.0 hours (SD 9.2) for DBD. Primary function (PNF) and delayed graft function (DGF) occurred in 2 (6.5%) and 3 (9.7%) recipients of living donor kidneys, respectively. In DBD donor kidneys, 2 (2.3%) recipients had PNF and 24 (27.3%) had DGF. Five year graft survival was 70.0% and 74.0% for living and DBD donors respectively (unadjusted p= .533). Death-censored 5 year graft survival was 87.7% and 82.3% for living and DBD, respectively (unadjusted p= .216). Five year patient survival was 78.2% and 84.6% for living and DBD, respectively (unadjusted p= .933). Five year death-censored graft survival of DBD donors with comparing the CIT categories; 0-18 (n=12), 19-24 (n=22), 25-36 (n=36), 37-57 hours (n=15), was respectively; 90.9%, 79.1%, 69.4%, and 92.9%, (unadjusted p= .473).

Conclusion: Five year follow-up showed that graft survival and patient survival were comparable between recipients of DBD and living donor kidneys. Furthermore, within the deceased donor kidney recipients, there was no significant difference in graft survival between the CIT categories. Although the numbers are still relatively small, these results are encouraging for the future of the trans-Atlantic program regarding the acceptance of grafts with high CIT.

P6

Eculizumab therapy monitoring: residual hemolysis in alternative pathway test is not caused by C5 activity

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Eculizumab is a C5-blocker used in the treatment of atypical hemolytic uremic syndrome (aHUS), paroxysmal nocturnal hemoglobinuria and generalized myasthenia gravis. Hemolytic assays are still used to monitor classical (CP) and alternative pathway (AP) blockade in these patients. The AP assay is known to show hemolysis in patient samples that attain target drug concentrations > 100 µg/mL. This suggests incomplete complement blockade and may lead to a change of therapy (higher dose or alternative drug). Therefore, we investigated whether the residual hemolysis detected by the AP assay in the presence of eculizumab is caused by incomplete C5 blockade by the drug.

Normal human serum (NHS) spiked with eculizumab (100, 200 and 500 µg/mL) and five aHUS samples containing 256-371 µg/mL of the drug had AP lysis of 17 - 48 %, whereas lysis of heat-inactivated NHS (dNHS) and NHS was < 10% and 89 ± 5%, respectively. The CP hemolytic assay and ELISA-based AP and CP Wieslab® tests showed blocked complement activity for all eculizumab samples.

AP assay performed in genetically C5 deficient serum gave lysis of 37 ± 4 %, in the range of eculizumab samples, whereas it was completely negative in CP and AP Wieslab® tests. Thus, hemolysis in AP occurs even in the absence of C5.

Furthermore, NHS spiked with 100 µg/mL of eculizumab and stimulated by 100 µg/mL of zymosan A for up to 60' produced significant time-dependent increase of C3 activation markers C3bBbP, C3bc and C3a but not of the C5 activation marker sC5b-9.

Thus, our data indicate that hemolysis seen in AP hemolytic assay under eculizumab treatment is not caused by the residual C5 activity. Alternatively, it may be caused by other factors, such as C3b opsonization, which may weaken the erythrocytes and make them more prone to hemolysis. ELISA-based methods are the better option to monitor C5 blockade during eculizumab treatment.

Poster session B

“Experimental Nephrology”

P7

Developmental aspects of renal membrane transporters

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Objectives:

The kidney has a critical role in disposition, efficacy and toxicity of drugs and xenobiotics. Developmental changes of renal membrane transporters have the potential to explain population variability in paediatric pharmacokinetics and -dynamics of drugs but data are missing. To further delineate the expression of human renal tubular transporters using immunohistochemistry (IHC) and study localization in paediatric kidney samples. To ultimately understand drug disposition in newborns and determine safe paediatric dosing guidelines.

Methods:

We planned to study the age-specific localization and semi-quantify expression levels of the transporters multidrug resistance-associated protein 4 (MRP4), multidrug resistance-associated protein 2 (MRP2) and organic cation transporter 2 (OCT2) with immunohistochemistry on 44 human neonatal and paediatric kidney samples. The staining intensity was semi-quantitatively scored by two independent observers.

Results:

MRP4 is found to be localized at the apical membrane of the renal proximal tubules at 27 weeks of gestational age (N=3) and no age-related changes of expression levels were detected. In a neonate of 24 weeks gestational age (N=1), no MRP4 was detected. The MRP2 and OCT2 staining did not meet the requirements to be scored and was rejected.

Discussion:

MRP4, the renal apical transporter in the membrane of the proximal tubules, is expressed from at least 27 weeks gestational age onwards and does not show developmental changes. The absence of MRP4 in premature neonates (<27 weeks) could potentially be linked with the 6 to 20-fold longer half-life of furosemide, an MRP4 substrate compared to half-life in adults and provides a mechanistic basis for adjusted dosing guidelines.

P8

Cultured glomerular cell lines as predictive tools for detecting circulating permeability factor(s) in steroid-resistant FSGS

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Objective

Many patients with steroid-resistant focal segmental glomerulosclerosis (FSGS) require kidney transplantation. However, heavy proteinuria often develops immediately after transplantation, and FSGS recurs. In some cases, plasma exchange therapy can reduce proteinuria. Therefore, one or more circulating plasma permeability factor(s) (CPF) are held responsible for recurrent FSGS. Despite many attempts, the identity of the CPF(s) remains unknown. In order to predict FSGS recurrence prior to transplantation, in order to aid in the identification of CPF, and eventually to develop better treatment to remove or neutralize the CPF, validated reproducible methods are required that are able to detect the presence of CPF in plasma of patients.

Methods

Plasmapheresis effluent of 11 patients with steroid-resistant FSGS was collected during active disease, recurrence after transplantation, or (partial) remission. Plasma of disease controls was obtained via either plasmapheresis (IgA nephropathy, minimal change disease) or whole blood plasma (healthy donors, non-recurrent FSGS). Conditionally immortalized human podocytes (hPod) and human and mouse glomerular endothelial cells (ciGEnC, mGEnC) were exposed to 10% plasma for 24 hours. Morphological changes were assessed microscopically. Cell granularity was assessed using flow cytometry (side scatter), and cell viability was measured using Cell Counting Kit-8.

Results

Using our hPod morphology scoring assay, 9 active disease plasmas significantly increased the amount of granules in hPod, compared with plasma of healthy donors. Moreover, effects of 3 paired active disease and (partial) remission plasmas on granularity correlated with proteinuria at the time of plasmapheresis. No effect of disease control plasmas was detected. However, also plasmas of 4 patients with post-transplant recurrent FSGS had no effect. Flow cytometry showed that hPod, ciGEnC, and mGEnC in parallel responded to presumably CPF-containing plasmas. Only samples in which very strong effects on granularity were detected, also reduced cell viability.

Conclusion

We established several *in vitro* models using glomerular cell lines that uniformly detect effects of plasmas presumably containing CPF. Our hPod granularity model showed the highest sensitivity and disease-specificity. However, not all presumably CPF-containing plasmas induced a response, suggesting heterogeneity in response or the presence of different CPFs altogether. These models will be implemented in our future research aimed at identification of the CPF(s) in patients with recurrent FSGS, and may aid in developing a diagnostic and/or pre-transplant predictive tool for detecting CPF in patients with steroid-resistant FSGS.

P9

Pioglitazone and Tolvaptan in a mouse model of Autosomal Dominant Polycystic Kidney Disease (ADPKD)

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Objective:
Autosomal dominant polycystic kidney disease (ADPKD) is a progressive debilitating disease, and is characterized by progressive renal cyst formation and fibrosis. ADPKD is caused by mutations in either the *PKD1* or *PKD2* gene, and affects 1 in 2500 individuals. Based on clinical trials, the lowering of intracellular cAMP levels is the most promising way so far to slow disease progression, and the vasopressin V2 antagonist tolvaptan has been licensed for ADPKD treatment as a result. However, due to unfavourable side effects, such as massive aquaresis, the drug is not well tolerated by all patients and there remains a need for alternative approaches. We hypothesize that the use of a combination treatment can result in a better therapy for ADPKD patients, as the complex ADPKD signalling is targeted from various angles simultaneously. For this, we conducted a preclinical study in an adult onset ADPKD model to investigate the effects of a combination treatment of tolvaptan and pioglitazone, a peroxisome proliferator-activated receptor gamma (PPARγ) agonist. This drug has recently been shown to slow disease progression in an ADPKD rat model, and is currently tested in a Phase II clinical trial with ADPKD patients.

Methods:
In vitro: Tolvaptan and/or pioglitazone were tested in a 3D-organoid model for PKD (mIMCD3-Pkd1KO cells) to evaluate their individual and combined efficacy.
In vivo: An inducible adult onset model for ADPKD (P18Ksp*Pkd1*^{del} mice) was used. Following *Pkd1* inactivation by tamoxifen (via oral gavage) on postnatal day 18, mice were fed with food pellets containing 0.1% tolvaptan and/or 30 mg/kg/day pioglitazone. Mice were treated until 50% of the untreated group had reached renal failure (blood urea levels > 20 mmol/L).

Results:
Tolvaptan and pioglitazone were both effective in slowing down cyst growth *in vitro* in a non-toxic manner. *In vivo*, tolvaptan was able to improve renal survival significantly (86% vs. 41% in the untreated group, $p < 0.01$) and a 2-fold reduction of the 2KW/BW ratio ($p < 0.001$). Pioglitazone did elevate plasma adiponectin levels (207% vs. untreated, $p < 0.001$), but did not have an effect on renal survival. Moreover, pioglitazone treatment did not change the expression of multiple PPARγ target genes, compared to the untreated group.

Conclusion:
Tolvaptan did slow down cyst growth *in vitro* and improved renal survival *in vivo*, confirming the relevance of our model systems. The cyst inhibiting properties of pioglitazone, previously shown in rats, were confirmed *in vitro*, but no therapeutic benefit was seen *in vivo*.

P10

Heparan sulphate oligosaccharides as novel biomarkers for glomerular diseases

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Objective

Kidney disease affects 10% of the world population. Filtration of the blood takes place in the glomerulus, where it first encounters the glycocalyx of the glomerular endothelial cells (GEnCs). This carbohydrate rich layer contains a large proportion of heparan sulphate (HS), a negatively charged glycosaminoglycan with diverse structural complexity. HS in the healthy glycocalyx contributes to endothelial barrier function and prevents the adhesion of inflammatory medicators, such as cells, cytokines and chemokines. A diseased glycocalyx leads to protein leakage and facilitates binding of inflammatory mediators. The chemical structure of HS is primarily defined during its biosynthesis in the Golgi. Post-synthesis modifications by sulphatases and heparanase may produce altered HS chain structures at the cell surface and free HS oligosaccharides, thereby modifying the HS in response to biological stimuli. These altered HS oligosaccharide species may thus serve as potential biomarkers of glomerular diseases.

Method

HS libraries have been established from GEnCs treated with different inflammatory mediators and conditions, such as TNFα, IL-1β, LPS, hyperglycemia and/or advanced glycation end products. HS was isolated and fractionated into size and charge species before structural characterisation by mass spectrometry. To generate highly sulphated HS domains suitable for MS analysis, fractions larger than a degree of polymerisation (dp) 12 were digested using heparinase III before size exclusion and strong anion exchange chromatographies were performed. Alongside, HS oligosaccharides were isolated from the plasma and urine of healthy controls and patients with renal inflammation, including: type I and type II diabetes with albuminuria, atypical hemolytic uremic syndrome, systemic lupus erythematosus, and chronic glomerular nephritis.

Results

GEnC HS oligosaccharides libraries have been generated for a range of inflammatory conditions. Some HS oligosaccharide species were increased and others decreased after treatment with the different conditions, suggesting that more than one HS oligosaccharide is important in the inflammatory process. LPS stimulation produced a unique HS oligosaccharide species, not found in the control, TNFα, or IL1β-stimulated cells. Patient plasma and urine samples from different renal conditions showed distinct HS oligosaccharide species not present in samples from other renal diseases, nor in healthy control samples. Notably, specific HS oligosaccharides were also detected for some patients without increased heparanase activity.

Conclusions

HS oligosaccharide biomarkers may be identifiable in a wide range of glomerular diseases, including those not associated with higher heparanase activity. Efforts continue to fully characterise these HS oligosaccharides as potential biomarkers elucidate their biological function during inflammation. Identification and characterisation of potential HS biomarkers will support the development of disease diagnostics for early intervention and increase understanding of the role of HS in renal inflammation.

P11

Interaction between drugs and endogenous metabolites for renal organic anion transport

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Objective: Recently, we showed that conditionally immortalized proximal tubule epithelial cells (ciPTECs) expressing organic anionic transporter 1 (OAT1) cultured on biofunctionalized membranes, actively secrete PBUTs, this being a first step towards the development of a bioartificial kidney (BAK)[1]. However, OAT1 also handles a wide range of drugs. We hypothesize that the medication that patients use to control ESKD-associated comorbidities would interact with the ciPTECs-OAT1 and, in the context of a ciPTECs-loaded BAK, the OAT1-mediated removal of drugs might compromise the clearance capacity for PBUTs by the BAK. In this study, we evaluated the interaction between commonly prescribed drugs in CKD (ACE inhibitors, ATII inhibitors, statins and furosemide) and an endogenous PBUT (indoxyl sulfate) with respect to OAT1-mediated uptake.

Methods: A panel of 9 drugs was screened for interactions in ciPTECs-OAT1 monolayers in the presence (+) and absence (-) of indoxyl sulfate (IS, at an uremic concentration [110µM]), as previously described [2]. To evaluate OAT1 function, fluorescein was used as substrate and its uptake was measured using a multi-plate reader.

Results: Our results show that ACE-inhibitors and cimetidine have either no effect or a slight effect (at non-therapeutical concentrations) on OAT1-mediated fluorescein uptake. On the contrary, ATII-inhibitors, statins and furosemide significantly reduced fluorescein uptake, with the highest potency for ATII-inhibitors. This trend was maintained in presence of IS, suggesting that these drugs could negatively influence secretion of PBUTs by ciPTECs. (**Table 1**).

Table 1. OAT1-mediated fluorescein uptake
IC_{50} (µM) in the absence (-) and presence (+) of IS *

Drug	**IC_{50} - IS**	**IC_{50} + IS**	**Drug**	**IC_{50} - IS**	**IC_{50} + IS**
ACE inhibitors			**Statins**		
Captopril	No effect	NT	Simvastatin	30.5 ± 4.94	27.23 ± 0.87
Enalaprilate	NT	NT	Pravastatin	478.6 ± 29.81	379.8 ± 18.95
Lisinoprol	No effect	No effect	**Others**		
ATII inhibitors			Furosemide	30.30 ± 4.53	158.75 ± 5.40
Losartan	14.37 ± 1.88	89.66 ± 3.62	Cimetidine	NT	No effect
Valsartan	24.55 ± 1.97	9.01±3.23			

* Values are given as mean of two independent observations. NT=non-therapeutical concentration.

Conclusions Further studies should address the effect of protein binding on the clearance capacity, as well as the transepithelial transport of PBUTs across the living membranes in the presence of drugs to understand the interaction between PBUTs and drugs on their secretion by a BAK system.

P12

Cleaved N-terminal histone tails distinguish between NADPH oxidase (NOX)-dependent and NOX-independent pathways of neutrophil extracellular trap formation

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Objectives. Neutrophil extracellular traps (NETs) act in various rheumatic diseases. Although NET formation was originally described as a NADPH oxidase (NOX)-dependent pathway, it appears there are also NOX-independent pathways of NET release. Currently no tools are available that can discriminate between both NET-forming pathways. We aimed to develop a serological method allowing the discrimination between NETs generated through NOX-dependent or NOX-independent pathways.

Methods. Histones from *in vitro* generated NOX-dependent and NOX-independent NETs were characterized with a panel of lupus-derived antibodies against N-terminal histone tails using immunofluorescence microscopy, Western blot and enzyme-linked immunosorbent assays (ELISA). NETs in patients with NET-associated diseases, i.e. rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriatic arthritis (PsA) and sepsis, were characterized in sandwich ELISAs employing antibodies against myeloperoxidase (MPO) and N-terminal histone tails as detecting and capturing antibodies, respectively. Functional responses of endothelial cells to NOX-dependent and NOX-independent NETs were assessed as well.

Results. Neutrophil elastase cleaves the N-terminal tails of core histones during NOX-dependent, but not during NOX-independent NET formation. Consequently, the detection of MPO - histone complexes with antibodies against N-terminal histone tails allows discrimination between NETs formed through a NOX-dependent or NOX-independent manner. Characterization of *in vivo* circulating NETs revealed the presence of NOX-independent NETs in RA, SLE and sepsis, but NOX-dependent NETs in PsA. NOX-independent NETs displayed an increased capacity to activate endothelial cells when compared to NOX-dependent NETs.

Conclusions. These results indicate heterogeneity in NET-forming pathways *in vivo* and highlight the need for disease-specific strategies to prevent NET-mediated pathology.

P13

Polycystin-1 dysfunction impairs electrolyte and water handling in a renal pre-cystic mouse model for autosomal dominant polycystic kidney disease

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Objective: The *PKD1* gene encodes polycystin-1 (PC1), a mechanosensor triggering intracellular responses upon urinary flow sensing in kidney tubular cells. Mutations in *PKD1* lead to autosomal dominant polycystic kidney disease (ADPKD). The involvement of PC1 in renal electrolyte and water handling remains unknown since the electrolyte and water balance in ADPKD patients has only been investigated in cystic ADPKD. We thus studied the role of PC1 in renal electrolyte and water handling in a renal pre-cystic context.

Methods: An inducible kidney-specific *Pkd1* knockout (iKsp-*Pkd1*$^{-/-}$) mouse model manifesting a pre-cystic phenotype was used to disclose the physiological consequences of PC1 dysfunction in the kidney. This model was generated after oral administration of tamoxifen to iKsp-*Pkd1*$^{lox/lox}$ mice at post-natal day 18 (PN18). Immunohistochemistry in specific nephron segments of control (*Pkd1*$^{+/+}$) and iKsp-*Pkd1*$^{-/-}$ mice at PN18 + 29 days was performed to accurately characterize the pre-cystic phenotype of the kidneys of iKsp-*Pkd1*$^{-/-}$ mice. The electrolyte content in the urine and feces of control and iKsp-*Pkd1*$^{-/-}$ mice was measured at PN18 + 22 and PN18 + 29 days. The serum electrolyte content was measured at PN18 + 29 days, day at which animals were sacrificed. At this day, gene expression of relevant genes for electrolyte handling in the kidney and intestine was measured by RTqPCR.

Results: Serum and urinary electrolyte determinations indicated that iKsp-*Pkd1*$^{-/-}$ mice display reduced serum levels of magnesium (Mg^{2+}), calcium (Ca^{2+}), sodium (Na^{+}) and phosphate (P_i) compared with control mice; and renal Mg^{2+}, Ca^{2+} and P_i wasting. In agreement with these electrolyte disturbances, downregulation of key genes for electrolyte reabsorption in the thick ascending limb of Henle's loop (TAL, *Cldn16*, *Kcnj1* and *Slc12a1*), distal convoluted tubule (DCT, *Trpm6* and *Slc12a3*) and connecting tubule (CNT, *Calb1*, *Slc8a1*, *Atp2b4*) was observed in kidneys of iKsp-*Pkd1*$^{-/-}$ mice compared with controls. Similarly, decreased renal gene expression of markers for TAL (*Umod*) and DCT (*Pvalb*) was observed in iKsp-*Pkd1*$^{-/-}$ mice. Conversely, mRNA expression levels in kidney of genes encoding solute and water transporters in the proximal tubule (*Abcg2* and *Slc34a1*) and collecting duct (*Aqp2*, *Scnn1a* and *Scnn1b*) remained comparable between control and iKsp-*Pkd1*$^{-/-}$ mice, though a water reabsorption defect was observed in iKsp-*Pkd1*$^{-/-}$ mice.

Conclusion: In conclusion, our data indicate that PC1 is involved in renal Mg^{2+}, Ca^{2+} and water handling, and its dysfunction resulting in a systemic electrolyte imbalance characterized by low serum electrolyte concentrations.

Poster session C

“Transplantation”

P14

Autoantibodies to Apolipoprotein A-1 as Independent Predictors of Cardiovascular Mortality in Renal Transplant Recipients

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Objectives: High levels of autoantibodies directed against apoA-1 (anti-apoA-1 IgG) are related to an independent increased cardiovascular disease (CVD) risk in different clinical settings and were found to be increased in end-stage renal disease. In renal transplant recipients (RTR), a vulnerable patient population with a largely increased risk of CVD mortality, traditional risk factors don't suitably predict CVD mortality. Thus, accurate CVD risk stratification in RTR represents an unmet clinical need in this constantly increasing patient population. Our aim is therefore to determine i) the prognostic value of anti-apoA-1 IgG for incidence of CVD specific mortality, all-cause mortality and graft failure in RTR and ii) to delineate the relationship of anti-apoA-1 IgG with HDL functionality.

Methods: 462 prospectively included RTR were followed for 7.0 years. Baseline anti-apoA-1 IgG were determined and associations with incidence of CVD mortality (n=48), all-cause mortality (n=92) and graft failure (n=39) were tested. HDL functionality was assessed in vitro by measuring cholesterol efflux capacity (CEC).

Results: Kaplan–Meier analyses demonstrated significant associations between tertiles of anti-apoA-1 IgG and CVD mortality (log rank test: P=0.048). Adjusted Cox regression analysis showed a 54% increase in risk for CVD mortality for each anti-apoA-1 IgG levels standard deviation increase (hazard ratio [HR]: 1.53, 95%ConfidenceInterval [95%CI]:1.14-2.05, p=0.005), and a 33% increase for all-cause mortality (HR: 1.33;95%CI:1.06-1.67,p=0.01), independent of CVD risk factors, renal and HDL function. The association with all-cause mortality disappeared after excluding cases of CVD specific mortality. The sensitivity, specificity, positive predictive value, and negative predictive value of anti-apoA-1 positivity for CVD mortality were 18.0%, 89.3%, 17.0%, and 90.0%, respectively. HDL functionality was not associated with anti-apoA-1 IgG levels.

Conclusion: This prospective study demonstrates that in RTR, anti-apoA-1 IgG are independent predictors of CVD mortality and are not associated with HDL functionality.

P15

Living donor transplantation leads to a major improvement in physical functioning, which is not paralleled by changes in physical activity and body composition

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Objective

Kidney transplantation (KTx) is expected to improve physical health. As prospective studies combining physical functioning (PF), physical activity (PA) and body composition (BC) after KTx are scarce, we aimed to study differences in these parameters between kidney transplant patients and their living donors, and study changes in recipients and donors in the first year after KTx/donation.

Methods

Twenty-two kidney transplant patients and 22 healthy kidney donors were included in this prospective longitudinal study with a follow-up until 12 months. PF was assessed by handgrip strength (HGS) and the physical domains of the Short form-36 questionnaire (PF (SF-36 PF) and physical component summary (PCS) score), BC was measured by the Body Composition Monitor©, PA was measured by the SenseWear^{TM} pro3.

Results

At baseline, KTx recipients had significantly lower HGS (after adjustment for sex and body weight), SF-36 PF, PCS, and PA as compared with their donors. In recipients HGS significantly increased in the first year after KTx, but PA did not change in the first six months after KTx. Body weight increased significantly twelve months after KTx, but no significant increase in lean tissue mass was observed. For healthy donors no significant changes in these parameters were observed, with exception of SF-36 PF, which declined in the first three months after donation, but equaled baseline values after twelve months.

Conclusion

PF showed major improvements after KTx reaching levels of kidney donors already six months post-KTx, which was not paralleled by an increase in PA or lean tissue mass. Therefore, reversal of the uremic state appears to have independent positive effects on PF. Moreover, after kidney donation, there was no prolonged decrease in any of the outcome parameters.

P16
Histopathological examination of removed kidney allografts: Is it useful?

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Objective: The rate of allograft nephrectomy after graft failure varies between 4.5% and 84.4%, reflecting the lack of a standard policy. The incidence and relevance of histological findings in removed grafts is unknown. In this study, we investigated the outcome of histopathological examination of removed grafts.

Methods: We performed a retrospective cohort study in patients who underwent a kidney transplantation between 1981 and 2015, and in whom the graft failed ≥ 3 months after transplantation. In these cases, our policy is to remove the graft only on indication (e.g. graft intolerance syndrome). We routinely send the removed graft for histopathological examination. In total, 198 allograft nephrectomies were performed. In 31 cases, no pathology report was available, leaving 167 cases for analysis. Pathology reports were especially reviewed for recurrences of primary disease and for unexpected findings. Moreover, the consequences for clinical management of these findings were examined in the patient charts.

Results: In 17 of the 167 examined grafts, gross necrosis due to infarction or thrombosis precluded adequate interpretation. Signs of acute and/or chronic rejection were reported in 137 of the remaining 150 allografts. Recurrence of the original disease was the main finding in 8 cases: primary focal segmental glomerulosclerosis (n=4) and membranoproliferative glomerulonephritis (n=4). In all cases, the recurrence was already known from prior kidney graft biopsies. Relevant secondary findings were present in 8 cases: renal cell carcinoma (n=2), urothelial cell carcinoma, candida pyelonephritis (n=2), PTLD, polyomavirus inclusions, and oxalate deposition. All these conditions were already diagnosed before the graft nephrectomy, with the exception of one case of papillary renal cell carcinoma of 0.8 cm. The clinical consequence of the latter finding was that retransplantation for this patient was withheld for 9 months.

Conclusion: As expected, acute and/or chronic rejection is the main histopathological finding in grafts that are removed after late graft failure. Unexpected secondary findings are very rare. We therefore question whether routine histopathological examination of removed kidney allografts is useful and cost-effective.

P17

Living donor and recipient specific associations of biomarkers of endothelial dysfunction, low-grade inflammation and advanced glycation endproducts

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Objective

Renal transplantation (rTx) has major survival benefits, mainly driven by reduction of cardiovascular risk. Endothelial dysfunction (ED), low-grade inflammation (LGI) and advanced glycation endproducts (AGEs) are associated with cardiovascular complications in end-stage renal disease (ESRD). However, literature about the effect of rTx and donation on biomarkers of ED, LGI and AGEs is scarce.

Methods

Seventeen recipients and 16 donors were included in a pre-rTx cross-sectional analysis. Fifteen recipients had complete follow-up data at baseline and three and six months post-rTx, and 13 donors had data at baseline and three months post-donation. Biomarkers of ED and LGI were measured with a single- or multiplex array detection system (Meso Scale Discovery). Standardized composite scores were calculated for ED (sVCAM-1, E-selectin, thrombomodulin, sICAM1) and LGI (hs-CRP, SAA, IL-6, IL-8, TNF-α, sICAM-1). AGE accumulation was assessed by measuring skin autofluorescence (SAF) with the AGE reader CU^{TM}.

Results

Pre-rTx, recipients had higher composite scores for ED (beta (95%CI) 1.32 (0.80; 1.91) SD), LGI (0.91 (0.29; 1.54) SD) and higher SAF (0.85 (0.42; 1.27) AU), as compared with donors. In recipients, sVCAM-1 ($p=0.004$), thrombomodulin ($p<0.001$) and TNF-α ($p=0.001$) decreased in the first three months post-rTx, and stabilized in the three months thereafter. In donors, sVCAM-1 ($p=0.028$), thrombomodulin ($p=0.001$) and TNF-α ($p=0.020$) increased in the first three months post-donation, and approached values obtained in recipients at this time period.

Conclusion

Pre-rTx, biomarkers of ED and LGI, and AGE accumulation were higher in rTx recipients, as compared with healthy donors. Biomarkers of ED and LGI improved after rTx, which may suggest that rTx has a positive effect on cardiovascular mortality and morbidity trough improvements of ED and LGI. In donors, an increase in biomarkers of ED and LGI was observed three months post-donation of which the clinical relevance is yet uncertain.

P18

The potential impact of hematocrit correction on evaluation of tacrolimus target exposure in pediatric kidney transplant patients

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Objective

Tacrolimus is an important immunosuppressive agent with high intra- and interindividual pharmacokinetic variability and a narrow therapeutic index. The optimal target exposure for tacrolimus in pediatric kidney transplant patients is mainly based on adult experience. As tacrolimus extensively accumulates in erythrocytes, hematocrit is a key factor in the interpretation of tacrolimus whole blood concentrations. However, as hematocrit values in children are generally different than in adults, translating whole blood concentration targets from adults to children without taking hematocrit into consideration, is theoretically incorrect. The aim of this study is to evaluate the potential impact of hematocrit correction on tacrolimus target exposure in pediatric kidney transplant patients.

Methods

Data were obtained from 36 pediatric kidney transplant patients. 255 tacrolimus whole blood samples were available, together responsible for 36 area under the concentration time curves (AUCs) and trough concentrations. First, hematocrit-corrected concentrations were derived using a formula describing the relationship between whole blood concentrations, hematocrit, and plasma concentrations. Subsequently, target exposure was evaluated using the converted plasma target concentrations. Ultimately, differences in interpretation of target exposure were identified and evaluated.

Results

In total, 92% of our patients had a lower hematocrit (median 0.29) than the adult reference value. A different evaluation of target exposure for either trough level, AUC or both, was defined in 42% of our patients, when applying hematocrit corrected target concentrations.

Conclusion

A critical role for hematocrit in therapeutic drug monitoring of tacrolimus in pediatric kidney transplant patients is suggested in this study. Therefore, we believe that hematocrit correction could be a step towards improvement of tacrolimus dose individualization.

P19

Urinary corticosteroid metabolites and 11β-HSD activity in renal transplant recipients

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Objective: Corticosteroids have been a mainstay of immunosuppressive therapy in renal transplant recipients (RTR), and the myriad of side-effects after prolonged exposure have sparked continuous interest in its underlying physiological effects. Besides the suppressive effects of (exogenous) corticosteroids on the hypothalamic-pituitary-adrenal (HPA) axis, a recent investigation presented evidence that it might also increase activity of 11β-hydroxysteroiddehydrogenase(HSD)-1, an enzyme involved in intracellular cortisol regeneration. The main goal of this study was to clarify the variation in corticosteroid metabolites and 11β-HSD-1 activity in RTR, and to explore potential differences between a group of RTR treated with and a group of RTR treated without corticosteroid maintenance therapy.

Methods: We performed a post-hoc analysis of an existing prospective, open label, randomized investigator driven trial in RTR. A group with corticosteroid-containing immunosuppression (CORT-group) was compared with a group that had early withdrawal of corticosteroid (maintenance) therapy (NON-CORT group). Twenty-four hour urine samples were collected and measurements of corticosteroid metabolites were performed using high-performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). The measured corticosteroid metabolites were: cortisol, cortisone, tetrahydrocortisone (THE), tetrahydrocortisol (THF) and allo-tetrahydrocortisol (Allo-THF), summated total corticosteroid metabolites, ratio cortisol/cortisol and ratio (THF+allo-THF)/THE, with the latter representing 11βHSD-1 activity. Differences in corticosteroid metabolites and ratios between the CORT and NON-CORT group were analyzed with a Mann-Whitney U test.

Results: The HPA-axis as measured by the 24 hour total urinary excretion of corticosteroid metabolites appeared significantly more suppressed in the CORT group as compared to the NON-CORT group at 3 months after renal transplantation ($P<0.001$). (THF+Allo-THF)/THE-ratio was not significantly different between both groups at 3 months, although the CORT group showed a progressive decrease in this ratio, which was sustained until the end of the 24 months observation period. Intra-individual variation of cortisol excretion of RTR treated with the same prednisolone dose differed greatly, from 3-fold (3.0 [1.9-3.5] in the NON-CORT group, to almost 4-fold (3.7[2.2-5.4]) in the CORT group. Inter-individual difference of cortisol excretion showed even larger variation at the same prednisolone dosage and time point, from 11-fold (11,4 [9.0-12,3] in the NON-CORT group to 39-fold (38,5 [22,5-64,7]) in the CORT group.

Conclusion: RTR show a great variation in intra- and inter-individual corticosteroid metabolite secretion, even when receiving identical prednisolone doses. Further research into the metabolism of corticosteroids is necessary to elucidate the underlying mechanisms in RTR.

Poster session D

“Dialysis”

P20

Longitudinal patterns of health-related quality of life and dialysis modality: a US cohort study

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Objectives: Health-related quality of life (HRQOL) varies among chronic dialysis patients who receive in-center hemodialysis versus home modalities. However, little is known about the association of dialysis modality with health-related quality of life over time. We describe longitudinal patterns of HRQOL among chronic dialysis patients by treatment modality.

Methods: In this retrospective cohort study, 886 adult patients who initiated home modality techniques (825 peritoneal dialysis and 61 home hemodialysis) were matched to 4234 clinically similar patients who initiated in-center hemodialysis between January 2013 and June 2015. All patients remained on the same modality for the first 120 days of the first two years and survived at least 485 days. HRQOL was assessed within the first 120 days of dialysis initiation (Period 1) and between 365 and 485 days (Period 2) after dialysis initiation.

Results: In-center and home modality patients had relatively similar demographic and clinical characteristics. At baseline, in-center dialysis patients had lower mean KDQOL scores across domains compared to home dialysis patients (Physical component score [PCS] 38.0 ±10.3 versus 41.1±10.5 and Burden of Kidney Disease 53.2±28.0 versus 58.0±27.6 (both $p<0.01$). For patients who remained on the same modality over time, there was no change in health-related quality of life. However, there were clinically meaningful changes in PCS for patients who switched from home to incenter therapies [41.7±10.4 to 38.0±10.4; $p<0.05$].

Conclusions: Among a US cohort of chronic dialysis patients, initial modality and modality change were associated with different patterns of HRQOL Providers and patients should be mindful of quality of life changes that may occur after dialysis modality change.

P21

Bone loss in patients with end-stage renal disease treated with kidney transplantation and different dialysis modalities.

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OBJECTIVES: Patients on dialysis have an increased risk of fractures due to mineral bone disorder. This disturbed mineral metabolism is normalized by kidney transplantation and improved by nocturnal hemodialysis, an intensified hemodialysis regimen. This study had three aims: to compare bone loss between kidney transplant recipients and patients on dialysis, to compare bone loss between different dialysis modalities, and to assess determinants of bone loss.

METHODS: In this prospective cohort, we measured trabecular bone mineral density (BMD) at the thoracic spine with computed tomography at inclusion and annually for up to 3 years, in 45 kidney transplant recipients and 78 patients on dialysis who were all transplantation candidates: 30 on nocturnal hemodialysis, 28 on conventional hemodialysis, and 20 on peritoneal dialysis.

RESULTS: Overall, mean age was 51 ±13 years, and 80 patients (65%) were male. BMD remained stable (increase 1.9 mg/cm^3 per year, 95% CI -1.4–5.2) in kidney transplant recipients, while it decreased with 6.4 mg/cm^3 per year (95% CI 3.7–9.2) in patients on dialysis ($P<0.001$ for difference). This decrease in BMD was not significantly different between patients on nocturnal hemodialysis, conventional hemodialysis, and peritoneal dialysis ($P>0.80$ for all differences). In kidney transplant recipients, determinants of bone loss were female sex and over 50 years old ($P=0.01$), higher BMD at inclusion ($P=0.01$), and receiving no calcium supplementation ($P=0.03$). In patients on dialysis, determinants were female sex ($P=0.01$) and low parathyroid hormone levels ($P=0.03$).

CONCLUSIONS: After kidney transplantation, patients have on average stable trabecular BMD at the spine, whereas those remaining on dialysis lose substantial amounts of trabecular bone during 3 years. This loss is similar in treatment with nocturnal hemodialysis, conventional hemodialysis, and peritoneal dialysis.

P22

Plasma Syndecan-1 in Hemodialysis Patients Associates with Survival and Reduced Volume Status

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Background – Syndecan-1, a transmembrane heparan sulfate proteoglycan, associates with renal and cardiovascular functioning. We earlier reported syndecan-1 to be involved in renal tubular regeneration. We now examined plasma values of syndecan-1 in a hemodialysis cohort and its association with volume, inflammatory and endothelial markers in addition to outcome parameters.

Patients and Methods – Eighty-four hemodialysis patients were evaluated for their plasma syndecan-1 levels by ELISA before, 60, 180 and 240 minutes after starting dialysis. Patients were divided into sex-stratified tertiles based on predialysis plasma syndecan-1 levels. We studied the association between plasma levels of syndecan-1 and volume, inflammation and endothelial markers and its association with cardiovascular events and all-cause mortality using Kaplan-Meier curves and cox regression analyses with adjustments for gender, age, diabetes and dialysis vintage.

Results – Predialysis syndecan-1 levels were two-fold higher in males compared to females (P=0.0003). Patients in the highest predialysis plasma syndecan-1 tertile had a significantly higher ultrafiltration rate (P=0.034) and lower plasma values of BNP (P=0.019), pro-ANP (P=0.024) and endothelin (P<0.0001) compared with the two lower predialysis syndecan-1 tertiles. No significant associations with inflammatory markers were found. Cox regression analysis showed that patients in the highest syndecan-1 tertile had significantly less cardiovascular events and better survival compared with the lowest syndecan-1 tertile (P=0.02 and P=0.005, respectively).

Conclusion – In hemodialysis patients, higher plasma syndecan-1 levels were associated with lower concentrations of BNP, pro-ANP and endothelin, and with better patient survival. This may suggest that control of volume status in hemodialysis patients allows an adaptive tissue regenerative response as reflected by higher plasma syndecan-1 levels.

P23

Role of von Willebrand factor in the association between residual GFR and mortality in dialysis patients

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Objective: Among dialysis patients, decreased residual kidney function is associated with increased mortality risk. The mechanism behind the increased mortality risk has not yet been elucidated. A potential mechanism could be that retained uremic solutes in decreased residual kidney function increase von Willebrand factor (VWF) levels by enhancing endothelial cell activation. VWF mediates platelet-platelet interaction and mediates platelet adhesion to the vessel wall and is prothrombotic. The aim of this study was to investigate the role of VWF in the association between residual glomerular filtration rate (rGFR) and mortality.

Methods: We prospectively followed 925 dialysis patients from the NECOSAD cohort. Residual GFR was calculated as the mean of creatinine and urea clearance and corrected for body surface area. VWF levels were measured using a semi-automated enzyme-linked immunosorbent assay. Cox proportional hazard analyses were used to calculate hazard ratios (HRs) with 95% confidence intervals (CIs) to investigate the association between quartiles of rGFR and two years all-cause mortality. HRs were adjusted for potential confounders. In a mediation analysis, we also adjusted for VWF levels to investigate the role of VWF in the association between rGFR and mortality.

Results: During a mean follow-up of 1.6 years, 195 dialysis patients died. The all-cause mortality rate was 136 per 1000 person-years. The mortality risk increased with decreasing rGFR. The lowest quartile of rGFR (mean rGFR 0.14 ml/min/1.73m^2) as compared with the highest quartile of rGFR (mean rGFR 6.51 ml/min/1.73m^2) was associated with a 1.6-fold (95% CI 1.1-2.4) increased all-cause mortality risk after adjustment for age, sex, dialysis modality, primary kidney disease, cardiovascular disease and systolic blood pressure. VWF levels increased with decreasing rGFR. The mean VWF level was 22.8 ug/mL in the group of patients with the lowest quartile of rGFR and was 18.6 ug/mL in the group of patients with the highest quartile of rGFR. After additional adjustment for VWF levels, the HR for all-cause mortality slightly attenuated to 1.4 (95% CI 0.9-2.2).

Conclusions: We showed that reduced rGFR was associated with higher levels of the prothrombotic VWF and an increased mortality risk in dialysis patients. A potential mechanism as explanation for the link between rGFR and mortality in dialysis patients could be endothelial cell dysfunction.

P24

Higher Pre-dialysis Serum Sodium Variability Associates with an increased risk of death: Results from a Cohort Study from the International Monitoring Dialysis Outcomes Initiative

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Objective

Low serum sodium (SNa) levels are associated with increased mortality chronic hemodialysis (HD) patients. Whereas variability of clinical parameters such as blood pressure was found to be related to adverse outcomes in HD patients, the association between variability in laboratory parameters such as SNa and outcome is not well established. In this study, we aimed to explore this association in a large population of international dialysis patients from the international MONitoring Dialysis Outcomes (MONDO) Initiative.

Methods: All adult incident patients from the MONDO database with more than 5 SNa measurements during the first year on dialysis were included, outcomes were recorded over a subsequent 2-years period. We computed average baseline SNa, and SNa variability as a) standard deviation (SD) of the residual of a fitted regression function for each patient, b) overall SD c) average real variability (ARV) and d) directional range (DR). Patients were stratified into 3 different groups of DR (-20 to -6, -6 to 6 exclusive and 6 to 20 mEq/l). Cox proportional models informing bivariate spline functions were developed to depict the joint effects of SNa with the variability metrics.

Results: In 20,216 analyzed patients a SNa<=135 mEq/L was the strongest predictor of increased mortality. Higher variability quantified as SD, ARV and DR also associated with an increased risk of death at all levels of SNa. Quantification of variability using the DR showed in both direction a relation with an increased risk of death. No association between variability quantified as the SD of the SNa residuals and mortality was found. Controlling the models for additional parameters showed consistent results.

Conclusion: Higher pre-HD SNa variability associates with increased all-cause mortality at all levels of SNa. The prognostic value differs between the computed variability metrics. The simplest variability metric (DR of SNa) showed a consistent predictive quality and may constitute a simple prognostic indicator, easily applicable at bedside in clinical practice.

Poster session E

“Clinical Nephrology”

P25

Determinants of intraregional differences in renal function in the Northern Netherlands: the LifeLines cohort study

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Background: Although interregional disparity in CKD prevalence has been reported globally, it is unclear which factors drive renal function clustering within regions. We aimed to study the intraregional distribution of renal function in the Northern Netherlands and identify factors associated with its geographic variability.

Methods: We included 130,545 participants in LifeLines, a population-based cohort in the Northern Netherlands. Spatial analysis was performed to identify regional clusters of eGFR (CKD-EPI), and multivariable logistic regression was used to identify demographic, clinical and environmental factors associated these clusters.

Results: Significant spatial clustering of high (hot spot) and low (cold spot) eGFR was found independent of age, sex, and BSA. Participants in cold spot areas(n=12,400) had lower eGFR (95.5±15.5 *vs.* 98.5±14.6 ml/min/1.73 m^2) and lower 24h creatinine clearance (126.8±35.5 *vs.* 130.9±33.7 ml/min) compared to those in hot spot areas(n=6,700) ($p<0.05$). In multivariable logistic regression, blood pressure per 5 mmHg($OR_{cold\ spots}$: 1.05 [95%CI: 1.03 to 1.07]), BMI per 5 kg/m^2 (1.16 [1.11 to 1.22]), cholesterol (1.06 [1.03 to 1.10]), serum potassium (0.41 [0.36 to 0.46]), glucose level (1.10 [1.05 to 1.15]), education level and urbanity were all independently associated with spatial distribution of renal function (model R^2=0.498). Subanalysis in 6,535 individuals showed that air pollution (NO_2, PM_{10}, $PM_{2.5}$) was independently associated with renal function.

Conclusions: We observed intraregional clustering of renal function in the Northern Netherlands and identified several potentially modifiable determinants. These data could guide better strategies for prevention and public health.

P26

Glomerular detection, segmentation and counting in PAS-stained histopathological slides using deep learning.

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Objective: Glomeruli are among the most extensively studied components in kidney histopathology. Researchers and clinicians often depend on quantitative measures for the assessment of glomeruli. Historically, these are obtained through manual counting or classical image processing techniques. These methods possess limited reproducibility, are insufficiently robust to inter-laboratory variations, and are infamous for their tedious nature. As an alternative, we trained a convolutional neural network (CNN) to detect, segment, and count healthy and sclerotic glomeruli in digitized Periodic acid-Schiff (PAS) stained tissue sections.

Methods: A CNN was trained using exhaustively annotated structures in rectangular regions in 50 whole-slide images (WSIs) of renal transplant biopsies. This resulted in annotations of 182 healthy and 18 sclerotic glomeruli. 40 WSIs were used for training and validation. Segmentation was assessed by calculating the Dice-coefficient on an unseen test set of 10 WSIs. To assess the network's ability to detect glomeruli in a larger composition of varying structures, we applied the CNN to 15 fully annotated nephrectomy WSIs. We calculated Pearson's correlation coefficients for glomerular counting (healthy and sclerotic glomeruli combined) in 82 renal transplant biopsies manually performed by three renal pathologists and the quantification by the CNN.

Results: We found a Dice-coefficient of 0.95 for healthy glomeruli and 0.62 for sclerotic glomeruli in the renal transplant biopsy test set. The CNN detected 93.4% of 1747 annotated healthy glomeruli in the nephrectomy samples, with 8.4% false positives. The CNN detected 76.4% of 72 annotated sclerotic glomeruli, with 45.5% false positives. Pearson's correlation coefficient for glomerular counting on 82 transplant biopsies of the CNN versus the pathologists was 0.924, 0.930 and 0.937 for pathologist 1, 2, and 3, respectively. The CNN counted on average 1.7 glomeruli more than the pathologists. The pathologists differed on average 0.78 glomerulus.

Conclusion: The network can accurately detect and segment healthy glomeruli. The CNN performs moderately well on segmenting sclerotic glomeruli, most probably due to the low amount of training data that was available for this class. The CNN's higher glomerular count can partly be explained by possible false positive detections of sclerotic glomeruli. Also, partially sampled glomeruli located at biopsy's edges are not counted by the pathologist, while they are included by the network. More training data for sclerotic glomeruli and additional post-processing techniques are needed to resolve this.

P27

Prediction of the effect of dapagliflozin on renal and heart failure outcomes based on short-term changes in multiple risk markers

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Aims: Sodium glucose cotransporter 2 inhibition with dapagliflozin reduces blood pressure, body weight and urinary albumin:creatinine ratio (UACR) in patients with type 2 diabetes (T2DM). We previously developed an algorithm, the PRE score, to predict how short term effects of a drug on risk factors may translate into long-term changes in clinical outcomes. We applied the PRE score to clinical trials of dapagliflozin to model the effect of the drug on renal and heart failure (HF) outcomes in T2DM patients with impaired renal function.

Methods: The relationships between multiple risk markers and long-term outcome were determined by means of a multivariable Cox model in a subgroup of T2DM patients derived from the ALTITUDE trial. These relationships were applied to short-term changes in risk markers observed in a pooled database of seven dapagliflozin clinical trials to predict the expected drug-induced changes to renal and HF outcomes. Patient characteristics within the background population were matched with respect to UACR and eGFR to those from the dapagliflozin trial participants. The renal outcome was defined as a composite of end-stage renal disease (ESRD) and a sustained doubling of serum creatinine. The heart failure outcome was defined as hospitalization due to HF.

Results: A total of 372 and 136 patients had UACR>30 mg/g and UACR>200 mg/g at baseline, respectively. The PRE score predicted a renal risk reduction of 39% (95% CI 17 to 61%) and 43% (95% CI 4 to 83%) with dapagliflozin 10 mg/day for the UACR>30 mg/g and UACR>200 mg/g subgroups. The predicted reduction in HF events was 21% (95% CI 8 to 35%) and 28% (95% CI 7 to 49%), respectively. Dapagliflozin decreased albuminuria by approximately 35% in both UACR subgroups. Simulation analyses showed that even with a smaller albuminuria lowering effect of dapagliflozin (10%), the estimated renal risk reduction was still 26.5% and 26.8% in the two UACR subgroups.

Conclusions:
The PRE score predicted clinically meaningful reductions in renal and HF endpoints associated with dapagliflozin therapy in patients with diabetic kidney disease. These results support a large long-term outcome trial in this population to confirm the benefits of the drug on these endpoints.

P28

Two interesting cases of prolonged classical pathway convertase activity: C4 Nephritic Factor and a non-autoantibody serum factor

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C3 glomerulopathy (C3G) is characterized by overactivation of the complement system, particularly of the alternative pathway. This can be caused by autoantibodies such as C3 nephritic factor (C3NeF) or by mutations in complement genes. Recent findings reveal presence of so-called C4 nephritic factors (C4NeFs) in some C3G cases. These may contribute to the complement dysregulation by stabilizing the C3 convertase of the classical pathway (CP). However, studies and routine diagnostic procedures are scarce. Using a recently described method for measuring convertase activity in whole serum, we detected C4NeF activity in a cohort of patients with complement-mediated renal diseases. Convertase stability was measured in a hemolytic assay using the C5-blocker eculizumab to separate the CP into two steps: formation of C3/C5 convertases by test sera in a time-variable step 1 and formation of lytic membrane attack complexes in a standardized step 2 for readout. Samples of 17 healthy controls and 47 patients with (suspected) C3G and closely related complement-mediated disorders were analyzed. Healthy control convertase activity profiles consistently showed maximal convertase activity after 0.5-2.5 min; activity returned to background levels at t=10. In contrast, 2/47 (4%) patients showed significant convertase activity at t=10. This prolonged activity indicates presence of CP convertase-stabilizing factors. We confirmed presence of stabilizing autoantibodies in the first patient, which was also positive for C3NeF. The Ig-fraction from the second patient's serum did not support convertase stabilization when added to control serum, indicating another non-autoantibody factor is present causing increased convertase half-life. The nature of this factor is under investigation. To conclude, prolonged CP convertase activity was found in two patients: one case was Ig-mediated, a C4NeF, and one was not. This study offers new opportunities for detection of (previously unrecognized) convertase-dysregulating factors of the CP in patients with various complement-mediated renal diseases.

P29
Dried Blood Spots in practice: clinical validation

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Background: Immunosuppressive drugs are successfully applied in kidney transplant patients to prevent allograft rejection. Sub therapeutic dosing leads to allograft rejection, overdosing leads to severe toxicity. To optimize dosing, monitoring of immunosuppressants blood drug concentrations and creatinine is mandatory requiring patients to frequently travel to the hospital to provide blood samples. Monitoring by Dried Blood Spot (DBS) provides patients the opportunity to sample a drop of blood from a fingerprick at home and apply it to a sampling card. This card can be send to the laboratory by mail thereby decreasing the need of the patients to travel to the hospital.

Methods: We performed a clinical validation in which we compared measurements from whole blood samples obtained by venapuncture with measurements from DBS samples simultaneously obtained by fingerprick. After exclusion of 10 DBS for poor quality, and 2 for other reasons, 199, 104 and 58 samples from a total of 181 patients were available for validation of creatinine and the immunosuppressants tacrolimus (TaC) and cycosporin A (CsA) respectively. Validation was performed by means of Passing & Bablok regression and bias was assessed by Bland-Altman analysis.

Results: For creatinine we found y = 0.73x – 1.55 (95% Confidence Interval [95%CI] slope 0.71,0.76), giving the conversion formula: [creatinine plasma concentration in µmol/L] = [creatinine concentration in DBS in µmol/L] / 0.73, with a non-clinically relevant bias of -2.1 µmol/L (95%CI -3.7,-0.5 µmol/L). For TaC we found y = 1.00x – 0.23 (95%CI slope 0.91,1.08), with a non-clinically relevant bias of -0.28 µg/L (95%CI -0.45,-0.12 µg/L). For CsA we found y = 0.99x – 1.86 (95%CI slope 0.91,1.08) and no significant bias. Therefore, for neither TaC nor CsA a conversion formula is required.

Conclusions: DBS sampling for the simultaneous analysis of immunosuppressants and creatinine can replace conventional venous sampling in daily routine.

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P30

Dietary sodium-induced systolic blood pressure rise is associated with a pro-inflammatory phenotype of classical monocytes – a randomized controlled trial in healthy human subjects

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Objective

High salt intake is associated with hypertension and cardiovascular disease. The mechanisms behind this are not fully elucidated, and may extend beyond the kidney. Proposedly, the mononuclear phagocyte system is involved. We investigated the effect of high salt intake on circulating monocytes and skin macrophages of healthy human subjects.

Methods

We performed a randomized cross-over trial in healthy males. All subjects pursued a 2-week low salt diet (LSD: <3 grams NaCl/day) and high salt diet (HSD: >12 grams NaCl/day) in randomized order. After each diet, body weight and blood pressure were measured, and blood and urine samples were obtained. Flow cytometry was used for phenotypic characterization of monocytes into classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$) and non-classical ($CD14^-CD16^+$) subtypes. We examined the expression of pro-inflammatory chemokine receptors (CCR2, CCR5, CCR7, CXCR1), anti-inflammatory chemokine receptors (CD206, CD200R) and molecules associated with migration (CD62L, CD49d, CD29, CD11b, CD11c, CD18). In addition, LPS-induced cytokine secretion (IL-6, IL-8, IL-10, IL-12, TNF) in whole blood was investigated. Furthermore, after each diet, skin biopsies were taken, to investigate skin macrophage density (CD163) and macrophages expression of the M1 marker HLA-DR and the M2 marker CD206.

Results

Eleven subjects were included in this study. HSD increased body weight and systolic blood pressure, whereas CRP and monocyte number did not differ between LSD and HSD. HSD increased CCR2 expression on classical monocytes (Figure 1A) and induced a trend towards decreased CD206 expression. There was no effect on the other chemokine receptors or on the molecules associated with migration. Furthermore, HSD increased LPS-induced IL-6 secretion without any effect on the other cytokines. Macrophage density significantly increased after HSD and showed increased HLA-DR expression but decreased CD206 expression.

Conclusion

In healthy human subjects, HSD-induced BP rise associates with a pro-inflammatory state of macrophages and of monocytes, including increased CCR2 expression and IL-6 secretion. Both factors have been shown to play a role in hypertension and cardiovascular disease in animal models, and may provide a link between salt and these deleterious outcomes in humans.