THE USE OF N-of-1 TRIALS WITH PROMs TO INDIVIDUALIZE TREATMENT IN PATIENTS WITH RENAL MAGNESIUM WASTING

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Background:
Patients with tubular disorders which cause chronic renal magnesium wasting and hypomagnesemia are rare. They require lifelong supplementation treatment with magnesium salts. Because of the paucity of randomized controlled trials in these rare disorders, the optimal treatment is not known and persistence of symptoms or side-effects are often encountered. In this study we used the N-of-1 methodology to compare different magnesium salts in the treatment of patients with therapy-resistant clinical symptoms attributed to the renal magnesium wasting.

Methods:
We performed randomized, double-blinded multi-crossover trials within individual patients which compared magnesium-gluconate, magnesium-aspartate and magnesium-lactate in four-week treatment blocks during a total of 36 weeks (3x3 treatment blocks). Main outcome parameters were patient reported outcome measures (PROMs). At two weeks intervals, patients completed a personalized questionnaire and a general quality-of-life questionnaire (Short-Form 36-Item Health Status Survey). In addition, laboratory investigations were performed with determination of serum magnesium, potassium and creatinine.

Results:
Thus far, we evaluated four patients with therapy-resistant renal hypomagnesemia. Based on PROMs, the N-of-1 trials were able to identify a superior treatment in three out of four patients. The three different magnesium salts did not result in significantly different serum magnesium levels, nor did serum magnesium correlate well with PROMs. The magnesium salt that was superior for the individual patient was not the same for all patients.

Conclusion:
N-of-1 trials are an elegant method to determine the best treatment strategy for the individual patient in an evidence-based fashion. They are particularly useful to assess treatment effects in stable and chronic diseases in relation to patient-reported outcome measures. In nephrology, this methodology for comparative effectiveness evaluation can directly impact on individual decision making, e.g. in rare renal tubular disorders.
EFFECTS OF THE HIGH FLOW ARTERIO-VENOUS FISTULA ON RIGHT VENTRICULAR CONTRACTILITY IN HEMODIALYSIS PATIENTS

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Abstract

Background: The presence of an arterio-venous fistula (AVF) in hemodialysis patients is a source of constant volume overload. The inability of the right ventricle (RV) to adapt to these conditions leaves it more susceptible to dysfunction as compared to the left ventricle (LV). Early recognition of RV failure in this population, especially in relation to the AVF flow, could contribute towards improved and personalized therapeutic strategies.

In this study we utilize tissue-tracking cardiac magnetic resonance to describe the relation between AVF flow and RV contractility. It is hypothesized that the RV contractility will be decreased in patients with a high flow AVF due to volume overload related RV dysfunction.

Methods: Hemodialysis patients (n=11) and age-matched controls (n=5) underwent CMR. Acquisitions were obtained prior to and after dialysis to distinguish between the effects of AVF flow and volume status (fluid overload). The patients were divided in Group 1 (low flow, <1000ml/min) and Group 2 (high flow, >1000ml/min) based on the AVF flow measured using ultrasonography. Global longitudinal (GLS), global circumferential (GCS) and global radial strain (GRS) of the RV were calculated with the tissue-tracking module of Circle Cardiovascular Imaging using short- and long-axis cine images.

Results: The contractility parameters between the groups were similar prior to dialysis. After dialysis, there were no significant changes 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 (-16.5±3.0% vs. -28.1±4.4%, P<0.05) and GRS (31.0±8.5% vs. 71.7±23.7%, P<0.05) after the dialysis session. No significant change was observed for the GCS between the control group and group 2 (-3.6±10.6% vs. -15.6±3.5%, P=0.06).

Conclusion: These findings suggest that patients with high AVF flow have significantly lower contractile parameters and thereby have at an increased risk for developing right ventricular dysfunction. Tissue-tracking analysis offers the possibility to detect subtle early changes in right ventricular contractility and could have significant therapeutic consequences in this patient group.

Keywords: x
2. **Morphology**

Control  < 1000ml/min  > 1000ml/min

* = Right Ventricle, + = Left Ventricle

**Strains**

Strain [%]

Time (ms)
IMPACT OF ARTERIOVENOUS 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
SEVERE VITAMIN C DEFICIENCY IN END-STAGE RENAL DISEASE PATIENTS

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Background
End-Stage Renal Disease (ESRD) patients suffer from various physical complaints and laboratory abnormalities. Vitamin C (vitC) deficiency may play a causal role and contribute to cramping, inflammation, iron deficiency, and immunity disorders. Unfortunately, there is scarce information on vitC deficiency and vitC intake in ESRD patients. Moreover, the prevalence of vitC deficiency and its related symptoms in ESRD patients have not yet been systematically examined. The goal of this study is to assess the prevalence of vitC deficiency in ESRD subgroups and to examine the relationship between vitC plasma level, dietary vitC intake, gender, age, and dialysis modality.

Methods
A cross-sectional observational study including 41 predialysis (Predx), 42 renal transplant (NTx), 41 conventional hemodialysis (CHD), 11 nocturnal in-center hemodialysis (NCHD), 10 peritoneal dialysis patients (PD), and 93 healthy controls (HC) of >18 years without active gastro-intestinal or oncologic diseases. Main study parameters are vitC plasma levels in the Predx, NTx, PD and HC groups, and vitC plasma and dialysate levels before, during, and after dialysis in the CHD and NCHD group. The vitC status was defined and divided into three groups: adequate (>35 µmol/L), inadequate (>10-35 µmol/L) and deficient (≤10 µmol/L). In addition, a 24-hours dietary recall of the participants was assessed. ≤75 mg vitC was considered as inadequate vitC intake. Multiple parametric and non-parametric tests were performed to examine the relationship between patient characteristics and vitC plasma level and dietary vitC intake.

Results

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Predx (n=41)</th>
<th>Ntx (n=42)</th>
<th>CHD (n=41)</th>
<th>NCHD (n=11)</th>
<th>PD (n=10)</th>
<th>P-value</th>
<th>Total ESRD (n=145)</th>
<th>HC (n=93)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>56%</td>
<td>57%</td>
<td>63%</td>
<td>82%</td>
<td>60%</td>
<td>0.597</td>
<td>61%</td>
<td>52%</td>
<td>0.167</td>
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<tr>
<td>Age (y)</td>
<td>63 ± 11</td>
<td>58 ± 12</td>
<td>62 ± 17</td>
<td>53 ± 14</td>
<td>63 ± 18</td>
<td>0.113</td>
<td>60 ± 14</td>
<td>60 ± 10</td>
<td>0.966</td>
</tr>
<tr>
<td>Vitamin C status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deficient ≤10 µmol/L</td>
<td>17%</td>
<td>7%</td>
<td>12%</td>
<td>9%</td>
<td>10%</td>
<td>0.718</td>
<td>12%</td>
<td>1%</td>
<td>0.002*</td>
</tr>
<tr>
<td>Inadequate &gt;10-35µmol/L</td>
<td>63%</td>
<td>41%</td>
<td>49%</td>
<td>64%</td>
<td>20%</td>
<td>0.063</td>
<td>50%</td>
<td>33%</td>
<td>0.013*</td>
</tr>
<tr>
<td>Adequate &gt;35 µmol/L</td>
<td>20%</td>
<td>52%</td>
<td>39%</td>
<td>27%</td>
<td>70%</td>
<td>0.006*</td>
<td>39%</td>
<td>66%</td>
<td>0.000*</td>
</tr>
<tr>
<td>Vitamin C intake (24-hours recall)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≤75 mg/day</td>
<td>56%</td>
<td>55%</td>
<td>68%</td>
<td>55%</td>
<td>90%</td>
<td>0.218</td>
<td>61%</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
VitC plasma significantly decreased during HD-treatment (p<0.001) and vitC is detected in dialysate. As reported before in the general population, men and smokers had significantly lower vitC plasma levels (respectively p=0.002 and p=0.023). Age showed no relationship with vitC plasma. A weak association between VitC intake and plasma was found (r=0.39; p=0.000).

Conclusion
Inadequate vitC-status and -intake is common in ESRD patients, with marked differences between mode of treatment. The high prevalence of vitC deficiency in Predx patients challenges the common belief that VitC loss during HD is the main source of poor vitC status. Whereas inadequate intake is common, it does not fully explain the high prevalence of inadequate of deficient vitC status. Further research is needed to examine the causes and mechanism of vitamin C deficiency in ESRD patients.
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 apha1 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.
LIVING DONOR TRANSPLANTATION LEADS TO A MAJOR IMPROVEMENT IN PHYSICAL FUNCTIONING, WHICH IS NOT PARALLELED BY CHANGES IN PHYSICAL ACTIVITY AND BODY COMPOSITION

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ABSTRACT

Background: Living donor transplantation is the most optimal method to reverse the uremic state and is also expected to lead to improvements in the physical health domain. Previous studies however have shown only limited changes in physical activity (PA) and a predominant increase in body fat mass, or were performed in patients with suboptimal renal function. As prospective studies combining physical functioning, PA and lean tissue mass (LTM) are scarce after renal transplantation (rTx), we aimed to study differences in these parameters between renal transplant patients and their living donors, and to study changes in recipients and donors in the first year after rTx/donation.

Methods: This was a cross-sectional and prospective longitudinal analysis of 22 renal transplant patients and 22 healthy kidney donors. Physical functioning was assessed by handgrip strength (HGS) and by the physical domains of health (physical functioning (SF-36 PF) and physical component summary (PCS) score) of the SF-36, LTM was measured by the Body Composition Monitor©, and PA was measured by a SenseWear™ pro3. Measurements were assessed prior to rTx/donation. rTx recipients had follow-up visits 1, 3, 6 and 12 months after rTx, donors had follow-up visits 3 and 12 months after rTx. PA was measured prior to and 6 months after rTx in the recipients, and prior to, 3 and 12 months after donation.

Results: At baseline, rTx recipients had significantly lower scores for HGS as compared with their donors after adjustment for differences in sex and body weight (-4.7 kg, 95% CI -9.3 to -0.1; p=0.047). HGS significantly increased in the first year after rTx (median ΔHGS: +4.5kg [0.8-8.3], p<0.001). SF-36 PF (46.1 [41.8-52.5] vs. 56.8 [52.0-56.8]), and PCS scores (46.3 [38.1-51.2] vs. 56.3 [54.3-57.8]) were significantly lower in rTx recipients (p<0.001). PA parameters, which were also significantly lower as compared with healthy donors: (number of steps: 6003.0 [3608.0-10429.0] vs. 12711 [9460.5-15194.0], p=0.004), did not change in the first 6 months after rTx. Body weight increased significantly 12 months after rTx (median: +1.7kg [-0.8-4.6], p=0.001), but no significant increase in LTM was observed. For healthy donors no significant changes in these parameters were observed, with exception of SF-36 PF, which significantly declined in the first year after donation (median ΔSF-36 PF: 0.0 [-2.1-0.0], p=0.049).

Conclusion: Physical functioning showed major improvements reaching levels of healthy kidney donors already 6 months after living rTx, which was not paralleled by an increase in PA or LTM. Therefore, reversal of the uremic state appears to have independent positive effects on physical functioning, with the potential of even further improvement by exercise training programs.
SKIN AUTOFLUORESCENCE LEVELS AND BIOMARKERS OF ENDOTHELIAL DYSFUNCTION AND LOW-GRAD 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 CU™. 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.
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.
EXCESSIVE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS HAVE A DIFFERENT ROLE IN THE PATHOGENESIS OF ANCA-ASSOCIATED VASCULITIS 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 (CitH3) 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.
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.
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.
HIGH PROTEIN INTAKE ACCELERATES KIDNEY FUNCTION DECLINE IN POST-MYOCARDIAL INFARCTION PATIENTS: THE ALPHA OMEGA COHORT STUDY

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Abstract:

Background: In post-myocardial infarction (MI) patients the rate of kidney function decline is doubled compared to the general population. Restriction of protein intake may retard kidney function decline in patients at risk for chronic kidney disease progression. Little is known about the effect of protein restriction or type of protein in post-MI patients with normal or mildly impaired kidney function. Therefore, the aim of this study was to assess the extent to which high total, animal, or plant-based protein intake is a risk factor for accelerated kidney function decline in older stable post-MI patients.

Methods: We included 2426 post-MI patients, aged 60-80 years (79% men), from the Alpha Omega Cohort. Dietary data were collected using a 203-item food frequency questionnaire. We estimated glomerular filtration rate (eGFR) based on serum cystatin C alone and combined serum creatinine-cystatin C at baseline and after 41 months, using the CKD-EPI equations. We investigated the association between protein intake (total, animal and plant) per g/kg ideal body weight and annual kidney function decline by multivariable linear regression.

Results: Of all patients, 16% were current smokers, 19% had diabetes, 56% had blood pressure ≥140/90 mmHg, and 23% were obese. Mean (SD) intake of total, animal and plant-based protein was 71 (19), 43 (15) and 27 (8) g per day, respectively, representing 16%, 10% and 6% of total energy intake. At baseline, mean (SD) cystatin C based eGFR was 81.5 (19.5) mL/min/1.73m². The mean annual cystatin C based eGFR decline was -1.30 (3.16) mL/min/1.73m². After multivariable adjustment, including age, sex, education, smoking, and total caloric and fat intake, each incremental g/kg daily intake of total, animal and plant-based protein was associated with an additional annual cystatin C based eGFR decline (95%-confidence interval) of -1.48 (-2.38; -0.58), -1.45 (-2.36; -0.54) and -1.76 (-4.11; 0.58) mL/min/1.73m², respectively. Results were consistent in subgroups based on baseline kidney function, use of renin-angiotensin system blockers, and diabetes. Results for eGFR based on combined creatinine-cystatin C were comparable.

Conclusion: Higher intake of total protein, plant as well as animal-based, was associated with more rapid kidney function decline in post-MI patients. Thus despite the fact that our patients received state-of-the-art drug treatment, we observed a beneficial effect of low-protein diet on kidney function.
ACUTE KIDNEY INJURY IN AMPHIA, IT IS AVOIDABLE.

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Background
Acute kidney injury (AKI) is characterized by a rapid reduction in kidney function with accumulation of waste products, oliguria and a failure of fluid, electrolyte and acid-base homeostasis. AKI is associated with increased length of hospital stay and a higher mortality rate. AKI is also a risk factor for the development of chronic kidney disease (CKD) and therefore an important factor to prevent.
We defined AKI according to KDIGO criteria, and investigated if AKI is avoidable.

Methods
We performed a retrospective, case-record study. Admissions of eleven medical specialties over a one week period were included. Incidence, severity, cause, and avoidance of AKI were analyzed.

Results
345 adult patients were hospitalized for at least one day (53% men). AKI incidence was 16.5%, of which 46% was present on admission, whereas 54% occurred during the hospital stay. The patients with AKI were older (median age 75y vs 68y, p<0.002), stayed longer (median hospital stay 7 vs 4 days, p<0.001), had more comorbidities such as hypertension, chronic renal failure, diabetes mellitus or heart failure (p<0.002), had a higher mortality rate (12% vs 3%, p<0.002) and had a decrease of their kidney function, 6-12 months after admission (median eGFR from 64 to 53 ml/min/1.73m², p<0.05). None of the patients required dialysis. AKI was primarily caused by prerenal factors (91%), which may be considered as potentially avoidable in 66.6%, because haemodynamically acting medication such as RAS-blockers, diuretics and NSAIDs were dosed too high or continued during hypovolemia.

Conclusion
AKI is common and often avoidable. More attention by all physicians for AKI is needed. Avoidance of RAS-blockers and nephrotoxic drugs and correcting volume depletion during acute illness are recommended to avoid AKI and prevent CKD.
Title:
An autologous cellularized in vivo engineered vascular graft capable of remodeling to a non-thrombogenic blood vessel upon arteriovenous grafting in adult goats.

Abstract
We have designed a method that allows the growth of cellularized tissue engineered blood vessels (TEBV's) to be grown in vivo, capable of remodelling towards a vascular phenotype upon vascular grafting. To induce a specific foreign body response, specifically designed polymeric rods are implanted subcutaneously in goats, resulting in the formation of an autologous fibrocellular tissue capsule (TC). One month after implantation, the polymeric rod is extracted whereupon TCs (length 6 cm, diameter 6.8 mm) were grafted as arteriovenous conduit between the carotid artery and jugular vein of the same goats. At time of grafting, the TCs were shown to have sufficient mechanical strength in terms of pressurised bursting pressure (2382 ± 129 mmHg), and suture retention strength (SRS: 1.97 ± 0.49 N). The arteriovenous grafts were harvested at 4 or 8 weeks after grafting. In an ex vivo whole blood perfusion system, the lumen of the vascular grafts was shown to be less thrombogenic compared to the initial TCs and ePTFE grafts, that were used as control. At explantation, the TEBV's were shown to be composed of desmin and myosin-heavy chain positive vascular smooth muscle cells that exerted a contractile phenotype as shown by a contractility assay. At 8 weeks after grafting, the entire graft was covered with an endothelial layer and abundant elastin fibers were present. Patency at 1 and 2 months was shown to be comparable to ePTFE. Here, we demonstrate the remodelling capacity of cellularized in vivo TEBV's, and their potential as replacements for synthetic AVG's.
A UREMIC GOAT MODEL CREATED BY SUBTOTAL RENAL ARTERY EMBOLIZATION

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BACKGROUND: There is an urgent need for a large animal model of (end stage) kidney disease for preclinical testing of novel renal replacement therapies. The aim of this study was to create stable uremia in goats via subtotal renal artery embolization.

METHODS: 3 Dutch white female goats were used because female goats are docile, have easily accessible neck veins and have body weights (70±4 kg) and distribution volumes comparable with humans. Polyvinyl alcohol particles were infused in branches of the renal artery aiming for embolization of ~75-90% of one kidney (Fig. 1) and complete embolization of the contralateral kidney. Gentamicin (10 mg/kg/day) was administered for 7-11 days to further increase urea and creatinine concentrations. Glomerular filtration rate (GFR) and estimated renal plasma flow (ERPF) were determined before and after embolization by measuring inulin and para-amino-hippuric acid clearances, resp. Plasma urea, creatinine and hemoglobin concentrations were monitored regularly for up to 11 months (range 3-11).

RESULTS: After an initial phase of acute kidney injury, plasma urea and creatinine concentrations stabilized within 2-3 weeks and remained elevated during follow up (pre vs. post-embolization: median 6 mM (range: 4.5-6.5) vs. 12 mM (range: 11-14) and 64 µM (range: 57-64) vs. 210 µM (range: 197-237), resp.). GFR and ERPF decreased by 51% (range: 22-64) and 59% (range: 54-70%), resp. Gentamicin induced (partially) reversible acute-on-chronic kidney injury with urea and creatinine concentrations rising to 42 mM (range: 28-56) and 1133 µM (range: 541-1724), resp., necessitating intermittent hemodialysis (3 sessions in 5 days) in 1 goat. After recovery, urea and creatinine concentrations stabilized at 15 mM (range: 10-19) and 298 µM (range: 227-370), resp. Hemoglobin concentrations decreased in all goats from 6.1 (range: 5.9-7.8) to 5.4 (range: 5.3-5.8) within 1-2 months and were responsive to erythropoietin and iron therapy.

CONCLUSION: A stable mildly uremic model was established in goats by subtotal renal artery embolization. Gentamicin can be used to temporarily aggravate uremia on demand.
Figure 1. Embolization of the inferior pole of the left kidney. Perfusion of blood vessels in the superior pole of the left kidney is visualized using x-ray imaging and a contrast material.
THREE-YEAR PATENCY AND RECURRENCE RATES OF REVISION USING DISTAL INFLOW WITH A VENOUS INTERPONATE FOR HIGH FLOW BRACHIAL ARTERY-BASED ARTERIOVENOUS FISTULA

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Abstract

BACKGROUND An upper arm arteriovenous fistula (AVF) occasionally develops high flow (>2L/min) which may be detrimental for cardiac function. Several operative techniques are available for flow reduction. Banding of the venous outflow tract seemed promising on the short term, but half of the banded patients developed recurrent high flow within one year. A more extensive surgical option is revision using distal inflow (RUDI) during which an interposition graft is placed between the venous outflow tract and an artery more distally located than the initial anastomosis. RUDI effectively reduces flow of a high flow access (HFA) in the short term and is also popularized for treatment of hemodialysis access-induced distal ischemia (HAIDI). The long term efficacy of RUDI is unknown. Aim was to report on three-year RUDI patency and recurrence rates for HFA with and without HAIDI.

METHODS Patients that underwent a RUDI using greater saphenous vein (GSV) interposition for HFA -defined as access flows >2L/min on at least two consecutive measurements using a dilution technique- between March 2011 and October 2017 in three facilities were eligible for study. HAIDI was diagnosed as proposed in a recent consensus meeting. Data were retrospectively gathered from local electronic patient files. Rates of patency and high flow recurrence were analysed using Kaplan-Meier analysis.

RESULTS During the six-years observation period, 21 patients were studied (seven females, 54 years ±3). Fourteen had uncomplicated HFA (HFA-group) whereas seven had additional complaints of HAIDI (HFA/HAIDI-group). Immediately postoperative, access flows decreased threefold (3120mL/min ±171 vs. 1170mL/min ±87, P<.001). Overall three-year primary patency was 48% ±12 (HFA, 55% ±15 vs. HAIDI/HFA, 29% ±17; P=.042, figure 1a.) whereas secondary patency was identical in both groups (overall, 84% ±9, figure 1b). During twelve percutaneous transluminal angioplasty sessions in nine patients, a total of sixteen significant stenoses was treated of which half were located in the GSV-interponate. Other interventions were thrombectomy (n=7, 3 patients) and revision with a new interponate (n=3). After three years, 51% ±12 were free of high flow (HFA, 32% ±13 vs. HAIDI/HFA, 100%, P=.018, figure 1c). High immediate postoperative access flow predicted recurrence (OR 1.004 [1.000-1.007]; P=.044). Furthermore, patients with recurrence were 12 years younger than those without (P=.055).
CONCLUSION  A RUDI-procedure with GSV interposition for HFA offers acceptable patency rates after three years although re-interventions are frequently required. High immediate postoperative access flows and young age are associated with recurrent high flow.
ENDOGLIN MEDIATES VEGF-A-INDUCED ENDOTHELIAL CELL ACTIVATION BY REGULATING AKT-SIGNALING

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Background
Diabetic nephropathy is characterized by microvascular injury driven by hyperglycemia and enhanced growth factor production. Increased VEGF-A expression causes an angiogenic imbalance, resulting in endothelial activation and dysfunction. Endothelial activation leads to adhesion and subsequent infiltration of inflammatory cells, which promotes renal damage. Endoglin is crucial for angiogenesis and vascular development, and is associated with endothelial activation and inflammation in animal models of renal disease. A link between endoglin and VEGF-A signaling has been proposed, but the mechanism is unknown. Here, we investigated whether reducing endothelial endoglin expression affects VEGF-A-induced endothelial activation and monocyte adhesion in vitro, and if so, which intracellular signaling pathways are involved. Furthermore, we investigated which glomerular cells express endoglin and whether glomerular endoglin expression is associated with endothelial activation in patients with diabetic nephropathy.

Methods
Immortalized endothelial cells with either wild type or reduced endoglin expression were used to investigate endothelial activation, VEGFR2 downstream signaling and monocyte adhesion after stimulation with VEGF-A. Double-labeled immunofluorescence was performed for endoglin and CD31 or VCAM-1 on biopsies of patients with diabetic nephropathy. Glomerular endoglin levels and expression of the endothelial activation marker VCAM-1 in biopsies were studied by immunohistochemistry. Data were analyzed using a Student’s t-test or a Mixed Model Regression Analysis. Differences were considered significant at p<0.05.

Results
Lowering endoglin expression in endothelial cells in vitro significantly impaired VEGF-A-mediated induction of activation markers VCAM1 and SELE, and significantly reduced monocyte adhesion (p<0.05). This was mediated by increased phosphorylation of Akt, thereby inhibiting ATF-2-induced transcription of VCAM1. In diabetic patients, endoglin was primarily expressed in glomerular endothelial cells, VCAM-1 expression co-localized with endoglin. Furthermore, glomerular VCAM-1 was significantly increased in patients with diabetic nephropathy (p<0.05) and correlated with glomerular endoglin levels (p<0.001).

Conclusion
Targeting endoglin function is a promising future strategy to inhibit endothelial activation in order to interfere with inflammation and the progression of diabetic nephropathy.
CLUSTERIN IS INCREASED IN GLOMERULI OF PATIENTS WITH DIABETIC NEPHROPATHY AND AFTER INDUCTION OF DAMAGE IN PODOCYTES IN VITRO

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Background

Clusterin is a glycoprotein which is ubiquitously expressed in many tissues, including the kidney. It is demonstrated that clusterin plays a role in apoptotic processes, and it is suggested to have protective properties on cells. The expression of clusterin has been reported to be up-regulated in diverse kidney injuries. In this study, we investigated whether clusterin is upregulated in glomeruli of diabetic nephropathy (DN), where clusterin is expressed in the glomeruli, and how clusterin is regulated under diabetic conditions.

Methods

Clusterin mRNA analysis was performed on kidney cortex and micro-dissected glomeruli from patients with DN (n=24), non-diabetic subjects were used as control (n=11). Clusterin protein expression was assessed by immunohistochemistry on renal tissue from patients with DN and non-diabetic subjects. Kidneys of streptozotocin-induced diabetic mice (n=10) and non-diabetic control mice (n=10) were sequentially stained for clusterin and WT1 (a podocyte marker). Furthermore, human podocytes (Moin Saleem, Bristol, UK) were cultured and incubated with glucose, VEGF-A, angiotensin II and puromycin aminonucleoside (PAN). qPCR was performed to investigate the regulation of clusterin under these diabetic conditions.

Results

Compared to non-diabetic subjects, clusterin mRNA expression was significantly increased in both glomeruli (2.3 times) and whole kidney lysates (3.6 times) of patients with DN (p<0.05). Clusterin protein levels were also increased in glomeruli of patients with DN compared to non-diabetic subjects(p<0.05). Similar results were found in glomeruli of diabetic mice compared to non-diabetic control mice (p<0.05). Interestingly, clusterin partly co-localised with WT-1 in glomeruli of mice. Glucose, VEGF-A, and angiotensin II stimulation did not increase the clusterin mRNA expression in podocytes, whereas PAN-stimulation significantly increased the clusterin mRNA expression (p<0.05) in vitro.

Conclusion

Our data show that clusterin is increased in glomeruli of patients with DN and in podocytes after PAN-induced damage in vitro. Further studies have to elucidate whether clusterin has protective effects on podocytes upon damage during the development of DN.
INCIDENCE OF BIOPSY-PROVEN RENAL DISEASES BETWEEN 1992 AND 2013 IN THE NETHERLANDS

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Background

In the Netherlands, renal diseases incidence data are lacking. As a step towards obtaining incidence data, we are the first to analyze the nation-wide incidence rate (IR) of biopsy-proven renal diseases. We questioned: “What are the five most common biopsy-proven renal diseases in the Netherlands and have their IR changed during the study period?” and “Has the percentage of biopsies rendering a diagnosis increased?”

Methods

We retrospectively classified all non-oncological and non-transplant renal biopsies performed in the Netherlands between 1992 and 2013, based on the pathology report in ‘The nationwide network and registry of histo- and cytopathology in the Netherlands’ (PALGA Foundation). We used linear models to investigate IR (cases per million of the population per year, pmp/y) change between 1992 and 2013.

Results

Our PALGA search retrieved 31,219 renal biopsies, from 30,178 patients, of which 58% were male, mean age of 50 years. IgA nephropathy (IgAN) had the highest mean IR: 9 pmp/y, followed by pauci-immune crescentic glomerulonephritis (GPA) 7 pmp/y, membranous glomerulopathy (MGN) 7 pmp/y, focal segmental glomerulosclerosis (FSGS) 7 pmp/y and tubulointerstitial nephritis (TIN) 6 pmp/y. All IR increased during the study period (slope/y: 0.18, 0.22, 0.09, 0.20 and 0.20, respectively). The biopsy IR also increased (slope: 0.75, mean 88.4 pmp/y), as did the percentage of representative biopsies and biopsies where a final diagnosis was rendered (slope/y: 0.28 and 0.14, mean 91% and 87%, respectively).

Conclusion

Our results show that in the Netherlands IgAN, GPA, MGN, FSGS and TIN are the five most common biopsy-proven renal diseases and that their incidence increased between 1992 and 2013. However, so did the biopsy rate, and the percentage of biopsies rendering a diagnosis.

We provide nation-wide incidence data for biopsy proven non-tumor renal diseases, from the largest described renal biopsy cohort worldwide. These data could help prioritize kidney research and renal disease prevention, ultimately improving kidney disease outcomes.
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-url)

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.

Figure 1. dp-ucMGP levels as boxplots in 36 kidney transplant recipients and 113 dialysis patients.
THE BIOBANK OF NEPHROLOGICAL DISEASES IN THE NETHERLANDS: CURRENT STATUS AND FUTURE PERSPECTIVES

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Background
Chronic kidney disease (CKD) affects over 10% of the Western population. Despite advancements in prevention and treatment of CKD, patients still progress to end-stage renal disease. The Biobank of Nephrological Diseases in the Netherlands (BIND-NL), part of the government-funded String of Pearls Initiative (PSI), is intended to facilitate CKD-related research, and to provide insight in routine care for clinical governance or quality improvement projects.

Methods
Participants were recruited in all 8 Dutch University Medical Centers. Study visits were scheduled in combination with routine outpatient clinic visits. Blood pressure was measured manually in sitting position. Socio-demographic characteristics, lifestyle behavior and medical history were assessed with self-administered questionnaires. Biochemical parameters were imported directly from the hospital information systems. Biobank samples were collected according to the Standard Operating Procedures (SOP) of the PSI initiative. Data are shown as mean±SD or as median [IQR] for normally distributed or skewed data, respectively.

Results
Between 2010 and 2016, 1947 participants were enrolled. Mean age was 56±16 years; 61% of the participants are male. Blood and DNA samples of 1839 participants and spot urine samples of 1810 individuals were stored for future analysis. Median eGFR at baseline was 43 [27-70] ml/min*1.73m², with a median urine protein excretion of 584 [233-2099] mg/24 hours. The most common etiologies of CKD (by ERA-EDTA diagnosis group) were glomerulonephritis (27.4%), cystic kidney disease (11.9%), renal vascular disease (11.4%), multisystem disease (10.6%), and interstitial nephritis (7.4%). The mean blood pressure was 136±20 / 81±11 mmHg. The majority of the cohort (85.2%) used antihypertensive drugs, with a median of 2 [1-3] different antihypertensive drugs. 69.5% of all participants used a RAAS-blocker. Mean BMI was 21±5 kg/m², and 14.8% of the cohort had type 2 diabetes mellitus. The mean total/HDL cholesterol ratio was 4.1±1.5, 44.5% used a statin, and 16.1% of participants were active smokers.

Conclusion
The String of Pearls Initiative (PSI) Biobank of Nephrological Diseases is a prospective cohort and biobank, uniquely reflecting the Dutch nephrology outpatient setting in University Medical Centers of the Netherlands. The available data and biomaterials may contribute to answering a wide variety of inquiries; investigators are invited to submit project proposals.
ADVERSE PREGNANCY OUTCOMES IN WOMEN WITH CHRONIC KIDNEY DISEASE

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Background: Pregnant women with chronic kidney disease (CKD) have an increased risk for adverse pregnancy outcomes (APO), such as prematurity, low birth weight, pre-eclampsia and renal function decline. Previous cohorts show that the risk for APO is inversely related to renal function and already rises in CKD stage 1. Pregnancy in women with CKD is becoming more common over the last decades underlining the need for reliable data on these adverse pregnancy- and renal outcomes. We therefore investigated the risk for APO in our cohort of women with CKD.

Methods: We conducted an observational, retrospective cohort study of subsequent pregnant women with CKD stage 1-3 from 2011 to 2017 (ongoing, partly included). Data on pregnancy, renal function and APO were collected from electronic health records. CKD stage was defined according to the KDIGO guidelines. Diagnosis of pre-eclampsia was made when the subject fulfilled the international criteria. CKD shift was defined as an upward shift in CKD stage due to renal function decline by comparing pre-conception and 3rd trimester serum creatinin.

Results: Present, we included 48 subsequent women with CKD stage 1-3. Results are shown in table 1. There were only small differences in birth weight, gestational age and other APO between CKD stage 1 and stage 2. However, women with CKD stage 3 showed worse maternal and fetal pregnancy outcomes with a significantly lower birth weight and more premature deliveries. NICU admission, caesarean section and pre-eclampsia occurred more frequently in the CKD stage 3 group.

Conclusion: These preliminary results from our expanding cohort show that women with CKD have an increased risk for APO. The incidence and severity increases with CKD stage. Our results are in line with previously reported cohorts of pregnant women with CKD. In the endeavor to improve this outcome we instituted a multidisciplinary approach, involving a specialized nephrologist and -obstetrician, to tightly monitor these high-risk pregnancies. Ongoing data collection and measuring of patient reported outcome and experience measures (PROMs and PREMs) through questionnaires will learn whether this approach can indeed improve maternal and fetal outcome as well as patient satisfaction in the future.

<table>
<thead>
<tr>
<th>CKD stage 1 (n=22)</th>
<th>Birth weight (mean, std)</th>
<th>Gestational age (mean, std)</th>
<th>Preterm delivery &lt; 37 weeks (%)</th>
<th>NICU admission (%)</th>
<th>Caesarean section (%)</th>
<th>Pre-eclampsia (%)</th>
<th>CKD stage shift (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3009 g (± 534)</td>
<td>37.9 wks (± 2.1)</td>
<td>6 (27)</td>
<td>2 (9)</td>
<td>8 (36)</td>
<td>5 (23)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>CKD stage 2 (n=16)</td>
<td>3075 g (± 591)</td>
<td>38.7 wks (±1.8)</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>3 (19)</td>
<td>4 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>CKD stage 3 (n=10)</td>
<td>2101 g (± 941)</td>
<td>35.1 wks (± 4.4)</td>
<td>6 (60)</td>
<td>3 (30)</td>
<td>5 (50)</td>
<td>4 (40)</td>
<td>1 (10)</td>
</tr>
</tbody>
</table>

Table 1 Adverse pregnancy outcomes in 48 subsequent women with CKD from 2011-2017 in the University Medical Center Utrecht
PIOGLITAZONE AND TOLVAPTAN IN A MOUSE MODEL FOR ADPKD
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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.
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 $\mu$g/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.
CONVERTASE-STABILIZING FACTORS IN PATIENTS WITH COMPLEMENT-MEDIATED RENAL DISEASES

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Background
Alternative pathway (AP) overactivation of the complement system is associated with the renal diseases atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G). C3 nephritic factors (C3NeF) are found in >50% of C3G patients and play an important role in pathogenesis by stabilizing the key enzyme of complement, the AP C3 convertase. In occasional C3G cases, also C4NeF, autoantibodies stabilizing classical pathway (CP) C3 convertases, have been detected. However, the reliability of currently used assays to detect C3NeF and C4NeF is limited. Therefore, we validated two prototype hemolytic methods that measure convertase stability in whole serum and optimized them for robust detection and characterization of convertase-stabilizing factors such as C3NeF and C4NeF in patient cohorts.

Methods
AP and CP convertase stability were measured in hemolytic assays using the C5-blocker eculizumab to separate the AP/CP into two steps: formation of C3/C5 convertases by test sera in a time-variable step 1 and formation of lytic membrane attack complexes in a standardized step 2 for readout. Samples of 15 controls and 27 C3G patients were analyzed for C3NeF. In addition, AP convertase stability was assessed in a family with complement Factor B (FB) mutation p.Lys323Glu and aHUS, a disease not linked to C3NeF. Screening for C4NeF was performed in a more heterogeneous cohort of 46 patients with (suspicion of) C3G, aHUS, or other complement-mediated renal diseases.

Results
Using healthy control sera, the normal AP and CP convertase activity profiles were determined: in all controls activity returned to background levels at t=30 min (AP) or t=10 min (CP). When serum or purified Ig fractions containing C3NeF or C4NeF were added to control serum, convertase stability was increased after 30 (AP) and 10 min (CP) of incubation. Thus, detectable convertase activity at t=30 min (AP) and t=10 (CP) were chosen as markers for presence of convertase-stabilizing factors such as C3NeF and C4NeF respectively. In the C3G cohort, 16 out of 27 (59%) patients showed increased AP convertase stability indicating C3NeF presence. Interestingly, prolonged AP convertase activity was also detected in an aHUS family and segregated with the FB mutation. Prolonged CP convertase activity was observed in 2 out of 46 patients (4%). One of them was also positive for C3NeF; we confirmed presence of stabilizing Igs in both the AP and CP convertase activity assay. Interestingly, the Ig fraction of the other positive patient did not show convertase stabilization, indicating another serum factor than C4NeF or a genetic aberration underlies the increased stability.

Conclusion
We present optimization of two robust and reliable assays to detect and characterize convertase-stabilizing factors including C3NeF, C4NeF, and some mutations in patients with various complement-mediated renal diseases. This study may give insight in disease pathogenesis and treatment strategies for these patients.
CONDITIONALLY IMMORTALIZED PROXIMAL TUBULE EPITHELIAL CELLS DO NOT POSSESS TUMORIGENIC POTENTIAL SUGGESTING A SAFE USE IN RENAL REPLACEMENT THERAPY

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Background: Novel renal replacement treatments, such as a bioartificial kidney (BAK), hold great promise for the improvement of current hemodialysis. However, when developing a BAK, availability of functional cells and their safety are frequently encountered problems. The aim of this study was to evaluate the tumorigenic potential in vitro and in vivo of conditionally immortalized human proximal tubule epithelial cells (ciPTEC) for use in BAK.

Methods: The urine derived ciPTEC line was characterized for loss of contact-inhibition by focus formation assay and proliferation, as well as anchorage-independent growth, over a period of 4 weeks. In addition, the expression of temperature-sensitive SV40 large T antigen (SV40T), used for conditional immortalization, and its effect on cell cycle distribution were addressed. ciPTECs were examined for apoptosis-resistance in function of SV40T, as well as for invading ability in Matrigel™ extracellular matrix. Genomic stability was determined by assessing transgene integration sites using targeted locus amplification (TLA) technology along with effects on cell function. Finally, tumorigenic potential in vivo was evaluated in athymic nude rats (Hsd:RH-Foxn1nu) after injecting 10^6 cells (ciPTEC or HeLa, positive control) subcutaneously in the flank.

Results: Focus formation assay showed that ciPTEC grow in stable monolayers maintaining contact-inhibition after 4 week culture (<1% foci compared to >50% by HeLa cells; p<0.001). Cell cycle analysis confirmed this as >85% (p<0.001) of cells were present in G0/G1. Anchorage-independent growth of ciPTEC indicates that the cells do require anchorage for efficient growth (less than 1 colony per field, compared to 15 colonies for HeLa cells; p<0.001). SV40T expression was downregulated by more than 80% (p<0.001) after culturing cells at non-permissive temperature for 7 days. Moreover, apoptosis analysis suggested that cells are not apoptosis-resistant and that exposure to the MDM2 inhibitor, nutlin-3a, induced apoptosis in cells at non-permissive temperature, by stabilizing p53 levels. Only 7% of ciPTECs were able to migrate through the Matrigel™ basement membrane matrix. The TLA analysis showed that transgenes integrated in the intronic regions of six distinct genes in ciPTECs, including the EEA1 (Early endosome antigen 1). EEA1 immunofluorescence staining and albumin uptake assay confirmed that EEA1 expression and function regarding endocytosis were not affected, compared to the parent cell line. Finally, the in vivo study showed that over the 5 months follow-up ciPTECs did not form tumors when injected subcutaneously in athymic nude rats, whereas 50% of the HeLa cells injected animals developed subcutaneous anaplastic carcinomas.

Conclusion: CiPTECs do not show typical cancer cell-like behavior in vitro, nor tumorigenic potential in vivo, suggesting a safe use in BAK and encouraging its further development and pre-clinical testing.
LASER CAPTURE MICRODISSECTION AND LIQUID CHROMATOGRAPHY TANDEM-MASS SPECTROMETRY FOR SMALL AMOUNTS OF FORMALIN-FIXED PARAFFIN EMBEDDED KIDNEY TISSUE

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Background: Liquid chromatography tandem-mass spectrometry (LC-MS/MS) is a sensitive and specific technique for in depth protein identification but diagnostic kidney material is scarce, limiting the number of protein identifications. Only a few studies have used this technique for protein identification in formalin fixed paraffin embedded (FFPE) glomerular material and only one used a practical amount that can be used for diagnostic biopsies. Therefore, we optimized a work-flow of laser capture microdissection (LCM) of glomeruli, followed by LC-MS/MS on FFPE tissue for a minimal amount of tissue with a maximum protein yield.

Methods: One FFPE tissue block of a normal human kidney was utilised. Cross-sections of randomly selected kidney and glomeruli were collected from 6 µm sections, mounted on a 1.0 PEN membrane, using LCM. Protein extraction and digestion was performed using filter aided sample preparation (FASP) with polyethylene glycol (PEG) as a carrier, adapted from previously described methods for microdissected colon tissue. Samples were purified using mixed anion exchange solid phase extraction (MAX-SPE) and analysed using LC-MS/MS.

Results: Using the PEG-FASP protocol, we established the optimum amount of tissue to be 3 nl, based on 605 protein identifications, compared to 216 and 693 identifications in 1 and 10 nl tissue respectively. In a reproduction analysis of 5 experiments, using both random kidney and glomerular tissue, an average of 457 and 228 proteins were identified respectively. The method was able to distinguish glomerular samples from random kidney samples, as demonstrated by differentially identified proteins and clustering of glomerular and random kidney samples.

Conclusion: The relative number of proteins detected was comparable to or higher than reported in previous studies using FFPE glomerular tissue. The presented work-flow expands the potential for novel protein identification in glomerular diseases, while keeping the amount of tissue small enough for diagnostic practicality. Therefore, the combination of LCM followed by LC-MS/MS, using PEG-FASP and MAX-SPE for sample preparation, is a suitable and promising technique for diagnostic applications, especially when specific proteins are overexpressed or abundant within glomeruli.
IN OBESE ZSF1 RATS, FEMALES SHOW INCREASED SALT-SENSITIVITY COMPARED TO MALES

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Background
The obese Zucker fatty/spontaneously hypertensive heart failure F1 hybrid (ZSF1) rat has been proposed as a viable animal model to study the metabolic cardiorenal syndrome as these rats spontaneously develop diastolic heart failure and chronic kidney disease in the presence of obesity, hyperglycemia and hypertension. Risk factors associated with the metabolic syndrome correlate strongly with salt-sensitivity of blood pressure. In Dahl-salt sensitive rats, a non-obese rat strain commonly used in salt-induced hypertension, males were more susceptible to develop high blood pressures during high salt intake than females. Therefore this study aimed to investigate the effects of obesity and sex on salt-sensitivity in the obese ZSF1 rat model and their lean counterparts. We hypothesized that obesity and male sex would both promote salt-sensitive hypertension.

Methods
Male and female ZSF1 rats, lean as well obese (n=4-8/subgroup), were either implanted with a deoxycorticosterone acetate (DOCA) pellet and fed a high salt diet (6% NaCl) or with a placebo pellet and fed a normal salt diet from 19 weeks of age. Every two weeks, from 18 (i.e. prior to pellet implantation) to 26 weeks of age, systolic blood pressure (SBP) and 24-hour natriuresis were measured.

Results
Obese ZSF1 developed signs of metabolic syndrome, with high cholesterol and triglyceride levels. However, only male obese ZSF1 became hyperglycemic. SBP was higher in both obese compared to lean DOCA+6% salt groups (p<0.0001). Natriuresis was higher in male obese vs. lean DOCA+6% salt groups (p<0.0001). The SBP response to high salt intake occurred in a stepwise manner in all four DOCA+6% salt groups (with constant SBP from 22 to 24 weeks). Comparison of slopes of the natriuresis-pressure relations using 18 and 26 weeks data (figure) showed differences between male obese and lean (p<0.01) and female obese and lean ZSF1 rats (p<0.01), suggesting that obesity promotes salt-sensitivity. Additionally, slopes between obese males and females differed (p<0.01) suggesting that salt-sensitivity was most marked in female obese ZSF1 rats.

Conclusion
Our results in ZSF1 rats indicate i) a phased blood pressure response to high salt intake, ii) an adverse effect of obesity on salt-sensitivity, and iii) a further increased salt-sensitivity in obese females vs. obese males.
Figure. Natriuresis-pressure relations in ZSF1 rats corrected for mm tibia length (TL).
HEMODIALYSIS VERSUS PERITONEAL DIALYSIS AND BLEEDING RISK

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Background

Bleeding as a manifestation and complication of renal failure was already recognized in the 18th century. Several studies showed that hemodialysis patients have an increased bleeding risk compared with the general population. However, there is limited information whether bleeding risks are different for peritoneal dialysis patients and hemodialysis patients. From a clinical point of view, there could be a preferred dialysis modality for patients with bleeding problems. Therefore, the aim of this study was to investigate the association between dialysis modality and bleeding risk.

Methods

In total, 1745 incident dialysis patients from the NECOSAD study were prospectively followed for major bleeding events within three years of dialysis. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated for hemodialysis as compared with peritoneal dialysis using Cox proportional hazard analyses. Hazard ratios were adjusted for age, sex, primary kidney disease, antiplatelet drug use, vitamin K antagonist use, EPO use, prior history of bleeding, cardiovascular disease, systolic blood pressure, residual GFR and albumin levels.

Results

Of the 1745 dialysis patients, 1211 (69.4%) started with hemodialysis and 534 (30.6%) started with peritoneal dialysis. A total of 183 patients had a bleeding event during a median follow-up of 2.2 years (interquartile range 1.0-3.0), of which 13 were fatal. The incidence rate of bleeding within three years of follow-up was 52.3 per 1000 person-years for the total cohort. The bleeding rate was 60.8 per 1000 person-years for hemodialysis patients and 34.6 per 1000 person-years for peritoneal dialysis patients. The fatal bleeding rate was 5.1 per 1000 person-years for hemodialysis patients and 0.9 per 1000 person-years for peritoneal dialysis patients.

The crude hazard ratio of bleeding was 1.7 (95% CI 1.2-2.5) for hemodialysis patients as compared with peritoneal dialysis. Hemodialysis patients as compared with peritoneal dialysis patients had a 1.5-fold (95% CI 1.0-2.2) increased bleeding risk after adjustment for age, sex, primary kidney disease, prior history of bleeding and cardiovascular disease. After additional adjustment for antiplatelet drug use, vitamin K antagonist use, EPO use, systolic blood pressure, residual GFR and albumin levels, the HR did not change 1.5 (95% CI 1.0-2.4).

Conclusion

In this large prospective cohort of incidence dialysis patients, hemodialysis as compared with peritoneal dialysis was associated with an increased bleeding risk. An explanation could be the use of heparin for hemodialysis sessions to prevent clotting of dialysis lines and dialyzers. Future studies should examine whether starting or switching to peritoneal dialysis could be beneficial for patients with bleeding problems.
HEALTHCARE COSTS OF CHRONIC KIDNEY DISEASE, DIALYSIS AND KIDNEY TRANSPLANT PATIENTS COMPARED TO MATCHED CONTROLS

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Background Patients with chronic kidney disease (CKD) and renal replacement therapy (RRT) contribute significantly to healthcare expenditures. In this study we assessed the annual healthcare costs for patients with kidney disease (i.e. CKD stage 4 and 5, dialysis or kidney transplantation) in comparison with age, sex and social economic status (SES) matched controls, separately for three different age groups.

Methods Using Dutch health claims data we identified adult patients with CKD and RRT (i.e. dialysis or kidney transplantation). These patients were subdivided into three age categories, i.e. 19-44 years, 45-64 years and ≥65 years. For each of the patients groups 2 age, sex and SES matched controls were selected out of the general population. Costs data are healthcare costs per calendar-year. As the high treatment costs for RRT patients hampers the comparison with the control group, we differentiated hospital costs into costs directly and non-directly related to the CKD or RRT treatment.

Results Average annual costs: Persons with CKD in the youngest age category had 7 times higher annual costs than their matched controls (€9,416 vs. €1,425). This cost ratio decreased to 6 in the age category of 45-64 years (€12,384 vs. €2,226) and to 3 in the age category ≥65 years (€11,627 vs. €4,221). Young dialysis patients had 77 times higher costs compared to controls (€92,686 vs. €1,201). This cost ratio decreased to 21 (€90,808 vs. €4,292) in the oldest age category. Transplant patients aged between 19-44 years had 14 times higher costs than controls (€18,176 vs. €1,277). This cost ratio decreased to 6 (€20,743 vs. €3,774) in the age category ≥65 years.

Hospital costs non-directly related to treatment: Costs ratios in CKD patients are practically similar across age categories. However, in dialysis patients the cost ratio decreased from 13 (€7,368 vs. €587) to 3 (€8,476 vs €2,591) and in transplant patients from 7 (€4,058 vs. €591) to 3 (€7,085 vs. €2,382) in the youngest versus the oldest age group.

Conclusion Although annual health care costs of CKD, dialysis and transplant patients were much higher than their matched controls in all age categories, these differences in costs decreased remarkably with age. Higher costs in RRT patients are mainly driven by high treatment costs. When excluding these treatment costs, cost differences decreased but the decline with increasing age persists.

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WORLDWIDE HEALTH INSURANCE CLAIMS DATABASES IN KIDNEY RESEARCH AND A NEW INITIATIVE: THE DUTCH KIDNEY ATLAS

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Background Health insurance claims data may be a valuable source of medical information and give rise to new research opportunities. Nowadays, a number of health insurance claims databases are available for renal research. We present an overview of known claims databases across the world and focus on their database characteristics. A number of publications in renal medicine and type of research are presented. We will introduce the Dutch claims database and a new initiative to use this database for the development of a Dutch Kidney Atlas.

Methods Claims databases and their published papers were identified by internet and literature search. Only papers using claims data as their primary data source were selected and were classified into four study categories: validation, descriptive epidemiological, cost and outcome studies.

Results We identified 7 claims databases in 6 different countries using their data for publications in renal research (Canada, France, Japan, South Korea, Taiwan, USA). Almost all databases have full coverage of inhabitants, except Medicare (USA) which only includes people aged 65 years or older and patients with certain conditions like end-stage renal disease. We identified 8 studies testing the validity of claims data in identifying renal patients (chronic kidney disease N=4, dialysis N=3, transplantation N=1). Few studies were descriptive epidemiological studies or costs studies. Published studies on outcomes research were mainly focusing on survival differences or the risk of cardiovascular disease.

In the Netherlands, the Vektis database contains all health insurance claims on 99% of inhabitants. The Vektis database offers a unique opportunity to study patients with renal diseases and to observe regional differences for incorporation in the Dutch Kidney Atlas.

Conclusion Health insurance claims databases may offer important opportunities for studies on large populations of patients with (kidney) diseases and health outcomes in a non-experimental setting. Characteristics of claims databases should be taken into account when making international comparisons. The Dutch claims database offers a unique opportunity to design the Dutch Kidney Atlas on (regional differences in) risk factors, diagnoses, treatment, outcomes and costs of patients with renal diseases.
CLARIFICATION OF GENETIC VARIANTS IN URINE-DERIVED RENAL EPITHELIAL CELLS AND BLOOD FROM CILOPATHY PATIENTS

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Background

Nowadays, performing whole-exome sequencing (WES) is standard diagnostic practice to determine the cause of hereditary diseases. For ciliopathies and renal disorders about 40% of WES analyses result in a clear diagnosis. The remainder can roughly be divided into two; one half cannot be genetically explained at this moment and the other half are so called variants of unknown significance (VUS) resulting in an inconclusive diagnosis. Functional tests evaluating the effect of genetic variants on protein and cellular level will clarify the pathogenicity of VUS and improve diagnostics. Ideally these functional tests are performed on patient-derived cells that can be obtained in a non-invasive manner, which lead us to the use of urine-derived cells. Ciliopathies are rare hereditary disorders caused by dysfunction of the cilium and often involve renal failure. Urine-derived renal epithelial cells (URECs) form primary cilia allowing us to study cilium morphology and functionality of patients with a (suspected) ciliopathy. The aim of this project is to clarify the pathogenicity of genetic variants by performing functional tests in patient-derived cells.

Methods

Urine is collected from patients and specifically cultured to stimulate the growth of renal epithelial cells. Once sufficient cells have been obtained functional tests are performed: (1) In URECs cilium morphology, length, and the intraflagellar transport (IFT) complex are visualized and analyzed by means of immunofluorescence cytchemistry and (2) URECs and blood cells are used to study mRNA splicing.

Results

Thus far, we have collected urine samples from ciliopathy patients (with or without renal involvement). We studied the intraflagellar transport system in a patient with two heterozygous variants in IFT140, i.e. one nonsense and one missense variant. IFT140 encodes a protein involved in retrograde IFT. URECs from this patient showed abnormal IFT in comparison to healthy controls. In addition, we studied the patient’s IFT140 variants in a CRISPR knock down model and could validate the UREC results. Together this data confirmed the pathogenicity of the variants and thus the diagnosis of the patient. In another example we studied mRNA splicing of DYNC2H1 in a patient who had two heterozygous variants in this gene, i.e. one nonsense and one silent variant. The mRNA splicing assay revealed exon skipping and termination of the gene due to the silent variant. This data confirmed the pathogenicity of the DYNC2H1 variants and explained part of the patient’s phenotype.

Conclusion

The increasing number of diagnostic WES analyses lead to a rise in variants of which the pathogenicity is uncertain. The next step in genome diagnostics is to further evaluate these variants by means of functional tests on protein and cellular level. Functional tests in urine-derived patient cells have proven to be an attractive non-invasive procedure to facilitate accurate diagnosis of ciliopathy patients.
CREATININE SYNTHESIS RATE AND PHYSICAL PERFORMANCE IN DIALYSIS PATIENTS: ASSOCIATIONS WITH MUSCLE STRENGTH AND SELF-REPORTED PHYSICAL HEALTH

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Background
Urinary creatinine excretion reflecting endogenous creatinine synthesis rate (CSR) is an established measure of muscle mass in the general populations and in patients with chronic kidney disease. In dialysis patients, CSR has scarcely been studied since it requires dialysate collection. There is increasing data to suggest that CSR not only reflects muscle mass, but also muscle function. We aimed to study whether CSR is associated with muscle strength and self-reported physical health in dialysis patients.

Methods
Total daily CSR (dialytic removal plus, if applicable, urinary excretion, all normalised to 24h), handgrip strength, and self-reported physical health according the subscales of the Checklist Individual Strength and the Short Form-36 were assessed in 50 dialysis patients. Determinants of CSR, and associations of CSR, indexed to body surface area-indexed, with handgrip strength, and self-reported physical health were studied using multivariable linear regression models.

Results
Median [interquartile range] age was 69 [60-78] years. Mean CSR was higher in men than in women (9.5±3.3 mmol/24h versus 6.8±1.9 mmol/24h resp., P=0.007). Age, BMI, and plasma albumin concentration were positively associated with CSR. CSR was positively associated with handgrip strength (adjusted (a·β: 0.44 [95% CI: 0.19 to 0.72]), self-reported physical functioning (a·β: 0.54 [95% CI: 0.19 to 0.87]), and vitality (a·β: 0.35 [95%CI 0.003 to 0.70]). CSR was inversely associated with self-reported physical inactivity (adjusted β: -0.71 [95% CI: -1.01 to -0.40], fatigue (adjusted β: -0.62 [95% CI: -0.94 to -0.29]), and role limitation due to physical health (a·β: 0.40 [95% CI: 0.05 to 0.75]).

Conclusion
In dialysis patients, a greater CSR is associated with higher muscle strength, better physical functioning, vitality, and more physical activity, and with less fatigue, and role limitation due to physical health. Thus, CSR not only measures muscle mass, but also reflects muscle function and self-reported physical health in dialysis patients.
THE INFLUENCE OF SCREENING TOOLS ON THE TREATING PHYSICIAN’S ADVICE FOR EITHER RENAL REPLACEMENT THERAPY OR CONSERVATIVE MANAGEMENT IN ELDERLY PATIENTS

Background:
In a new Dutch guideline that should support healthcare providers in choosing between renal replacement therapy (RRT) and conservative management (CM) in elderly patients, it is recommended to perform a structured assessment of the patient before giving advice. This prospective observational cohort study examined whether the results of validated screening tools have an influence on the physician’s advice in favor of RRT or CM.

Methods:
Validated screening tools (MMSE, clock-drawing test, Fried frailty criteria, iADL, ADL and 6-month mortality on HD calculator) were used for structured assessment once between May and October 2017 in patients with progressive chronic kidney damage stage 4 and 5 and an age >70 years (n=17) or younger than 70 years with suspected frailty (n=3) in the RadboudUMC. For each patient the treating nephrologists in training and their supervisor completed a locally developed questionnaire about their advice for RRT or CM, including a 10-point Visual Analogue Scale (VAS) both before and after being informed of the results of the screening tools. The numeric advice on the VAS scale before and after the screening tools was analyzed by non-parametric statistical tests.

Results:
Among the 20 patients, both the nephrologists in training and their supervisor changed 30% of their advice after seeing the results of the screening tools, mostly in favor of RRT. When their advice was not changed, the physicians still felt that the results of the tests would support their advice to the patient. The screening tools most frequently provided better insight into cognition, and secondly in functional status and frailty. Knowledge of the results of the screening tools led to a significant difference (P=0.011) between the advice of the nephrologists in training and the advice of the supervisor more often favoring RRT above CM. All physicians indicated that they would like more information on the quality of life (QOL) and the specific wishes of the patient to substantiate their advice for RRT or CM.

Conclusion:
Screening tools strengthen the physician’s advice to the elderly patient who has to choose between CM and RRT. Because of the major impact of the advice from the treating physician on the patient’s choice, it is imperative to involve at least two physicians in the treatment advice. For an even better advice of the physician, it seems important to include a questionnaire on QOL in the structured assessment of the elderly patient as well as a standardized test for evaluation of the patient’s wishes, e.g. the same VAS that was used in our questionnaire. By comparing the VAS results of the treating nephrologist with the VAS filled by the patient, discrepancies between expected and perceived benefits of RRT over CM would easily be recognized.
NLRX1 DOES NOT PLAY A ROLE IN DIABETES NOR THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Background:

Diabetic Nephropathy (DN) remains the leading cause of end-stage renal disease (ESRD) in the developed world. Inflammation and oxidative stress are key players in the progression of DN. NLRX1 is a mitochondrial member of the Nucleotide binding leucine-rich repeat containing (NLR) family, which function within the innate immune system as a Pattern Recognition Receptor (PRR). NLRX1 is unique in the fact that besides influencing inflammation it reduces oxidative stress in the kidney by regulating mitochondrial activity¹. Given this, the current study aims to determine whether NLRX1 plays a role in the development of diabetes and DN.

Methods:

Male WT and NLRX1 deficient C57BL/6 mice (n= 14 per group) were subjected to multiple low dose Streptozotocin (STZ)-induced diabetes for 20 weeks. The diabetic state and renal function were determined by analyses in plasma and urine. The kidneys were extracted for (immuno)-histochemical and Real Time q-PCR analysis of renal damage, fibrosis, inflammation, and oxidative stress. Non-diabetic male WT and NLRX1 deficient C57BL/6 mice were used as controls (n= 14 per group).

Results:

Upon STZ treatment, both WT and NLRX1 deficient mice exhibited the hallmark features of diabetes, as seen by increased blood glucose, HbA1c (glycated hemoglobin), Urinary albumin excretion (UAE), and decreased pancreatic beta cell function (HOMA-beta index). Interestingly, renal macrophage influx was significantly increased in diabetic WTs, but decreased in diabetic NLRX1 deficient mice. Oxidative stress, as measured by the quantification of the lipid peroxidation product 4-HNE, was significantly increased in both diabetic groups. This model resulted in a very mild DN phenotype, in which renal function was not affected and neither renal damage nor fibrosis could not be clearly identified. In all analyses, NLRX1 deficient diabetic mice did not differ from diabetic WT mice.

Conclusion:

A diabetic phenotype was successfully achieved by STZ in both groups as seen by the diabetic parameters. NLRX1 deficiency did not affect the development of diabetes nor the severity of diabetic nephropathy, which led us to conclude that in contrast to other PRR family members there is no evidence for the role for this innate immune receptor in the development of type I diabetes or DN.

References:

KIDNEY TRANSPLANTATION IN ADULTS KNOWN WITH COMMON VARIABLE IMMUNODEFICIENCY

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ABSTRACT

Background

Common Variable Immunodeficiency (CVID) patients are characterized by primary antibody failure and hypogammaglobulinemia with a high prevalence of infections and malignancies. Immunosuppression regimens in transplantation may amplify this risk. There are only 2 cases reported of CVID patients with renal transplantation and both have a short-term bad outcome. We present 2 renal transplant patients with CVID transplanted with a low immunosuppressive regimen and favorable short-term outcome.

Cases

Case 1: A 43-year old female known with ESRD due to reflux nephropathy, frequent urinary and upper airway infections and multiple PD-peritonitis. CVID was diagnosed by low total IgG (5.2g/l, normal IgG2 and IgG2), absent IgA and IgM, and mild lymphopenia with normal account of T-cells and NK-cells. In August 2015 she received a 43-year old DBD-renal transplant. Postoperative course was uncomplicated besides delayed graft function. Immunosuppression consisted of tacrolimus and low dose steroids. Protocol biopsy at month 3 was normal and steroids were withdrawn. Posttransplant, she had a few recurrent urinary and respiratory infections for which prophylactic azithromycin was given. At 1 year she was in good clinical condition without malignancies and with a good renal function (eGFR 50ml/min) without rejection in protocol biopsy. 2.5 years posttransplantation patient is still doing well with a persistent good renal function (eGFR 44ml/min).

Case 2: A 61-year old male with end-stage renal disease (ESRD) due to polycystic kidney disease and recurrent severe abdominal infections. CVID was diagnosed by decreased total IgG, IgG1, IgG2, and IgG4 (resp. 3.66, 2.56, 0.69, and 0.037g/L), normal IgG3, IgA and IgM, decreased B-cells in whole blood and impaired response to pneumococcus vaccination. After substitution of IVIG he had no recurrent infections. In May 2016, he was transplanted with a 64-year old DCD donor kidney. Postoperative course was uncomplicated besides IGF. Immunosuppression consisted of tacrolimus, low dose steroids for 10 days and mycophenolate mofetil for 3 months. He had no acute rejection nor infection besides twice an asymptomatic cystitis (Enterococcus faecalis at time of JJ-catheter and later Hafnia alvei). One year posttransplantation he was in good general condition without any malignancy. Renal function was 27.5ml/min. Protocol biopsy showed borderline cellular rejection, which was left untreated. Until now, 1.5 years after transplantation, he has a persistent renal function of 28.3ml/min.

Conclusion

The clinical outcome of our patients is far better than previous publications, probably due to a low immunosuppressive regimen. This rare population of ESRD patients with CVID should not be kept away from the benefits of kidney transplantation brings along compared to dialysis. Nephrologist should consider to transplant without induction immunosuppression in this specific group.
**Dyslipidemia in nephrotic rats associates with increased hepatic Proprotein Convertase Subtilisin/Kexin type 9 (PCSK9) and changes in heparan sulfate**

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

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

**Results** – Rats treated with adriamycin showed increased proteinuria, decreased creatinine clearance and increased serum triglycerides and cholesterol (all \(p<0.001\)) compared to control group without differences in the protein and mRNA expression of hepatic lipoprotein receptors LDLR, LRP-1 and syndecan-1. However, the sinusoidal localization of liver PCSK9 was increased significantly in adriamycin treated rats compared to control animals (\(p<0.001\), immunofluorescence), but not at mRNA and total protein levels (qRT-PCR and Western blot). Non-significant increment in serum PCSK9 was found in nephrotic condition compared to the controls. Importantly, besides being a binding partner for LDLR, hPCSK9 protein was found to interact strongly with heparin/heparan sulfates, and the interaction was found to be dependent on the sulfation of heparanoids and their chain length. Profiling of sinusoidal HS by anti-HS mAbs and ligand binding assays revealed loss of 6-O sulfate dependent FGF2 binding (\(p<0.02\)), despite lower hepatic SULF2 mRNA expression (\(p=0.06\)) and might be related to competitive sinusoidal HS binding with PCSK9. Serum cholesterol correlated with serum and liver PCSK9 (\(r=0.82,\) \(p=0.0001\); \(r=0.80,\) \(p=0.001\) respectively) and inversely with hepatic HS staining (\(r=-0.61;\) \(p<0.03\)).

**Conclusions** – These data suggest loss of TRL clearance capacity under nephrotic conditions, probably related to increased interaction of PCSK9 with LDLR and syndecan-1/HS, thereby hampering proper TRL binding and hepatic degradation.
PRE-IMPLANTATION GENETIC DIAGNOSTICS FOR RENAL DISEASE: A CLINICAL CASE AND THE NATIONWIDE EXPERIENCE

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Background

Preimplantation genetic diagnosis (PGD) has been developed for couples at risk of passing on a monogenic disease or a chromosomal anomaly to their children. It entails performing genetic testing on one or a few cells of an IVF/ICSI-embryo and only transferring an unaffected embryo. As monogenic renal diseases are generally considered severe enough to currently qualify for PGD, this technology can be offered to this population, after genetic counselling.

One of the renal diseases PGD is performed for, is hereditary focal segmental glomerulosclerosis (FSGS). Genetic mutations are one of the underlying causes of this clinical-morphological syndrome, but without genetic testing it is difficult to distinguish these from non-genetic FSGS. Here we present the case of a patient with ‘secondary FSGS’, which was diagnosed to be genetic by gene panel sequencing. Additionally, we provide an overview of the nationwide PGD experience for genetic renal diseases.

Methods

Clinical case: A 30-year old man with end-stage renal disease was diagnosed as having ‘secondary FSGS of unknown etiology’ based on renal biopsy. Family history was negative. Sequencing of a 20 gene FSGS gene panel showed a heterozygous known pathogenic mutation (c.217G>A) in the INF2 gene, which is a major cause for autosomal dominant FSGS. After counseling, the patient and his partner opted for PGD, with which they will start in early 2018.

Results

National PGD experience: Genetic testing of the biopsied cells is performed at the Maastricht UMC+, the national license holder. In the UMCs Utrecht, Groningen and the AMC patients can go through the IVF/ICSI procedure. These centers work together in “PGD the Netherlands”. From 1995 to 2016, 146 couples were referred for PGD because of a genetic renal disease. Of these, n=65 (65/146, 45%) started a PGD cycle, leading to n=26 (26/65, 40%) ongoing pregnancies.

The referred couples were at risk for one of 23 genetic renal conditions; 11 syndromic renal diseases, three severe pediatric onset diseases (e.g. ARPKD), two childhood (renal) malignancy syndromes and seven renal diseases with adult presentation (e.g. FSGS). The expansion to perform PGD for adult-onset diseases is one of recent years. Interestingly, most patients are referred because of ADPKD, underscoring the relevance of PGD for adult-onset diseases.

Conclusion

We present a case with end-stage renal disease at age 30, for whom the diagnosis of a monogenic cause of FSGS had great impact: the couple opted for PGD to try to prevent passing the mutation in the gene on to a child. PGD has become increasingly available for a variety of genetic renal diseases,
including adult-onset conditions, with over 145 referrals for renal disease to date. However, the process, from counselling, to firmly establishing a molecular diagnosis via validating the single cell genetic test for the specific indication and the actual PGD procedure, is time-consuming and patients should thus be referred timely.
Background
The advances in chronic kidney disease (CKD) care have led to pregnancy being attainable for many young, female CKD patients. Notwithstanding, it is still a high risk endeavor and it poses not only technical considerations, but also ethical ones. Here we examine the medical implications of pregnancy in CKD that should be discussed in pre-pregnancy counseling, to illustrate the ethical deliberations that may emerge. We also address how to safeguard decision-making in daily practice.

Methods
First, that the risk of pregnancy complications is increased including hypertensive pregnancy diseases, such as pre-eclampsia. There is also risk of renal function deterioration ('CKD shift') in the third trimester and in advanced CKD, not recovering post-partum. The fetus has a high risk of prematurity and low birthweight, which can lead to long-term neurodevelopmental delay. With advancement in CKD stage, the chance of complications raises significantly, especially above CKD4. Therefore adequate timing of a pregnancy is crucial. Nevertheless, recent work has shown that with intensive dialysis, a relatively safe pregnancy can be attained in selected cases. Also, one should discuss that the disease possibly has a genetic cause (as is the case in ~25% of cases with onset <25 years) and genetic counseling should be considered timely. Accurate diagnosis of a genetic disease is essential to estimate the risk that they may pass the disease on to their children, even more so because they may be eligible for preimplantation genetic diagnostics (=embryoselection).

Results
Abovementioned topics can lead to a myriad of ethical questions. Central to these is the weight of ‘respect for reproductive autonomy’, which is people’s liberty to decide whether or not to have children, versus the principle to do no harm (primum non nocere) to the patient and her future child. In this discussion, it is important to not use paternalism (acting in the assumed interest of a person), which is too much of an infraction on maternal autonomy in the majority of cases. As pregnancy is inherently a high risk situation, the chances of complications only trump the reproductive autonomy in patients who have an exceptionally high complication risk, even for CKD standards. Naturally, these are hardly ever clear-cut decisions. And thus they should be made in a specialized and multidisciplinary setting, especially in advanced CKD.

Conclusion
In conclusion, pregnancy is attainable in CKD, but patients should be counseled on the risk of maternal and fetal complications and possibilities of genetic counseling. These discussions are influenced by the ethical dilemma of valuing maternal reproductive autonomy versus the principle to do no harm to both mother and future child. Deliberating on these matters should be done in a multidisciplinary setting, to avoid paternalism. Through shared decision-making, patient and physician(s) can come to a case-specific informed decision.
GLOMERULAR ENHANCER OF ZESTE HOMOLOG-2 (EZH2) HISTONE METHYLTRANSFERASE REDUCES GLOMERULAR ENDOTHELIAL GLYCOCALYX DURING DIABETIC NEPHROPATHY BY REGULATING HYALURONAN SYNTHESIS

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

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

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

Conclusion
In conclusion, our data suggests that EZH2-mediated epigenetic changes reduce endothelial glycocalyx via reduction of hyaluronan in DN.
FUTURE SCREENING TOOL FOR PRE-DIALYSIS PATIENTS THAT MAY HELP IN THE DECISION-MAKING PROCESS PRO OR AGAINST RENAL REPLACEMENT THERAPY.


**Background:** Prevalence of end stage renal disease increases, partially because of senescence. Most of these elderly patients are frail and have multiple comorbidities, low performance scores, and cognitive dysfunction. In this group, renal replacement therapy (RRT) may possibly not increase survival and might, therefore, not be the correct treatment option. This study investigates whether a screening tool may help in the decision-making process pro or against RRT.

**Methods:** In this prospective observational study 90 elderly pre-dialysis patients were screened with the Charlson co-morbidity Index (CCI), Davies comorbidity score, Groningen Frailty Indicator (GFI), Karnofsky score and the mini-mental state examination (MMSE) in combination with the clock drawing test to determine eligibility for RRT. Age and serum-albumin levels of the patients, as well as a surprise question (“Would you be surprised if this patient died within the next year?”) answered by the treating nephrologist were taken into account to determine the advisable treatment option.

**Results:** After the complete screening, 31 (34.4%) patients were advised not to start with RRT. This is an increase of 19 (21.1%) compared to the standard treatment decision making process, which is based on the opinion of the physician and wishes of the patient (NcNemar: p < 0.001)

**Conclusions:** After a complete screening, significantly more patients were advised not to start with RRT. The 77 patients from this study that started RRT will be followed for 3-5 years to determine whether this screening method correctly predicts eligibility for RRT, based on outcome.

**Keywords:** elderly / ESRD / treatment option / screenings method
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.
Conservative Care as a treatment option for patients aged 75 years and older with CKD stage V: A National survey in The Netherlands

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Abstract

Background and objectives
Conservative care for patients aged 75 years and older with CKD stage 5 as a treatment option besides dialysis was proposed officially in the Netherlands in October 2016. This national survey showed the current implementation of this option in Netherlands nephrology departments.

Design, setting, participants and measurement
A web based survey was sent to medical managers of 60 nephrology departments in the Netherlands in August 2016.

Results
Twenty-one medical managers (35%) completed the survey. The term “conservative care” is frequently used and well known. The estimated number of patients in whom the decision for maximal conservative care was made in 2015 was 310 of 2249 patients with CKD stage 5 age 75 years and older (range 5-50 patients per department). 164 patients became symptomatic and received no dialysis. There is no official registration for this treatment option and patient category.

The practice patterns vary widely. Only one of 21 respondents reported a conservative care outpatient clinic. Formal training or education regarding conservative care are not available in most of departments.

95% of respondents discussed this treatment option with their patients. General practitioners are always being informed about their patient’s decision. Their main role is providing or organizing palliative care support at the end of life and discussing advance care planning.

Most of respondents (86%) considered to include their patients in a prospective multicentre observational study, conservative care versus dialysis.

Conclusions
Conservative care as a treatment option for patients with CKD stage 5 aged 75 years and older is well established. The practice patterns are varied in the Netherlands. Follow up studies are needed to see whether the new multidisciplinary guideline facilitate harmonization of practice pattern. Funding is needed to optimize the implementation of conservative care.
APOPTOSIS CAUSED BY 24P3R-MEDIATED HEMOGLOBIN UPTAKE IN THE DISTAL NEPHRON IS PREVENTED BY ENDOGENOUS HEPcidIN SYNTHESIS

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Background Reactive forms of iron, such as heme, are increasingly associated with renal injury. Hemolysis and subsequent hemoglobinuria have been related to renal injury in various pathologies including paroxysmal nocturnal hemoglobinuria, favism and sickle cell anemia, but also as potential post-operative complication of cardiopulmonary bypass. Currently, there is no treatment available and its pathophysiology is not completely understood. However, recent studies have indicated that the iron-regulatory hormone peptide hepcidin may have protective effects against heme-mediated kidney injury. The present study was conducted to get more insight in the molecular pathways that are involved in renal hemoglobin (Hb) handling and subsequent injury in the distal nephron (DN) and the potential modification of these processes by locally synthesized hepcidin.

Methods Mice were administered i.v. Hb weekly for 8 weeks. Kidney injury markers were measured in urine with ELISA and in kidney tissue using qPCR. Mouse cortical collecting duct (mCCD<sub>cl1</sub>) cells were incubated with 1µM and 10µM Hb or hemin for 4h and 24h. Hepcidin and 24p3R in mCCD<sub>cl1</sub> cells was silenced using siRNA. Injury and cell stress markers were measured with qPCR. Oxidative stress and cell death were measured with specific staining.

Results Involvement of the DN in Hb kidney injury was suggested by the induction of renal hepcidin synthesis (p<0.001) in mice repeatedly injected with Hb. Moreover, the hepcidin induction was associated with a decline in urinary kidney injury markers 24p3/NGAL and KIM-1, suggesting a role for hepcidin in protection against Hb kidney injury. We demonstrated that uptake of Hb in the mCCD<sub>cl1</sub> cells is mediated by multi-protein ligand receptor 24p3R, as indicated by a significant 90% reduction in Hb uptake (p<0.001) after 24p3R silencing. Moreover, incubation of mCCD<sub>cl1</sub> cells with Hb or hemin for 4h or 24h resulted in hepcidin synthesis and increased mRNA expression of markers for oxidative (Ho-1, Hif1α), inflammatory (IL-6) and ER (Chop) stress, but no cell death as indicated by apoptosis (Annexin V-FITC) staining. A protective role for cellular hepcidin against Hb-induced injury was demonstrated by aggravation of oxidative stress and induction of apoptosis after 4h Hb or hemin incubation in hepcidin silenced mCCD<sub>cl1</sub> cells.

Conclusion Renal hepcidin synthesis protects the DN against heme-mediated injury.
Title: The NET-effect of combining Rituximab with Belimumab in severe systemic lupus erythematosus

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Objective In systemic lupus erythematosus (SLE) patients, excessive formation of neutrophil extracellular traps (NETs) is observed and their degradation is impaired. In vitro, immune complexes (ICx) trigger NET formation while NET-derived DNA is a postulated autoantigen for anti-nuclear autoantibodies (ANAs), found in SLE. Based on these self-perpetuating mechanisms in SLE, this study investigates whether interfering with ICx formation using a combination of rituximab (RTX) and belimumab (BLM) could decrease NET formation and ameliorate disease.

Methods A phase 2A, open-label, single arm proof-of-concept study was performed wherein 16 SLE patients with severe, refractory disease were treated with a combination of CD20-mediated B cell depletion with rituximab and sustained inhibition of B cell activating factor with belimumab. Besides safety, the study’s endpoints were chosen to address the concept of autoantibodies in relation to excessive NET formation.

Results We demonstrated that SLE-derived immobilized IgG, but not soluble IgG, induced excessive NET formation, confirming ex vivo that ICx mediate excessive NET formation in SLE. We showed that therapeutic intervention with RTX+BLM led to specific reductions in ANAs and regression of excessive NET formation. RTX+BLM appeared to be safe and achieved clinically significant responses:
low lupus disease activity state was achieved in 10 patients, renal responses in 11 patients and concomitant immunosuppressive medication was tapered in 14 out of the 16 patients.

**Conclusion** This study provides novel insights into clinical beneficence of reducing excessive NET formation in SLE by therapeutic targeting ANA production with RTX+BLM. Altogether putting forward a new treatment concept that specifically ameliorates underlying SLE pathophysiology.

**Trial registration.** ClinicalTrials.gov NCT02284984
RENAL ALLOGRAFT TRANSCRIPTOME ANALYSIS WITH NANOSTRING®
NCOUNTER® ANALYSIS SYSTEM REVEALS SIMILAR SIGNATURE OF ACUTE
T CELL MEDIATED REJECTION IN PATIENTS TREATED WITH TACROLIMUS
OR BELATACEPT

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Introduction: Identification of biomarkers of acute kidney allograft rejection (AR) can potentially lead to improved diagnostics. Here, we analyzed the expression of 209 genes in biopsies of kidney transplant patients with AR with the NanoString® nCounter® analysis system. With this novel technique, only low quantities of RNA from formalin fixed paraffin embedded (FFPE) biopsies are required and no amplification is needed. Therefore, residual material used for histopathological diagnosis can be analyzed. The objectives of this study were: i) to examine the gene expression profile in biopsies of patients with acute T cell-mediated rejection (aTCMR) versus patients without aTCMR and ii) to compare the gene expression profiles in patients with aTCMR treated with tacrolimus versus patients treated with belatacept.

Materials and Methods: Biopsies from 21 kidney transplant were studied. Seven biopsies from patients with aTCMR (Banff 1b-3, without C4d) treated with tacrolimus as maintenance immunosuppressive therapy, 9 biopsies from patients with aTCMR treated with belatacept, and 5 negative controls (for-cause biopsies without histomorphological changes) were included. Patients were matched for age, days after transplantation and Banff 2015 category. RNA was extracted from FFPE biopsies and gene expression was analyzed using the NanoString® nCounter® analysis system. P values were corrected for false discovery.
Results and Discussion: A distinct pattern was seen in biopsies with aTCMR compared to biopsies without rejection. Comparison of aTCMR and controls identified 97 genes with higher expression (FDRPV <0.05 to 2E-6). The most significant were T cell associated genes, CD3, CD8, and CD4 (p < 10E-5), and interferon (p = 2x10E-3) inducible genes (CXCL9, CCL5, TBX21 p< 10E-3), plus effector genes (GNLY, ITGAX p<10E-3). This overall pattern is that of aTCMR. Interestingly, pairwise estimates showed no significant differences between belatacept or tacrolimus treated subjects with aTCMR.

Conclusion: Gene expression analysis on FFPE biopsies with the novel technique NanoString® nCounter® analysis system can distinguish kidney transplant biopsies showing aTCMR from those of without aTCMR. Interestingly, we found no differences in gene expression profiles in renal allograft biopsies showing aTCMR in subjects receiving tacrolimus or belatacept-based immunosuppressive regimens.
POLYCYSTIN-1 DYSFUNCTION IMPAIRS ELECTROLYTE HANDLING IN A RENAL PRE-CYSTIC MOUSE MODEL FOR ADPKD

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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⁻/⁻) was induced upon oral administration of tamoxifen to inducible kidney-specific Pkd1 knockout (iKsp-Pkd1lox/lox) 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⁻/⁻ 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⁻/⁻ kidneys, resulting in a systemic electrolyte imbalance characterized by low serum electrolyte concentrations.
DISCREPANCY IN PHYSICIANS’ PREFERENCES ON VASCULAR ACCESS MANAGEMENT AFTER KIDNEY TRANSPLANTATION: RESULTS OF A MULTI-NATIONAL SURVEY.

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BACKGROUND: Arteriovenous fistulas (AVFs) for hemodialysis access burden the cardiovascular system. After successful kidney transplantation, prophylactic AVF ligation may improve cardiac outcomes, but evidence is scarce. This survey investigates physicians’ preference for management of AVFs and identifies factors associated with preference for AVF ligation or maintenance.

METHODS: A survey was sent to members of eight national and international Nephrology and Vascular Surgery societies. The survey comprised eight case vignettes of asymptomatic patients with a functioning AVF after kidney transplantation. Respondents were asked to state preference to maintain or ligate the AVF. Linear mixed-effects models were used to investigate the association of treatment preference with AVF flow, left ventricular ejection fraction (LVEF) and patient age.

RESULTS: 585 surveys were returned. A reduced LVEF of 30% (beta 0.60, 95% confidence interval 0.55 ; 0.65) and a high flow of 2500ml/min (beta 0.46, 95% confidence interval 0.41 ; 0.51) were associated with a higher preference for AVF ligation. Disagreement among respondents was significant, as in four out of eight cases less than 70% of respondents agreed on the AVF management strategy.

CONCLUSION: Although respondents recognize a reduced LVEF and high AVF flow as risk factors, the high disagreement on treatment preferences suggests that evidence is inconclusive to recommend routine AVF ligation or preservation after kidney transplantation. More research is needed to determine optimal AVF management after successful kidney transplantation.
[Figure] Associations of patient factors on the tendency to maintain or ligate AVFs. Intercept values indicate the treatment preference in absence of the factor, and range from 1 (strong preference to maintain AVF) to 4 (strong preference to ligate AVF). Beta values indicate the change in treatment preference in the presence of the factor, and range from -3 (factor strongly associated with AVF maintenance) to +3 (factor strongly associated with AVF ligation).
VALIDATION OF THE PROGNOSTIC VALUE OF THE HISTOPATHOLOGICAL CLASSIFICATION OF ANCA-ASSOCIATED GLOMERULONEPHRITIS: A META-ANALYSIS

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Background: In 2010, a histopathologic classification of antineutrophil cytoplasmic autoantibody (ANCA)-associated glomerulonephritis (AAGN) was proposed by an international consortium of renal pathologists and nephrologists. It comprises four biopsy classes: focal, crescentic, mixed and sclerotic, the order of which was shown, in the initial publication, to correspond to increasing severity of renal impairment during follow-up. The aim of this meta-analysis was to evaluate the prognostic value of these phenotypical classes by means of validation studies that have been published since.

Methods: A literature search was performed using Web of Science, Google Scholar, PubMed and Embase in March 2017, selecting studies that associated histopathological class to renal outcome in adult patients with AAGN. The risk of developing end-stage renal disease (ESRD) during follow-up was compared between classes using a meta-analysis with random effects model. Weighted relative risks (RR) with 95% confidence intervals (95% CI) were reported.

Results: Nineteen studies were included with a total of 2,408 patients. Using sclerotic class as a reference category, ESRD risk was lower in the crescentic class (RR 0.53, 95% CI 0.43-0.64); RR in focal was lower than in crescentic class (RR 0.27 95% CI 0.20-0.37). RR in crescentic compared to mixed class was 1.18 (95% CI 0.95-1.45); RR in focal compared to mixed class was 0.34 (95% CI 0.25-0.47).

Conclusion: Our meta-analysis shows that the risk for developing ESRD increased with more severe histopathological lesions. We found no difference between the crescentic and mixed classes, pointing towards a comparable risk profile with regard to ESRD. We are currently performing an individual patient data meta-analysis, as this technique is better equipped to deal with study heterogeneity. For the moment, this meta-analysis confirms the use of the histopathological classification system as a predictor of renal outcome in the prognostication of patients with AAGN.