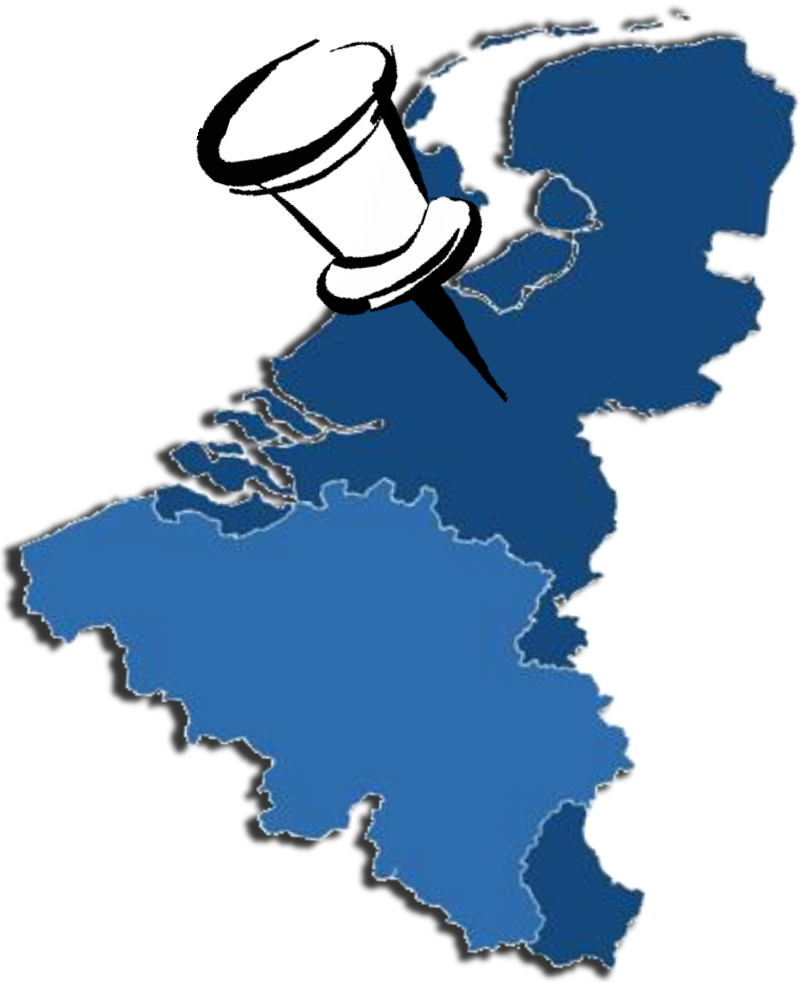
BENELUX KIDNEY MEETING 2019

Abstract Book



Dutch Federation for Nephrology

SBVN

ORAL PRESENTATIONS (O1-O14)

O1

Kidney-centered radiotherapy attenuates renal ischemia-reperfusion injury in mice

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Introduction

Whole-body irradiation has been associated with renal ischemic preconditioning in mice. Here, we investigate the functional and fundamental impact of radiotherapy centered on the kidneys before renal ischemia/reperfusion (I/R) in mice.

Materials and Methods

Experience1: Animals (n=5) were anesthetized and placed in the irradiator. Two beams of X-rays (225Kv, 13 mA) specifically targeted both kidneys to delivered a dose of 8,56Gy. One month later, a right nephrectomy was performed, and a left renal ischemia was induced for 30min. After 48 hours of reperfusion, the left kidney was collected, as well as blood. Control group (n=6) underwent a similar renal I/R procedure, with no prior irradiation. Experience 2: Unilateral irradiation of left kidneys (8.56 Gy) was performed on mice (N=11). One month later, the left (irradiated) kidney was collected. Additionally, kidneys were collected from non-irradiated mice (N=5). Total RNAs were extracted from irradiated and control kidneys to perform comparative high-throughput RNA-Seq. BaseSpace Sequence Hub Illumina was used. Functional enrichment analysis was performed using DAVID program.

Results

Following kidney I/R, blood urea nitrogen (BUN) levels were significantly lower in pre-irradiated mice (148.4±93.1) compared to controls (495.7±33.3, $p<0.01$). The number of PCNA-positive proliferating cells was significantly lower in pre-irradiated mice (130.8±52.7) compared to controls (545.4±257.3, $p<0.001$). The renal infiltration by inflammatory CD11b-positive cells (90.2±32.2) *vs.* (414.5±148.6) and F4-80-positive macrophages (80.6±22.9) *vs.* (178.5±68) was significantly reduced in pre-irradiated animals. Comparative transcriptomics showed a significant up-regulation of signaling pathways involved in angiogenesis (*HMOX1*) and stress response (*HSPA1A*, *HSPA1B*), and a down-regulation of oxidoreduction (*NOX4*).

Conclusion

Kidney irradiation induces ischemic preconditioning in mice, with improved renal function and decreased inflammation following renal I/R. The aforementioned signaling pathways may play a role in irradiation-associated kidney resistance to I/R.

O2

Circulating long noncoding RNAs are associated with diabetic nephropathy (DN) and normalize after simultaneous pancreas-kidney transplantation (SPKT)

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Objective: SPKT replaces kidney function and restores endogenous insulin secretion. Long noncoding RNAs (Lnc-RNAs) are promising biomarkers in multiple diseases and can provide insight into pathogenesis. Several Lnc-RNAs have been reported in the context of diabetic nephropathy and cardiovascular disease. However little is known about these markers after simultaneous pancreas kidney transplantation (SPKT).

Methods: We performed a pilot study of 40.173 Lnc-RNAs in healthy controls and patients with diabetic nephropathy. Based on a these results and a literature search we selected 14 promising Lnc-RNAs of which 9 were detectable in plasma samples in DN (n=14), SPKT (n=38) and healthy controls (n=15). In 14 DN patients samples were obtained before and 1, 6 and 12 months after SPKT. All results from qPCR were normalized using the housekeeping gene Actine. Lnc-RNAs that were detected in less than 95% of the samples were excluded from the study.

Results: Normalized MALAT1 was significantly higher (p=0.002) in patients with DN (median 13.8, IQR 2.9-16.3) compared with healthy controls (median 0.16, IQR 0.1-1.3). The first month after SPKT median normalized MALAT1 declined significantly (p=0.012) from 13.8 to 0.3 and nearly reached physiological levels. Normalized EPHA6, LIPCAR and G003293 showed a similar significant decline after SPKT (resp. p=0.01, p=0.1 and p=0.04)

Conclusion: Normalized MALAT1 correlates with DN and decreases after SPKT. A rapid decline of MALAT1, EPHA6, LIPCAR and G003293 is seen after SPKT. These circulating LNC-RNAs are potentially interesting biomarkers for disease progression of diabetic nephropathy and can be useful for a better insight in the pathophysiology.

O3

Multicenter randomized controlled trial of vitamin K antagonist replacement by rivaroxaban with or without vitamin K2 in hemodialysis patients with atrial fibrillation: the Valkyrie study

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Objectives.
Vitamin K antagonists (VKA), although commonly used to reduce thromboembolic risk in atrial fibrillation, have been incriminated as probable cause of accelerated vascular calcification (VC) in hemodialysis patients. Functional vitamin K deficiency may further contribute to their susceptibility for VC. We investigated the effect of vitamin K status on VC progression in 132 hemodialysis patients with atrial fibrillation treated with VKA or qualifying for anticoagulation.

Methods.
We conducted an investigator-driven, randomized, prospective, open-label interventional clinical trial, conducted at 3 sites in Belgium (AZ Sint-Jan Brugge, OLV Ziekenhuis Aalst, ZNA Antwerpen). Patients were randomized to VKA with target INR 2-3, rivaroxaban 10 mg daily, or rivaroxaban 10 mg daily plus vitamin K2 2000 µg thrice weekly during 18 months. Systemic dp-ucMGP levels were measured to assess vascular vitamin K status. Cardiac and thoracic aorta calcium scores and pulse wave velocity were measured to evaluate VC progression.

Results.
Baseline dp-ucMGP was severely elevated in all groups. Initiation or continuation of VKA further increased dp-ucMGP, while levels decreased in the rivaroxaban group and to a larger extent in the rivaroxaban+vitamin K2 group, but remained nevertheless substantially elevated. Changes in coronary artery, thoracic aorta and cardiac valve calcium scores and pulse wave velocity were not significantly different among the treatment arms. All cause death, stroke, cardiovascular event rates and bleeding episodes were not significantly different between the groups. The incidence of life-threatening and major bleeding was significantly lower in the pooled rivaroxaban arms than in the VKA arm, with a RR (95% CI) of 0.43 (0.22-0.83).

Conclusions.
Withdrawal of VKA and high-dose vitamin K2 improve vitamin K status in hemodialysis patients, but have no significant favorable effect on VC progression. Severe bleeding complications may be lower with rivaroxaban than with VKA.

O4

Laminar flow profoundly affects Glomerular Endothelial Cell morphology and functionality

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Objective: Glomerular Endothelial Cells (GEnC) are continuously exposed to shear stress *in vivo* as a result of the blood flow. However, *in vitro* studies investigating GEnC, are generally performed under static cell culture conditions. By lacking the cardinal influence of laminar flow on GEnC, *in vitro* studies performed under static cell culture conditions might poorly resemble the *in vivo* behaviour of GEnC. The objective of this study was therefore to characterize the influence of long term laminar flow on the morphology and functionality of GEnC.

Methods: *In vitro* experiments were performed using conditionally immortalized mouse glomerular endothelial cells (mGEnC). mGEnC were cultured under either static conditions or laminar flow of 5 dyn/cm2 for 7 days. Flow was applied using the Ibidi pump flow system. Gene, Heparan Sulfate (HS) and protein expression, using qPCR and IF stainings of mGEnC cultured under static or flow conditions was compared after 7 days of culture. Nitrix Oxide (NO) production by mGEnC was analysed using the NO sensitive dye DAF-FM diacetate (10µM).

Results: mGEnC cultured under flow partially aligned in the flow direction and rearranged their actin cytoskeleton. Moreover, cells cultured under flow showed increased expression of the shear stress responsive genes Krüppel-like factor-2 (KLF2) and Cytochrome P450 1B1 (CYP1B1). Flow altered the composition of the endothelial glycocalyx as characterized by the increased expression of the HS domain recognized by MoAb JM403 and a decreased expression of the HS-degrading enzyme Heparanase. In addition, RNA and protein expression of the NO producing enzyme endothelial Nitric Oxide Synthase (eNOS) was increased in response to shear stress, which was accompanied by increased synthesis of NO. Moreover, the expression of the anti-oxidant genes NAD(P)H dehydrogenase quinone 1 (NQO1) and Nuclear factor erythroid 2-related factor (Nrf2) was increased in mGEnC cultured under laminar flow. Furthermore, flow resulted in an altered inflammatory state, which was characterized by decreased expression of intracellular adhesion molecule 1 (ICAM-1) in unstimulated cells, whereas TNFα stimulation resulted in highly increased ICAM-1 expression levels relative to the static condition.

Conclusion: Culture of GEnC under laminar flow results in glycocalyx alterations, increased NO production, and an altered inflammatory and anti-oxidant response, compared to GEnC cultured under static conditions. These findings suggest that GEnC cultured under flow have a more representative phenotype compared to static culture.

O5

Organoids as a model to study renal electrolyte transport: optimization protocol for DCT enrichment

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Objective: The lack of reliable models representing the renal in-vivo situation brings a limit to study molecular mechanisms of electrolyte disorders. The past decade has witnessed an increasing interest in kidney organoids, which are multicellular self-organizing 3D kidney structures. However, to date no information is available regarding their functional electrolyte transport properties/characteristics. In order to assess their suitability as a novel tool to study distal convoluted tubule (DCT) physiology, we aim to define a new protocol to enrich the DCT population of cells in induced pluripotent stem cells (iPSCs)-derived kidney organoids. In addition, we aim to identify suitable markers for DT identification and sorting in iPSCs-derived kidney organoids.

Methods: iPSCs-derived kidney organoids were generated following the published protocol by Takasato et al. 2014. Subsequently, the protocol was adapted by adding growth factors and/or other compounds during differentiation. Expression of different nephron segment markers was assessed by quantitative real-time PCR (RT-qPCR) and immunostaining. Furthermore, whole organoids were immunostained with mucin-1 (MUC1) and various nephron markers to study co-localization.

Results: RT-qPCR analysis demonstrated the existence/presence of proximal tubule, loop of Henle, distal convoluted tubule and collecting duct, as well as the expression molecular markers of staminality in iPSC-derived kidney organoids. Immunostaining showed the expression of GATA-binding protein (GATA3), Na-K-Cl cotransporter (NKCC2), E-cadherin (ECAD), nephrin and Loctus Tetragonolobus Lectin (LTL), confirming the presence of different tubular segments. During organoid development, growth of iPSCs for 3 days in the presence of the GSK3 inhibitor CHIR99021 followed by 4 days incubation with fibroblast growth factor 9 and heparin resulted in a slightly increase in the expression of markers characteristic of the distal convoluted tubule at day 25 of culture. Addition of growth factors (CHIR99021, epithelial growth factor or R-spondin) at day 12 can induce differential expression of segment specific markers at day 25. Immunostaining of whole organoids demonstrated colocalization of MUC1 with ECAD (marker distal convoluted tubule), partial colocalization with NKCC2 (marker Loop of Henle), and no colocalization with LTL, a proximal tubule marker.

Conclusion: Differentiation of iPSC-derived kidney organoids can be influenced by the addition of specific growth factors to allow enrichment of specific subpopulations of tubular cells. Identification of MUC1 as DCT marker is key for future work using fluorescence-activated cell sorting to obtain DCT cells from dissociated kidney organoids that can be used for subsequent functional studies like electrolyte transport assays.

O6

ANCA- and B-cell-status predict relapses after induction therapy with rituximab but not with cyclophosphamide in ANCA-associated vasculitis

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Objective: Tailored rituximab (RTX) maintenance treatment guided by anti-neutrophil cytoplasmic antibodies (ANCAs) and B-cells was demonstrated to prevent relapses in ANCA-associated vasculitis (AAV) patients after remission-induction with cyclophosphamide (CYC). However, guidelines recommend CYC or RTX as remission-induction therapy. Yet, how tailored ANCA- and B-cell-guided maintenance treatment performs in real-life after these regimens is unknown. Therefore, this single-center study aimed to investigate how ANCA and B-cell-status related to relapses in AAV patients treated with RTX or CYC.

Methods: From 1990-2018 ANCA-positive AAV patients who received remission-induction treatment for active disease were eligible, which identified 174 cases, including 111 RTX- and 63 CYC-treated patients. ANCA-, B-cell-status and relapses were assessed over two years.

Results: Patients that achieved and maintained PR3-ANCA-negativity after remission-induction treatment with RTX (n=26) or CYC (n=17) had few relapses (resp. 8%;4%). Persistent PR3-ANCA-positivity associated with more relapses in both RTX- (n=54) (37%;p=0.007) and CYC-treated patients (n=10) (17%;p=0.02). After achievement of ANCA-negativity, the reappearance of PR3-ANCA associated with relapses only in RTX-treated (n=10) (42%;p=0.004) but not in CYC-treated patients (19) (13%;p=0.1). After RTX, both incomplete depletion (n=10) and repopulation of B-cells (n=59) associated with relapses (resp. 60%,p=0.006 and 40%;p=0.03). Combined immunomonitoring of ANCA- and B-cell-status in RTX-treated patients demonstrated that reappearance of PR3-ANCA with B-cell return (n=8) strongly associated with relapses (62%;p=0.03) while relapses in persistent MPO-ANCA-positive patients occurred only upon B-cell return (n=10) (60%;p=0.09).

Conclusion: ANCA- and B-cell-status predict relapses after remission-induction therapy with RTX, but not with CYC. Thus, tailored ANCA- and B-cell-guided maintenance treatment could be beneficial after remission-induction with RTX in AAV patients.

O7

DIMINISHED EFFICACY OF THE ANGIOTENSIN RECEPTOR BLOCKER LOSARTAN DURING HIGH POTASSIUM INTAKE IN CKD PATIENTS

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Objective: High potassium intake increases natriuresis and lowers blood pressure (BP). Whether these beneficial effects are also present in chronic kidney disease (CKD) patients and whether potassium intake affects BP and proteinuria-lowering efficacy of angiotensin receptor blockade (ARB) is unknown. We set out to address the effect of potassium intake on BP and proteinuria response to losartan in non-diabetic proteinuric CKD patients.

Methods: We performed a post-hoc analysis of a placebo-controlled interventional cross-over study in which 33 non-diabetic proteinuric patients (mean baseline proteinuria 3.8 g/d) were treated for 6 weeks with placebo, losartan 100mg, and losartan/hydrochlorothiazide (HCT) 100mg/25mg, respectively. Patients underwent the 3 interventions during both a habitual (~200 mmol/d) and low-sodium diet (<100 mmol/d), in randomized order. To analyze the effects potassium intake, we categorized patients based on median split of 24-hour urinary potassium excretion, reflecting potassium intake.

Results: Mean potassium intake was stable during all 6 treatment periods. Patients with high potassium intake (≥100 mmol/day) had similar BP reductions across all treatments as compared to low potassium intake (<100 mmol/day), whereas a lower proteinuria reduction (p=0.014) was observed for all treatments. Proteinuria reduction to losartan monotherapy and to losartan/HCT, respectively, was significantly lower during high potassium intake (20% vs. 41%, p=0.011; and 48% vs 64%, p=0.036). These differences in antiproteinuric response abolished when adding a low sodium diet. In multiple regression analysis, potassium intake was a significant independent predictor of the antiproteinuric response to losartan monotherapy (-21%, 95%CI -36, -6%), but not to losartan combined with HCT.

Conclusion: In proteinuric CKD patients, the proteinuria, but not BP-lowering response to losartan was hampered during high potassium intake. Differences disappeared after sodium status change by low-sodium diet.

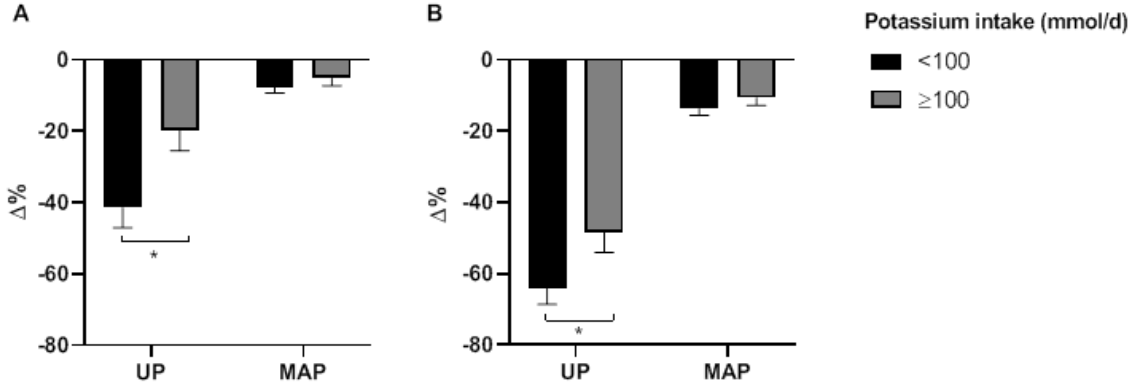

Figure 1 A. The antiproteinuric response to losartan monotherapy was higher in patients with a low potassium intake compared to patients with a high potassium intake (p=0.011). **B.** This difference became smaller after adding HCT (p=0.036). UP: proteinuria, MAP: mean arterial pressure. Values are mean±SEM. *p<0.05

O8

Selective binding of heparin/heparan sulphate oligosaccharides to Factor H and Factor H-related proteins have therapeutic potential for C3 glomerulopathies

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Objective: The complement system is vital for defence against pathogens and removal of apoptotic debris. Deregulated alternative complement activation is a hallmark of complement component 3 glomerulopathy (C3G), a severe glomerular disease. Factor H (FH), an important inhibitor of the alternative pathway, contains two heparan sulphate (HS) binding sites and FH-HS interactions underpin host-pathogen differentiation. Complement FH-related proteins (FHRs) are a group of 6 proteins that are highly homologous to FH, but lack the N terminal complement regulatory domain. Competition-increasing mutations in FH and FHRs, or disturbed glomerular HS structure may affect the FH/FHR balance, resulting in the development of C3G. Indeed, C3G patient renal biopsies show increased FHR deposits, suggesting that the mechanism responsible for complement deregulation likely involves FHRs. Due to the presence of homologous HS binding domains in FHRs FH/FHRs may compete for host tissue HS, with increased FHR binding promoting local deregulation of complement.

Methods: FHR-mediated deregulation and specific HS sulphate modifications that determine FH/FHR binding were investigated via competition assays, ELISAs and flow cytometry using human glomerular endothelial cells and C3G patient plasmas.

Results: In the presence of C3b, FHRs differentially bound to glomerular endothelial cells. Importantly, cellular C3 depositions from C3G patient sera were significantly increased in comparison to normal human serum, supporting deregulation of the alternative pathway in disease. Since the HS sulphation pattern mediates ligand binding, we hypothesised that distinct HS oligosaccharides differentiate between FH and FHR binding, potentiating the development of novel competitive HS-based drugs. Using selectively desulphated heparins and size-defined oligosaccharides, the contribution of relevant HS modifications for FH/FHR binding were assessed. FHR binding was primarily mediated by N-sulphation, whereas FH required N-, 2-O and 6-O sulphation. Importantly, distinct O-desulphated heparin species significantly decreased C3 deposition from C3G patient sera on endothelial glomerular cells, potentiating these derivatives as novel C3G therapeutics.

Conclusion: Heparan sulphate/heparin derivatives have therapeutic potential for complement-mediated renal disease.

O9

Prevalence, progression and implications of breast artery calcification in patients with chronic kidney disease across stages of disease

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Background:
Breast artery calcification (BAC) is increasingly recognized as a specific marker of medial calcification. In this retrospective observational cohort study, we aimed to define the prevalence and progression rate of BAC in CKD patients across disease stage, to identify clinical and biochemical correlates of BAC and to explore the association of BAC with incident cardiovascular morbidity and overall mortality.

Methods:
Presence and extent (as quantified by summation of the lengths of BAC, referred as BAC score) were determined on mammograms in 311 females (58.7 ± 10.8 yrs, Caucasian) with CKD across disease stage (CKD 2-5D n=133; transplant recipients [Tx]: n=178). In a subset of 88 patients (CKD5D n=14, Tx n=74), a repeat mammography was performed after a mean interval of 3.5 ± 2.2 years, allowing to calculate the annualized BAC rate. Relevant clinical and laboratory parameters, including parameters of mineral metabolism and inflammation, and outcomes were extracted from electronic files. Survival analysis was performed in the TX group by Kaplan-Meijer analysis.

Results:
BAC was observed in 34.7% of the patients. Prevalence and extent of BAC increased parallel to the decline of kidney function. In the overall cohort, patients with BAC were older, suffered more from CVD and inflammation, had higher pulse pressure, and borderline higher prevalence of diabetes . The BAC progression rate was significantly higher in patients with CKD5D as compared to Tx patients (2.2 ± 1.2 vs 1.0 ± 0.4 mm/yr, mean ± SE; p=0.02). Progressors were characterized by more inflammation, worse kidney function and higher BAC score at baseline. In the Tx subcohort, progressors moreover showed higher serum phosphate levels at baseline. Presence of BAC associated with major adverse cardiovascular event free survival (Log-Rank p=0.002) in Tx.

Conclusion:
BAC is common among CKD patients, progresses at a slower pace in Tx as compared to CKD5D, and associates with dismal cardiovascular outcomes. BAC score, kidney function and serum phosphate at baseline are important determinants of progression. Measurement of BAC may offer a personalized, non-invasive approach to risk-stratify CKD patients for cardiovascular disease at no additional cost or radiation since a majority of women over the age of 40 undergo regular breast cancer screening.

O10

ENDOTHELIAL GLYCOCALYX HYALURONAN IS REQUIRED FOR GLOMERULR INTEGRITY AND IS DETERMINED BY SHEAR STRESS REGULATED GLUCOBIOSYNTHESIS

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Objective. Conditional endothelial loss of glomerular hyaluronan (HA) resulted in mesangiolysis and capillary ballooning and albuminuria, which over time develops into glomerular capillary rarefaction and glomerulosclerosis. Laminar shear stress is required to preserve glycocalyx expression. We investigated how downstream cellular regulation of production and maintenance of glycocalyx hyaluronan occurs.

Methods and Results. Both *in vitro* as well as in *in vivo*, HA expression on the endothelial surface is increased upon laminar shear and reduced when exposed to oscillatory flow, which is regulated by KLF2. Using a CRISPR-CAS9 edited small tetra cysteine tag to endogenous HAS2, we demonstrate increased expression and translocation of HAS2 to the endothelial cell membrane during laminar shear. HA production by HAS2 was shown to be further driven by availability of the HA substrates UDP-glucosamine and UDP-glucuronic acid. KLF2 inhibits endothelial glycolysis and allows for glucose intermediates to shuttle into the hexosamine- and glucuronic acid biosynthesis pathways, as measured using NMR analysis in combination with ^{13}C labelled glucose.

Conclusion. Endothelial glycocalyx function and functional adaptation to shear is coupled to KLF2 mediated regulation of endothelial glycolysis and HAS2 expression. As such, glomerular endothelial hyaluronan is a hitherto unrecognised key ECM component required for glomerular structure and function, which is lost in diabetic nephropathy.

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O11

Development and characterization of human induced pluripotent stem cell-derived kidney organoids to model idiopathic and congenital nephrotic syndrome.

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Objective. Recent advances in human stem cell-derived kidney organoid models have opened new avenues to accurately model podocytopathies in 3D *in vitro*. The aim of this study is to develop and characterize human induced pluripotent stem cells (iPSC)-derived 3D kidney organoids as a first step in modeling idiopathic and congenital nephrotic syndrome-in-a-dish.

Methods and Results. Human skin fibroblasts (wildtype) were successfully reprogrammed into iPSC. Cells were differentiated into ureteric bud and metanephric mesenchyme lineage in 2D for 7 days. At day 7, both lineages were merged and 3D organoids were differentiated using a Transwell™ air-liquid interface for an additional 18 days using our optimized protocol. Gene expression analysis and immunocytochemistry showed clear nephrin, podocin, synaptopodin, Wilm's tumor-1 and collagen IV expression in podocytes, surrounded by numerous CD31-positive endothelial capillaries. Electron microscopy (EM) and immuno-EM confirmed the presence of slit diaphragms and expression of nephrin and CD31, respectively. Organoids exposed to protamine sulphate showed clear podocyte cytoskeleton rearrangements and injury was rescued by heparin, illustrating functional 3D podocytes. The protamine sulphate effect was podocyte specific as proximal tubules were not affected, as confirmed by EM. Organoids exposed to active FSGS plasma for 36h showed cytoskeleton rearrangements and increased CXCL-12 gene and protein expression, a chemokine associated with kidney injury and its endogenous mRNA transcripts were confirmed in podocytes using RNAscope. Effects caused by FSGS plasma could be partially reversed using prednisolone. Remission FSGS plasma increased CXCL-12 expression levels to a lesser extend as compared to active plasma. Peripheral blood mononuclear cells derived from a patient suffering from congenital nephrotic syndrome (*NPHS2* mutation) were successfully reprogrammed and cultured into organoids. Podocyte abnormalities can be detected and associated disturbed molecular mechanisms are under investigation.

Conclusion. We successfully developed human iPSC-derived kidney organoids that will serve as a state-of-the-art tool to accurately study podocytopathies in a dish.

O12

Ex vivo gene therapy rescues the cellular phenotype in cystinotic urine-derived kidney progenitor cells: a prospect on cell-based gene therapy

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Objective Nephropathic cystinosis is a rare lysosomal storage disorder, caused by bi-allelic mutations in the lysosomal cystine/proton cotransporter cystinosin (*CTNS*). The kidney is the first and most severely affected organ, characterized a general proximal tubular dysfunction early in life, followed by rapid progressive glomerular damage leading to end-stage renal disease at adolescence.
Recently, we demonstrated an excessive loss of proximal tubular cells (PTEC's) and podocytes in urine of cystinosis patients. Therefore, we hypothesized that in compensation for this cell loss, ongoing regeneration might happen – however maladaptive - which could be reflected by the presence of kidney progenitor cells in the urine of cystinosis patients.

Methods Kidney progenitor cells were isolated and cultured from fresh urine samples of cystinosis patients prior to kidney transplantation (cystinotic uKPC's). Quantification of uKPC's in urine was performed via qPCR, and characterization was performed by qPCR and FACS. Specific uKPC clones differentiated to PTEC's and podocytes, as demonstrated by qPCR and IF, followed by functional assessment. Complementation of CTNS in cystinotic uKPC's was performed via transduction with a self-inactivating lentiviral vector containing a *CTNS-3HA* transgene, and intracellular cystine levels and distribution of the LAMP1 lysosomal compartment were assessed as read-out for successful restoration of the healthy phenotype.

Results We demonstrated a significant increased loss of kidney progenitor cells in urine of cystinosis patients compared to controls. Potent uKPC clones showed expression of nephron progenitor and mesenchymal stem cell markers, of which some demonstrated the capacity of exclusive differentiation towards a functional PTEC or Podocyte. Moreover, complementation with *CTNS* via LV transduction was shown to be successful as demonstrated by a significant reduction in cystine levels and redistribution of the LAMP-1 lysosomal compartment, while transduced uKPC's retain their differentiation potential.

Conclusion We demonstrated the presence of kidney progenitor cells in the urine of cystinosis patients which can differentiate in a functional PTEC or Podocyte. Following complementation with CTNS, we demonstrated the rescue of the cellular phenotype. We conclude that these uKPC's might serve as a source for cystinosis kidney organoid development and disease modelling, drug screening, and a base for the exploration of feasibility for an autologous cell-based gene therapeutic approach to provide a cure for the renal phenotype in nephropathic cystinosis.

O13

Dietary salt modifies the blood pressure response to renin-angiotensin inhibition in experimental CKD

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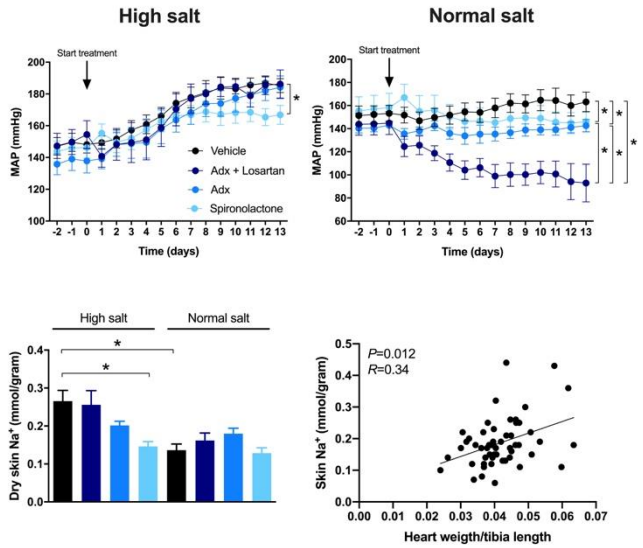
Objective: Salt-sensitive hypertension is a hallmark of chronic kidney disease (CKD). The role of dietary salt and the renin-angiotensin system (RAS) in the pathogenesis of salt-sensitive hypertension in CKD is incompletely understood. Our aim was to dissect the role of dietary salt and the RAS in a rat model of hypertension and CKD.

Methods: Sprague Dawley rats were subjected to 5/6th nephrectomy, allowed to recover for four weeks, and subsequently subjected to one of four treatments: (1) vehicle, (2) adrenalectomy (Adx), (3) Adx + losartan (30 mg/kg/d), or (4) spironolactone (80 mg/kg/d). These interventions were performed either under normal dietary salt (0.4% NaCl) or high salt (4% NaCl) conditions. Mean arterial pressure (MAP) was measured by radiotelemetry, GFR by transcutaneous FITC-sinistrin clearance, and skin sodium (Na^+) by flame photometry after dissolving skins in nitric acid and hydrogen peroxide.

Results: 5/6th nephrectomy reduced GFR from 1.3 ± 0.4 to 0.4 ± 0.1 ml/min/100g body weight. On a high salt diet, BP was resistant to RAS intervention, except for an attenuated BP rise in rats receiving spironolactone (Figure). On a normal salt diet, BP kept increasing with vehicle, but stabilized with Adx and spironolactone. Adx + losartan reduced BP remarkably. On a high salt diet, spironolactone prevented Na^+ accumulation in skin. For all groups, skin Na^+ correlated positively with heart weight.

Conclusion: High salt increases BP in CKD in part via direct effects on the mineralocorticoid receptor. Under normal salt conditions, however, hypertension in CKD depends on the combined effects of angiotensin II and aldosterone. Dietary salt modifies the BP response to RAS interventions in CKD, and accumulates in the skin. Our observations may have both therapeutic and prognostic implications for human CKD.



O14
The immunometabolic fingerprint of the aged kidney.

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Background: The aged kidney undergoes structural and functional changes which are associated with epigenomic changes, especially in genes of the innate immune response. Innate immune receptors are known to induce effector functions in cells through a metabolic rewiring. However, the crosstalk between, innate immunity, metabolism and phenotypic changes in the aged kidney are currently unknown. In this explorative study we aim to understand whether the renal phenotype is determined by the metabolic profile cells display and whether this might be under the control of innate immune sensors. Understanding these changes might generate novel hypothesis to reverse or slow down the ageing process, through the immunometabolic reprogramming.

Methods: Young (12 weeks old) and Old (24 months old) C57BL/6J mice were purchased from Janvier (St. Berthevin, France) and housed in standard environmental conditions (light, temperature and humidity). Animals received standard chow diets (#2018, containing 18% protein, 50% carbohydrate and 6.0% fat; Harlan Laboratories, Madison, WI, USA) and were sacrificed after overnight fasting. Kidneys were harvested and processed for histology and RNA isolation.

Results: Aged animals displayed increased glomerulosclerosis and interstitial fibrosis/tubular atrophy, assessed by PASD score. Analysis of innate immune sensors expression by RT-PCR revealed a significant up-regulation of *Nlrp3, Trem-2, Rage, Tlr8 and Tlr9*. Additionally, the inflammatory markers *Cxcl1, Ccl2* and *Il1b* as well as infiltration of F4/80+ macrophages, increased in the aged kidney compared to young ones. Expression of rate-limiting glycolytic and oxidative phosphorylation enzymes, revealed a metabolic reprogramming of the aged kidney towards glycolysis. Analysis of the glycolytic protein PKM2 by IHC confirmed this switch, and revealed an aberrant glycolysis primarily in renal tubular cells. Finally, chronic inflammation and metabolic reprogramming were associated with upregulation of tubular injury markers (*Kim1* and *Ngal*), increased α-SMA+ cells and lipid deposits.

Conclusion: We report that the expression of specific innate immune sensors is associated with metabolic changes during ageing, leading to chronic inflammation, tubular damage and trans-differentiated phenotype. These results would suggest that innate immune receptors are critical factors linking changes in tubular cell metabolism to cell phenotype and outcome during ageing.

POSTERS (P1-P29)

❢ signifies POSTERS SELECTED for POSTER HIGHLIGHTS SESSION

P1

A ten-year review of revascularization in patients with renal artery stenosis.

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Objectives

Renal artery stenosis is usually caused by atherosclerosis. It can cause renal failure and hypertension.[1] In recent years there has been discussion about whether these patients should be treated with medication or revascularization. Two major studies have been conducted on this subject. In 2009, the ASTRAL researchers stated that there is no difference in outcome when treating patients with medication or revascularization.[2] However, this study showed that there were more complications in the revascularization group, which leads to the belief that medication would be the better option. In 2014, the CORAL researchers investigated the usefulness of stenting for the prevention of serious renal and cardiovascular events.[3] They found no significant differences in adverse events in this study, but also no benefit from revascularization. However, there was a significant difference in systolic blood pressure between the two groups. Both studies seem to conclude that the use of revascularization is not beneficial. Various guidelines have difficulty reaching a consensus on which patients will benefit. Moreover, there are no protocols in our hospital about which patients should undergo revascularization.

Methods

We have performed a retrospective search in our database of patients who have undergone revascularization in our hospital in the past 10 years. A total of 47 out of 60 patients were selected. Exclusion criteria were fibromuscular dysplasia, renal arterial dysfunction after trauma and poor visualization of the stenosis. The primary endpoint was whether there was improvement of renal function and or hypertension. Secondary endpoints were the occurrence of complications such as rapidly decreasing renal function, bleeding and even death.

Results

We discovered that there is a benefit of revascularization in a selected group of patients. Patients with a rapidly decreasing renal function, recurrent cardiac asthma attacks, and uncontrolled hypertension, seem to benefit from revascularization. In patients with slowly decreasing renal function no benefit of revascularization was found. Moreover, these patients had more complications. In addition, some patients with slowly declining function or normal function with an accidentally found renal artery stenosis suffered major complications, such as dialyses and death. Patients with only one functional kidney were particularly more at risk of adverse events.

Conclusion

Revascularization in patients with renal artery stenosis should not be taken lightly and should only be performed in carefully selected patients. If not carefully selected, patients can suffer from serious complications.

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P2

Implementation of a structured care pathway for resistant hypertension in secondary care: patient profile after 1 year

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Objective: Cardiovascular diseases remain a major cause of death. Arterial hypertension is an important risk factor, and although it is very frequently screened for, it is often suboptimally treated and adequate blood pressure control is not reached. By implementing a structured care pathway for treatment-resistant hypertension in secondary care, we aimed to improve diagnosis, optimize treatment and identify patients with secondary hypertension as well as patients at high risk for developing cardiovascular and renal complications.

Methods: Patients can be referred to the hypertension clinic for treatment-resistant hypertension despite 3 classes of antihypertensive drugs in an adequate dose, or for hypertension diagnosed before the age of 40. All patients receive 24h blood pressure monitoring and screening lab tests. Patients with inadequately treated hypertension (>130/80 mmHg/24h) are then further evaluated via a structured pathway with a multidisciplinary approach including cardiac and renal ultrasound, lifestyle and nutritional education and additional screening for secondary causes of hypertension.

Results: In the first year after implementing our structured care pathway, 51 patients were referred: 28 patients because of hypertension diagnosed before the age of 40 and 23 patients because of treatment-resistant hypertension.
Mean age was 51 (range 17-81). 55% were overweight or obese, 16% smoked, 25% had diabetes and 22% used lipid-lowering drugs.
24-hour blood pressure monitoring identified 45 patients (88%) with inadequately treated hypertension. Their treatment regimen was intensified.
Screening for secondary hypertension suggested primary hyperaldosteronism in 3 patients. Fibromuscular dysplasia was diagnosed in 1 patient.
Patients with treatment-resistant hypertension were on average taking 3,3 different classes of antihypertensive drugs, with ACEi/ARB being used by 96% of patients, and calcium-antagonist by 78%. Patients with hypertension before the age of 40 were on average taking 1,2 different classes, with ACEi/ARB being used by 11% of patients, and calcium-antagonist by 18%.
Cardiac ultrasound revealed left ventricular hypertrophy in 17 patients (33%). 4 patients (8%) had renal insufficiency (eGFR<45 ml/min/1,73m²), 3 (6%) had proteinuria.
In patients with treatment-resistant hypertension, the median 10 year risk of cardiovascular death (based on SCORE risk charts) was 4,25% (range 1-30%). The median 10 year risk of cardiovascular morbidity (based on the Framingham general CVD risk score) was 26,18% (range 7,12-62,6%).

Conclusion: Implementing a structured care pathway in secondary care for treatment-resistant hypertension that includes 24-hour blood pressure monitoring is an important tool to identify patients that need antihypertensive treatment optimization and to diagnose patients with secondary hypertension. Furthermore, it can identify patients with end-organ damage and with a high cardiovascular disease risk.

P3

Nephro-geriatric assessment in routine care: Pathway for OLDer patients with Endstage Renal disease (POLDER)

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Affiliates: [1]LUMC, [2]St. Antonius Ziekenhuis, [3]MUMC, [4]UMCG, [5]Haga Ziekenhuis

Objective: To study the feasibility of incorporating a care pathway specifically for older patients with ESRD in routine care which includes a yearly geriatric assessment. To capture these data in a national database and collect biomaterial for future research. Moreover, determinants of successful implementation will be assessed and determinants of adverse outcomes will be explored.

Methods: A consensus-based nephro-geriatric test set was designed to assess frailty, cognitive and functional status in routine predialysis care for older patients with ESRD, in accordance with (inter)national guidelines. First, selection criteria were set based on experience from national best-practices, second, examples were presented in an expert-meeting, concluded by a final round of comments and the possibility to gather input, after which the test set was definite for pilot testing.
A multicentre prospective observational pilot study will be conducted in 11 Dutch hospitals to study the feasibility of implementation of the nephro-geriatric test set. Dutch speaking patients >70 years of age with an eGFR < 20 ml/min/1.73 m^2 (based on CKD-EPI) will be included. In this study, we will yearly perform a nephro-geriatric assessment, and the analyses of the pseudonymised test results will be stored in a national database. In addition, in four hospitals serum samples will be collected and stored. Feasibility of implementation is defined as when 20 patients in each of a minimum of 10 hospitals have successfully completed the nephro-geriatric test set, and their data is gathered in the database.

Results: The geriatric assessment encompasses the different geriatric domains of functionality (Katz ADL, Lawton iADL, Hand grip strength), cognitive function (MOCA, LST, 6-CIT), mood (GDS-2, Optimism check), nutritional status (PG-SGA), clinical judgement (surprise question, clinical frailty score, Charlson co-morbidity index), patient preferences (SF-12, DSI). The assessment takes 30-45 min, and an additional 20-30 min for the patient to fill in the questionnaire. We aim to include 200 patients by the end of 2019, in order to study determinants of adverse outcomes, implement interventions and improve outcomes for chronic care in older patients with ESRD. First outcomes of the pilot study are expected by the end of the year 2020.

Conclusion: A nephro-geriatric test set is designed for routine care. Feasibility of use in routine care will be tested in a pilot study in 11 Dutch hospitals, next to collection of clinical and geriatric data and biomaterial in a national registry. We hypothesize that implementation of a nephro-geriatric assessment and a national registry in chronic care for older patients with ESRD will provide insights in determinants of adverse outcomes and will benefit decision making trajectories for RRT and improve outcomes for these patients.

P4

Perspectives and experiences of patients and healthcare professionals with geriatric assessment in CKD: a qualitative study.

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Affiliates: [1] Nephrology, LUMC; [2] Clinical Epidemiology, LUMC; [3]St. Antonius Ziekenhuis;

Objective: To explore the perspectives and experiences of patients and health-care professionals with a nephro-geriatric assessment (NGA; i.e. a screening of all geriatric domains using standardized tests) in (pre)dialysis care.

Methods: A qualitative study was conducted using six semi-structured focus groups with purposive heterogeneous samples of patients (n=18, aged 67 to 88 years), caretakers (n=4, children or partner) and professionals (n=25, including nephrologists, geriatricians, nurse (practitioners), social workers, and dieticians). Participants had experience with different NGA instruments during (pre)dialysis or - transplant care. Benefits and burden of NGA's were discussed as well as barriers and facilitators for implementation into routine care. Transcripts were analysed thematically.

Results: Both patients and professionals experienced the NGA as beneficial. Patients had both positive and negative strong associations with school examination and braingames. For some patients outcomes were confrontational, whilst for others the assessment was seen as a challenge and patients strived to perform at their best. Patients believed that outcomes did not directly influence treatment decisions. However, they did recognize the value of repetitive NGA and its potential to support treatment (choice). Caretakers acknowledged the importance of NGA for safety and competence reasons.

Professionals believed the NGA was an important tool to enhance their knowledge of the patient, to initiate a dialogue on treatment decision and -goals, and to provide explanations for targeted interventions. Moreover, NGA improves awareness to (re)consider different treatment options. Professionals also noticed that frailty and cognitive problems were identified more often. Involvement of geriatric expertise was valuable: professionals believed that patients appreciated the extra time and were able to share their fears and concerns with another medical doctor.

Perceived barriers by patients to implementation of NGA included lack of information about the purpose and goal of the NGA, the impact of outcomes of NGA, frequent and multi-hour duration of assessment, poor legibility, and illiteracy. Multidisciplinary cooperation was considered a major success factor. Cooperation between geriatric- and nephrology departments and involvement of a social worker or occupational therapist were considered indispensable. Organisational barriers included limited time, high turnover of doctors, poor planning of care, limited geriatric knowledge and unavailability of geriatricians.

Conclusions: Both patients and professionals were positive about the use of NGA in routine nephrology care. Moreover, it benefits treatment decisions and is possible to implement into routine care. Future research should enhance knowledge about the influence of NGA on treatment choices.

P5

Glucocorticoid precipitated attack of Hypokalemic Periodic Paralysis complicated by severe bradyarrhythmia: a case report

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Here we report the case of a 40-year-old male with Hypokalemic Periodic Paralysis (HPP) who presented with acute tetraparesis which was complicated by severe bradyarrhythmia. The patient, with no remarkable medical history, had gone to bed in good general condition and woke up at night noticing complete inability to move all four limbs. A few days before admission he was bitten by an insect, causing pain and swelling of his left upper arm. He had taken one tablet of methylprednisolone 32 mg on the evening prior to the attack. Initial physical examination revealed symmetrical hypotonia and hyporeflexia of all four limbs while sensibility remained preserved. Arterial blood gas analysis showed a potassium level of 1.8 mmol/l. CT scan and lumbar puncture, performed prior to the finding of hypokalemia, were unremarkable. Electrocardiogram (ECG) on admission showed significant bradycardia with prolonged QT_c interval (550 ms). Following lumbar puncture, ECG rhythm evolved into an asystolic pause, requiring short-term cardiopulmonary resuscitation and intravenous injection of atropine. The patient was given high-dose intravenous potassium supplements through a central venous line and he was subsequently admitted to the ICU for further monitoring and follow-up of blood potassium levels. After normalization of hypokalemia, ECG changes resolved and muscle strength was fully regained. The patient was informed about potential HPP provoking factors and instructed in actions to be taken in case of relapse. In addition, he was notified of the contraindication for future use of QT-prolonging medication.

Hypokalemic Periodic Paralysis is a rare and incompletely understood neuromuscular disorder caused by one of several possible mutations in genes encoding skeletal muscle ion channels (e.g. CACNA1S, SCN4A and KCNJ2). Patients may experience sudden severe muscle weakness or paralysis of all four limbs (but typically sparing head, neck and respiratory muscles) which may last several minutes to several days. Attacks are often triggered by strenuous exercise or carbohydrate-rich meals and result from a shift of extracellular potassium into muscle cells. It is assumed that initial small decreases in serum potassium levels –induced by aforementioned precipitating factors– cause paradoxical depolarization of the sarcolemma, which sets off a vicious cycle of hypokalemia and further depolarization, ultimately leading to severe muscle paralysis. ECG changes typical for hypokalemia (e.g. QT_c prolongation) may be present but severe bradycardia (sinus arrest) as witnessed in our patient is unusual. The diagnosis can be confirmed through provocative, electromyographic and/or genetic testing (which is currently being performed). Moderate to severe episodes usually require oral or intravenous potassium administration with close serum monitoring as (arrhythmogenic) rebound hyperkalemia may ensue following the attack. HPP precipitating factors should be avoided to prevent recurrence. Carbonic anhydrase inhibitors or potassium-sparing diuretics can be used when lifestyle changes are not sufficient. In case of concomitant hypokalemia-induced QT_c prolongation, future use of QT-lengthening drugs is contraindicated.

P6

CKD detection might benefit from an ethnic specific screening approach – results from the HEalthy Life In an Urban Setting (HELIUS) study

B.J.M.V. Huisman, B.J.H. van den Born, C.O. Agyemang, M.B. Snijder, L.Vogt

Objective. Screening for chronic kidney disease (CKD) is currently recommended for patients with a history of diabetes mellitus, hypertension or cardiovascular disease (CVD). This approach may not identify all individuals with CKD. Ethnicity, age, and socio-economic status have been described to affect CKD risk and these factors may influence CKD detection in the general population. We therefore studied whether the addition of criteria for age and socioeconomic status (SES) may improve CKD detection in a multiethnic population.

Methods. Baseline data from the HELIUS study, a multi-ethnic cohort study conducted in the city of Amsterdam, were used. Analyses were conducted among 21,421 participants (mean age 44 years, 43% male) of Dutch (n=4539), South-Asian Surinamese (n=3027), African Surinamese (n=4114), Ghanaian (n=2297), Turkish (n=3576) and Moroccan (n=3868) ethnic origin. Detection success of three screening approaches was investigated in each ethnic group. Respectively, approaches I, II and III consisted of the traditional approach (i.e., screening when a history of diabetes mellitus, hypertension or CVD was present); the traditional approach combined with age >50 yr; and the traditional approach combined with low SES (i.e, no or elementary schooling only). These three approaches were investigated in all above mentioned ethnicities. We defined CKD as eGFR (CKD-EPI formula, < 60mL/min/1.73m^2) and/or albuminuria (≥3 mg/mmol).

Results. In our cohort, 2284 (10.6%) participants had CKD. Overall, compared to approach I, approach II increased detection success with 6.7% and approach III with 6.3%. Detection success of approach I among the ethnic groups varied from 38.8 tot 70.3%. Detection improvement by using approach II and III showed ethnic specific differences. In participants of Dutch origin, approach II significantly increased the detection rate with 15.1%, while in participants of Turkish and Moroccan origin approach III significantly increased detection rates (8.9 and 14.1%, respectively).

CKD cases detected by ethnicity per approach

	Approach I	Approach II	Approach III	P-value
Dutch *(n=257)*	50.1%	65.2%	52.5%	<0.001
South-Asian Surinamese *(n=398)*	70.3%	75.4%	71.6%	0.25
African Surinamese *(n=452)*	68.4%	75.4%	70%	0.054
Ghanaian *(n=267)*	67.5%	70.6%	75.5%	0.13
Turkish *(n=405)*	44.8%	49.5%	53.7%	0.04
Moroccan *(n=505)*	38.8%	45.8%	52.9%	<0.001

Conclusion. Addition of age >50 yr and low SES to the currently advised screening approach results in higher CKD detection. This improvement varies however among ethnic groups. Addition of an age criteria improved detection in Dutch participant, while adding a SES criterion improved detection in Turkish and Moroccan participants. Our results prompt for development of a different set of CKD screening criteria in a multiethnic population.

P7

Assessing the complex causes of kidney allograft loss: an observational single center study.

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Introduction
Although graft loss is a primary endpoint in many clinical studies in kidney transplantation and a broad spectrum of risk factors for graft failure has been identified, the eventual causes of graft failure in individual cases remain ill studied. When studied, often only histological diagnoses are reckoned, creating a bias towards alloimmune phenomena as predominant causes of loss.

Methods
We performed a single-center observational cohort study in1000 renal allograft recipients, transplanted between March 2004 and February 2013.

Results
In total, 365 (36.5%) graft losses were identified, of which 211 (57.8%) were due to recipient death with a functioning graft and 154 (42.2%) to graft failure defined as return to dialysis or retransplantation. The main causes of recipient death were malignancy, infections and cardiovascular disease. The main causes of graft failure were distinct for early failures, where structural issues and primary nonfunction prevailed, compared to later failures with a shift towards chronic injury. In contrast to the main focus of current research efforts, pure alloimmune causes accounted for only 14.9% of graft failures and only 6.3% of overall graft losses.

Conclusion
In conclusion, this study provides better insight in the eventual causes of graft failure, and their relative contribution, highlighting the weight of nonimmune causes. Future efforts aimed to improve outcome after kidney transplantation should align with the relative weight and expected impact of targeting these causes.

P8 ❗

Impact of immunosuppressive drugs on endothelial barrier

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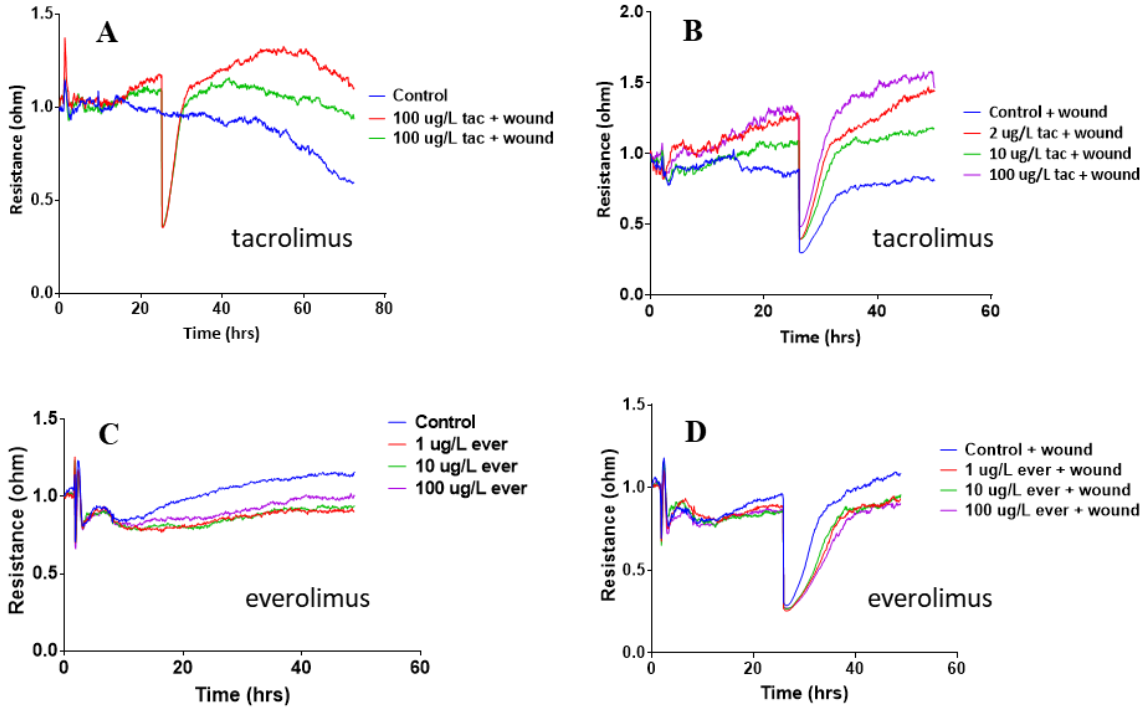
Objective. Cardiovascular disease is still the major cause of death in renal transplant recipients. This group of patients has an extremely increased cardiovascular risk as compared to the general population with high prevalence of traditional and non-traditional risk factors. Both success and limitation of transplantation are primarily determined by the immunosuppressive medication. Apart from amplification of traditional risk factors, these drugs may also have a direct detrimental effect on endothelium. In this study we aim to investigate the effects of several immunosuppressive drugs on the endothelial barrier.

Methods. To analyze the effects of immunosuppressive drugs on the endothelial barrier function we used quantification of trans-endothelial electrical resistance using the Electrical Cell-substrate Impedance Sensing (ECIS) device. ECIS measurement was performed of human umbilical vein endothelial cells (HUVEC's) pre-incubated with several drugs with different concentrations (prednisolon, cyclosporin, tacrolimus and everolimus). Regeneration of integrity after artificial electrical wounding was also measured. A comparison was made with a control condition.

Results. With the ECIS measurements tacrolimus seems to induce a concentration dependent improved barrier function as compared to the control condition (A). After wounding the tests also suggest an improved regeneration (B). Everolimus, however, seems to induce endothelial barrier dysfunction (C) with disturbed regeneration after wounding (D). Cyclosporin did not show any effects on endothelium. Prednisolon revealed conflicting data.



Conclusion. Our data suggests the possibility of opposite effects of tacrolimus and everolimus on endothelial barrier function, Data validation, however, is necessary.

P9

Laminaria japonica fucoidan protects glomerular endothelial heparan sulfate by inhibition of heparanase-1 activity and reducing leukocyte adhesion

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Objective: An important cause for chronic kidney diseases are inflammatory glomerular diseases, like diabetic nephropathy and glomerulonephritis. Filtration of the blood takes place in the glomerulus, where it first encounters the carbohydrate rich glycocalyx of the glomerular endothelial cells (GEnCs). The glycocalyx contains a large proportion of heparan sulphate (HS), a negatively charged glycosaminoglycan with a diverse structural complexity. HS in the glycocalyx contributes to endothelial barrier function and under healthy conditions prevents the adhesion of inflammatory components (such as cells, cytokines and chemokines). We showed previously that heparanase-1 (HPSE1) activity is increased in the vast majority of glomerular diseases (1), thereby impacting glomerular HS in the glycocalyx and contributing to albuminuria.
Fucoidans are a class of marine fucose-based carbohydrates. *Laminaria japonica* is a seaweed, which contains a branched fucoidan that is structurally similar to HS. As an analogue, *L. japonica* fucoidan derivatives have the potential to modulate biological mechanisms that involve HS. Therefore, we hypothesised that *L. japonica* fucoidan may inhibit activity and leukocyte adhesion to glomerular endothelial cells possess anti-inflammatory activities with HS ligands that support healthy HS-mediated events in disease.

Methods: LPS stimulation of GEnCs was used as an *in vitro* model system for the expression of active HPSE1 and for the binding of leukocytes in glomerular inflammatory conditions. Fucoidan and HPSE1 activity were measured via indirect ELISAs. Cell surface HS glycocalyx was visualized by immunocytochemistry. HS glycocalyx release and fucoidan-reactive antibodies were assessed using competition ELISAs.

Results: Fucoidan significantly inhibited both human recombinant and cell-derived HPSE1 activity *in vitro* and also reduced leukocyte binding to GEnCs under inflammatory conditions. Cell surface HS glycocalyx was preserved by fucoidan treatment in LPS-stimulated GEnCs and the release of HS glycocalyx was also reduced. Importantly fucoidan attenuated HPSE1 activity in plasma of patients with glomerular disease.

Conclusion: Together, our data suggests that fucoidan protects endothelial HS glycocalyx by inhibition of HPSE1, thereby reducing leukocyte adhesion. Fucoidan may have therapeutic potential in the treatment of inflammatory glomerular diseases

Reference:

[1] Rabelink, TJ; *et al* (2017) *Nat Rev Nephrol.* (4):201-21

P10

MANNAN-BINDING LECTIN (MBL) INDUCES RENAL EPITHELIAL CELL CYTOTOXICTY VIA TO THE BASOLATERAL SIDE OF THE CELLS IN RENAL ISCHEMIA/REPERFUSION

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Renal ischemia reperfusion injury (IRI) is inevitable in transplantation and influences short- and long-term graft survival. High serum levels of mannan-binding lectin (MBL), the initiator of the lectin pathway of complement activation, have been associated with inferior renal graft survival. In a rat model of renal IRI, inhibition of MBL protected against renal dysfunction and tubular cell death, indicating a novel therapeutic target to improve graft survival. Here we investigated the mechanism of MBL-mediated cytotoxicity on renal epithelial cells. MBL was isolated from human serum via mannan- or polyclonal anti-human MBL affinity chromatography. Both types of serum-derived MBL (sdMBL) were still associated with different molecules, including mannan-binding lectin serine protease 1 and 2 (MASP1, MASP2), alpha-2 Macroglobulin (A2M) and serum amyloid P (SAP), as shown by mass spectrometry, western blotting and complex-ELISAs. When administered to subconfluent cultures of human proximal tubular epithelial cells (HK2), both sdMBL preparations induced altered cell morphology, cell detachment and cell death after 24 hours, as shown by fluorescent live/dead staining and increased LDH release. In contrast, these changes were not induced by recombinant human MBL (rMBL), which was free of associated proteins. Interaction of MBL with HK2 was evaluated using flowcytometry. Binding of rMBL to HK2 was completely abolished in the presence of EDTA, and strongly reduced by D-mannose. This suggests binding of MBL via its calcium-dependent carbohydrate recognition domain (CRD). In contrast, sdMBL bound in a calcium-independent manner to these HK2 cells, suggesting a role for associated molecules to facilitate the binding to HK2. To further characterize the interaction of MBL with HK2, cells were seeded on a collagen-1 coated transwell insert. Addition of active sdMBL at the apical side had no functional consequence, while basolateral exposure did induce cell death and a loss of barrier function. In conclusion we have shown that sdMBL has a direct cytotoxic effect on renal epithelial cells, and that this feature seemed to be linked to the presence of associated molecules that affect MBL binding. In future research we will further explore the mechanisms of MBL interaction to epithelial cells and study in more depth the composition of pathological MBL to develop inhibitory strategies to reduce ischemia reperfusion injury during transplantation.

P11

Minimal Residual Autoimmunity after Rituximab in ANCA-associated Vasculitis Patients

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Objective: B-cell depletion with rituximab (RTX) is an effective treatment for ANCA-associated vasculitis (AAV) patients. Repeated RTX upon B-cell repopulation or return of ANCAs improved therapeutic efficacy, which indicates the presence of minimal residual autoimmunity (MRA) after RTX. Therefore, this study aimed to perform in-depth phenotypic and functional analyses of B and plasma cells after RTX in AAV.

Methods: EuroFlow-based highly sensitive flow cytometry (HSFC) was used during longitudinal follow-up of RTX-treated AAV patients (n=12). To investigate MRA in the memory B-cell compartment after RTX, peripheral blood mononuclear cells (PBMCs) were stimulated with CpG, IL-2 and IL-21 *in vitro* to induce plasma cells (PCs) and ANCA-IgG and -IgM were measured in these supernatants and in paired serum samples by ELISA.

Results: By employing HSFC we demonstrated that 12 weeks after RTX, low but significant numbers of circulating $CD19^+$ B cells (0.21^*10^6 cells/L) could still be detected (reduction of -99.7%). While naïve B-cells, memory B-cells and $CD20^+$ plasmablasts (PB) were rapidly depleted, $CD20^-$ PCs were reduced slower and depleted incompletely. Residual $CD20^-$ PCs were 0.05^*10^6 cells/L (-95.8% from baseline), whereof 57% were mature $CD138^+$ PCs. Early repopulation at 12 weeks was dominated by $CD20^-CD138^-$ PCs, followed by $CD20^+$ PBs at 24 weeks while memory and naïve B cells remained suppressed. Simultaneously, serum ANCA IgG, IgM and IgA, produced by autoreactive PCs, decreased but did not disappear after RTX. Interestingly, 24 weeks after RTX, serum anti-MPO IgM increased in 3/4 patients, which associated with repopulating $CD20^+$ PBs. This suggested remaining autoreactive B cells despite RTX treatment, which was further studied by *in vitro* PBMC cultures. In these supernatants both anti-MPO-IgG and -IgM were detected at baseline, whereas anti-MPO IgG disappeared after RTX, in contrast to anti-MPO IgM, which was detected 24 weeks after RTX.

Conclusion: RTX results in a strong but not complete B cell depletion. In-depth analysis demonstrated that both ANCA-producing PCs and ANCA-memory B cells can be detected after RTX, indicating residual B-cell autoimmunity in AAV patients. Further identification of MRA could be worthwhile for guiding personalized treatment in AAV patients.

P12

CLASSICAL PATHWAY ACTIVATION IN A PATIENT WITH LIGHT CHAIN DEPOSITION DISEASE AND ANGIOEDEMA

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Objective: B-cell lymphoproliferative disorders are often associated with acquired angioedema (AAE). In particular, paraproteins have been suggested to play a role in pathogenesis via activation of classical pathway (CP) and depletion of C1 inhibitor. Serum analyses of C1 inhibitor and C4 are important in the diagnosis of AAE. Here we describe a male patient with light chain deposition disease (LCDD), renal insufficiency and angioedema. Initial laboratory workup revealed increased concentrations of monoclonal serum IgG lambda (20 g/L; normal 8 - 16), lambda free light chains (196 mg/L; normal 10 - 34) and normal functional and quantitative testing of C1 inhibitor. C1 inhibitor complex was not increased (22 CAU/mL; normal <56), however C4d (430 CAU/mL; normal <164) and other complement activation markers (C3bBbP (31 CAU/mL; normal <15), C3bc (31 CAU/mL; normal <12) and sC5b-9 (2.0 CAU/mL; normal <0.5)) were strongly elevated. We further investigated whether paraprotein could have caused CP activation in this patient, despite conflicting results of C1 inhibitor and C4d and downstream markers.

Results: We purified paraprotein-containing immunoglobulin (Ig) fraction from patient plasma using Protein A/G column. When incubated with normal human serum (NHS), Ig from the patient induced a profound time-dependent generation of both C1 inhibitor complex and C4d, while these markers in NHS and NHS supplemented with Ig from pooled normal plasma did not increase. Furthermore, generation of alternative pathway markers C3bBbP, C3bc and sC5b-9 in the presence of patient Igs was also more pronounced.

Conclusion: Our results indicate that paraprotein in the LCDD patient with angioedema causes activation of CP. This may lead to the local C1 inhibitor consumption which was not reflected in initial laboratory workup and via this way contribute to AAE and possibly renal damage in this patient. Analysis of purified Ig may provide evidence for the role of paraproteinemia in pathogenesis of AAE with initially normal C1 inhibitor tests and facilitate choice of treatment.

P13 ❢

National observational study monitoring the restrictive regimen of eculizumab therapy in aHUS in the Netherlands

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Objective
Nowadays eculizumab is the cornerstone of treatment in atypical hemolytic uremic syndrome (aHUS). The optimal treatment strategy in aHUS is still unknown. In October 2016, a Dutch guideline promoting a restrictive eculizumab regimen in aHUS, was implemented. Here we report the preliminary results of the CUREiHUS study, monitoring this guideline.

Methods
The data of pediatric and adult Dutch aHUS patients included in CUREiHUS study from 1-10-2016 till April 2019 were evaluated. Patients were divided in two groups; a historical cohort containing aHUS patients already on eculizumab treatment before October 2016 (n=13) and aHUS cohort who started with eculizumab after October 2016 (n=24).

Results
In the historical cohort eculizumab could be withdrawn in 5 of the 13 aHUS patients (median duration treatment 6,6 months). Four patients are treated with interval elongation ranging from 3 to 6 weeks). Three patients are on two-weekly interval dosage. In two of the 24 patients who started eculizumab after October 2016 aHUS was diagnosed <3 months and are receiving the standard two-weekly regimen according to the Dutch guideline. In 22 of these 24 aHUS patients the follow up is > 3 months. In 13 patients the eculizumab therapy could be withdrawn. The median duration of treatment was 3,2 months (range 0,3-10 months). The median follow-up after the last gift of eculizumab was 17 months (range 1-35 months). Tapering of eculizumab was seen in 6 patients (interval 3-6 weeks). Additional three patients are treated with the standard two weekly regimen treatment. Relapse of HUS occurred in 14 patients and were treated immediately with eculizumab. In total 8/34 (24%) are at the moment treated with eculizumab biweekly. Eculizumab therapy is withdrawn in 38% (5/13) and 62% (13/21) in patients with onset of aHUS before and after implementation of the guideline, respectively.

Conclusion
The majority of aHUS patients do not need eculizumab biweekly and can be safely switched to an extended interval or withdrawal of therapy. Continuous monitoring and further determination of contributing or predictive factors to relapse(s) are necessary.

P14 ❢

The impact of dietary fiber intake on gut-derived uraemic toxins in paediatric CKD

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Introduction

Chronic kidney disease (CKD) in children, is a pro-inflammatory and invalidating systemic condition that leads to an unacceptably high morbidity and mortality. Central is the accumulation of organic metabolic waste products, coined as uraemic toxins (UTs). Several of these UTs are protein-bound and gut-derived. Accruing adult evidence points out distinctly dysbiotic gut microbiota in CKD, resulting in a state of increased proteolytic fermentation, that might be counteracted by dietary fiber. Several attempts to alter UT generation with pre-, pro- and synbiotics yielded contradictory results. This is unexplored in the paediatric population. Therefore, we aimed to define the relationship between dietary fiber intake and protein-fiber ratio (PFR) versus UT concentrations.

Methods

In this 2-year prospective observational longitudinal study, we included 262 visits of 47 predialysis CKD 2-5 or transplant patients [9.1 (1.0-17.0) years)]. Total and free levels of 6 protein-bound UTs (indoxyl sulfate (IxS), p-cresyl sulfate (pCS), p-cresylglucuronide (pCG), hippuric acid (HA), indole-acetic acid (IAA) and 3-carboxy-4-methyl-5-propyl-furanpropionic acid (CMPF), coupled to in-depth diet histories were collected. Linear mixed models were used to assess the relationship between the UTs versus dietary fiber and PFR, considering eGFR.

Results

Total dietary fiber intake was low, especially in advanced CKD: 13.3 g/day/BSA (7.9-19.8) in CKD1-3 versus 8.8 (1.7-11.6) in CKD 4-5 ($p = 0.018$). While no association between fiber intake and UT concentrations was found, a significant association between PFR with total serum IxS [$e= 0.039$ (0.0014 – 0.076) $p = 0.042$] was seen.

Conclusion

Our data show that fiber intake in paediatric CKD is low, and that a higher PFR is associated with higher levels of IxS. The neglect of current CKD nutrition guidelines for dietary fiber intake thus seems unjustified. They further illustrate the complexity in restoring eubiosis and suggest a multifactorial approach, in which the interaction between fiber and protein intake might be a piece of the puzzle.

P15 ❢

GENERATING A HUMAN *APOL1* G2/G2 PODOCYTE CELL MODEL TO STUDYING APOL1-RELATED KIDNEY DISEASE MECHANISM

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Background
APOL1 risk variants (RVs) G1 and G2 confer an increased risk of various nondiabetic kidney diseases in African population. To date the precise mechanisms by which *APOL1* RVs induce podocyte and other kidney cells injury remain unclear. To explain the mechanism whereby *APOL1* RVs expression induces damage in kidney cells, most of *in vitro* studies used animal or cell models created by gene editing.

Objective
In the present study, we aimed to generate a cell model derived directly from humans carrying *APOL1* high risk genotype (HRG), which can be used to study the podocyte disease *in vitro*.

Methods
From African population living in Belgium, DNA was extracted and the genotyping for *APOL1* was performed. Freshly voided urine was collected from participants in whom *APOL1* HRG has been detected and exfoliated cells were cultured. Cells were immortalized using a temperature-sensitive SV40-TERT viral system and sub-cloned.

Results
We have successfully generated an *APOL1* G2/G2 podocyte cell model isolated from urine of a human donor carrying *APOL1* HRG (G2/G2). After overexpressing APOL1, G2/G2 podocyte cell lines exhibited various functional features, including alteration of podocyte cytoskeleton and reduction of autophagy flux.

Conclusion
The cell model generated may contribute to the investigation of cellular function of *APOL1* and the podocyte disease *in vitro* in order to unravel the mechanism of *APOL1*-induced podocyte injury.

Key words: *APOL1 HRG, human podocyte cell model, podocyte injury, molecular mechanism, CKD*

P16

Ontogeny and cross species comparison of pathways involved in drug absorption, distribution, metabolism, and excretion in neonates (Review): KIDNEY

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Objective: The kidneys play an important role in many processes, including urine formation, water conservation, acid-base equilibrium, elimination of waste. The anatomical and functional development of the kidney has different maturation time points in humans versus animals, with critical differences between species in maturation before and after birth. Absorption, distribution, metabolism and excretion (ADME) of drugs varies depending on age and maturation, which will lead to differences in toxicity and efficacy. When neonate/juvenile laboratory animal studies are designed, a thorough knowledge of the differences in kidney development between newborns/children and laboratory animals is essential. The human and laboratory animal data must be combined to obtain a more complete picture of the development in the kidneys around the neonatal period and the complexity of ADME in newborns and children. This review examines the ontogeny and cross-species differences in pathways involved in ADME in the developing kidney in preterm and term laboratory animals and children. It provides an overview of insights into ADME functionality in the kidney by identifying what is currently known and which gaps still exist

Methods: A thorough literature search was performed in PubMed/Medline, Web of Science and EMBASE databases using a comprehensive list of keywords related to maturation of the kidney and the role of the kidney in the ADME processes. The review was centred around humans and the most predominant used toxicology laboratory animal species being rat, mouse, dog, pig and monkey.

Results: Comparative ontogeny tables between human, mouse, rat, dog, monkey and pig were drafted for anatomical and functional development, glomerular filtration rate, renal blood flow, concentration ability, transporter maturation and drug metabolism.

Conclusion: Important renal function properties such as glomerular filtration rate, renal blood flow and ability to concentrate are generally well known, detailed knowledge about transporter and metabolism maturation is growing, but still lacking. Preclinical data in those properties is limited to the rat and the mouse and generally covers only the expression levels of transporter or enzyme-encoding genes. More knowledge on a functional level is needed to predict the kinetics and toxicity in neonate/juvenile toxicity and efficacy studies.

P17
The Inverse Relationship between Vitamin D and Kidney Function

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Background: A low vitamin D concentration is commonly found in patients with chronic kidney disease (CKD). However, the relationship between vitamin D and estimated glomerular filtration rate (eGFR) is unclear. Higher plasma creatinine concentrations due to higher muscle mass might falsely indicate lower eGFR. Therefore, we investigated the relationship between plasma 25-hydroxyvitamin D [25(OH)D] with baseline eGFR, prevalent and incident CKD and eGFR over 12 years follow-up, taking muscle mass into account as measured by 24-hour urinary creatinine excretion.

Method: The PREVEND Observational Study included 8,095 participants aged 28-75 year with four follow-up examinations spaced three years apart. Plasma 25(OH)D was measured using liquid chromatography-tandem mass spectrometry. Kidney function was estimated by serum creatinine and cystatin C using three equations to estimate GFR and 24-hour urinary creatinine excretion was used as indicator of muscle mass. We used various regression analyses to study the cross-sectional and longitudinal relationships adjusted for potential confounders including season, parathyroid hormone and urinary creatinine excretion.

Results: Mean age was 49.3±12.3 years and 50.3% of the participants were women. Mean plasma 25(OH)D was 57.9±23.8 nmol/l and mean eGFR 83.9±15.5 ml/min/1.73m^2. We observed a robust inverse association between plasma 25(OH)D with eGFR and prevalent CKD ($eGFR_{creat+cysC}$ <60 ml/min/1.73m^2) odds ratio: 1.47 (1.24, 1.74). Longitudinally, vitamin D remained inversely associated with reduced eGFR over 12 year follow-up taking all five examinations into account. Also, 25(OH)D was significantly associated with incident CKD with a relative risk per 1-SD increment 25(OH)D (23.7 nmol/L) of 1.19 (95% CI 1.04, 1.36), and was consistent for all eGFR equations. However, results were less pronounced with the cystatin C only equation, although still statistically significant indicating some confounding by creatinine. Adjusting for 24-hour urinary creatinine did not change the associations.

Conclusion: This study indicates a robust inverse association between plasma 25(OH)D and eGFR, which could not be explained by muscle mass as measured by urinary creatinine excretion. Since vitamin D supplementation is widely prescribed, further research is needed to explore whether higher vitamin D is directly contributing to CKD progression.

P18

VITAMIN D, RESIDUAL RENAL FUNCTION AND BONE HEALTH IN END STAGE KIDNEY DISEASE

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Background*:* Low $1.25(OH)_2D$ levels are considered a hallmark of end stage kidney disease (ESKD) and have been repeatedly associated with poor outcomes. Recent insights into vitamin D regulation and catabolism suggest that CKD is a state of stagnant vitamin D metabolism characterized by reduced $1.25(OH)_2D$ production (mediated by CYP27B1) and catabolism (mediated by CYP24A1). The present study aimed to clarify whether this is caused by insufficient delivery of substrate or low nephron mass. As a secondary aim we investigated seasonal variation and long term trends of vitamin D levels in patients with ESKD

Methods: We analyzed serum levels of $1.25(OH)_2D$ (LC MS/MS), 25(OH)D (RIA), along with other parameters of mineral metabolism (including PTH, FGF23, sclerostin), markers of inflammation (C-reactive protein [CRP], interleukin 6 [il6]) in 518 adult patients (age 54.7 ± 12.8 yrs, males 60.6%) with ESKD, referred for single kidney transplant at the University Hospitals Leuven between April 23, 2006 and December 21, 2013. Data on residual renal function (RRF) were available in 330 patients: 115 patients were anuric (24h urine output < 100 ml) and 21 patients were anephric.

Results: Median 25(OH)D and 1.25(OH)2D levels in the overall cohort were 35.9 [24.0 – 48.6] µg/L and 26.8 [18.2 – 36.9] ng/L, respectively. 25(OH)D <30 µg/L and 1.25 $(OH)_2D$ < 20 ng/L were observed in 38,4 and 29.1 %, respectively. 25(OH)D levels showed seasonal variation and increased by 16% along the study period (2006-2013), most probably as a result of more intense supplementation. A parallel 31% increase of $1.25(OH)_2D$ levels was noted. In regression analysis, only high 25(OH)D and low phosphate and sclerostin independently associated with high $1.25(OH)_2D$ levels. 25(OH)D was the most important determinant of $1.25(OH)_2D$ levels, explaining 30% of its variability. RRF did not correlate with 1.25(OH)2D levels. Remarkably, 1.25(OH)2D levels were only slightly lower in anephric patients (20.3 vs 27.3 ng/L, median, p=0.02). This proves that non-renal tissues may contribute substantially to circulating $1.25(OH)_2D$ levels. In anuric patients, 25(OH)D levels, but not mineral metabolism hormones or inflammation were associated with $1.25(OH)_2D$ levels. In the overall cohort, neither vitamin metabolite associated with bone turnover markers or bone mineral density. Remarkably, in the subgroup of patients with adequate 25(OH)VitD levels, 25(OH)D levels negatively associated with BMD at the femoral neck, independent of classical determinants.

In **conclusion**, $1.25(OH)_2D$ levels in patients with ESKD are manifestly dependent on the delivery of 25(OH)D. Extrarenal CYP27B1 activity may sustain normal $1.25(OH)_2D$ levels, even in anephric patients. Substrate delivery thus seems to be much more important than nephron mass in maintaining adequate $1.25(OH)_2D$ level. Seasonal fluctuations and long term kinetics illustrate that both vitamin D generation in the skin and nutritional vitamin D supplements contribute to 25(OH)D stores in ESKD. Our date, furthermore, indicate a complex relationship between vitamin D status and bone health in ESKD.

P19

STUDYING THE LINK BETWEEN NEPHROPATHIC CYSTINOSIS AND TUBULAR ACIDOSIS

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Introduction: Recently, a 23-month old girl presented with rickets, metabolic acidosis, signs of renal Fanconi syndrome and increased granulocyte cystine levels. The suspected diagnosis was cystinosis, a disease caused by mutations in the *CTNS* gene (cystine transporter), leading to the lysosomal accumulation of cystine, causing organ damage, particularly the kidneys. However, genetic testing revealed no mutation in *CTNS*, but compound heterozygous pathogenic mutations in the *ATP6V1B1* gene. *ATP6V1B1* encodes the B1 subunit of the lysosomal V1 ATPase, which is deficient in distal renal tubular acidosis type 1B, but with an unknown link to cystinosis. The present study aimed to determine the link between renal tubular acidosis and nephropathic cystinosis.

Methods: CRISPR/Cas9 technology was used to knock-out the *ATP6V1B1* gene in conditionally immortalized proximal tubular epithelial cells (ciPTEC) and cell characteristics were compared to isogenic *CTNS*-/- cells. An untargeted metabolomics approach based on UHPLC-MS/MS was applied for the intracellular quantification of metabolites differentially expressed in knock-out and control cells. Fluorescence-based imaging assays were applied to monitor the lysosomal-autophagy dynamics (TFEB, LC3, and DQ-BSA) in ciPTEC.

Results: The *ATP6V1B1-/-* isogenic ciPTEC showed a significant increase in cystine accumulation compared to healthy control cells (0.26 vs. 0.13 nmol/mg protein; $p<0.05$). But this was significantly lower as compared to cystine accumulation in *CTNS*-/- cells (6.32 vs. 0.26 nmol/mg protein; $p<0.05$). Like the *CTNS*-/- cells, *ATP6V1B1-/-* cells demonstrated an abnormally increased autophagy as shown by the increased TFEB nuclear translocation (2-fold; $p<0.05$), increased accumulation of LC3 (2.3-fold; $p<0.05$), and increased lysosomal degradation of DQ-BSA (2-fold; $p<0.05$). Moreover, using metabolomics, we identified several metabolites and pathways that were altered ($p<0.05$) in both renal acidosis and cystinotic cells.

Conclusion: We successfully developed a new genetically engineered renal tubular acidosis cell model with isogenic controls. These cells provide a novel versatile tool to study the pathology of renal tubular acidosis. Metabolomics allowed us to bridge the gap between cystinosis and renal acidosis, with the future aim of finding druggable targets.

P20 ❢

Investigating The Pathophysiology And A Potential Therapeutic Approach For Cystinosis

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Introduction: Nephropathic cystinosis is a severe genetic disorder caused by mutations in *CTNS* gene (cystine transporter), leading to the lysosomal accumulation of cystine and progressive organ damage. To date, no appropriate *in vitro* isogenic cystinotic cell models exist, a pre-requisite to study the link between the *CTNS* gene and the disease, and to investigate potential therapeutic strategies. Hence, our aim was to generate a cystinosis phenotype in human kidney cells using CRISPR/Cas9 and study cystinosis pathology.

Methods: We selectively knocked-out the *CTNS* gene in conditionally immortalized proximal tubular epithelial cells (ciPTEC). An untargeted metabolomics approach based on UHPLC-MS/MS was applied for the intra- and extracellular quantification of cystine and other metabolites differentially expressed in knock-out and control cells. Various assays were applied to monitor the lysosomal-autophagy dynamics (TFEB, LC3-II and DQ-BSA) in ciPTEC.

Results: The *CTNS*-/- isogenic cell line of ciPTEC showed a significant increase in cystine accumulation compared to healthy control cells (6.32 *vs.* 0.05 nmol/mg protein; $p<0.001$). Upon treatment with cystine depleting drug cysteamine, *CTNS*-/- cells showed a significant reduction in cystine levels (0.74 nmol/mg protein; $p<0.01$). Using metabolomics, we identified that not only cystine but also >25 metabolites and 9 metabolic pathways were affected ($p<0.05$) in cystinotic cells. *CTNS*-/- cells demonstrated an abnormally increased autophagy, confirmed by the increased TFEB nuclear translocation (2-fold; $p<0.05$), increased accumulation of LC3-II (2.3-fold; $p<0.05$) and increased lysosomal degradation of DQ-BSA (2-fold; $p<0.05$). Of note, cysteamine had no effect on the restoration of autophagy, which might explain its limited effect on treating renal Fanconi syndrome. However, a promising registered drug molecule was found to be effective either alone or in combination with cysteamine in resolving cystinotic manifestations.

Conclusion: We developed a genetically engineered cystinotic cell model with isogenic controls. These cells provide a novel versatile tool to study the pathology of cystinosis and develop screens for drugs with the potential to reverse the symptoms. Metabolomics allowed an unbiased analysis of potential new targets for treatment of cystinosis.

P21

Correlation between point-of-care salivary potassium and serum potassium: a proof of principle study

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Objective: Electrolyte abnormalities such as hypokalemia or hyperkalemia are frequently seen in patients with e.g. renal tubular disorders and patients with impaired renal function. It is not yet possible for these patients to measure their potassium concentration [K] at home. Clinical studies suggest that measurement of salivary concentration of electrolytes could be useful in reflecting serum levels. Analysis of salivary electrolytes can be done non-invasively and quickly with the use of a point-of-care (POC) portable device. Here, we present a proof of principle pilot study of the relation between the potassium concentrations in saliva and in blood.

Methods: We conducted a validation study of the LAQUAtwin K-11 meter, an ion selective electrode device (ISE) measuring [K]. Using standardized potassium solutions and salivary samples from healthy volunteers, the hand-held device was compared with a reference analyzer (C8000) at the laboratory. Subsequently salivary and blood samples were collected simultaneously from patients with kidney disease. We aimed to include a broad range of blood potassium levels. Therefore blood samples were taken from patients undergoing hemodialysis (pre- and postdialysis) and outpatients. Potassium concentrations are determined before and after dialyses.

Results: We found a linear relationship between standardized potassium solutions as well as saliva [K] measured using the hand-held device versus the C8000 analyzer. Pearson coefficient of correlation between two methods was 0,968. The mean concentration of salivary potassium in healthy volunteers was 26,8 ± 6,4 mmol/l. From the 49 saliva samples retrieved from patients, 43 were from dialysis patients and 6 were taken during out-patient visits. In the pre-dialysis samples the mean concentration of salivary potassium was 35,5 ± 14,9 mmol/l. A lower salivary concentration of potassium was observed in the post dialysis samples 27.8 ± 7.2 mmol/l (P = 0,04). No statistically significant correlation was discernable between salivary [K] and serum [K] in renal patients (Pearson coefficient was 0,397; Figure 1).

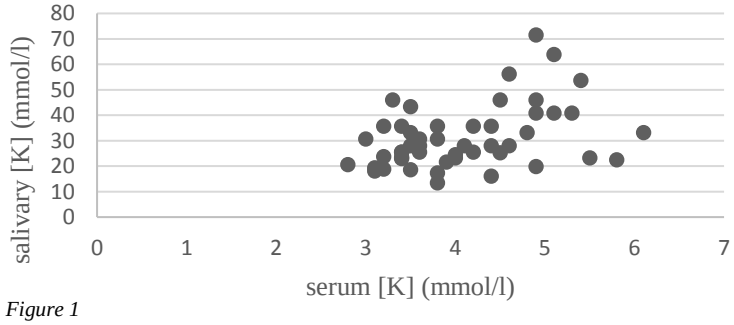

Figure 1

Conclusion: The LAQUAtwin K-11 device was proven to be highly accurate in measuring the potassium concentration in aqueous solutions as well as in saliva. However, there was no clear correlation found between the POC [K] in saliva and serum [K]. Therefore, POC salivary potassium levels are no alternative to approximate serum [K]. Hand-held devices for electrolyte analyses may be useful for other applications.

P22 ❢

PANNEXIN-1 MEDIATES FLUID SHEAR STRESS-SENSITIVE PURINERGIC SIGNALING AND CYST GROWTH IN POLYCYSTIC KIDNEY DISEASE

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Objective: Tubular ATP release is regulated by mechanosensation of fluid shear stress (FSS). The molecular mechanism mediating this process is poorly understood. Polycystin-1/polycystin-2 (PC1/PC2) functions as a mechanosensory complex in the kidney. Extracellular ATP is implicated in polycystic kidney disease (PKD), where PC1/PC2 is dysfunctional. This study aims to provide new insights into renal ATP signaling under physiological conditions and PKD.

Methods: Microfluidic setups, pharmacologic inhibition of mTORC1 and pannexin-1, and CRISPR/Cas9 loss-of-function approaches were combined to assess ATP release in mouse distal convoluted tubule 15 (mDCT15) cells. To study the relevance of the mechanisms disclosed *in vivo*, inducible kidney-specific *Pkd1* knockout mice (iKsp-*Pkd1*$^{-/-}$) and zebrafish *pkd2* morphants (*pkd2*-MO) were used as models for PKD.

Results: mDCT15 cells subjected to FSS displayed an increased ATP release. Pannexin-1 inhibition and knockout decreased the FSS-modulated ATP release by these cells. Likewise, healthy human volunteers displayed increased urinary ATP excretion when exposed to acute water loading. In iKsp-*Pkd1*$^{-/-}$ mice, elevated renal pannexin-1 mRNA expression and urinary ATP were observed. Similarly, in *Pkd1*$^{-/-}$ mDCT15 cells, elevated ATP release was observed upon FSS mechanosensation compared to wild-type mDCT15 cells. In these cells, mTORC1 inhibition failed to enhance ATP extrusion, but increased pannexin-1 mRNA expression was observed compared to wild-type mDCT15 cells. Importantly, pannexin-1 inhibition in *pkd2*-MO decreased renal cyst growth.

Conclusion: Our results demonstrate that pannexin-1 channels mediate ATP release into the tubular lumen due to pro-urinary flow. We present pannexin-1 as novel therapeutic target to prevent renal cyst growth in PKD.

P23

Not all calciproteins are the same: differences in size, number of particles and calcification potency.

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Objective

Chronic kidney disease (CKD) is associated with an increased risk for vascular calcification and cardiovascular disease. Recently, circulating calciprotein particles (CPP), precipitates of calcium and phosphate with incorporated proteins, were identified as drives of the calcification process in CKD. Currently, many different protocols to make CPP *in vitro* are used to study the effects of CPP on vascular calcification. However, it is unknown how the synthesis of CPP affects their morphology and function. Therefore, the present study aims to compare four differently synthesized CPP on morphology, composition, number of particles and calcification potency.

Methods

The CPP were synthesized in mixtures containing 4.4 mM (CPP-A and B) or 6 mM (CPP-C and D) phosphate and 2.8 mM (CPP-A and B) or 10 mM (CPP-C and D) calcium. As a protein source fetal bovine serum was used in CPP-A, B and D and CPP-C was made with fetuin A only. The mixtures were incubated for 7 (CPP-A), 14 days (CPP-B) or 12 hours (CPP-C and D) before isolation.

Results

Transmission electron microscopy showed no visual differences between the CPP in density, only CPP-C are larger (306±19 versus 192±8, 195±7 and 205±6 nm for CPP-A, B and D, respectively). Additionally, the number of particles of CPP-C ($2.9x10^{11}$) was significantly increased compared to CPP-A, B and D (1.5, 2.0, $6.4x10^{10}$ respectively). By incubating human vascular smooth muscle cells (hVSMC) with CPP equivalent to 100 μg/ml calcium, calcification was induced. hVSMC calcification was significantly increased using CPP-B (655±192) and CPP-C (624±147), compared to control (6±1), CPP-A (214±43) and CPP-D (53±8 μg Ca^{2+}/mg protein). However, when normalizing CPP on particle number rather than calcium content, calcification was comparable among CPP-A, B and C. In both experiments CPP-D was not potent to calcify hVSMC.

Conclusion

Differently synthesized CPP are morphological comparable, however calcification potency varies between CPP. Additionally, our results question the current practice of quantifying the amount of CPP based on calcium content.

P24 ❗

The EDITH Kidney patient survey on modality choice among more than 8000 European dialysis and transplant patients

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Objective. Renal replacement therapy (RRT) modality selection may be challenging for both patients and nephrologists. Within the EDITH project we surveyed adult European dialysis and kidney transplant patients on factors influencing modality choice and their satisfaction with the modality choice made.
Methods. The EDITH kidney patient survey (online and on paper) was translated into 30 languages. European adults with end-stage kidney disease treated by dialysis or kidney transplantation were eligible to participate between November 2017 and November 2018.
Results. 8133 patients from 40 European countries participated. Age, gender and modality characteristics (56% male, mean age 59 years (SD 14), 66% on haemodialysis (HD), 6% on peritoneal dialysis (PD), 29% on transplantation (Tx)) reflected the European RRT population in the ERA-EDTA Registry. A quarter of the patients did not receive any information on any modality before the start of RRT. 44% received no information on home haemodialysis (HHD), 24% nothing on PD and resp. 23% and 20% nothing on living and deceased kidney donor Tx. The majority of those who received information, were (very) satisfied with the information (range 57% for HHD to 86% for deceased kidney donor Tx). Two-thirds of the patients reported that decision making was shared with their doctor and most patients (83%) were satisfied with way the decision was made. The main reasons for patients not having a particular treatment are listed in Table 1. Most important factors influencing modality choice were quality of life, survival and safety (resp. 97.3%, 96.6% and. 92.2% rated as (very) important). Results were similar by age group, sex, educational level and start of RRT time period.
Conclusion. Though most patients seem to be satisfied with the information provision and modality choice, there remains room for improvement as a quarter of all patients did not receive any information on treatment modalities. Better education may also influence patients to choose a home-based form of dialysis or empower them to find a living donor.

Table 1: Main reasons not to have a certain treatment

HHD
Don't want treatment at home (34%)
Treatment is not available in my hospital (26%)
Discomfort with no supervision (24%)
PD
Don't want treatment at home (34%)
Dislike of abdominal catheter (23%)
Fear of peritonitis (22%)
Living Tx
No living kidney donor available (37%)
Don't want to ask potential donors (31%)
Concerns about the health of the donor (18%)
Deceased Tx
Not healthy enough (25%)
Currently on waiting list (22%)
Too old (18%)

P25

INTERDIALYTIC HOME MEASUREMENTS OF BLOOD PRESSURE AND VOLUME STATUS IN MAINTENANCE HAEMODIALYSIS PATIENTS

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Objectives – The aim of this study was to evaluate blood pressure (BP) and volume status in the interdialytic period of haemodialysis (HD) patients. We hypothesized that the interdialytic BP and VS might be associated to intradialytic haemodynamic characteristics.

Methods – A cross-sectional two-centre study in maintenance HD patients was performed. BP and VS were measured during a mid-week HD session and subsequently at home during the interdialytic interval by the researcher. Hypo- and hypertension in the short-interdialytic period were defined as a systolic blood pressure (SBP) < 90mmHg, > 140mmHg or a diastolic blood pressure < 60 mmHg, > 90mmHg, respectively. Volume status was assessed using the body composition monitor and categorized as normohydration [fluid overload/extracellular volume (FO/ECW) < 6% or > -6%], dehydration (FO/ECW ≤ -6%), or overhydration (FO/ECW ≥ 6%).

Results – A total of 66 HD patients with a mean age of 70.8 years were enrolled, among whom 67.6% were male and 45.6% diabetic. Prevalence of interdialytic hypotension was 16%, whereas 39% of the subjects had interdialytic hypertension. Interdialytic SBP was significantly correlated with pre- and postdialysis SBP ($r = .585$, $p < .001$; $r = .588$, $p < .001$ resp.). Subjects with interdialytic hypotension had statistically significant lower absolute pre- and postdialysis SBP values ($p = .005$ and $p = .006$ resp.) compared to those with interdialytic hypertension. Interdialytic SBP was not correlated with pre- or postdialysis volume status nor relative intradialytic SBP changes. 17% of the subjects was interdialytic dehydrated and 32% was interdialytic overhydrated. Interdialytic volume status was significantly correlated with pre- and postdialysis volume status ($r = .514$, $p < .001$; $r = .511$, $p < .001$ resp.).

Conclusion – Home measurements of BP and volume status during the interdialytic interval provide valuable information to the knowledge gap in HD. Innovation in renal medicine such as remote monitoring of haemodynamics by wearable devices holds great potential.

P26 ❢

Rise of plasma sodium induces syndecan-1 shedding during hemodialysis

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Objectives: In hemodialysis (HD) patients, endothelial dysfunction (ED) contributes to atherosclerosis. A major hallmark of ED is loss of glycocalyx evidenced by shedding of syndecan-1 into the blood stream. Release of syndecan-1 is seen in pro-inflammatory and pro-oxidative conditions such as in HD patients. HD by the Hemocontrol biofeedback system (HHD) is characterized by initially higher dialysate and plasma sodium levels. Using HHD as a model for an acute increase in plasma sodium, we investigated associations between courses of plasma sodium and syndecan-1 during HHD and standard HD (SHD).

Methods: Plasma syndecan-1 was measured by ELISA in blood samples obtained from a cohort of 29 prevalent HD patients before, during and after HHD and SHD (randomized sequence). Wilcoxon signed-rank test or paired student's t-test was used to compare syndecan-1 levels between SHD and HHD. Intradialytic shedding of syndecan-1 was determined by area under the curve analyses. Associations with the intradialytic course of syndecan-1 were analyzed with a mixed effects repeated-measures model.

Results: Compared to predialytic values (~139 mmol/L), plasma sodium increased at 30 and 60 min after the start of HD, with more pronounced values in HHD than in SHD (~142 mmol/L in HHD versus ~140 mmol/L in SHD; $p<0.001$). During HHD, plasma syndecan-1 increased after 30 minutes of HD ($P=0.007$) and stayed significantly increased until after 180 minutes ($P<0.0001$). During HHD, the plasma syndecan-1 increase coincided with the rise in plasma sodium levels. Plasma syndecan-1 also increased significantly during SHD but at a later point (at 120 minutes; $P=0.003$). At 120 min of dialysis, the rise in plasma syndecan-1 levels was significantly higher in HHD as compared to SHD (+42.9% vs. +17.2%; $P=0.021$). The total amount of shed syndecan-1 was higher during HHD than during SHD, albeit at borderline significance ($p=0.0595$). Lower plasma sodium and osmolality before dialysis were independent predictors of plasma syndecan-1 increase during dialysis ($P=0.001$ for both groups). In HHD, a higher cumulative UF volume was associated with greater intradialytic syndecan-1 increase at the end of dialysis with a borderline significance of $P=0.05$.

Conclusion: Plasma syndecan-1 levels, as a measure of glycocalyx shedding, increased significantly during both HHD and SHD. In HHD, this rise was significantly greater and lasted longer than in SHD. This may reflect ED resulting from increased sodium load. Further research to assess long term effects and clinical implications of high intradialytic salt exposure is needed.

P27

Glycosaminoglycan modified-dialysis membranes improve blood biocompatibility *in vitro*

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Objective: The number of patients requiring renal replacement therapies is increasing with an estimated number of 5.4 million a year in 2030. Most patients use (hemo)dialysis (HD) therapy. Major drawbacks of HD are: (i) poor removal of toxic larger middle-sized molecules and protein-bound uremic solutes; (ii) large fluctuations in water balance and uremic waste, potassium and phosphate of the patients,since it is non-continuous (iii) not fit for prolonged use due to clogging and coagulation of the membranes. Recently, we showed in vitro that combining dialysis and adsorption in one step using mixed matrix membranes (MMM) improves removal of protein-bound uremic solutes from human plasma as compared to conventional dialysis membranes. Although the results with MMM are promising, for continuous use further optimization is required. Due to the well-known contribution of glycosaminoglycans (GAGs) to the barrier and anti-fouling properties of the natural filtration barrier in the kidney, this work aimed to improve hemo- and biocompatibility of MMM by application of novel GAGs either as coatings post membrane fabrication or by incorporation of the GAG into the membrane polymer via blending.

Methods: Flat MMM were coated or blended with the following GAG sources: Heparin, GAGs from porcine intestine (GPI), heparan sulphate (HS) isolated from cultured glomerular endothelial glycocalyx, HS from bovine kidney (HSBK), and heparinase III digested HSBK. Water permeance, and a panel of anti-coagulation and platelet adhesion assays were studied in all cases.

Results: Both GAG coating and blending showed a high stability on the MMM. The new MMM with 3 out of 5 GAGs have higher water permeance in comparison to non-modified MMM whereas heparin and GPI modified MMM were superior in their anti-coagulation and platelet adhesion properties.

Conclusions: GAG-modified MMM have superior biocompatible properties that may improve current dialysis treatment and ultimately incorporation into a future portable artificial kidney device.

P28

IS VASCULAR ACCESS ASSOCIATED WITH MORTALITY IN HEMODIALYSIS?

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Objective
The aim of our study was to assess if there is a difference in survival according to vascular access type.

Methods
Patients that started chronic hemodialysis treatment between 1/1/2007 and 31/12/2016 at the 'Universitair Ziekenhuis Brussel' were retrospectively studied. The time to death was studied as a function of the two main vascular access types using survival analysis, considering the type of vascular access at the start of dialysis or as time varying, and accounting for the available baseline characteristics.

Results
Of 374 patients 309 (82.6%) started hemodialysis with a catheter, while 65 patients started with an arteriovenous access. Vascular access type during follow up did not change in 74% of all patients. A Kaplan Meier plot did not suggest a survival dependent on the vascular access type. A Cox proportional hazard analysis showed that age, congestive heart failure and cancer were associated with higher mortality. Vascular access type was not, even when analysed as a time changing variable.

Conclusions
In this retrospective cohort study, hemodialysis vascular access type was not independently correlated with patient survival, even after taking into account change of vascular access over time.

P29

The 2019 ERBP/VAS Clinical practice guideline on peri- and postoperative care of arteriovenous fistulas and grafts for haemodialysis in adults

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Objective

A vascular access makes life-saving haemodialysis possible. For that, the access needs to function properly, allowing adequate blood flow for uraemic retention solute removal, while minimizing the risk of systemic infection. In response to emerging evidence and increasingly stringent standards for guideline development, European Renal Best Practice (ERBP; the guidance body of the European Renal Association-European Dialysis and Transplant Association (ERA-EDTA)) set out to update its vascular access guideline in collaboration with various stakeholders within the field, including representatives of the Vascular Access Society (VAS), nephrologists, vascular access surgeons, radiologists, dialysis nurses, researchers, patients and their care givers.

Methods

Development of the guideline followed a rigorous process evidence review and appraisal, based on systematic reviews of results from clinical trials and observational data where necessary. The structured approach was modelled after the GRADE system, which ascribes grades to the certainty of the overall evidence and strength for each recommendation. Where appropriate, the guideline development group issued ungraded advice for clinical practice, which was not part of a systematic evidence review. An attempt to adhere to increasingly stringent guideline development methodology has required certain sacrifices in terms of scope.

Results

The guideline consists of 12 chapters specifically covering peri- and postoperative aspects of arteriovenous (AV) fistulas and grafts: medical, surgical, endovascular, and self-administered treatments for promoting AV fistula maturation and maintaining patency; perioperative antibiotic prophylaxis, timing of first cannulation; cannulation techniques and needle types; AV access surveillance; timing and type of intervention for AV fistula thrombosis. Despite the scarcity of high-certainty evidence for most areas in vascular access, ERBP was committed to developing a high-quality guideline, giving guidance where possible, and listing research recommendations where it was not. Of the 32 graded statements, only 5 were strong recommendations; none could be supported with Level A and only four with Level B evidence. The remaining 28 recommendations are supported by lower-grade evidence, offering less certainty as to the expected benefits and harms of different strategies.

Conclusions

The ERBP/VAS guideline on vascular access is the result of a group effort by a multidisciplinary team to comprehensively review and interpret current evidence on several high-priority topics. ERBP hopes the guideline will assist the professional community in making decisions about vascular access processes, pathways and care; help patients and carers gain insight; and facilitate joint decision-making in this field.