HEMODIALYSIS INDUCES DECLINE IN CEREBRAL BLOOD FLOW IN ELDERLY PATIENTS.

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Background
The initiation of hemodialysis treatment is associated with an accelerated decline of cognitive function and an increased incidence of cerebrovascular accidents and white matter lesions. It has been hypothesized that the repetitive circulatory stress of HD induces ischemic cerebral injury, but the mechanism is unclear. Despite the sophisticated regulation of the brain to keep the cerebral blood flow (CBF) steady; hemodialysis might induce a fall in CBF. We studied the acute effect of conventional hemodialysis on cerebral blood flow, measured by [¹⁵O]H₂O PET-CT, which is considered the gold standard for measuring CBF.

Methods
Twelve patients aged ≥65 years (5 females, 7 males) with a median dialysis vintage of 3.8 years completed the study. During a single hemodialysis session, three [¹⁵O]H₂O-PET-CT scans were performed: Before, early after the start, and at the end of HD. For each PET-CT scan a bolus injection of [¹⁵O]H₂O was administered intravenously in the non-dialysis access arm, and arterial blood was continuously sampled from the arteriovenous fistula. Dialysate temperature was 36.5°C. Linear mixed models were used to study global and regional CBF change during hemodialysis.

Results
Mean (±SD) arterial blood pressure declined from 101±11 before hemodialysis to 93±17mmHg at the end of hemodialysis. Global CBF declined significantly by 10±15% from a mean of 34.5 to 30.5 mL/100g/min (difference -4.1 mL/100g/min [-7.3 to -0.9] P=0.03) at the end of hemodialysis. CBF decline (-20%) was symptomatic in one patient. Regional CBF declined in all volumes of interest: The frontal, parietal, temporal, and occipital lobes, cerebellum and thalamus. Higher tympanic temperature, ultrafiltration volume and rate, and pH were significantly associated with lower CBF.

Conclusion
Conventional hemodialysis induces a significant reduction in global and regional CBF in elderly patients. Repetitive intradialytic decreases in CBF may be one of the mechanisms by which hemodialysis induces cerebral ischemic injury.
TRENDS IN MORTALITY DUE TO MYOCARDIAL INFARCTION AND STROKE IN DIALYSIS PATIENTS

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Background
In the last decades, important improvements have been made in the prevention and treatment of atherosclerotic disease (myocardial infarction and stroke). However, it is unknown whether mortality rates of myocardial infarction and stroke in dialysis patients as compared with the general population have improved with time. The aim of this study was to assess the mortality rates due to myocardial infarction and stroke in a large European cohort of dialysis patients as compared with the general population for three times periods.

Methods
We included incident dialysis patients in 11 European countries providing data to the ERA-EDTA Registry who started dialysis between 1994 and 2011 and followed them for three years. The causes of death in dialysis patients were compared with the causes of death in the general population in the same time period. Mortality due to myocardial infarction and stroke were classified according to the coding system of the ERA-EDTA in dialysis patients and to ICD-codes in the general population. We calculated age- and sex-standardized mortality rate ratios (SMRs) with 95% confidence intervals (CIs) by dividing mortality rates in dialysis patients by mortality rates in the general population for three time periods (1994-1999, 2000-2005 and 2006-2011).

Results
Of the 201,918 dialysis patients, 79,327 patients died during follow-up of whom 8,181 (10.3%) died due to myocardial infarction and 5,204 (6.6%) due to stroke. In the general population, 19,058,469 persons died during follow-up of whom 1,400,833 (7.4%) died due to myocardial infarction and 1,170,222 (6.1%) due to stroke.

Compared with the general population, the SMRs of myocardial infarction for dialysis patients were 11.4 (95% CI 10.8-12.0) (25.9 per 1,000 person-years [py] among dialysis patients versus 1.2 per 1,000 py in the general population) between 1994 and 1999, 12.1 (95% CI 11.5-12.8) (22.4 per 1,000 py among dialysis patients versus 0.9 per 1,000 py in the general population) between 2000 and 2005 and 14.0 (95% CI 13.2-14.8) (19.9 per 1,000 py among dialysis patients versus 0.7 per 1,000 py in the general population) between 2006 and 2011.

The SMRs for stroke were 11.0 (95% CI 10.3-11.9) (15.2 per 1,000 py among dialysis patients versus 0.9 per 1,000 py in the general population) between 1994 and 1999, 11.7 (95% CI 10.8-12.6) (14.5 per 1,000 py among dialysis patients versus 0.7 per 1,000 py in the general population) between 2000 and 2005 and 12.4 (95% CI 11.5-13.3) (13.4 per 1,000 py among dialysis patients versus 0.6 per 1,000 py in the general population) between 2006 and 2011.

Conclusion
Mortality rates for myocardial infarction and stroke were higher in dialysis patients than in the general population and SMRs increased with time. Dialysis patients seem to benefit less of improvements made in the prevention and treatment of myocardial infarction and stroke than non-dialysis patients which may be related to the severity of the atherosclerotic disease.
PERFORMANCE OF STROKE RISK SCORES IN DIALYSIS PATIENTS

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Background
Dialysis patients have an increased ischemic stroke risk. Stroke risk scores including the CHADS2, ATRIA and CHA2DS2VASC have been developed to identify patients with an increased stroke risk in patients with atrial fibrillation allowing for personalization of vitamin K antagonist prescription. In the original articles, CHADS2 (C-statistic 0.82), ATRIA (C-statistic 0.73) and CHA2DS2VASC (C-statistic 0.67) had reasonable predictive abilities. However, the predictive performances of these stroke risk scores have not been validated in dialysis patients. Therefore, the aim of this study was to validate existing stroke risk scores in dialysis patients.

Methods
A total of 755 incident dialysis patients from the NECOSAD study were prospectively followed for ischemic stroke within five years of dialysis. Hazard ratios with 95% confidence intervals (CIs) were calculated using Cox proportional hazards analyses for high and intermediate risk scores as compared with low risk scores for the CHADS2 (low risk= 0, intermediate risk=1-2 and high risk= ≥3) and ATRIA (low risk= 0-5, intermediate risk=6 and high risk= ≥7) and high as compared with intermediate risk score (no events in low risk score) for the CHA2DS2VASC (low risk= 0, intermediate risk=1 and high risk= ≥2). Furthermore, we evaluated the discriminative performance of these bleeding risk groups by calculating Harrell’s C-statistics.

Results
During a median follow-up of 2.0 years (interquartile range 0.9-3.7), 58 first ischemic stroke events occurred. Of the 755 patients, 14% were classified as high risk by the CHADS2, 28% by the ATRIA and 72% by the CHA2DS2VASC. A high risk score was associated with a 8.2-fold (95% CI 1.1-63.1) increased ischemic stroke risk for the CHADS2, a 2.2-fold (95% CI 1.3-3.9) increased risk for the ATRIA and an 8.7-fold (2.1-35.7) increased risk for the CHA2DS2VASC risk score (Table 1). The C-statistics were 0.59 for the CHADS2, 0.61 for the ATRIA and 0.62 for the CHA2DS2VASC.

Conclusion
In this prospective cohort with validated data on ischemic stroke, we showed that high stroke risk scores were associated with an increased ischemic stroke risk. However, there were large differences between the different stroke risk scores in the identification of high risk patients. Furthermore, we showed that the CHADS2, ATRIA and CHA2DS2VASC had poor predictive abilities. Therefore, these stroke risk scores may not be useful for guiding individual decision-making in dialysis patients.

Table 1. Stroke risk scores and stroke risk

<table>
<thead>
<tr>
<th>Stroke risk score</th>
<th>N</th>
<th>Incidence rate per 1000 py</th>
<th>HR</th>
<th>(95% CI)</th>
<th>C-statistic</th>
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<tbody>
<tr>
<td><strong>CHADS2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Low</td>
<td>73</td>
<td>7.0</td>
<td>1</td>
<td>(ref)</td>
<td>0.59</td>
</tr>
<tr>
<td>Intermediate</td>
<td>573</td>
<td>32.8</td>
<td>4.8</td>
<td>(0.7-34.8)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>109</td>
<td>56.9</td>
<td>8.2</td>
<td>(1.1-63.1)</td>
<td></td>
</tr>
<tr>
<td><strong>ATRIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>461</td>
<td>23.7</td>
<td>1</td>
<td>(ref)</td>
<td>0.61</td>
</tr>
<tr>
<td>Intermediate</td>
<td>83</td>
<td>45.0</td>
<td>1.9</td>
<td>(0.9-4.0)</td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>211</td>
<td>53.6</td>
<td>2.2</td>
<td>(1.3-3.9)</td>
<td></td>
</tr>
<tr>
<td><strong>CHA2DS2VASC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>32</td>
<td>0.0</td>
<td>-</td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>Intermediate</td>
<td>178</td>
<td>5.1</td>
<td>1</td>
<td>(ref)</td>
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</tr>
<tr>
<td>High</td>
<td>545</td>
<td>44.1</td>
<td>8.7</td>
<td>(2.1-35.7)</td>
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</tbody>
</table>
CITRATE-ACIDIFIED DIALYSATE IMPROVES THE CALCIFICATION PROPENSITY OF HEMODIALYSIS PATIENTS: A MULTICENTER PROSPECTIVE RANDOMIZED CONTROLLED CROSS-OVER TRIAL

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Background
The prescription of dialysate calcium concentration (dCa) has been suggested to affect vascular calcification, but evidence is scarce. The use of citrate-acidified dialysate (dCit) may have a beneficial effect on the calcification tendency. The aim of this study was to compare the intradialytic and short-term effects of dCa of 1.50 mmol/l (dCa1.50) and dCa of 1.25 mmol/l (dCa2.25), as well dCit (with a dCa of 1.50 mmol/l) on calcification propensity in serum.

Methods
Nineteen chronic hemodialysis patients (mean age 66.6 years) from two centers were randomized into a cross-over sequence of dCa1.25 or dCit for one week, the alternate treatment was provided after a washout week with dCa1.50. Calcification propensity of serum was assessed by time-resolved nephelometry where the T50 reflects the transition time between formation of primary and secondary calciprotein particles.

Results
Intradialytic change (Δ) in T50 is increased in dCit (119.6±35.2min) compared to dCa1.25 (76.6±40.9min, p<0.001) and dCa1.50 (62.8±43.9min, p<0.001). ΔT50 was inversely correlated to Δphosphate in all treatments (dCa1.50: r=-0.68, p<0.01; dCa1.25: r=-0.69, p<0.01; dCit: r=-0.46, p=0.06), and to Δionized calcium in dCa1.25 (r=-0.61, p=0.01), dCit (r=-0.46, p=0.05) and dCa1.50: r=-0.45, p=0.06). During the treatment week, predialysis T50 increased significantly for dCit (278.5±50.5 to 295.8±55.2min, p=0.001) with a tendency for dCa1.25 (287.6±58.5 to 303.4±57.9min, p=0.07), but not for dCa 1.50 (287.0±65.8 to 297.6±54.9min, p=0.47).

Conclusion
Calcification propensity, as measured by the change in T50, improved significantly during treatment in dCit compared to dCa1.25 and dCa1.50, with effects potentially lasting beyond the dialysis treatment. Changes in ionized calcium during dialysis may also effect calcification propensity. Long term studies are needed to investigate whether dCit and modifying dCa could have a beneficial effect on patient prognosis and vascular calcification.
EXCESSIVE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS HAVE A DIFFERENT ROLE IN THE PATHOGENESIS OF ANCA-ASSOCIATED VASCUITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Renal involvement in ANCA-associated vasculitis (AAV) and systemic lupus erythematosus (SLE) manifests as autoimmune-mediated glomerulonephritis (AIGN) with respectively crescentic lesions and a pauci-immune immunofluorescence versus endo- and extracapillary proliferative lesions and a full-house immunofluorescence. Although these are clinically divergent autoimmune diseases, neutrophil extracellular traps (NETs) are thought to be involved in their pathogenesis. NETs are immunogenic, extracellular DNA structures harbouring relevant ANCA- and nuclear auto-antigens. However, it is still unclear how and if NETs can act as a common pathway for both AAV and SLE. To increase our understanding of the potential pathogenic role of NETs in AAV and SLE, the aim of the present study was to compare the characteristics of AAV- and SLE-induced NET formation.

Methods: Ex vivo NET formation was quantified by a novel, highly-sensitive NET quantification assay using 3D-confocal microscopy (Kraaij et al. 2016) in 82 AAV patients, 56 SLE patients and 10 healthy controls (HC). Live cell imaging visualized the morphology and kinetics of NET formation. Qualitative characteristics were investigated by immunofluorescence microscopy that detected co-localisation of NET-markers, and the presence of IgG, IgM or IgA autoantibodies. Autoantibodies as trigger of NET formation were investigated by depleting serum from IgG and NET inhibition assays were performed using (peptidylarginine deiminase-4) PAD4 and NADPH inhibitors.

Results: Quantifying ex vivo NET formation demonstrated excessive NET formation in both AAV and SLE compared to HC. AAV-induced NET formation was significantly higher compared to SLE-induced NET formation. Secondly, live cell imaging revealed lytic NET formation in AAV peaking after 2-4 hours while in SLE non-lytic NET formation with neutrophil clustering occurred within minutes. Thirdly, the presence of citrullinated histon-3 (CitrH3) was significantly higher on AAV-induced NETs, whereas SLE-induced NETs contained significantly more high mobility group box protein-1 (HMGB1). AAV-NETs were triggered independent of IgG, in contrast to IgG dependence in SLE NETs. Intriguingly, immunofluorescence staining of immunoglobulins revealed a pauci-immune expression on AAV-NETs compared to a full-house expression of IgG, IgM and IgA on SLE NETs. Both PAD4 and NADPH were involved in AAV- but not in SLE-induced NET formation. Lastly, we found that SLE NETs were enriched for oxidized mitochondrial DNA as demonstrated by TOMM20 and MitoSOX.

Conclusions: This study demonstrates that excessive NET formation in AAV is intrinsically different to NET formation in SLE. AAV NETs are characterized by a suicidal lytic PAD4- and NADPH-dependent expulsion of citrullinated NETs, whereas SLE NETs are characterized by rapidly-induced clusters with HMGB1, enrichment for mitochondrial DNA and immune complex formation altogether supporting a pro-inflammatory role of NETs in the pathophysiology of immune-complex mediated, full-house lupus nephritis.
Background

Autosomal Dominant Polycystic Kidney Disease (ADPKD) affects 4 in 10,000 individuals, caused by PKD1 or PKD2 mutations, leads to thousands of kidney cysts and renal failure. Patients require lifelong treatment, precluding interventions with poor safety profiles. Tolvaptan, a vasopressin type II receptor antagonist that lowers intracellular cAMP levels, slows ADPKD progression in patients. However, side effects of Tolvaptan include massive diuresis. We hypothesize that combination treatment could lead to improved benefit/risk ratios by targeting complex ADPKD signaling from various angles simultaneously. We conducted a preclinical trial with adult onset PKD mice to study the effects of Tolvaptan treatment combined with Pioglitazone, a relatively well tolerated anti-diabetic drug that targets the peroxisome proliferator-activated gamma receptor (PPARY). Pioglitazone has been shown to slow disease progression in PKD rats, and is currently tested in a phase II clinical trial with ADPKD patients. If proven successful, Tolvaptan/Pioglitazone combination therapy could be implemented in clinical trials relatively easy.

Methods

In vitro: Tolvaptan and/or Pioglitazone were tested for efficacy to slow cyst growth in a 3D-organoid model for PKD.

In vivo: Tamoxifen inducible kidney specific Pkd1lox,lox mice (iKsp-Pkd1del) were used as an adult model for PKD. Gavage of Tamoxifen at post natal day 18,19 leads to adult onset PKD. Manually made foodpellets contained 0.1% Tolvaptan (Spray-dried formulation from Otsuka pharmaceuticals) and/or 0.01875% Pioglitazone. Plasma adiponectin levels, used as surrogate drug-marker for Pioglitazone, were measured by ELISA to establish clinically relevant dosages. Treatment started 3 weeks after Pkd1 inactivation and continued until the end of the experiment when 50% of the untreated mice had renal failure (based on blood/urea levels).

Results

Both Tolvaptan and Pioglitazone were effective at non-toxic dosages to slow cyst growth in vitro. In PKD mice, Tolvaptan improved renal survival (survival at end of experiment was 86% in the Tolvaptan group versus 41% in the untreated group, \( P<0.01 \)) and reduced kidney weight by 2-fold (\( P<0.001 \)). Pioglitazone did not slow PKD progression. The combination Pioglitazone/Tolvaptan was not more effective than Tolvaptan alone.

Conclusions

Tolvaptan effectively slowed cyst growth and improved renal survival, indicating the relevance of our mouse model to human ADPKD. However, although the previously reported cyst inhibiting properties of Pioglitazone in rats could be confirmed in vitro, Pioglitazone alone or in combination did not elicit additional therapeutic benefit. The ongoing clinical trial with Pioglitazone may determine if this treatment could be beneficial to ADPKD patients.
C4D DEPOSITIONS IN FSGS BEFORE THE DEVELOPMENT OF SEGMENTAL GLOMERULOSCLEROSIS

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Background: Immune deposits of complement components are occasionally seen in patients with FSGS. These deposits are non-diagnostic and are often considered as nonspecific entrapment in sclerotic lesions. However, deposits of IgM and C3 have also been observed in non-sclerotic glomeruli. Moreover, recent animal studies demonstrated a role for the complement system in the pathogenesis of FSGS. Here, we investigated the pattern of complement deposition in glomeruli of experimental and human FSGS, using the complement activation biomarker C4d.

Methods: Kidney sections of Munich Wistar Frömter (MWF) rats of 4 (no proteinuria), 8 (only proteinuria) and 24 (proteinuria with glomerulosclerosis) weeks of age were stained for C4d. Age-matched spontaneously hypertensive rats (SHR) with no proteinuria were used as controls. Also, we performed a C4d staining on 40 kidney biopsies of patients with FSGS and 46 control biopsies of patients with minimal change disease (MCD) who have proteinuria without segmental glomerulosclerosis. Prevalence and localisation of C4d deposition in glomeruli were investigated.

Results: The percentage of C4d positive glomeruli was significantly higher in MWF rats at 8 and 24 weeks of age compared to controls (p<0.001 and p<0.01 respectively). C4d deposits were also more frequently observed in rats of 4 weeks of age, yet not significant. At 24 weeks, 94% of sclerotic glomeruli were C4d positive, whereas 50% of C4d positive glomeruli showed segmental glomerulosclerosis. In human biopsies, glomerular C4d deposits were observed in 75% of FSGS and 35% of MCD cases (p<0.001). Of positive cases, 40% of glomeruli were positive in FSGS compared to 33% in MCD. In FSGS, C4d was co-localized with segmental sclerosis in 57% and was present in non-sclerotic glomeruli or non-sclerotic parts of sclerotic glomeruli in 45%.

Conclusion: Here, we show that in the MWF rat model for FSGS, C4d deposits are present before the development of glomerulosclerosis. Similarly, C4d deposits were present in non-sclerotic glomeruli of patients with FSGS and in patients with MCD who have proteinuria without segmental glomerulosclerosis. These results indicate that complement activation could be involved in the development of FSGS.
POLYCYSTIN-1 DYSFUNCTION IMPAIRS ELECTROLYTE HANDLING IN A RENAL PRE-CYSTIC MOUSE MODEL FOR ADPKD

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# Equally contributed to this study

Background

The Pkd1 gene encodes polycystin-1 (PC1), a mechanosensor for urinary flow triggering intracellular responses in kidney tubular cells. Mutations in Pkd1 lead to autosomal dominant polycystic kidney disease (ADPKD), a major cause of renal failure. In contrast to cystic ADPKD, renal electrolyte handling in pre-cystic ADPKD remains uncharacterized. Identification of electrolyte disturbances in pre-cystic ADPKD may be relevant for early symptom detection and may disclose a role for PC1 in renal electrolyte handling. In this study, we assessed the electrolyte balance in pre-cystic kidney-specific Pkd1 knockout mice.

Methods

A Pkd1 knockout (Pkd1<sup>−/−</sup>) was induced upon oral administration of tamoxifen to inducible kidney-specific Pkd1 knockout (iKsp-Pkd1<sup>lox/lox</sup>) mice at postnatal day 18 (PN18). The electrolyte content in 24-hour urine and serum samples was determined via inductively coupled plasma mass spectrometry at PN18 + 22 and PN18 + 29 days respectively. Gene expression in the kidney and intestine was analyzed via quantitative PCR. Immunohistochemistry was performed to determine the pre-cystic phenotype of the kidneys.

Results

Serum and urinary electrolyte determinations indicated that Pkd1<sup>−/−</sup> mice suffer from reduced serum concentrations of magnesium, calcium, sodium and phosphate with renal magnesium, calcium and phosphate wasting. In agreement with the electrolyte disturbances, downregulation of key genes for electrolyte reabsorption in the thick ascending limb of Henle's loop (TAL, i.e. Cldn16, Kcnj1 and Slc12a1), distal convoluted tubule (DCT, i.e. Trpm6 and Slc12a3) and connecting tubule (CNT, i.e. Calb1, Slc8a1 and Atp2b4) was observed. Similarly, decreased renal gene expression of markers for TAL (i.e. Umod) and DCT (i.e. Pvalb) was observed. Conversely, no changes in genes encoding solute and water transporters in the proximal tubule (i.e. Abcg2 and Slc34a1) and collecting duct (i.e. Aqp2, Scnn1a and Scnn1b) were observed. Furthermore in the intestine, upregulation of duodenal Trpv6, involved in calcium handling, was observed.

Conclusion

Our data illustrates that PC1 dysfunction impairs renal electrolyte handling by TAL, DCT and CNT in pre-cystic Pkd1<sup>−/−</sup> kidneys, resulting in a systemic electrolyte imbalance characterized by low serum electrolyte concentrations.
DERMAL TISSUE REMODELING AND NON-OSMOTIC SODIUM STORAGE IN RENAL PATIENTS

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Background. In Western world, high dietary intake of sodium is associated with increased cardiovascular risk. Recent data indicate that excess dietary sodium is not only excreted by the kidneys, but also stored by non-osmotic binding with glycosaminoglycans in connective tissue of the skin, and is associated with dermal inflammation and lymphangiogenesis. We hypothesize that in renal patients dermal tissue remodeling is accompanied by increased storage of sodium.

Methods. Abdominal skin tissue of 12 end-stage renal disease patients (4 on dialysis) and 12 healthy kidney donors was obtained during renal transplant surgery. Skin biopsies were processed for dermal sodium measurement by atomic absorption spectroscopy, and evaluated for tissue remodeling markers (CD68+ macrophages, CD3+ T-cells, collagen I, podoplanin+ lymph vessels, and heparan sulfate glycosaminoglycans) by qRT-PCR and immunohistochemistry. Data were evaluated by Mann-Whitney-U test. Associations on healthy controls and non-dialysis renal patients (totally n=20) were done by Spearman Rank correlation analysis.

Results. Compared to controls, renal patients showed dermal tissue remodeling evidenced by increased CD68+ macrophages and CD3+ T-cells, increased levels of Collagen I alpha1 chain, and increased expression for podoplanin, both at mRNA and protein level. Also, both N- and O-sulfation of heparan sulfate glycosaminoglycans were increased (all dermal parameters: healthy vs renal patients: p<0.05). These skin remodeling events were most outspoken in renal patients on dialysis. Dermal sodium content of renal patients did not differ from healthy individuals, but was inversely associated with plasma sodium values (p<0.05), likely reflecting dietary sodium intake. Plasma and urinary sodium associates with dermal lymph vessel number (both p<0.03), whereas loss of GFR, proteinuria and systolic blood pressure associated with dermal macrophages density (all three p<0.02).

Conclusion. Our data indicate that renal failure associates with dermal inflammation, whereas sodium intake associates with dermal lymph vessel formation and loss of dermal sodium storage capacity.
CHANGES IN THE URINARY EXTRACELLULAR VESICLE PROTEOME ARE ASSOCIATED WITH NEPHRONOPHTHISIS-RELATED CILIOPATHIES

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**Background:** Nephronophthisis is one of the leading genetic causes of end-stage renal disease in childhood. Early diagnostics and prognostics for nephronophthisis are currently limited. Consequently, nephronophthisis is typically diagnosed in advanced stages of chronic kidney disease, when kidney damage is irreversible. Extracellular vesicles are secreted in urine by cells lining the kidney and urinary tract. These vesicles can be considered ‘liquid biopsies’ because their protein content reflects the physiological state of the cells of origin. We aimed to identify non-invasive protein biomarkers for nephronophthisis in urinary extracellular vesicles to improve early diagnostics and prognostics.

**Methods:** Extracellular vesicles were isolated from urine of 12 patients with a nephronophthisis-related ciliopathy and 12 age- and gender-matched controls, followed by label-free LC-MS/MS proteomics analysis for protein identification and quantification by spectral counting.

**Results:** Supervised cluster analysis of proteomic profiles separated patients from controls (Figure 1). We identified 156 differentially expressed proteins with fold change ≥4 in patients compared to controls (P<0.05). Importantly, expression levels of discriminating proteins were correlated with chronic kidney disease stage, suggesting possible applications for urinary extracellular vesicle biomarkers in prognostics for nephronophthisis. Enrichment analysis of gene ontology terms revealed GO terms including signaling, actin cytoskeleton and endocytosis among the downregulated proteins in patients, whereas terms related to response to wounding and extracellular matrix organization were enriched among upregulated proteins.

**Conclusion:** Proteomic profiles of urinary extracellular vesicles differentiate nephronophthisis-related ciliopathy patients from healthy controls. Further research is needed to determine specificity of the candidate biomarkers. Our findings represent the first step towards a non-invasive diagnostic test for nephronophthisis.
Figure 1. Supervised hierarchical clustering of normalized protein counts (P< 0.05) separates NPH-RC patients (red) from controls (black). Three separate clusters (marked 1, 2 and 3) can be discerned. Blue represents downregulated proteins and orange represents upregulated proteins. NPH: isolated nephronophthisis; BBS: Bardet-Biedl syndrome; CED: cranioectodermal dysplasia (Sensenbrenner syndrome); JBTS: Joubert syndrome.
THE EQUAL COHORT STUDY: KIDNEY FUNCTION AND SYMPTOM TRAJECTORY OVER TIME IN PREDIALYSIS ADVANCED CKD PATIENTS

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Background The limited value of kidney function alone in defining the optimal moment to start renal replacement therapy (RRT) is a major concern. Initiation of RRT often is the result of symptoms related to chronic kidney disease (CKD) progression, in combination with the kidney function. However, the timing of symptom onset is difficult to predict. Therefore, we aimed to study the association between kidney function decline and development of disease-related symptoms in advanced CKD patients during predialysis.

Methods Patients with a kidney function ≤ 20 mL/min/1.73m², based on the MDRD equation, and of ≥ 65 years were followed for one year during predialysis in the European Quality (EQUAL) study. Clinical and laboratory data were assessed each 6 months. Linear regression on individual slopes was used to assess the association between kidney function decline and the development of disease-related symptoms. These symptoms were assessed by number and severity using the Dialysis Symptom Index.

Results 591 patients had at least two kidney function estimates and symptom number. A subset of 535 patients had at least two kidney function estimates and symptom severity. At cohort entry, mean (standard deviation) kidney function was 19.5 (5.2) mL/min/1.73m², number of symptoms was 12.3 (6.3) and symptom severity was 34.7 (20.5). The mean (95% confidence interval [CI]) kidney function decline was 1.42 (0.93; 1.90) mL/min/1.73m² per year. The mean (95% CI) overall increase in the number of symptoms was 0.63 (-0.03; 1.28) per year. The additional increase in annual number of symptoms was 0.09 (95% CI: -0.02; 0.20) per additional mL/min/1.73m² decrease in kidney function during predialysis. The overall increase in symptom severity was 2.70 (95% CI: 0.64; 4.75) per year. The additional increase in annual symptom severity score was 0.37 (95% CI: 0.04; 0.70) per additional mL/min/1.73m² decrease in kidney function. This means that the faster the kidney function decline, the steeper the increase in number and severity of symptoms. There was no cross-sectional association in level of kidney function and symptoms.

Conclusion Number and severity of symptoms increased over time during predialysis. In addition, though there is no cross-sectional association in level of kidney function and symptoms, the changes over time are correlated: faster kidney function decline is associated with a steeper increase in both number and severity of symptoms over time during predialysis.

![Figure 1. Trajectories of kidney function and either number or severity of symptoms over time during predialysis in advanced CKD patients.](image)

During predialysis, the steeper the increase in number and severity of symptoms. A subset of 535 patients had at least two kidney function estimates and symptom severity.
URINARY TIMP-2 AND IGFBP7 IN ACUTE AND CHRONIC KIDNEY DYSFUNCTION

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Background. Given the limitations of serum creatinine as a marker of renal injury or repair, interest has grown in alternative biomarkers in both acute and chronic kidney dysfunction. Urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) have been proposed to detect acute kidney injury. We investigated TIMP-2 and IGFBP7 as potential biomarkers for the duration of functional delayed graft function (fDGF) in donation after circulatory death (DCD) kidney transplant recipients and in patients with chronic tubular pathology such as autosomal-dominant polycystic kidney disease (ADPKD).

Methods. TIMP-2 and IGFBP7 were measured by enzyme-linked immunosorbent assay in stored urine samples. In 76 DCD kidney transplant recipients, concentrations were measured in samples obtained from day 0-10 after transplantation and at week 6 and month 6. One sample per participant was analysed in 296 patients with ADPKD and 71 healthy controls. Renal function was estimated using the CKD-EPI equation.

Results. Elevated urinary TIMP-2 adequately identified patients with fDGF after transplantation (ROC AUC 0.89, 95% CI 0.78-0.99), whereas IGFBP7 did not (AUC 0.63). The AUC was even higher after correcting TIMP-2 for urine osmolality (AUC 0.91). Furthermore, high TIMP-2 levels at day 10 predicted prolonged duration of fDGF (AUC 0.77). A decrease in TIMP-2 preceded the resolution of fDGF. TIMP-2 and IGFBP7 did not differ between patients with ADPKD and healthy controls.

Conclusion. Urinary TIMP-2, but not IGFBP7, is a promising biomarker to monitor the resolution of ischemic-reperfusion injury in DCD kidney transplant recipients. These biomarkers are not useful for risk stratification in chronic renal injury, such as ADPKD.
SKIN AUTOFLUORESCENCE LEVELS AND BIOMARKERS OF ENDOTHELIAL DYSFUNCTION AND LOW-GRADE INFLAMMATION ARE HIGHER IN END-STAGE RENAL DISEASE, BUT ARE NOT CONSISTENTLY AFFECTED BY DIALYSIS INITIATION

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Abstract

Background: Patients with end-stage renal disease are at risk of cardiovascular disease related morbidity and mortality. Advanced glycation end products (AGEs) contribute to endothelial dysfunction (ED) and low-grade inflammation, which are associated with cardiovascular disease (CVD). However, the effect of starting dialysis on these parameters, or the difference between stage 5 chronic kidney disease (CKD5) non-dialysis vs. dialysis patients has not been studied in great detail.

Methods: Skin AGEs were assessed by measuring skin autofluorescence (SAF) with the AGE Reader CÙ™. Large panels of biomarkers reflecting ED and low-grade inflammation involved in the progression of vascular disease were measured by single or multiplex assays (Meso Scale Discovery). Thirty-two CKD5 non-dialysis patients, 24 dialysis patients, and 20 age-matched healthy controls were included in a cross-sectional analysis. For 29 of these CKD5 non-dialysis patients 6 month follow-up data for SAF and biomarkers were available after the start of dialysis. After 12 months SAF data was available for 25 patients. For the cross-sectional analyses, standardized composite scores were calculated for ED (sVCAM-1, E-selectin, thrombomodulin and sICAM-1) and low-grade inflammation (hs-CRP, serum amyloid A (SAA), IL-6, IL-8, TNF-α and sICAM-1). The longitudinal change in the biomarkers could only be evaluated at the level of individual biomarkers. Reported associations were evaluated with linear regression analyses and adjusted for age, sex and diabetes.

Results: As compared with controls, SAF was 0.77 (95%CI 0.42; 1.12) arbitrary units (AU) higher in CKD5 non-dialysis patients and 1.19 (0.77; 1.60) AU higher in CKD5 dialysis patients in cross-sectional analyses. CKD5 non-dialysis and dialysis patients had higher composite scores for ED (betas 1.62 (1.23; 2.02) standard deviation (SD) and 1.73 (1.26; 2.20) SD, respectively) and low-grade inflammation (betas 1.37 (0.95; 1.80 SD) and 1.36 (0.84; 1.86) SD, respectively). In CKD5 non-dialysis patients with follow-up data SAF was similar 6 months after dialysis initiation (n=29), and 12 months after dialysis initiation (n=25). Further, as compared with predialysis levels, sVCAM-1 (ratio 1.07 (95%CI 1.02; 1.13)), thrombomodulin (ratio 1.09 (1.02; 1.17)) and TNF-α (absolute difference 1.68 (-0.30; 3.67)) increased, but hs-CRP (ratio 0.72 (0.50; 1.03)) and SAA (ratio 0.64 (0.41; 0.99)) decreased in the first 6 months after dialysis initiation.

Conclusion: SAF and levels of biomarkers of ED and low-grade inflammation are higher in CKD5 non-dialysis and dialysis patients as compared with healthy controls. SAF did not significantly change during the first year after dialysis initiation, whereas the biomarkers of ED and low-grade inflammation followed
no consistent pattern of change in the first 6 months after dialysis initiation. These findings may suggest that these risk factors for CVD for a large part already develop in earlier stages of CKD.
URINARY BIOMARKERS PREDICT THE RATE OF RENAL FUNCTION LOSS IN ADPKD

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Background: Markers currently used to predict the likelihood of rapid disease progression in patients with autosomal dominant polycystic kidney disease (ADPKD) are limited in sensitivity, expensive or time consuming to assess. New and easy to measure markers are therefore needed that alone or in combination with conventional risk markers can predict the rate of disease progression. We previously showed in a small group of ADPKD patients that urinary excretion of tubular damage and inflammation markers holds promise in this respect. In the present study we investigated the predictive ability of these markers in an independent cohort of ADPKD patients.

Methods: At baseline albumin, IgG, KIM-1, β2MG, H-FABP, NGAL and MCP-1 were measured in 24-hr urine samples of patients participating in the open-label DIPAK-1 study. Kidney function was estimated (eGFR) using the CKD-EPI formula, and total kidney volume was measured on MRIs with the manual tracing method and adjusted for height (htTKV). Change in eGFR and htTKV during follow-up was calculated using mixed modeling taking into account 12 eGFR and 3 htTKV values. Urinary biomarkers were studied as continuous variable using linear regression or as dichotomized variable with optimal cut-offs based on ROC curves using logistic regression.

Results: Included were 302 patients of whom 53.3% were female, with an average age of 48±7 years, eGFR of 52±12 ml/min/1.73m² and a htTKV of 1083 (736-1669) ml/m. Cross-sectionally, all markers were associated with eGFR and htTKV in univariable analyses. For the longitudinal analyses only patients randomized to standard care were considered (n=150). Longitudinally, urinary excretion of KIM-1, H-FABP and MCP-1 were associated with eGFR slope after adjustment for conventional risk markers (st. β=-0.24 p=0.006, st. β=-0.26 p=0.004 and st. β=-0.21 p=0.02 respectively). Notably, none...
of the urinary markers was associated with hTKV slope. Including urinary KIM-1 as well as H-FABP excretion in a model containing conventional risk markers improved the explained variability of eGFR slope ($R^2=0.16$ vs. 0.25, $p=0.001$). When patients were classified as having fast or slow progressing disease (eGFR slope $\leq$ or $> -3.5$ml/min/1.73m$^2$) the predictive value (AUC) of urinary KIM-1 plus H-FABP with a cut-off value of 1.23 and 25.0 μg/24hr respectively was 0.67, $p<0.001$. Including urinary KIM-1 as well as H-FABP in a model containing conventional risk markers improved the prediction of fast progressing disease (AUC 0.72 vs. 0.78, $p=0.07$). The net reclassification improvement (NRI) was estimated at 15.7% ($p=0.04$).

**Conclusion:** Urinary KIM-1 and H-FABP excretion are associated with eGFR decline and both have ability beyond conventional risk markers to predict the rate of renal function loss in ADPKD. Urinary damage and inflammation markers can therefore be used to predict the rate of ADPKD disease progression and to select patients for disease modifying treatment.
Iron Deficiency, Erythropoietin, and Fibroblast Growth Factor 23 in the General Population
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Background. Elevated plasma levels of the phosphaturic hormone fibroblast growth factor 23 (FGF23) are known to be an independent risk factor for death in the general population. Hence, it is important to establish which factors regulate FGF23 levels. Recently, in experimental models and human studies with chronic kidney disease, it has been put forward that iron deficiency and erythropoietin (EPO) are prominently involved in FGF23 physiology. However, the interplay between iron status, EPO, and FGF23 in the general population has not been assessed.

Methods. Total plasma FGF23 levels were measured in plasma EDTA samples with a C-Terminal specific ELISA (Quidel) in the Prevention of Renal Vascular Endstage Disease (PREVEND) study. Serum EPO levels were measured on Immulite 2000 assay. Hepcidin was measured with ELISA. Soluble transferrin receptor was measured using Immunonephelometry. Blood samples were collected between 2001 and 2003. Statistical analyses were performed using univariable linear regression followed by multivariable linear regression.

Results. We included 6172 community-dwelling subjects (age 53±12 years; 49% males) with a median [IQR] FGF23 level of 70 (57-87) RU/ml. In univariable analysis, ferritin (β=-0.35, P<0.001), TSAT (β=-0.27, P<0.001), hepcidin (β=-0.35, P<0.001), and soluble transferrin receptor (β=0.39, P<0.001) were associated with FGF23 levels. Similarly, EPO was univariably associated with FGF23 levels (β=0.33, P<0.001). In multivariable linear regression, ferritin (β=-0.46, P<0.001), TSAT (β=-0.23, P<0.001), hepcidin (β=-0.38, P<0.001), soluble transferrin receptor (β=0.35, P<0.001), and EPO (β=0.27, P<0.001) remained major determinants of FGF23, independent of adjustment for potential confounders including age, sex, eGFR, serum phosphate, serum calcium, parathyroid hormone, C-reactive protein, and hemoglobin levels.

Conclusion. Iron deficiency and elevated EPO levels are major independent determinants of FGF23 levels in individuals in the general population. Since elevated FGF23 levels jeopardize the overall survival in the general population, particular attention to iron status and EPO levels seems warranted.
THE EFFECT OF B CELL TARGETED THERAPIES ON AUTOANTIBODIES AND EXCESSIVE NEUTROPHIL EXTRACELLULAR TRAP FORMATION IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Introduction

Systemic lupus erythematosus (SLE) is a severe systemic autoimmune disease characterized by immune-complexes which cause systemic inflammation and damage. Neutrophil extracellular traps (NETs) are an important source of autoantigens in SLE patients leading to the production of autoantibodies. Functionally, SLE-specific autoantibodies as immune-complex are important triggers of excessive NET formation. As such, effective targeting of pathogenic autoantibodies in SLE are subject to several promising experimental treatment strategies. Recently, the combination of Rituximab (RTX) and Belimumab (BLM) in severe SLE patients led to a strong decrease of autoantibodies and diminished excessive NET formation as well as improvement of clinical disease. A consortium was formed to study different experimental treatment strategies that target the humoral autoimmune system, including RTX, Bortezomib (BTZ) or combination of RTX and BLM. The present study aimed to investigate the effects of B cell targeted therapies on relevant autoantibody levels and excessive NET formation in severe SLE patients.

Methods

This study involved three cohorts of anti-dsDNA positive, severe SLE patients that were eligible to experimental treatment with RTX (n=16), BTZ (n=6) or RTX+BLM (n=16). A cross-sectional cohort of 35 anti-dsDNA+ SLE patients served as a control cohort. A panel of SLE relevant autoantibodies against dsDNA, histones, nucleosomes and C1q were measured by ELISA before and after treatment. As a functional result of autoantibody levels, NET formation was quantified by our novel highly-sensitive NET quantification assay using 3D confocal microscopy (Kraaij et al. 2016).

Results

Comparing three regimens, RTX+BLM resulted in the strongest reduction of anti-dsDNA (median ratio of baseline; 0.32 vs 0.78 vs 0.65; p=0.08), -histone (0.36 vs 0.51 vs 0.53; p=0.45), -nucleosome (0.38 vs 0.61 vs 0.58; p=0.15), and significantly the strongest reduction of -C1q antibodies (0.55 vs 0.91 vs 1.00; p=0.016) compared to RTX and BTZ. Excessive NET formation diminished significantly with a ratio of 0.66 [0.49 – 0.93] after RTX (p=0.005) and 0.25 [0.15 – 0.47] after RTX+BLM (p=0.0002), however was not reduced after BTZ with 1.37 [0.90 – 1.61]. As such, excessive NET formation correlated with disease activity (p=0.001), except for BTZ. Importantly, the regression of excessive NET formation was associated with reduction of anti-C1q antibodies. In an independent cohort we confirmed that the presence of anti-C1q antibodies correlated with excessive NET formation (p=0.03).

We further observed that the presence of three or more autoantibody specificities associated with excessive NET formation (p=0.02).

Conclusions

This study demonstrates a synergetic effect of RTX+BLM compared to RTX or BTZ on the reduction of relevant autoantibodies in SLE patients which associated with significant regression of NET
formation. The reducing effects of RTX+BLM, RTX and BTZ on anti-C1q antibodies underpinned the observed, immunological effects on humoral autoimmunity.
IMPACT OF ARTERIO-VENOUS FISTULA FLOW ON VENTRICULAR CONTRACTILITY IN HEMODIALYSIS PATIENTS – A CARDIAC MAGNETIC RESONANCE STUDY

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Abstract

Background: The arterio-venous fistula (AVF) in hemodialysis patients often leads to a substantial increase in cardiac output. The resulting high-output state can have detrimental effects in the long term. In this study the relation between AVF flow and ventricular contractility parameters was investigated using cardiac magnetic resonance imaging (CMR).

Methods: CMR was performed in 11 hemodialysis patients and 5 age-matched controls. CMR acquisitions were obtained prior to and after dialysis to differentiate between the effects of AVF flow and volume status (fluid overload). AVF flow, measured using ultrasonography, was used to subdivide the patients in Group 1 (low flow, <1000ml/min) and Group 2 (high flow, >1000ml/min). Short- and long-axis cine images were used for calculating global longitudinal strain (GLS), global circumferential strain (GCS) and global radial strain (GRS) with the tissue-tracking module of Circle Cardiovascular Imaging.

Results: There were no significant differences in the contractility parameters between the three groups prior to dialysis. Following dialysis, no significant changes in contractility were observed between Group 1 (n=5) and the control group (n=5). In comparison to the control group, patients in group 2 (n=6) had a significantly lower GLS (-14.2±2.3% vs. -20.4±3.3%, P<0.05), GCS (-13.5±1.6% vs. -22.3±2.1%, P<0.05) and GRS (23.3±4.7% vs. 45.0±8.4%, P<0.05) after the dialysis session.

Conclusion: These findings suggest that patients with high AVF flow are at an increased risk for developing ventricular dysfunction. Tissue-tracking analysis can be used to detect subtle early changes in contractility and could improve the diagnosis and prognosis of this patient group.

Keywords: x
THE ROLE OF RENAL REPLACEMENT THERAPY AND PHOSPHATE Binder USE ON VITAMIN K STATUS IN PATIENTS WITH END-STAGE RENAL DISEASE

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BACKGROUND: Cardiovascular disease is the leading cause of death in end-stage renal disease and is strongly associated with vascular calcification. Both kidney transplantation and phosphate binders aim to lower the risk of vascular calcification. Vascular calcification is actively inhibited by vitamin-K-dependent matrix γ-carboxyglutamic acid protein (MGP). Whether kidney transplantation or phosphate binders affect vitamin K status is currently unknown. Therefore, we studied the influence of kidney transplantation and phosphate binder prescription on vitamin K status in kidney transplant recipients and chronic dialysis patients.

METHODS: In this cross-sectional study of recent kidney transplant recipients (n=36), patients on chronic hemodialysis (n=82) and peritoneal dialysis (n=31), we measured plasma desphospho-uncarboxylated MGP (dp-ucMGP), a marker of vitamin K status, using a sandwich immunoassay. With medication inventory we assessed phosphate binder prescription. With linear regression, we assessed the influence of kidney transplantation and phosphate binder prescription on natural-log-transformed dp-ucMGP, adjusting for potential confounders. Kidney transplantation and no phosphate binders were considered reference groups.

RESULTS: Mean age was 51.9 ±13.4 years and 102 patients (68%) were male. Plasma dp-ucMGP in kidney transplant recipients was significantly lower compared to dialysis patients (regression coefficient -0.61, 95% confidence interval [CI] -0.84; -0.37; Figure 1).
Phosphate binders were prescribed to 89 chronic dialysis patients. Prescription of any phosphate binder was not associated with dp-ucMGP levels compared to no phosphate binders (regression coefficient 0.25, 95% CI -0.04; 0.53), while 26 patients were prescribed sevelamer monotherapy, which was associated with higher dp-ucMGP levels (regression coefficient 0.35, 95% CI 0.02; 0.68) after adjusting for age, sex and vitamin K antagonist use.

CONCLUSIONS: Recent kidney transplantation is associated with lower dp-ucMGP levels suggesting rapidly improved vitamin K status. Sevelamer monotherapy is associated with higher dp-ucMGP levels suggesting lowering of vitamin K status. This warrants attention to vitamin K status in dialysis patients, as vitamin K is necessary for protection against vascular calcification.
THE ROLE OF HEME IN THE PATHOPHYSIOLOGY OF SHIGA TOXIN-ASSOCIATED HEMOLYTIC UREMIC SYNDROME

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Background
The exact pathogenesis of an infection with Shiga toxin (Stx) producing E. coli (STEC) resulting in hemolytic uremic syndrome (HUS) is only partially understood. During extensive hemolysis, as present in patients with STEC-HUS, heme is released into the extracellular space. In the early stages of the disease, heme is directly scavenged by hemopexin and degraded by heme oxygenase 1 (HO-1) to prevent accumulation of heme and limit heme-mediated toxicity. However, during the progression to thrombotic microangiopathy (TMA), the scavengers are rapidly depleted, leading to accumulation of heme, which could ultimately contribute to an amplification of the cascade that leads to TMA. In this study, we measured levels of extracellular heme and hemopexin in a cohort of STEC-HUS patients. Furthermore, the effects of heme in combination with Stx2 on macro- and microvascular endothelium was assessed in vitro.

Methods
Circulating heme levels were quantified by spectrophotometric assay in plasma of 50 pediatric STEC-HUS patients admitted in the acute phase to Radboudumc between 1990-2016. Clinical, diagnostic and follow-up data were collected retrospectively. Plasma hemopexin levels were determined by a sandwich enzyme-linked immunosorbent assay (ELISA). Human umbilical vein endothelial cells, Human Microvascular Endothelial cells (HMECs), human blood outgrowth endothelial cells, and immortalized Glomerular Microvascular Endothelial Cells (iGMVECs) were exposed to heme, Stx2, or a combination of both, and HO-1 activity was assessed.

Results
Significantly elevated heme levels up to 26 µM were observed in STEC-HUS patients compared to the healthy controls (mean concentration of 1.58 µM; p<0.001). These elevated heme levels correlated with low hemopexin levels (R²=0.6037; p<0.0001). Endothelial cells internalized heme in vitro, resulting in oxidative stress and the induction of a pro-inflammatory response. Moreover, HO-1 was upregulated both on mRNA and protein level after exposure to heme. Interestingly, HMEC and iGMVECs showed a clear decrease in HO-1 after co-stimulation with Stx2.

Conclusion
Heme levels are elevated in the acute phase in STEC-HUS patients compared to controls. These elevated heme levels activate endothelial cells and cause endothelial injury in vitro. Moreover, Stx2 seems to attenuate HO-1 expression in HMEC and GMVECs, suggesting that this decrease in protection against heme plays a role in STEC-induced HUS. Future research has to provide further insight into heme-mediated toxicity in STEC-HUS.
CRISPR-MEDIATED CTNS KNOCKOUT PROXIMAL TUBULE EPITHELIAL CELLS OFFER A VERSATILE TOOL FOR STUDYING NEPHROPATHIC CYSTINOSIS

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Introduction
Nephropathic cystinosis is a rare, but severe genetic disorder that presents itself early in life and leads to progressive organs damage, particularly the kidneys. Cystinosis is caused by mutations in CTNS, a gene that transcribes the lysosomal transporter cystinosin, and results in lysosomal accumulation of cystine throughout the body. 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 investigate novel therapeutic strategies. Hence, our aim was to generate a cystinosis phenotype in human kidney cells using CRISPR/Cas9 system and study cystinosis pathology.

Methods
CRISPR/Cas9 was used to selectively knock-out the CTNS gene in conditionally immortalized proximal tubular epithelial cells (ciPTEC). A sensitive HPLC-MS/MS method was developed for the intracellular quantification of cystine. In addition, an untargeted metabolomics approach based on UHPLC-MS/MS was applied for the intra- and extracellular quantification of metabolites differentially expressed in knock-out cells as compared to controls. Fluorescence-based imaging assays were applied to monitor the autophagy regulator proteins, mTORC1 and transcription factor EB (TFEB), and autophagic flux in ciPTEC.

Results
Using CRISPR/Cas9 technology, heterozygous (CTNS+/−) and homozygous (CTNS−/−) isogenic cell lines of ciPTEC were generated. Consistent with a cystinotic phenotype, CTNS−/− but not CTNS+/− cells displayed a significantly high level of cystine as compared to control cells (6.32 ± 0.9 vs. 0.05 ± 0.02 nmol/mg protein; p<0.001). Upon treatment with cysteamine (100 μM), a cystine depleting agent, CTNS−/− cells showed a significant reduction in cystine levels (0.74 ± 0.05 nmol/mg protein; p<0.01). Using metabolomics, we identified that not only cystine but also over 25 metabolites and 9 metabolic pathways are affected (p<0.05) in cystinotic cells. Immunostaining revealed that mTORC1 was dislocated from lysosomes and inactivated in cystinotic cells, resulting in 2 and 2.5-fold increase (p<0.001) in TFEB nuclear translocation in CTNS−/− and CTNS+/− cells, respectively, when compared to control cells. This suggests an abnormal induction of autophagy in cystinosis, which was confirmed by the increased production of LC3, an autophagy end-product protein in both CTNS−/− and CTNS+/− cystinotic cells (p<0.001). Of note, cysteamine had no effect on the restoration of autophagy, which might explain its limited effect on treating renal Fanconi syndrome.

Conclusion
We successfully developed a new 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. Moreover, metabolomics approach allowed an unbiased analysis of the metabolites affected and provided a better understanding of the metabolic networks involved in cystinosis.
URINARY EXOSOMES AS A NOVEL TOOL TO STUDY THE SODIUM CHLORIDE COTRANSPORTER IN HYPERTENSION

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Background
The sodium chloride cotransporter (NCC) is located at the apical membrane of epithelial cells lining the distal convoluted tubule of the kidney, and plays an instrumental role in blood pressure regulation by fine-tuning renal sodium excretion. Renal salt transporters such as NCC are excreted in urinary exosomes after internalization into multivesicular bodies. The aims of this study were to investigate the effect of thiazides on the abundance of NCC in urinary exosomes in patients with essential hypertension, and to assess whether NCC abundance in exosomes can predict the blood pressure response to thiazides.

Methods
The abundance of NCC was determined in exosomes isolated from urine of patients with essential hypertension (n=23) before and after thiazide treatment (Group 1). In Group 2, NCC abundance was compared in urinary exosomes both before and after treatment in hypertensive kidney transplant recipients who did or did not respond to thiazides. Responders to treatment were defined as patients with a significant anti-hypertensive response to thiazides (≥5 mmHg, n=10), while non-responders had a minimal or no response (<5 mmHg in blood pressure, n=8). In Groups 1 and 2, urinary exosomes of each subject were loaded according to the urine creatinine concentration onto the gel. To analyze whether normalization by urinary creatinine resulted in a similar number of exosomes loaded on a gel, the abundance of the exosomal-marker CD9 was measured.

Results
Despite the inhibitory action of thiazide on NCC, immunoblot analysis of exosomes showed increased abundance of NCC in Group 1 (>2.5-fold, P<0.05). The increase in NCC abundance in urinary exosomes after thiazide treatment correlated with the blood pressure response and change in plasma potassium levels (R²=0.22, P<0.05; R²=0.19, P<0.05, respectively). In Group 2, abundance of NCC in urinary exosomes before treatment was significantly higher in responders compared to non-responders (>6-fold, P<0.05). Moreover, after thiazide treatment, the increase in abundance of NCC in urinary exosomes and decrease in plasma potassium levels was stronger in responders compared to non-responders (P<0.05). In both groups, no significant differences in CD9 abundance were observed between the two experimental groups, suggesting comparable urinary exosomal numbers.

Conclusion
Our studies highlight that NCC is upregulated by thiazides and this increase correlates with the blood pressure response to thiazides and the change in plasma potassium levels. Additionally, we show that higher abundance of NCC prior to treatment with thiazides predicts the blood pressure response to thiazides. This implies that assessment of NCC in urinary exosomes could represent novel method to guide anti-hypertensive therapy in hypertensive patients.
A MINIATURE ARTIFICIAL KIDNEY FOR PERITONEAL DIALYSIS – WEAKID

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BACKGROUND
We have developed a miniature artificial kidney (WEAKID – H2020 SC1) for peritoneal dialysis (PD), that recirculates the peritoneal dialysate via a tidal mode using a single lumen peritoneal catheter. The dialysate is continuously regenerated by the system containing sorbents, maintaining a large plasma–dialysate concentration gradient, thereby enhancing blood purification while reducing the number of exchanges. Application is envisaged at night as a bedside device (nighttime system). Optionally, a wearable system (~1.5 kg) can provide additional clearance during the day. The removal of potassium, phosphate, urea and creatinine from spent peritoneal dialysate was studied.

METHODS
The night- and daytime WEAKID systems were tested separately (n=3 per system). In each system, 2 L of spent peritoneal dialysate was continuously recirculated via a tidal mode (mean flow rate: 100 and 50 ml/min, respectively) in a closed-loop system over a sorbent cartridge for 8 h. To simulate the in vivo situation, potassium, phosphate, urea and creatinine were added to the peritoneal effluent each hour. Samples were taken hourly. Cumulative removal was quantified during the 8 h experiment. Modeling was used to estimate cumulative removal during 24 h and time averaged plasma clearances applying one Extraneal exchange per day (Table 1). Both systems were equilibrated at physiological calcium and magnesium concentrations, hypotonic sodium concentration, and lactate concentration of 35 mM.

RESULTS
Cumulative removal of potassium, phosphate, urea and creatinine by the nighttime system was 26±5, 11±2.7, 206±29 and 10±2.4 mmol in 8 h, and by the daytime system 0±0, 6.5±1.7, 35±4.4, 5.3±0.5 mmol in 8 h, respectively, at peritoneal dialysate [K+] of 3.7±0.5 mM, [PO₄³⁻] 1.3±0.5 mM, [urea] 21±5.1 mM and [creatinine] 0.6±0.2 mM (Table 1). Equilibration of the night- and daytime system could prevent calcium and magnesium removal and sodium release. Cumulative glucose release by the nighttime system was 79±23 mmol in 8 h. Cumulative lactate release with the night- and daytime system was 54±3.9 and 33±15 mmol, respectively.
Table 1. Daily cumulative removal and plasma clearance based on modeling

<table>
<thead>
<tr>
<th></th>
<th>Nighttime (8 h)*</th>
<th>Nighttime (8 h) + Daytime (8 h)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cumulative removal (mmol/day)</td>
<td>Clearance* (ml/min)</td>
</tr>
<tr>
<td>Potassium</td>
<td>41±5.3</td>
<td>7.8±1.8</td>
</tr>
<tr>
<td>Phosphate</td>
<td>11±2.2</td>
<td>7.0±3.3</td>
</tr>
<tr>
<td>Urea</td>
<td>271±39</td>
<td>9.2±1.3</td>
</tr>
<tr>
<td>Creatinine</td>
<td>11±2.4</td>
<td>14±6.6</td>
</tr>
</tbody>
</table>

*Assuming 1 exchange per day (Extraneal) and an ultrafiltration volume of 1.5 L/day.
**Time averaged clearance per 24 h.

CONCLUSIONS
Clinically relevant removal of potassium, phosphate, urea and creatinine from peritoneal dialysate by a miniature artificial kidney for peritoneal dialysis, was shown in vitro. Time-averaged plasma clearances of phosphate, urea and creatinine based on modeling suggest superior performance compared to conventional PD. The evaluation of the WEAKID system in a uremic large animal model is warranted to study uremic toxin removal, glucose release, and influence on electrolyte and acid-base balance in vivo.
THE CALCIUM-DEPENDENT PROTEASE CALPAIN-1 LINKS TRPC6 ACTIVITY TO PODOCYTE INJURY

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Background
The hallmark of podocytopathies like Focal Segmental Glomerular Sclerosis (FSGS) is podocyte injury resulting in proteinuria. Transient Receptor Potential channel C6 (TRPC6) is a calcium-conducting ion channel expressed at the slit diaphragm. TRPC6 gain-of-function mutations and glomerular TRPC6 overexpression are associated with proteinuria. The pathway(s) linking TRPC6 to podocyte injury, characterized by loss of the slit diaphragm protein nephrin, activation of several intracellular pathways including calcineurin-NFAT signaling, and cytoskeletal rearrangement, remain elusive. We hypothesized that the calcium-dependent protease calpain-1 mediates TRPC6-dependent podocyte injury.

Methods
Activity, expression and localization of calpain, expression of TRPC6 and the podocyte cytoskeletal protein and calpain substrate Talin-1, and calcineurin activity were determined in kidneys of human FSGS patients. Healthy rats were injected with adriamycin, as a model for human FSGS in which TRPC6 expression is known to be increased, and were treated with a calpain inhibitor. Urine, blood and kidneys were harvested, urinary as well as cortical calpain activity was assessed, and in situ zymography was used to localize calpain. Furthermore, glomerular calpain-1, TRPC6, nephrin and Talin-1 expression were determined.

Immortalized mouse podocytes were transfected with either scrambled or inducible TRPC6 and calpain-1 knockdown constructs. Podocytes were injured using adriamycin or treated with the TRP channel activator 1-oleoyl-acetyl-sn-glycerol (OAG), the calpain inhibitor calpeptin or the TRPC channel blocker 2-APB, and TRPC6-dependent calcium influx was measured. Calpain activity and Talin-1 expression were determined.

Results
In kidneys of FSGS patients we demonstrated increased TRPC6 expression, increased calpain and calcineurin activity and reduced expression of the calpain target Talin-1, which links the actin cytoskeleton to integrins and is critical for podocyte cytoskeletal stability. In the adriamycin model for human FSGS, increased glomerular and urinary calpain activity was associated with reduced Talin-1 abundance, enhanced calcineurin activity and increased proteinuria. Treatment with the calpain inhibitor calpeptin prevented the above effects. TRPC6-dependent calcium influx in cultured podocytes increased calpain-1 activity, which resulted in loss of Talin-1 and activation of calcineurin. Calpain activation, Talin-1 loss and calcineurin activation were inhibited by silencing TRPC6 and knockdown of calpain-1.

Conclusion
We elucidated a novel mechanism that links TRPC6 activity to calpain-1 activation and, through e.g. Talin-1 loss and calcineurin activation, ultimately to the podocyte injury characterizing FSGS. Therefore, calpain-1 and/or TRPC6 inhibition could be future therapeutic options to treat patients with FSGS or other podocytopathies.
IMMUNOLOGICAL TRAINING OF ENDOTHELIAL CELLS IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies against nuclear components. The accumulation of apoptotic material in combination with autoantibodies may lead to activation of immune cells and tissue damage, especially in the kidney. Patients with SLE suffer from recurrent periods of high disease activity termed flares, which are characterized by increased inflammatory processes and activated endothelial cells. Apoptotic microparticles (MPs) and neutrophil extracellular traps (NETs) are endothelial stimuli, which are abundantly present during disease flares. We hypothesize that MPs and NETs can ‘train’ endothelial cells during periods of flares leading to hyper reactive endothelial cells with increased responses to activating stimuli.

We hypothesize that MPs and NETs can ‘train’ endothelial cells during periods of flares leading to hyper reactive endothelial cells with increased responses to activating stimuli. Training of innate immune cells has recently been shown to increase the immune response in a process termed ‘trained innate immunity’. ‘Trained innate immunity’ is mediated by epigenetic changes involving specific histone marks that regulate immune response genes. In this study we aim to investigate the training capacity of endothelial cells in the context of SLE.

Methods: Human umbilical vein endothelial cells (HUVECs) were treated with MPs and NETs for 24 hours followed by removal of stimuli and a resting phase for 3 days. Subsequently, HUVECs were restimulated with the same or an unrelated inflammatory stimulus for 24 hours. mRNA expression of activation markers and secretion of pro-inflammatory cytokines were analyzed using realtime PCR and ELISA, respectively. Histone modifications (H3K27ac, H3K27me3 and H3K4me3) at immune response genes were analyzed using chromatin immune precipitation.

Results: Expression of activation markers VCAM and ICAM as well as secretion of pro-inflammatory cytokines MCP-1 and IL-8 were increased upon training with both MPs and NETs. NETs and MPs, in particular, induced an increase in H3K27ac and a decrease in H3K27me3 at promoter regions of immune response genes, indicating increased activity of the regulated genes. H3K4me3 marks remained stable upon stimulation.

Conclusion: We show for the first time that endothelial cells can be ‘trained’ by MPs and NETs and upon restimulation show increased responsiveness. Training with SLE-specific stimuli leads to increased expression of activation markers and increased secretion of pro-inflammatory cytokines. This hyper reactive state is mediated by epigenetic changes especially involving the H3K27 histone marks. Thus, endothelial cells can be trained during disease flares in patients with SLE, thereby leading to an enhanced inflammatory response when re-challenged with MPs, NETs or other inflammatory stimuli.
SINGLE-TUBULE RNA-SEQ REVEALS THAT THE INDUCTION OF LITHIUM-INDUCED NEPHROGENIC DIABETES COINCIDES WITH ACTIVATION OF AN INFLAMMATORY RESPONSE IN PRINCIPAL CELLS

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Background: Lithium salts, used for treatment of bipolar disorder, frequently induce nephrogenic diabetes insipidus (NDI). The associated polyuria limits therapeutic success and is associated with a loss of expression of the aquaporin-2 (AQP2) water channel in collecting duct (CD) principal cells, but the transcriptional regulatory mechanisms and signaling pathways responsible for lithium-induced repression of AQP2 gene transcription remain unknown. The chief limiting factor in the investigation of lithium-induced NDI is that collecting duct principal cells make up only a small fraction of the kidney. Thus, standard biochemical and systems biology techniques applied at a tissue level are not effective as a way of detecting responses in collecting duct principal cells. Recently, we have introduced single-tubule RNA-Seq techniques that are capable of quantifying the entire transcriptome in samples of microdissected renal tubules from rat kidney consisting of less than 2000 cells. Here, we employed the same technique addressing the question, “What signaling pathways are activated or inactivated during development of lithium-induced NDI?”

Methods: To answer this question, we used the methods of systems biology in a well-established rat model of lithium-induced NDI to identify signaling pathways activated at the onset of polyuria. Using single-tubule RNA-Sequencing, full transcriptomes were determined in microdissected cortical CDs of vasopressin-treated rats 72 hours after initiation of lithium treatment (vs. time-controls without lithium).

Results: Treatment of rats with clinically-relevant lithium concentration revealed a consistent significant downregulation of AQP2 at 72hrs after starting the treatment. Following single tubule isolation after 72hrs of treatment, mapping of transcriptome-wide changes in mRNA abundances to gene sets associated with curated canonical signaling pathways showed evidence for activation of NF-κB signaling with induction of genes coding for multiple chemokines as well as most components of the MHC Class I antigen presenting complex. It also confirmed prior evidence of a shift from quiescence into the cell cycle with arrest. Time course studies demonstrated an early (12hr) increase in multiple immediate early transcripts including the transcription factors Nfkb2 and Relb, simultaneous with the initial fall in AQP2 gene expression.

Conclusion: Integration of new data with prior data about lithium effects at a molecular level leads to a signaling model in which lithium inhibits the protein kinase GSK3β and increases ERK activation leading to induction of NF-κB signaling and an inflammatory-like response that represses AQP2 gene transcription.
A NOVEL HYPOKALEMIC-ALKALOTIC SALT-LOSING TUBULOPATHY IN PATIENTS WITH CLDN10 MUTATIONS


*equal contribution

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Background
Hypokalemic alkalosis can result from either acquired causes, frequently drug-induced, or several rare (genetic) renal tubular disorders, often due to mutations affecting transcellular sodium reabsorption. We identified a novel hypokalemic salt-losing nephropathy in patients with mutations in CLDN10, encoding the tight junction protein Claudin-10 expressed in the renal tubule.

Methods
We carefully phenotyped two unrelated patients with a hypokalemic-alkalotic salt-losing phenotype and their family members, including tubular function testing, and performed whole exome sequencing. Thereafter, we characterized the identified sequence variations in vitro.

Results
The first patient was diagnosed with Bartter syndrome (BS) over 30 years ago. Re-evaluation demonstrated hypocaliuria and hypercalcemia, suggesting Gitelman syndrome (GS). However, serum magnesium was in the upper-normal to hypermagnesemic range, thiazide responsiveness was not blunted, and genetic analyses did not show mutations in genes associated with either GS or BS. A reduced urinary concentrating ability with a preserved aquaporin-2 response to desmopressin was demonstrated, with an intact to exaggerated response to furosemide. These findings were not in line with any known salt-losing nephropathy. Whole exome sequencing revealed compound heterozygous CLDN10 sequence variants.

A second unrelated patient was thereafter identified demonstrating a similar phenotype and compound heterozygous CLND10 sequence variants. Both patients’ phenotypes resembled a mouse model lacking distal tubular Claudin-10. These mice demonstrated a reduced TAL paracellular sodium permeability leading to a urine concentrating defect, and enhanced paracellular magnesium and calcium permeability. Cell surface biotinylation and immunofluorescence experiments in cells expressing the Claudin-10 mutants showed that the phenotype is not explained by mere lack of Claudin-10 membrane localization or tight junction strand formation.

Apart from renal tubular cells, Claudin-10 is also expressed by e.g. glandular tissue and epidermis. Our patients also exhibited hypo/anhydrosis, reduced saliva production and plantar keratoderma.

Conclusion
This is the first report of pathogenic CLDN10 mutations causing a novel tight junction disease, possibly affecting TAL paracellular ion transport, characterized by a non-Bartter, non-Gitelman autosomal recessive hypokalemic-alkalotic salt-losing phenotype.
Restrictive Eculizumab Regimen in Patients with Atypical HUS in the Netherlands: An Annual Update from the CUREiHUS Study

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Background
With the introduction of eculizumab, the first drug targeting the complement system in 2011, a new era began for patients with atypical hemolytic uremic syndrome (aHUS). Although the results were highly promising, the introduction of eculizumab gave rise to a worldwide debate regarding the optimal treatment strategy. Without evidence, lifelong treatment with this highly expensive orphan drug was advised.

In the Netherlands, an unique and challenging initiative was undertaken by the national working group aHUS, comprising a delegation of (pediatric) nephrologists of all academic hospitals. In 2016, the new Dutch guideline for the diagnostics and treatment of aHUS, drafted by this working group, was implemented. This guideline advocates a restrictive treatment regimen and is monitored by the national, observational, CUREiHUS study. Within this annual report, the first preliminary results are presented.

Methods
All known aHUS patients who (1) were treated with eculizumab on 1st of January 2016 and (2) all patients with suspected aHUS who presented from 1st of January 2016 up till September 2017 and had been discussed in the national aHUS working group, were included in this annual report. Based on clinical and laboratory assessment, consent to continue (1-1-2016) or to start with eculizumab (>1-1-2016) was discussed. Patients received plasma therapy and/or eculizumab following the guideline and therapy was evaluated and tapered when patients were stable and in remission. Follow up data were only available of the aHUS patients included in the CUREiHUS study.

Results
In total, 69 patients with thrombotic microangiopathy (TMA) have been discussed: 25 patients with aHUS who were already receiving eculizumab treatment at 1st of January 2016 and 44 new patients with TMA. In 27 of them aHUS was suspected. With consent of the working group eculizumab therapy was initiated in 25 patients. In the remaining 17 patients, other diagnoses explanatory for the TMA were present, hence no eculizumab treatment was started. Of the in total 52 patients with strong suspicion of aHUS, 32 were included in the CUREiHUS study. In all but one the therapy was tapered or even withdrawn. In total, 6 of the 32 patients experienced a relapse following therapy adjustment. After rapid initiation of eculizumab, eculizumab therapy was tapered again in 5 patients with a safe and good clinical outcome. In total, a costs reduction of 55% was accomplished (€9,300,000).

Conclusion
The new Dutch guideline on the treatment of aHUS regarding a restrictive eculizumab regimen appears safe and effective. Moreover, by critically evaluating eculizumab therapy, aHUS treatment can be economized. Although this first annual report showed promising results, the majority of patients is within their first year of follow up and continuous careful monitoring remains warranted.
FGF23 IS INDEPENDENTLY ASSOCIATED WITH INCIDENT CKD AND MORTALITY IN THE GENERAL POPULATION

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* Authors contributed equally to this work

Background
The phosphaturic hormone Fibroblast Growth Factor 23 (FGF23) plays a key role in the regulation of phosphate and vitamin D. In patients with pre-existing kidney disease, high FGF23 levels are associated with progression of kidney disease progression and premature mortality. It is, however, unclear whether FGF23 is also associated with incident CKD and mortality in the subjects of the general population without pre-existing kidney disease. We therefore investigated these potential associations in the general population-based Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort.

Methods
Total (C-terminal) FGF was measured by ELISA in plasma samples of 4171 subjects with no prior history of CKD. Samples were collected between 2001 and 2003. Incident CKD was defined as new-onset eGFR < 60ml/min*1.73m² or 24-hour urinary albumin excretion > 30mg. Data is shown as mean±SD or median [IQR]. Associations between FGF23, incident CKD, and all-cause mortality were studied using Cox proportional hazards regression.

Results
Median age was 50 [IQR 42-59] years; 44% were male. Baseline FGF23 was 68.4 (56.1-85.5) RU/ml. FGF23 levels were correlated with baseline eGFR (r -0.158, P<0.001), age (r 0.089, P<0.001), plasma phosphate (0.128, P<0.001), PTH (0.097, P<0.001), and calcium (0.068, P<0.001).

During follow-up for 8.3 [7.8-9.0] years, 392 participants developed CKD and 151 participants died. High FGF23 was significantly associated with both an increased risk of incident CKD (HR 1.19 [1.09-1.30] per SD increase, P<0.001) and mortality (HR 1.16 [1.01-1.35], P=0.038). In a multivariable model including age, sex, BMI, eGFR, UAE, systolic blood pressure, use of anti-hypertensive medication, total cholesterol, smoking status, diabetes status, plasma phosphate, calcium, and PTH, FGF23 remained significantly associated with both incident CKD (HR 1.18 [1.06-1.31] per SD increase, P=0.003) and mortality (HR 1.21 [1.02-1.46] per SD increase, P=0.03). In sensitivity analyses, the association of FGF23 with incident CKD also remained when the eGFR and the UAE endpoint were analyzed separately (fully adjusted model: P=0.04 and P=0.002, respectively).

Conclusion
In the general population, in subjects without pre-existing CKD, high levels of FGF23 are associated with increased risk for both incident CKD and all-cause mortality, independent of baseline renal function and traditional cardiovascular risk factors. Future studies should investigate whether FGF23 may be of added value in predictive models for the development of CKD or mortality in the general population.
HEALTHCARE COSTS OF PATIENTS ON DIFFERENT RENAL REPLACEMENT MODALITIES – ANALYSIS OF DUTCH HEALTH INSURANCE CLAIMS DATA

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Background Patients with end-stage renal disease contribute significantly to healthcare expenditures, because renal replacement therapy (RRT) is an expensive therapy. Comprehensive cost estimates of RRT in the Netherlands are based on one study from the 1990s. More recent studies in other countries lack a complete overview of all RRT modalities, especially of living donor kidney transplantation (LDKT). The aim of this study is to present average annual healthcare costs for Dutch patients for 7 treatment modalities, including LDKT.

Methods Dutch health insurance claims data were used to identify adult RRT patients. Patients with a health insurance claim for dialysis treatment in 2014 or a claim for kidney transplantation in 2012-2014 were selected. Costs data are healthcare costs per calendar-year and comprise of costs incurred in the hospital, primary care, mental healthcare, medication, transportation and remaining healthcare costs. The average annual healthcare costs were analyzed for 5 dialysis modalities (in-center haemodialysis (CHD), home haemodialysis (HHD), continuous ambulatory peritoneal dialysis (CAPD), automated peritoneal dialysis (APD), multiple dialysis modalities in a year (mix dialysis group) and 2 transplant modalities (LDKT and deceased donor kidney transplantation (DDKT)).

Results The total average annual healthcare costs ranged from €77,566 for CAPD patients to €105,833 for patients in the mix dialysis group. Costs for kidney transplant recipients were €85,127 in the year of transplantation and rapidly declined in the first and second year after successful transplantation (resp. €29,612 and €15,018). Transplantation with a DDKT resulted in higher costs (€99,450) in the year of transplantation compared to an LDKT (€73,376).
Conclusion This study presents comprehensive cost estimates of 7 different RRT modalities, including different dialysis and kidney transplantation modalities, using Dutch health claims data. CAPD patients have the lowest costs compared to other dialysis modalities. Costs in the year of an LDKT are 25% lower than those of a DDKT. Notably, after successful transplantation, annual costs decline substantially to a level that is approximately 14-19% of annual dialysis costs.
ALTERED CARRIER DISTRIBUTION OF CIRCULATING MICRORNAS ASSOCIATES WITH VASCULAR INTEGRITY IN DIABETIC NEPHROPATHY

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Background: We previously demonstrated an association between total plasma levels of specific microRNAs (miRNAs) and microvascular injury in patients with diabetic nephropathy (DN). These circulating miRNAs are carried by extracellular vesicles (EVs), RNA-binding protein Argonaute2 (Ago2) or high-density lipoprotein (HDL). We hypothesized that identification of the carrier specificity of selected miRNAs can enhance the biomarker potential of these miRNAs and that carrier-specific transfer of miRNAs to vascular cells may play a causal role in vascular injury.

Methods: We assessed the plasma carrier distribution of miRNAs in DN (n=21), diabetes mellitus (DM; n=15; eGFR of ≥ 30 mL/min) patients and healthy controls (n=15). EVs, HDL and Ago2 were isolated using size exclusion chromatography, KBr density gradient ultracentrifugation and immunoprecipitation, respectively. MiRNA expression was determined using TaqMan® miRNA Arrays and correlated to markers of vascular injury, including angiopoietin-2 (Ang2), soluble thrombomodulin (sTM) and capillary tortuosity. In vitro studies were performed to assess transfer and function of specific miRNA-carrier complexes.

Results: Specific miRNA-carrier complexes were identified to be associated with DN and vascular injury. Most notably, we found EV-miR-21 and Ago2-miR-660 levels to display a significant increase in both DM and DN groups compared to healthy controls and correlated with capillary tortuosity and sTM, respectively. Furthermore, HDL-miR-132 levels decreased in DN and correlated with levels of Ang2. Mechanistically, in vitro studies demonstrated that these specific miR-complexes are transferred to endothelial cells, repress validated target genes and ultimately affect angiogenic capacity and barrier function.

Conclusion: Our data suggest that miRNAs in specific carriers could improve their sensitivity as biomarkers for vascular injury in DN and are not just by-products of disease progression but can play an active, and modifiable role in the regulation of vascular integrity.
COMBINED LOW STATUS OF VITAMIN D AND VITAMIN K IS ASSOCIATED WITH ALL-CAUSE MORTALITY AND PREMATURE GRAFT FAILURE IN STABLE KIDNEY TRANSPLANT RECIPIENTS


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3) Department of Internal Medicine, Division of Nephrology, University of Groningen, University Medical Center Groningen, Groningen

Introduction: Kidney transplant recipients (KTR) are often low in micronutrient status, including vitamin D and K. The long-term implications of combined low status of vitamin D and K in KTR are unknown. We investigated vitamin D and K status in relation to all-cause mortality and premature graft failure in stable outpatient KTR.

Methods: We studied 461 KTR from a single-center after median 6.1 years (interquartile range 3.0-11.9) after transplantation. At baseline, vitamin D and K status were measured by 25-hydroxyvitamin D [25(OH)D] and dephosphorylated uncarboxylated matrix gla protein (dp-ucMGP) respectively. Vitamin D and vitamin K were categorized by 25(OH)D <50/≥50 mmol/L and median dp-ucMGP <996/≥996 pmol/L. We used Cox regression analyses to estimate hazard ratios (HR) and 95% confidence intervals.

Results: At baseline, mean age was 52±12 years, and 122 KTR (26%) had combined low vitamin D and K status. During median 9.8 years (interquartile range 8.7-10.3) follow-up, 128 patients (28%) died and 48 (10%) developed graft failure. Combined low vitamin D and K status was associated with 2.46 (95% CI 1.30-4.63) greater risk of all cause-mortality and a 2.93 (95% CI 1.05-8.13) greater risk of graft failure. Of all KTR, 45 (10%) used vitamin D supplements, mainly alfacalcido (80%). Plasma dp-ucMGP was more strongly associated with all-cause mortality and graft failure in vitamin D supplement users than in no vitamin D supplement users: HR per 500 pmol/L ducMGP 1.11 (1.04-1.19) and 1.41 (1.08-1.84), (See Figure 1, P-for interaction 0.06 and 0.108, respectively adjusted for age and sex).

Conclusions: Combined low vitamin D and K status is highly prevalent in stable KTR. The joint association of low vitamin D and K status is associated with greater risk of premature mortality. Vitamin D supplement users with low vitamin K status have a strong risk of premature mortality and graft failure compared to non-users. Future studies should address whether combined vitamin D and K supplementation may lead to improved outcomes after kidney transplantation.
Figure 1: Continuous associations for dp-ucMGP with all-cause mortality and transplant failure stratified by vitamin D supplement users.
ULTRASTRUCTURAL EXAMINATION OF GLOMERULAR FIBRILLARY DEPOSITS IN DIABETIC NEPHROPATHY

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1. Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
2. Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands

Background: In diabetic nephropathy (DN), glomerular fibrillary deposits have been observed but there are no large-scale, ultrastuctural examinations in the literature about the nature of these deposits. We here report our investigation of fibrillary deposits found by transmission electron microscopy (TEM) in DN, and compare results to those in fibrillary glomerulonephritis (FGN).

Methods: Twenty-two patients with autologous biopsy confirmed DN were selected and routine light microscopic evaluation, classification and clinical data were reviewed. TEM was performed and fibril diameter was calculated from 60-90 measurements per glomerulus. For comparison, 7 non-diabetic FGN patients were selected.

Results: For detailed results we refer to Table 1. There were 7 cases with class II, 10 with class III and 5 with class IV DN. Irrespective of class, small, randomly organised fibrils with a diameter of 7-14 nm were present in glomeruli of all cases, while additionally, straight or curved, organised fibrils with a diameter of 17-37 nm were present in 11 cases. No renal comorbidity at time of biopsy or at clinical follow up that could explain fibrillary deposits was present.

FGN patients had similar fibrillary deposits that were either small and randomly organised or had a more organised aspect and a diameter of 13-32 nm. None of the patients had diabetes. IF findings did not contribute to differentiate between DN and FGN, but patients with DN did have a significantly thicker GBM than those with FGN.

Conclusion: Fibrillar deposits found in glomeruli of all 22 DN patients are morphologically similar to those in FGN patients. The widespread presence of fibrils in DN was an unexpected finding; their similarity to fibrils in FGN may complicate the histological diagnosis, especially in patients with clinically overlapping symptoms. Therefore, we are currently investigating their composition using mass spectrometry.

Table 1. Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>DN (n=22)</th>
<th>FGN (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>55 ± 14</td>
<td>55 ± 10</td>
</tr>
<tr>
<td><strong>Gender = male</strong></td>
<td>17 (77%)</td>
<td>3 (43%)</td>
</tr>
<tr>
<td><strong>DM type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>5 (23%)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Type 2</td>
<td>16 (73%)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>MODY</td>
<td>1 (5%)</td>
<td></td>
</tr>
<tr>
<td><strong>DM duration (years)</strong></td>
<td>15 ± 14</td>
<td>Not applicable</td>
</tr>
<tr>
<td><strong>Clearance (ml/min/1.73m²)</strong></td>
<td>43 ± 28</td>
<td>41 ± 27</td>
</tr>
<tr>
<td><strong>Proteinuria (g/day)</strong></td>
<td>6 ± 3</td>
<td>7 ± 6</td>
</tr>
<tr>
<td><strong>Follow-up (years)</strong></td>
<td>3 [0-19]</td>
<td>0 [0-7]</td>
</tr>
</tbody>
</table>

Values are depicted as mean ± SD, median [min-max] or as number of patients(%).
Beinvoerde afdrukmateriaal de hemostasetijd na verwijdering van dialysenaald bij arterio-veneuse shunts?
Margreet ter Meer OLVG West

Inleiding
Dialysepatiënten geven aan dat korte hemostasetijd voor hen belangrijk is (1) Richtlijnen geven veelal praktisch advies hoe de handeling uitgevoerd dient te worden. (2) (3) (4). Afdrukken is een fijne balans tussen genoeg en overmatige compressie. Een gemiddelde dialysepatiënt ondergaat zo driemaal per week 2 puncties, wat neerkomt 312 puncties per jaar. Belangrijk voor de patiënten is een zo kort mogelijke afdruktijd. Actuele richtlijnen adviseren een afdruktijd die varieert tussen de 8 en 12 minuten en om tussentijds niet te kijken. Vroegtijdige controle kan het hemostaseproces onderbreken en bloeding herstarten. Soms is er na 10 minuten de hemostase niet compleet. Naast het steriel gaas zijn er andere ondersteunende materialen in omloop, de keuze voor een bepaald materiaal verschilt sterk tussen de dialysecentra en wetenschappelijke data zijn nauwelijks voorhanden (5) (6) (7). Is er verschil in afdruktijd met hemostase bevorderende verbandmiddelen?

Methode.
Een single centrum cross-over bij 80 hemodialysepatiënten waar bij in vijf weken vijf verschillende verbandmaterialen zijn getest. (per patiënt 6 metingen per week). De periode van onderzoek was van 23 mei tot 26 juni 2016. Alle patiënten zijn geïnformeerd d.m.v. brief (bij communicatieprobleem is familie gebeld voor uitleg) er is klinische les voor personeel gehouden. Iedere week is de patiënt geïnformeerd over betreffende verband materiaal d.m.v. digitale presentatie in de wachtkamer. Om tijd te meten in de hectiek van afsluiten is gebruik gemaakt van keukenwekkers. De afdruktijd is gemeten in tijdseenheid van 5 min (1 = < 5 min, 2 = 5-10 min, 3 = 10-15 min en 4 = > 15 min) Na en thuisbloedingen zijn geregistreerd, zo ook de patiënt tevredenheid t.a.v. het product en de kosten van verbandmateriaal. Om de gegevens te kunnen presenteren is er toestemming gevraagd en aan de ACWO commissie.

De volgende materialen zijn getest.
Week 1 Een steriel gaas
Week 2 Spongostan met steriel gaas
Week 3 Woundclot
Week 4 Plaster XL
Week 5 TipStop

Inclusie:
- 80 patiënten van de ochtend en avond shift, met Arterio-Veneuze-Fistel (AVF) en Arterio-Veneuze-Graft (AVG).

Exclusie:
- Patiënten met nachtdialyse
- Patiënten met Centraal Veneuze Katheter
- Patiënt (1) met thorax PTFE

Resultaat
In de periode van 23-05-2016 tot 26-6-2016 zijn bij 80 HD patiënten (44, 55% mannen en 36, 45% vrouwen) vijf verschillen verband materialen getest. De gemiddelde patiënten leeftijd was 68,6 jaar (29-94 jaar) SD 14,46. De gemiddelde shuntleeftijd was 57 maanden (3-306 maanden) SD 52,70. En de gemiddelde therapiëleefgtijd was 5,4 jaar (0-25) SD 5,23. Bij 47 Arterio-veneuze-Fistel (AVF) en 33 Arterio-veneuze-graft (AVG).
**Na en thuisbloedingen in %**

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na bloeding</td>
<td>12,5</td>
<td>2,56</td>
<td>9,21</td>
<td>17,33</td>
<td>10,29</td>
</tr>
<tr>
<td>Thuisbloeding</td>
<td>8,75</td>
<td>14,1</td>
<td>15,78</td>
<td>18,63</td>
<td>14,7</td>
</tr>
</tbody>
</table>

Patiënttevredenheid is gemeten met schaal -2 tot +2 waarbij 0 neutraal was (Week 1 mean 0,76,(-2 tot 2) Week 2 mean 0,96, (-1 tot 2) Week 3 mean 1,17, (0 tot 2) Week 4 mean 0,77,(-1,5 tot 2) Week 5 mean 0,95 (-1 tot 2).

**Kosten,**

<table>
<thead>
<tr>
<th></th>
<th>Prijs per stuk</th>
<th>X 2</th>
<th>Per jaar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaas</td>
<td>€ 0,29</td>
<td>€ 0,58</td>
<td>€ 90,48</td>
</tr>
<tr>
<td>Gaas + Spons</td>
<td>€ 0,29 &amp; € 0,40</td>
<td>€ 1,38</td>
<td>€ 215,28</td>
</tr>
<tr>
<td>Woundclot</td>
<td>€ 2,12</td>
<td>€ 4,24</td>
<td>€ 661,44</td>
</tr>
<tr>
<td>PlasterXL</td>
<td>€ 0,45</td>
<td>€ 0,90</td>
<td>€ 140,40</td>
</tr>
<tr>
<td>TipStop</td>
<td>€ 1,20</td>
<td>€ 2,40</td>
<td>€ 374,40</td>
</tr>
</tbody>
</table>

**Voorlopige conclusie.**

Het lijkt dat hemostase bevorderende verbandmiddelen kortere afdruk tijd hebben. Patiënttevredenheid bij woundclot geen negatieve score.

Wat is het afkap punt, 10 min mogelijk overschatte tijd.

Pilot verder onderzoek nodig.

Momenteel worden beïnvloedende variabelen t.o.v. materiaal onderzocht

- Shuntflow
- Leeftijd patiënt (>65)
- Soort Naald (staal/katheter)
- Dikte dialysenaald
- Anticoagulantia gebruik
- Type Shunt(graft/fistel)
Litertuurlijst:


Dialyse hypotensie

J.Kuipers¹, W Paans², W Krijnen², R.Westerhuis¹, C.F.M. Franssen³
¹Dialyse Centrum Groningen (DCG), ²Hanzehogeschool Groningen, ³Universitair Medisch Centrum Groningen (UMCG)

Inleiding
Dialyse hypotensie wordt beschouwd als één van de meest voorkomende complicaties van de dialysebehandeling. Het gaat vaak gepaard met klachten en symptomen. In de literatuur wordt aangegeven dat dialyse hypotensie bij 20 tot 50% van de dialysepatiënten regelmatig voor komt. Dit zijn echter vaak oudere studies. Sindsdien zijn de dialysetechnieken verbeterd en zijn nieuwe technieken geïntroduceerd om hypotensie te voorkomen. Aan de andere kant is de gemiddelde leeftijd van de dialysepatiënt gestegen en daarmee ook het aandeel van patiënten met aanzienlijke co-morbiditeit (diabetes mellitus, hartfalen). Deze patiënten hebben een groter risico op het ontstaan van dialyse hypotensie. In onze populatie hebben we onderzocht hoe vaak dialyse hypotensie voor komt en of er verschillen zijn in bloeddrukbeloop tussen de eerste en overige dialyses van de week. Ook is onderzocht of er een relatie is tussen Kwaliteit van Leven (KvL) en dialyse hypotensie. Tenslotte hebben we een literatuur studie gedaan om een overzicht te krijgen van studies waarbij daadwerkelijk onderzocht is hoe vaak dialyse hypotensie voor komt.

Methoden
Een literatuurstudie en een prospectief onderzoek gedurende 3 maanden waarbij gedetailleerd gegevens zijn verzameld en geanalyseerd van 3818 hemodialyse behandelingen. Patiënten hebben elke dialyse geëvalueerd m.b.v. een scoreformulier. De onderzoeksvragen:
- Hoe vaak komt dialyse hypotensie volgens de EBPG richtlijnen (European Best Practice Guidelines) voor in onze populatie
- Zijn er verschillen in bloeddrukbeloop tussen de eerste en overige dialyses van de week
- Hebben de variabelen van dialyse hypotensie (een systolische bloeddrukdaling van 20mmHg, symptomen, interventies) invloed op de KvL van de HD patiënt

Resultaten
Hypotensie volgens de EBPG definitie komt in onze populatie voor in 6,7% van de hemodialyse behandelingen. Zowel de predialyse als de intradialytische bloeddruk tijdens de eerste dialyse van de week zijn het hoogst en blijven ook het hoogst ondanks een groter UF volume. Het optreden van dialyse hypotensie lijkt niet gerelateerd aan de KvL. Wel is er een relatie tussen de patiënten scores en de KvL, een goede score geeft ook een hogere KvL score. In de literatuur worden veel verschillende definities van dialysehypotensie gehanteerd. Hoe vaak dialyse hypotensie voor komt is afhankelijk van de gebruikte definitie.

Conclusies
Dialyse hypotensie gedefinieerd volgens de EBPG richtlijn, komt in onze dialyse populatie minder vaak voor dan in de literatuur wordt aangegeven. De systolische en de diastolische bloeddruk bleef beter op peil tijdens de 1e HD dan tijdens de 2e en 3e HD van de week. Er is consensus nodig over de definitie van dialysehypotensie. En er is meer aandacht nodig voor de ‘dialysebeleving’ van de patiënt en wat we daar als verpleegkundigen in kunnen verbeteren.
DIALYSE IN SAMENWERKING MET EEN VVT-ORGANISATIE

José Koemeester, Ronald Visser, Anneke Nauta

1. Achtergrond

In Nederland worden ongeveer 300 patiënten behandeld met hemodialyse in de thuissituatie (THD). THD wordt traditioneel door de patiënt zelfstandig, met hulp van partner of solo uitgevoerd. In andere gevallen kan een dialyseverpleegkundige ondersteuning bieden. Maatschappelijke veranderingen zorgen voor een verschuiving van CHD naar THD. De vergrijzing veroorzaakt een stijging van de gemiddelde leeftijd met als gevolg een grotere vraag naar verpleegkundige ondersteuning. Het aantal THD-patiënten met ondersteuning ligt op dit moment op bijna 60%. In 2014 was dit 42%.

2. Methode

Binnen een VVT organisatie woont een dialysepatiënt met interesse voor THD. Deze patiënt gaat 3x per week naar het dialysecentrum. Patiënt heeft de wens op de woninglocatie te kunnen dialyseren. Echter, de kamers in een VVT-instelling zijn moeilijk in te richten voor THD. Taxivervoer naar het dialysecentrum heen en terug is tijdrovend, vermoeiend en is een bron van frustratie voor dialysepatiënten. Niet alleen moet de woning geschikt zijn voor THD, het betekent meestal voor deze patiënt ook dat er verpleegkundige ondersteuning moet zijn. Dit is gezien deze personele inzet moeilijk kostendekkend te krijgen. Hierdoor komt deze patiëntengroep doorgaans niet in aanmerking voor THD. THD geeft weinig rompslomp en is eenvoudig te realiseren. De inrichting is goedkoop, makkelijk aan te leggen en weer te verwijderen. In samenwerking met het verzorgingshuis is een dialyse-HUB, gerealiseerd in een ruimte in de instelling zodat de patiënt in de eigen omgeving kan dialyseren. Deze HUB, met twee dialyse stations, is in nauwe samenwerking met patiënt en familie tot stand gekomen. Kwaliteit en veiligheid van behandeling binnen de HUB moet gelijk zijn aan het centrum, wat heeft geleid tot een standaarduitvoering van een PRI bij de opstart van het traject en een tweejaarlijkse audit als onderdeel van de kwaliteitsbewaking.

3. Resultaat

Patiëntsatisfactie: Het dialyseren in de eigen woonomgeving wordt door de patiënten als zeer positief ervaren: patiënt A: “ik hoef niet meer zo vroeg op te staan en kan wandelend naar mijn dialysestation” Patient B: “het is zo fijn dat mijn man tijdens de dialyse gezellig met mij koffie kan drinken”.

Efficiëntie: Snel na de start van de eerste patient is een 2e patient gestart die juist om de dialysefaciliteit in de instelling is gaan wonen. 2 patiënten tegelijk dialyseren met ondersteuning van 1 verpleegkundige is efficiënter dan 1 patient door 1 verpleegkundige.

Naar aanleiding van dit proefproject zijn wij in gesprek met zorgverzekeraars om de besparingen op taxikosten en huisaanpassingen om te zetten naar investeringen voor het ontwikkelen van nieuwe HUB’s. Het verzorgingshuis ziet een mogelijkheid zichzelf te onderscheiden als organisatie waar een dialysefaciliteit aanwezig is. Een HUB in huis trekt verzorgenden aan met extra carrièreopties om opgeleid te worden voor de ondersteuning bij de dialysebehandeling.

4. Conclusie

Een dialyse-HUB binnen een VVT-organisatie biedt de kans aan bewoners om dicht bij huis behandeld te worden. Ideaal als je oud en kwetsbaar bent. Met het oog op veiligheid is het als organisatie echter van groot belang je te realiseren dat je je strikt moet houden aan de gestelde criteria voor THD en bij afwijkingen de patiënt terugsturen naar het dialysecentrum. Heldere communicatie met patiënt, familie en het verzorgingshuis tijdens het proces is dan ook heel belangrijk.
AUDITTEN MIDDELS EEN PATIËNTEN TRACER.

Inleiding: In 2016 is de HKZ normenset herzien waarbij de patiënt met zijn behoeften en verwachtingen centraal als uitgangspunt is genomen. De Nederlandse dialysecentra zijn inmiddels al heel wat jaren ge(her)certificeerd. De oude manier van ‘systeem’ audits sluit op den duur niet meer aan op hoe we de patiënt centraal stellen. In 2016 is binnen de Ziekenhuisgroep Twente de gehele auditsystematiek voor het ziekenhuis omgezet naar patiënt- en systeemtracers. De opbrengst van deze audits is wezenlijk anders dan wat in het verleden werd getoets/bevonden. De dialyseafdeling bleef auditten volgens de standaard procedure van interne audits.

Methode: Eind 2017 hebben we een eerste patiënten tracer gelopen op het HD proces binnen ZGT. De K&V groep bestaande uit 3 interne auditoren en een procesbewaker werden verdeeld in 2 groepen. 2 hemodialyse zalen werden geselecteerd waarbij op elke zaal met 2 patiënten werd meegekeken van binnenkomst-aansluitproces- tot afsluiten. In tussentijd werden professionals en patiënten bevraagd op ‘live’ gebeurtenissen, de ‘wat als’ situaties, maar ook de beleving van de patiënt in dit geheel. Tijdens de tracer wordt een open sfeer gecreëerd waarbij de patiënt ook ziet dat de professionals zich toetsbaar opstellen.

Resultaten: De vragenlijst en de resultaten zijn opgenomen in iProva van Infoland. Hierin zijn zowel de NIAZ vragen gebruikt als de normenset van de HKZ als vraagstelling verwerkt. De resultaten bestrijken slechts één audit, maar de uitkomsten zijn door de gezichtspunten van de patiënt afwijkend van eerdere audit bevindingen. Voorbeeld van bevinding onder toegevoegd.

<table>
<thead>
<tr>
<th>Ja</th>
<th>Deels</th>
<th>Nee</th>
<th>NVT</th>
<th>Opmerkingen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Ontvangt u uw cliënten bij de dialyse met respect voor privacy en vertrouwelijkheid?</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Conclusie: De wijze van een audit middels een patiënten tracer betreft de patiënt meer bij de te verkrijgen informatie. De output behelst niet alleen of het protocol wordt gevolgd, maar ook het handelen indien er afwijkende situaties optreden, zo mogelijk ook de risicovolle situaties. Protocollair zijn deze vaak wel afgedicht maar hoe gaat de professioneel op de werkvloer hier mee om? Deze vragen kunnen middels een tracer beter worden beantwoord. Voor het auditteam is het een nieuwe wijze van informatie vergaren, maar het enthousiasme na de eerste smaakt naar meer!
DIANIA STUDIE.
 Wat is het effect van Niacinamide suppletie op het serumfosfaatgehalte bij hemodialysepatiënten?

Inez Jans1,6, Willemien Baan2,6, Arend Jan Smilde3,6, Geert W Feith4,6, Julia M Hofstra4,6, Nicole M de Roos5,6


Inleiding
Hyperfosfatemie is een blijvend probleem bij dialysepatiënten en geassocieerd met cardiovasculaire complicaties en verhoogde mortaliteit. Daarom is het behaaldeloos bereiken van normofosfatemie. Patiënten krijgen hiervoor vaak veel fosfaatbindende medicijnen voorgeschreven, in te nemen bij elke fosfatrijke (tussen)maaltijd. Naast de grote kostenpost leidt dit, mede door de bijwerkingen, tot een verminderde kwaliteit van leven. Vaak is ook de therapietrouw voor deze medicatie slecht.

Sinds 2004 bestaan aanwijzingen dat suppletie met vitamine B3 het serumfosfaat verlaagt. Dit door remming van het fosfaattransport via de natriumfosfaatkanalen (NaPi-2b) in de darm. Groot voordeel is de inname 1x per dag, onafhankelijk van de maaltijd. Er zijn 2 vormen van Vitamine B3: Nicotinezuur (of Niacine) en Niacinamide (of Nicotinamide).

In 2012 hebben we een interventiestudie uitgevoerd bij 29 hemodialyse patiënten, waarbij gedurende 8 weken extra Nicotinezuur is geslikt. Resultaat was een daling van serumfosfaat met 0.36 mmol/L (21%). Hierbij echter een drop out van 66% l.g.v. ervaren bijwerkingen (m.n. jeuk en flush) zodat brede toepassing niet haalbaar lijkt. Zowel het positieve effect als de bijwerkingen zijn ook door anderen beschreven (Müller et al, CJASN 2007).

In de huidige studie onderzoeken wij of Niacinamide -de andere vorm van B3- beter wordt verdragen met behoud van het fosfaatverlagerende effect.

Methoden
Wij hebben een dubbelblinde cross-over interventiestudie verricht met Niacinamide (250 mg, Orthica). 35 hemodialyse patiënten werden geïncludeerd (19 M en 16 V, 65 jaar ± SD 16, baseline gem. serumfosfaat 1.49 ± SD 0.29 mmol/l) en gerandomiseerd voor interventie A of B. Groep A start met Niacinamide, groep B met placebo. De eerste 4 weken 1 tablet, daarna 8 weken 2 tabletten. Na 12 weken kreeg groep A het placebo en groep B Niacinamide.

Resultaten
Van de 35 patiënten hebben 26 patiënten het onderzoek voltooid (drop out 26%). Slechts 2 patiënten (6%) hebben de studie voortijdig beëindigd vanwege bijwerkingen (diarree en jeuk); 7 patiënten zijn om andere redenen gestopt. Het serumfosfaat was na de interventieperiode gemiddeld 0.20 mmol/L (13%) lager.
Conclusie
1. Niacinamide wordt in tegenstelling tot Nicotinezuur goed verdragen.
2. Niacinamide leidt tot een significante fosfaatdaling van 0,20 mmol/L. Er zijn echter grote interindividuele verschillen.
3. Het effect van Niacinamide lijkt kleiner dan van Nicotinezuur, maar kan dosis gerelateerd zijn.

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DIAGNOSTIC ACCURACY OF THE MALNUTRITION SCREENING INSTRUMENTS MUST AND SNAQ IN PATIENTS WITH CHRONIC KIDNEY DISEASE (CKD) AND THE ALTERNATIVE OPTION OF THE SHORT FORM OF THE PG-SGA.

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Introduction
Early recognition of malnutrition or protein energy wasting (PEW) is important because early intervention for disease-related malnutrition can limit suffering of patients and save costs by reducing complications, duration of hospital stay, and mortality. Therefore, screening is routinely performed at admission, but it is likely that the widely used MUST (Malnutrition Universal Screening Tool) and SNAQ (Short Nutritional Assessment Questionnaire) fail to recognize many malnourished CKD patients, because fluid retention can mask the weight loss that is a major component in these tools. This study evaluated the diagnostic accuracy of the MUST and SNAQ with the PG-SGA (Patient Generated-Subjective Global Assessment) as golden standard. We also determined whether the PG-SGA SF is a better alternative screening tool in CKD patients.

Methods
Cross-sectional observational study in 123 CKD patients: 68 outpatients and 55 inpatients (64 treated with dialysis). All patients were screened in outpatient setting or at admission for malnutrition by the MUST, SNAQ, and PG-SGA SF. Nutritional status was determined by PG-SGA global rating and classified into two categories ‘well nourished’ or ‘malnourished’ (a combination of moderate and severely malnutrition, or ≥9 points for the scored PG-SGA). For the PG-SGA SF a cutoff point of ≥6 was used as criterion for ‘malnourished’.

Results
According to PG-SGA global rating 47% of the patients were malnourished (with scored PG-SGA 52%). The MUST≥2 and SNAQ≥3 had sensitivities of 22% and 36%, respectively, to detect malnourishment. The PG-SGA SF score ≥6 had a sensitivity of 79%.

Conclusion
The PG-SGA SF with cutoff point ≥6 had a much higher diagnostic accuracy (79%) for detecting malnourishment in patients with CKD than the MUST and SNAQ that failed to recognize more than 64% of the malnourished CKD patients. The PG-SGA SF should therefore be used as a first screening tool for malnourishment in CKD patients including dialysis patients.
DE BEoordeling van de Voedingstoestand Middels PG-SGA Bij Hemodialyse en Peritoneaal Dialyse Patiënten
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Inleiding
Ondervoeding komt bij 35-45% van de dialysepatiënten voor en staat in verband met een hoger risico op infecties, een toename van complicaties, morbiditeit en mortaliteit. Het screenen en diagnosticeren van ondervoeding is belangrijk binnen deze populatie. In 2015 is de vakgroep diëtetiek van Dialyse Centrum Groningen gestart met een pilot van het gebruik van de Patient-Generated Subjective Global Assessment (PG-SGA) als assessmentinstrument. De PG-SGA scoort punten op de 5 onderdelen: gewicht, voedsinginname, symptomen en activiteit ingevuld door de patiënt, en professional ingevuld door diëtist op de onderdelen: ziekte, metabole stress, lichamelijk. Het doel van dit onderzoek was om te beoordelen of PG-SGA geschikt is om de voedingstoestand te monitoren en of PG-SGA het bewustzijn t.a.v. de eigen voedingstoestand verhoogt bij dialysepatiënten.

Methoden
De PG-SGA wordt elk half jaar uitgevoerd bij zowel hemodialyse- (HD) als peritoneaal dialysepatiënten (PD) van ≥ 18 jaar die de Nederlandse taal beheersen. De PG-SGA score is onderverdeeld in 3 categorieën: A is goed gevoed (0 – 8 punten); B is matig gevoed of verdenking op ondervoeding (>8 – 14), C is slecht gevoed (>14). Bij de eerste PG-SGA meting zijn relevante parameters voor de voedingstoestand verzameld. Voorafgaand aan de 1e meting en na de 3e meting is een vragenlijst afgenomen over het begrip voedingstoestand en hebben patiënten hun eigen voedingstoestand gescoord.

Resultaten
De populatie bestond in totaal uit 208 dialysepatiënten (173 HD en 35 PD). Het verband tussen PG-SGA score en parameters staat in de tabel:

<table>
<thead>
<tr>
<th></th>
<th>Totaal</th>
<th>PG-SGA Categorie A</th>
<th>PG-SGA Categorie B en C</th>
<th>P waarde</th>
</tr>
</thead>
<tbody>
<tr>
<td>PG-SGA puntenscore (meting 1)</td>
<td>n=208</td>
<td>n=173</td>
<td>n=35</td>
<td></td>
</tr>
<tr>
<td>Streefgewicht (kg)</td>
<td>75 [16,4]</td>
<td>75 [16,8]</td>
<td>77 [14,9]</td>
<td>.739</td>
</tr>
<tr>
<td>PCR (gram)</td>
<td>71,6 [29,8]</td>
<td>72,8 [26,8]</td>
<td>63,6 [35,3]</td>
<td>.099</td>
</tr>
<tr>
<td>nPCR streefgewicht (g/kg/dag)</td>
<td>0,98 [0,32]</td>
<td>0,98 [0,30]</td>
<td>0,90 [0,41]</td>
<td>.140</td>
</tr>
<tr>
<td>Creatinine (umol/L)</td>
<td>740 [316]</td>
<td>759 [312]</td>
<td>659 [317]</td>
<td>.038*</td>
</tr>
</tbody>
</table>

De waarden zijn weergegeven in mediaan [interquartile range]. Het albumine en creatinine zijn significant lager als er respectievelijk meer punten voor ‘professional’ en ‘activiteit’ gescoord worden. Uit de vragenlijst bleek dat na de 3e PG-SGA meting een hoger percentage (84,8% vs. 43,7%) van de patiënten weet waar de diëtist op let bij het beoordelen van de voedingstoestand.

Conclusie
De PG-SGA is een veelbelovend assessmentinstrument in de dialysepopulatie, de PG-SGA laat een verband zien met albumine en creatinine en lijkt daardoor de problemen van de voedingstoestand goed weer te geven. Ook lijkt PG-SGA het inzicht van patiënten in hun eigen voedingstoestand en bijbehorende aspecten te vergroten. Er is meer onderzoek nodig om de causaliteit van de verbanden verder te onderzoeken.
Inleiding

Uit eerder onderzoek vanuit het Catharina Ziekenhuis was aangetoond dat de handknijpkracht een van de meest valide parameters is om de spiermassa in relatie tot de voedingstoestand bij dialysepatiënten te bepalen. Diëtisten in het Catharina Ziekenhuis hebben hiervoor geen referentiewaarden wat een objectieve beoordeling van de handknijpkrachtmeting bemoeilijkt.

Doelstelling

1. Beïnvloeden leeftijd, geslacht, dialyseduur en dialysesoort de handknijpkrachtwaarde bij dialYSerende patiënten van het Catharina Ziekenhuis?
2. Staat een lage handknijpkracht in verband met mortaliteit?

Methode

Alvorens er begonnen werd met het veldonderzoek is er eerst een literatuur onderzoek gedaan. Vervolgens is er gestart met veldonderzoek door handknijpkrachtmetingen van dialyserende patiënten uit het Catharina Ziekenhuis te verzamelen in een zelf opgestelde datamatrix middels SPSS (versie 23.0 voor Windows). De handknijpkrachtmetingen zijn verkregen uit de Elektronische Patiënten Dossiers (EPD). De handknijpkrachtmetingen zijn gemeten binnen zes maanden na de start van de dialyse en werden in principe elk half jaar herhaald middels de SOP procedure van de handknijpkracht. In dit onderzoek zijn tevens overleden patiënten meegenomen in de datamatrix om doelstelling 2 te kunnen beantwoorden. Voor de statistische analyses is gebruik gemaakt van een lineaire regressieanalyse, scatterplot en Pearson’s correlatiecoëfficiënt.

Resultaten

Literatuuronderzoek

Uit het literatuuronderzoek was naar voren gekomen dat ouderdom geassocieerd is met een lagere spierkracht. Ook was uit meerdere wetenschappelijke onderzoeken gebleken dat mannen een hogere handknijpkracht hebben dan vrouwen. Verder was gebleken uit literatuur dat PD patiënten vaak jonger zijn dan HD patiënten en dat PD patiënten vaak gezonder zijn bij aanvang. Ook was gebleken dat dialysepatiënten met een restfunctie van de nier een hogere handknijpkracht hebben dan dialysepatiënten zonder restfunctie. Tenslotte is gebleken dat er een relatie is tussen een lage handknijpkracht en mortaliteit.

Veldonderzoek

In totaal waren er 237 patiënten, waarvan 67 overleden meegenomen in de berekeningen van dit onderzoek waarbij er een significant verschil is gevonden tussen de waarde van de handknijpkracht en leeftijd (p=0,00), de waarde van de handknijpkracht en geslacht (p=0,00) en de waarde van de handknijpkracht en dialysesoort (p=0,03). Er is geen significant verschil tussen de waarde van de handknijpkracht en dialyseduur (p=0,60). Tevens is er geen significant verschil tussen de overleven en de handknijpkracht (p=0,42).

Conclusie

Leeftijd, geslacht en dialysesoort beïnvloeden de handknijpkrachtwaarden. Dialyseduur doet dit niet. Er is geen verband tussen een lage handknijpkracht en mortaliteit, terwijl uit literatuur blijkt dat hier wel een verband tussen is.

Aanbeveling

Het advies voor de praktijk luidt als volgt. Voor zowel het kunnen opstellen van referentiewaarden als voor het onderzoeken van de mortaliteit zal er meer onderzoek gedaan moeten worden. 67 patiënten is namelijk te weinig om een duidelijk verband aan te tonen met betrekking tot de mortaliteit. Tevens dient er geavanceerder statistiekmethoden te worden toegepast in de vorm van een mixed model. Wanneer dit wordt gebruikt kan er ook gekeken worden of de vier parameters onderling elkaar ook beïnvloeden en in welke mate dat gebeurt. Ten slotte zijn er ook meer patiënten nodig om referentiewaarden te kunnen opstellen. Om deze reden is er een datamatrix opgesteld, waarin de diëtisten van het Catharina Ziekenhuis de handknijpkrachtwaarden de komende jaren bijhouden. Hiermee kan er een nieuw onderzoek gestart worden.
**TPE(TOTAL PLASMA EXCHANGE) MET CITRAAT SPECIALISTENWERK!**

**Inleiding**

In april 2012 zijn we op de dialyseafdeling van het Radboudumc gestart met TPE met heparine antistolling door middel van filtratietechnieken (plasmafiltratie).

Hier werd veel filterstolling gezien. Vaak waren er meerdere filters nodig per behandeling. Patiënten met verhoogde bloedingsneiging werden bij voorkeur niet behandeld met de filtratietechniek maar met de centrifuge techniek (plasmaafwerking) op de Hemaferese afdeling. Daar maakte men gebruik van citraat.


**Methoden**

Wij zijn begonnen met het formeren van een werkgroep. Hier zaten de volgende personen in:
- Nefroloog
- Twee dialyseverpleegkundigen
- Medewerker Firma Baxter

Er heeft een werkbezoek plaatsgevonden op de dialyseafdeling van het UMC Leiden. Hierna is het protocol van Leiden aangepast zodat deze inzetbaar was voor het Radboudumc. Alle verpleegkundigen zijn theoretisch geschoold. De eerste patiënt is behandeld volgens dit protocol. Na de behandeling werd geëvalueerd met patiënt, verpleegkundige en betrokken arts. Aan de hand van de bloeduitslagen werd besloten om bij de volgende behandeling de flowinstellingen te verhogen.

Hierna is men gestart met het inwerken van verpleegkundige in de praktijk, “training on the job”.

**Resultaten**

TPE met citraat laat aanmerkelijk minder stolling in het filter zien. Het aanbod van patiënten is laag en onvoorspelbaar. Hierdoor was het niet mogelijk om een team van 45 verpleegkundigen praktisch te scholen en hun vaardigheid te laten behouden met een patiënten aanbod van 148 per jaar. In de praktijk moesten patiënten geregeld worden overgedragen aan de afdeling hematologie of moest men noodgedwongen TPE met heparine uitvoeren. Met het team is besloten dat er een team van tien verpleegkundige wordt gevormd om deze behandeling uit te voeren. Dit team heeft extra theoretische en praktische scholing gehad. Met de nefroloog is afgesproken dat de TPE met citraat zoveel mogelijk overdag of in de vroege avond plaatsvindt.

**Conclusie**

TPE met citraat is geen complexe behandeling maar men moet deze behandeling regelmatig om de kennis en vaardigheden te kunnen onderhouden. Onze aanbeveling is om dit met een klein specialistisch team binnen de dialyseafdeling te doen. Wij zijn nog aan het onderzoeken hoeveel behandelingen een verpleegkundige per jaar moet uitvoeren om de vaardigheid te behouden. De behandel duur van de TPE met citraat hebben wij terug kunnen brengen tot minimaal 1 1/2 uur en een substitutiesnelheid van maximaal 3000ml/uur. Sneller wisselen is niet wenselijk in verband met citraat stapeling in het lichaam.