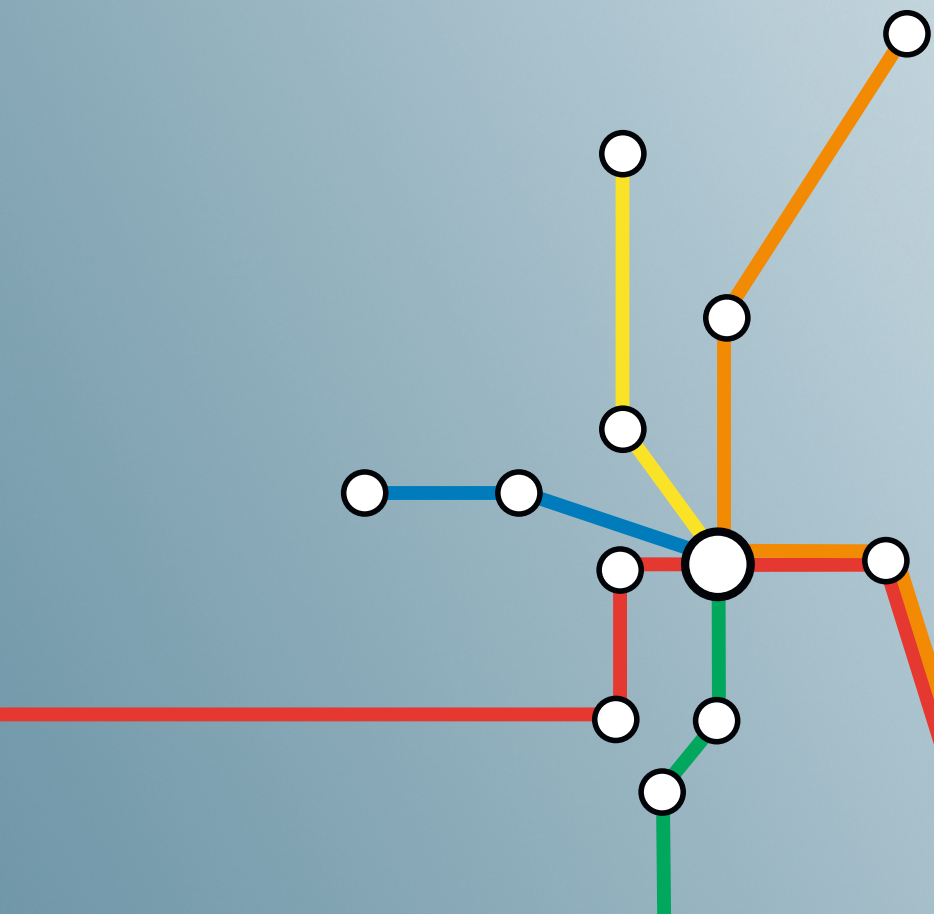
Metabolomics to predict progression in chronic kidney disease

Dekker, S.E.I.



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Urinary tissue inhibitor of metalloproteinases-2 and insulin-like growth factor-binding protein 7 do not correlate with disease severity in ADPKD patients

Shosha E.I. Dekker[1], L. Renee Ruhaak[2], Fred P.H.T.M. Romijn[2], Esther Meijer[3], Christa M. Cobbaert[2], Johan W. de Fijter[1], Darius Soonawala[1,4]; DIPAK Consortium.

1. Department of Nephrology, Leiden University Medical Center, Leiden, the Netherlands
2. Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands
3. Department of Nephrology, University Medical Center Groningen, Groningen, the Netherlands.
4. Department of Internal Medicine, Haga Teaching Hospital, The Hague, The Netherlands

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Abstract

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive cyst formation and variable renal function decline that frequently leads to end-stage renal failure. With the advent of renoprotective treatment, there is renewed interest in non-invasive biomarkers to help identify patients at risk of rapid disease progression at early stages. Urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) have been validated as early markers of acute kidney injury. Because these markers are associated with tubular damage, we studied the performance of both markers in a cohort with chronic tubular pathology. We investigated whether these biomarkers may be useful to evaluate disease severity in ADPKD.

Methods

In a cross-sectional analysis, we measured TIMP-2 and IGFBP7 in stored spot urine samples of patients with ADPKD with various stages of chronic kidney disease (CKD) and healthy controls by enzyme-linked immunosorbent assay. Renal function was estimated using the CKD-Epidemiology Collaboration equation. Patients were stratified according to the Kidney Disease Outcomes Quality Initiative classification for CKD. In a subset of patients, total kidney volume (TKV; using magnetic resonance imaging [MRI]) was measured.

Results

In 296 patients with ADPKD (45.5 ± 11.5 years, 51.0% female, serum creatinine 106 [85 – 147] mmol/L), urine levels of TIMP-2 and IGFBP7 were not increased or tended to be lower as compared with 71 healthy controls (46.5 ± 18.5 years, 72.6% female). The levels did not differ across CKD stages, which remained so after correcting for urine creatinine or osmolality, and for age, sex, and urine protein in multivariable analyses.

Conclusion

Urinary levels of TIMP-2 and IGFBP7 were not higher in patients with ADPKD and did not correlate with disease severity.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease with a prevalence of 1:2500 to 1:3300.[1] The disease is caused by mutations in the *PKD1* (85% of cases) or *PKD2* gene (15% of cases) and characterized by progressive development of multiple cysts in both kidneys and various extra-renal manifestations, such as liver cysts and intracranial aneurysms.[2] The course of disease progression is highly variable. ADPKD frequently leads to end stage renal disease (ESRD) between the fourth and seventh decades of life.[3] In clinical practice, measurement of serum creatinine and estimated glomerular filtration rate (eGFR) is used to monitor kidney function and progression of disease. However, progressive cyst formation and loss of nephrons precedes a decline in eGFR.[4] Therefore, in the earlier stages of disease, serum creatinine is a poor predictor of disease progression in ADPKD.

With the advent of treatment that slows the rate of disease progression, there is renewed interest in alternative, non-invasive biomarkers to identify patients at high risk for rapid progression in the early stage of disease and to evaluate therapeutic efficacy. Although several markers have been studied in ADPKD, including neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), monocyte chemotactic protein-1 (MCP-1) and copeptin (reviewed in cited literature),[5] their predictive value is limited. Total kidney volume adjusted for height (htTKV) and age determined by computed tomography (CT) or magnetic resonance imaging (MRI) is more accurate in predicting renal disease progression in patients with typical morphology of ADPKD.[6, 7] However, in many hospitals the use of a CT or MRI scan to measure total kidney volume (TKV) is not yet standard practice due to limitations pertaining to time and cost. Furthermore, follow-up TKV measurements seem to be less suitable for the evaluation of long-term treatment effects.[8]

Urinary tissue inhibitor of metalloproteinases-2 (TIMP-2) and insulin-like growth factor-binding protein 7 (IGFBP7) have been validated as early biomarkers of acute kidney injury (AKI). In AKI, these markers outperform other urinary markers.[9] In chronic kidney disease (CKD) data regarding TIMP-2 and IGFBP7 are sparse. The exact role of TIMP-2 and IGFBP7 in renal physiology remains to be determined. Abundant secretion of IGFBP7 in proximal tubular cells and distal tubular cells and TIMP-2 in distal tubular cells is associated with early tubular damage in an ischemia-reperfusion injury model.[10] Whether these tubular damage markers also play a role in chronic renal dysfunction is unclear. Since the pathophysiology of ADPKD involves tubular dysfunction, measurement of urinary TIMP-2 and IGFBP7 in patients with CKD due to ADPKD might be of interest.

We performed a cross-sectional analysis of a well-characterized cohort of patients with ADPKD with various stages of CKD to determine whether these markers correlated with disease severity. Disease severity was categorized according to eGFR and htTKV. We hypothesized that in ADPKD, progressive cyst growth and compression of surrounding tissue causes a snowball effect with multiple repetitive acute ischemic events, which may result in an increase in urinary TIMP-2. Since tubular injury is not limited to distal tubules and the collecting duct, we thought urinary IGFBP7 may also increase.

Methods

Study population

Patients Baseline spot urine samples from patients with ADPKD were obtained from 4 distinct cohorts: (i) second morning fasting samples from patients taking part in a multicentre trial, the DIPAK 1 study (Developing Interventions to halt Progression of Autosomal dominant polycystic Kidney disease) (n = 137). Participants in this trial were included from July 2012 through March 2015, are 18 to 60 years of age and have an eGFR between 30 and 60 mL/min/1.73m². Their htTKV was measured at baseline.[11] (ii) In addition, second morning fasting samples were obtained from adult patients (age 19 – 74 years, eGFR 13 – 134 mL/min/1.73m²) taking part in the DIPAK observational study (2013 – 2015, n = 189), a multicentre observational study investigating renal disease progression (annualized change in eGFR and htTKV). (iii) Non-fasting samples were also obtained from a single-centre observational study conducted between 2007 and 2009 (n = 82). In this cohort (age 19 – 67 years, eGFR 11 – 136 mL/min/1.73m²), Meijer et al. have previously analyzed other urinary biomarkers.[12] (iiii) Non-fasting samples were collected from patients (age 19 – 77 years, eGFR 8 – 120 mL/min/1.73m²) participating in the centralized Dutch Parelsnoer Institute/CuraRata biobank initiative (2010, n = 56). Subjects included in these studies were diagnosed with ADPKD based upon the modified Ravine criteria.[13]

Healthy controls Stored non-fasting spot urine samples were used as controls. These samples were obtained from the following sources: (i) baseline samples from a single-centre study on healthy volunteers (age 18 – 81 years) taking part in a vaccination study (n = 60) conducted in 2008.[14] (ii) Healthy people (age 32 – 75 years, eGFR 71 – 114 mL/min/1.73m²) who came to our centre for medical examination prior to donating a kidney (2014, n = 17). (iii) Healthy employees (age 33 – 59 years) of the outpatient clinic at our centre who donated samples anonymously (2015, n = 17).

The relevant national and institutional Ethics Committees approved all procedures in all studies and subjects gave informed consent before donating samples. Consent included storage and use in future studies such as ours.

Sample collection, storage and measurement

Aliquots were stored at -80°C and thawed prior to analysis. The samples underwent 2 freeze/thaw cycles prior to analysis. Urine concentrations of TIMP-2 and IGFBP7 were determined using sandwich enzyme-linked immunosorbent assay (ELISA, DTM200, R&D systems, Minneapolis, MN for TIMP-2 and EK0991, Boster Biological Technology, Pleasanton, CA for IGFBP7) according to the manufacturer's instructions. Sample dilutions used were checked to be within the linear range and the same lot numbers (P115276 for TIMP-2 and 637121261219 for IGFBP7) were used throughout the entire study. Two internal quality control (QC) samples (QC1 and QC2), consisting of pooled urine, were analyzed in triplicate on each sample plate to assess the stability of the assay. For TIMP-2 total analytical imprecision, expressed as CV%, was 2.8% at 183 pmol/L for QC1 (n = 21) and 3.6% at 238 pmol/L for QC2 (n = 21). For IGFBP7 total imprecision during the study was 11.8% at 945 pmol/L for QC1 (n = 17) and 16.1% at 2237 pmol/L for QC2 (n = 18). The product of these 2 markers (TIMP-2 x IGFBP7) is the concentration of TIMP-2 (pmol/L) multiplied by the concentration of IGFBP7 (pmol/L) divided by 1,000 ([pmol/L²]/1000).

Urine osmolality was determined using the Osmo-Station, ARKRAY Inc., Kyoto, Japan. Two internal quality control samples (Lyphocheck Quantitative Urine Control Normal and Abnormal, Bio-Rad laboratories, Irvine, CA) were used. Here the mean and CV values obtained were 320 mOsm/kg and 0.5% for the normal QC (n = 12) and 868 mOsm/kg and 0.9% for the abnormal QC (n = 11). The urinary concentrations of total protein, albumin and creatinine were measured on a Modular P800 (Roche Diagnostics) according to instructions from the manufacturer.

Because urine osmolality is associated with disease severity in patients with ADPKD, biomarker concentrations might be affected by dilution of urine in the tubule. This makes urine osmolality a potential confounder. Therefore, TIMP-2 and IGFBP7 were normalized to urine osmolality and expressed as a ratio of the biomarker divided by osmolality. Both markers were also corrected for urine creatinine and expressed as the biomarker divided by creatinine.

Renal function was derived from serum creatinine based eGFR applying the CKD-Epidemiology Collaboration equation[15] and using an isotope dilution mass spectrometry (IDMS)-standardized, selective enzymatic creatinine method. We stratified patients with

ADPKD according to the CKD classification, based on 5 categories of eGFR, proposed by the Kidney Disease Outcomes Quality Initiative (KDOQI).[16] Stages 4 and 5 were combined into 1 group (CKD 4 – 5). CKD 1: ≥ 90 ml/min/1.73m²; CKD 2: 60 – 89 ml/min/1.73m²; CKD 3: 30 – 59 ml/min/1.73m²; CKD 4 – 5: < 30 ml/min/1.73m².

MRI data of the DIPAK 1 study were obtained at the same time point as the urine samples and were used to measure TKV in ADPKD patients. Abdominal MRI was performed using a standardized imaging procedure without the administration of intravenous contrast. The protocol includes T2-weighted single-shot fast gradient spin-echo sequence with fat-saturation.[11] TKV was analyzed by means of a validated stereology technique.[17, 18] An online imaging calculator was used to estimate htTKV and to stratify patients into five subclasses (1A-1E by htTKV and age) according to the Mayo Classification.[19] This model predicts disease progression in patients with typical ADPKD.[6]

Statistical analysis

Continuous variables with normal distribution are expressed as mean ± standard deviation (SD) and nonparametric variables are summarized by median and interquartile range (IQR). Categorical data are given as proportions. Normality of variable distribution was evaluated by the Kolmogorov-Smirnov test. The independent *t* test and one-way ANOVA were used for analyses when data were normally distributed. In case of skewed distribution of data, Mann-Whitney *U* test and Kruskal-Wallis test were used to compare continuous variables. Multivariable regression analyses were used to investigate the associations between the urinary biomarkers and eGFR or htTKV, with sequential adjustment for potential confounding factors (age, sex, urine creatinine, urine osmolality and urine protein concentration). For all analyses, 2-sided *P* values < 0.05 were considered to indicate statistical significance. Analyses were performed using SPSS, version 23.0 (SPSS Inc.) and GraphPad Prism, version 7.0.2.

Results

Study cohort

A total of 464 ADPKD patients and 94 controls were identified. The study population consisted of 296 ADPKD patients (mean age 45.5 ± 11.5 years, 51.0% female) and 71 controls (mean age 46.5 ± 18.5 years, 72.6% female) for whom sufficient urine material was available for analyses. As expected, age, body mass index (BMI) and renal function significantly differed across the CKD stages in ADPKD patients, as indicated in **Table 1**.

Table 1. Patient characteristics

Variable	ADPKD	Stages of CKD			
		1	2	3	4-5
n	296	60	95	112	29
Female sex, *n* (%)	151 (51.0)	30 (50.0)	56 (58.9)	50 (44.6)	15 (51.7)
Mean age, yr (SD)	45.5 (11.5)	33.1 (9.4)	44.5 (8.9)*	51.5 (9.3)*	51.0 (8.7)*
Median BMI, kg/m² (IQR)	25.8 (23.1 – 28.9)	22.5 (20.9 – 27.5)	26.1 (23.1 – 29.3)*	26.3 (24.2 – 29.0)*	26.3 (23.9 – 31.5)*
Median serum creatinine, µmol/L (IQR)	106.0 (85.3 – 146.8)	73.5 (67.3 – 85.0)	93.0 (84.0 – 106.0)*	140.0 (119.0 – 159.8)*	229.0 (200.0 – 304.0)*
Median eGFR, mL/min/1.73m² (IQR)	60.7 (42.9 – 84.7)	102.0 (92.9 – 110.6)	70.4 (63.7 – 81.8)*	45.9 (37.7 – 53.4)*	21.6 (18.5 – 28.1)*

ADPKD, autosomal dominant polycystic kidney disease; BMI, body mass index; CKD, chronic kidney disease; eGFR, estimated GFR; IQR, interquartile range. * P < 0.005 versus stage 1.

Table 2. Urinary biomarker levels in controls and patients with ADPKD

Variable	Controls	ADPKD	Stages of CKD			
			1	2	3	4-5
n	71	296	60	95	112	29
TIMP-2/Creat, pmol/mmol	15 (10 – 20)	12 (8 -18)*	10 (3 – 20)	13 (7 – 17)	11 (8 – 17)	14 (10 – 22)
IGFBP7/Creat, pmol/mmol	121 (80 – 164)	103 (84 – 130)*	104 (81 – 145)	104 (86 – 145)	113 (77 – 122)	122 (91 – 148)
TIMP-2xIGFBP7, pmol/L²/1000	48 (15 – 214)	48 (10 – 124)	34 (3 – 254)	60 (10 – 167)	50 (18 – 99)	38 (21 – 80)
Creatinine, µmol/L	5289 (3166 – 10410)	5898 (3350 – 8826)	5394 (2410 – 11658)	6235 (3393 – 9728)	6091 (4075 – 8451)	5061 (3671 – 6408)
Total protein, g/L	0.05 (0.04 – 0.08)	0.07 (0.04 – 0.12)*	0.07 (0.04 – 0.11)	0.06 (0.04 – 0.10)	0.07 (0.04 – 0.12)	0.08 (0.04 – 0.22)

ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic kidney disease; TIMP-2, tissue inhibitor of metalloproteinase-2; IGFBP7, insulin-like growth factor-binding protein 7. Data are given as median (interquartile range). * P < 0.05 versus controls.

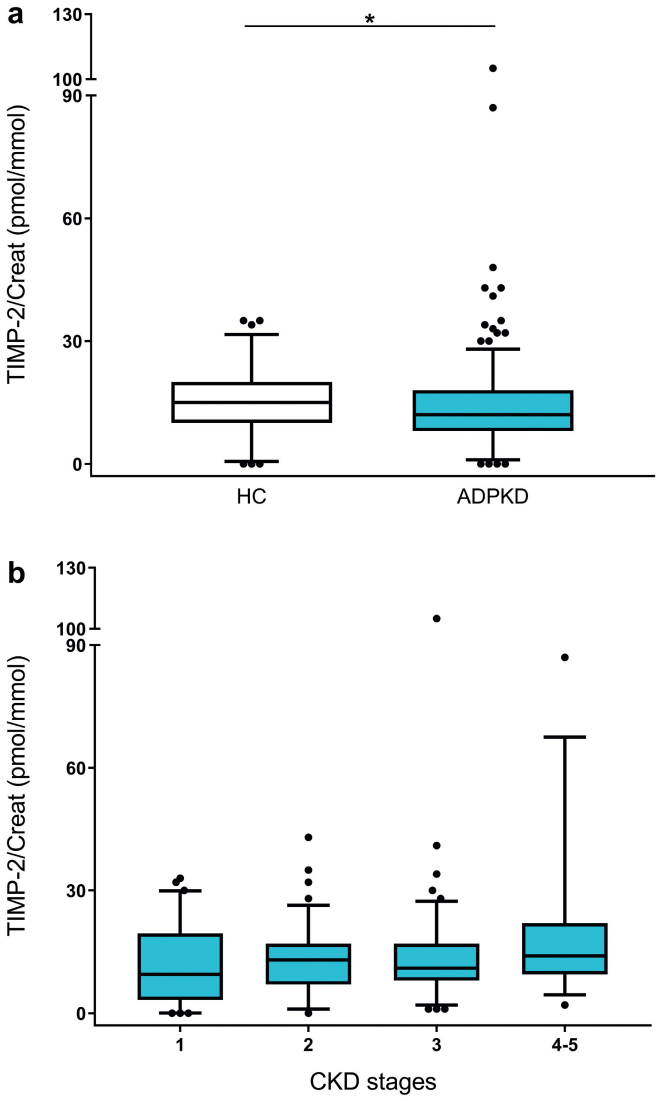

Figure 1: Urine tissue inhibitor of metalloproteinases-2 (**TIMP-2)/creatinine (Creat) in autosomal dominant polycystic kidney disease (ADPKD) according to chronic kidney disease (CKD) stage and healthy controls (HC)**. TIMP-2/Creat levels were significantly lower in patients with ADPKD as compared with HC (a, *P = 0.02) and not significantly different across CKD stages (b, P = 0.10). Boxplots represent median, interquartile ranges and their 5 – 95 percentiles.

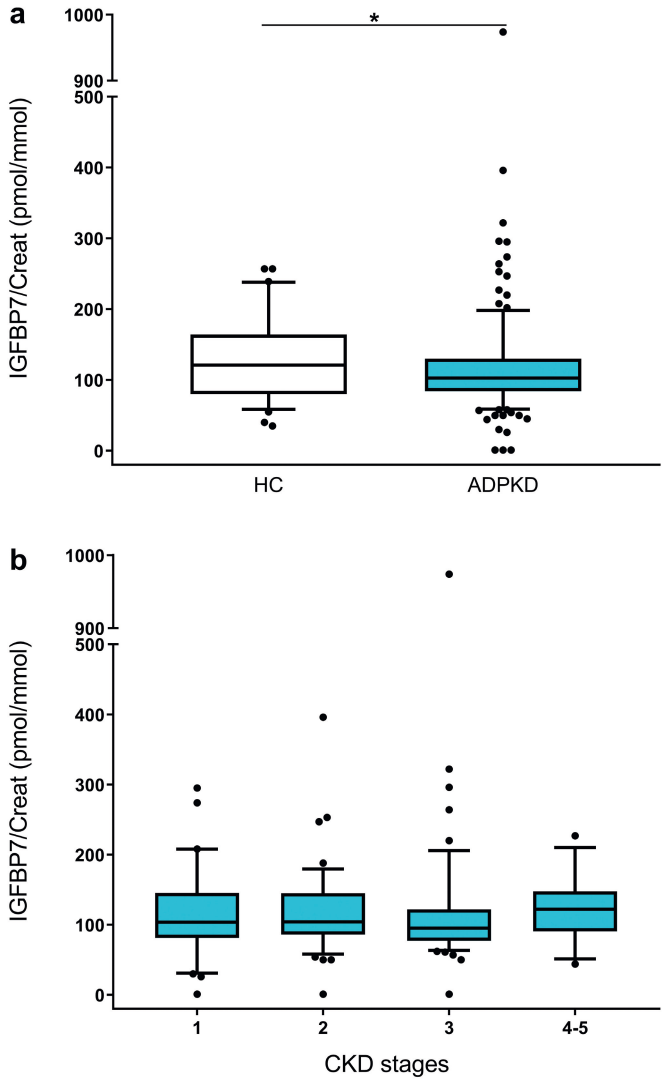

Figure 2: Urine insulin-like growth factor-binding protein 7 (IGFBP7)/creatinine (Creat) in autosomal dominant polycystic kidney disease (ADPKD) according to chronic kidney disease (CKD) stage and healthy controls (HC). IGFBP7/Creat levels were significantly lower in patients with ADPKD as compared with HC (a, *P = 0.03) and not significantly different across CKD stages (b, P = 0.07). Boxplots represent median, interquartile ranges and their 5 – 95 percentiles.

Correlation of urinary TIMP-2 and IGFBP7 with ADPKD and stages of CKD

Levels of TIMP-2 (P = 0.24) and IGFBP7 (P = 0.40) were comparable between ADPKD patients and healthy controls, as was the product of both markers (TIMP-2 x IGFBP7, P = 0.33). Normalized to urine creatinine, both TIMP-2 (P = 0.02) and IGFBP7 levels (P = 0.03) were significantly lower in ADPKD patients as compared with controls (**Table 2**, **Figures 1A** and **2A**). Urine protein levels were higher in ADPKD patients than in controls (median 0.07 (IQR 0.04 – 0.12) g/L vs 0.05 (IQR 0.04 – 0.08) g/L, respectively, P = 0.003), as shown in **Table 2**.

In ADPKD patients, there were no differences in uncorrected urinary TIMP-2 (P = 0.73), IGFBP7 (P = 0.50) and TIMP-2 x IGFBP7 levels (P = 0.75) between the various CKD stages. Comparable results were found after correcting TIMP-2 and IGFBP7 for urine creatinine (TIMP-2/Creat, P = 0.10; IGFBP7/Creat, P = 0.07) (**Table 2**, **Figures 1B** and **2B**). Osmolality was lower in ADPKD patients with later-stage CKD (P = 0.03, across CKD stages) (**Figure 3**). Median levels of urine osmolality ranged from 475 (IQR 231 – 614) mOsm/kg in patients with preserved renal function to 336 (IQR 307 – 397) mOsm/kg in patients with CKD stage 4-5. Seeing that urine is more diluted in later-stage CKD, we corrected for this effect on the urine biomarker level (TIMP-2/Osm and IGFBP7/Osm). **Figure 4** shows that after correcting for osmolality, neither TIMP-2 (**Figure 4A**) nor IGFBP7 (**Figure 4B**) differed across the four CKD stages, P = 0.11 and P = 0.57, respectively.

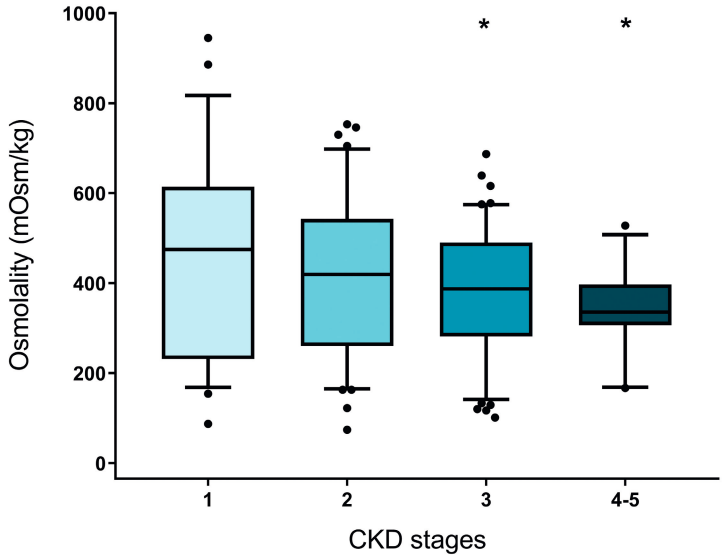

Figure 3: Urine osmolality in autosomal dominant polycystic kidney disease (ADPKD) according to chronic kidney disease (CKD) stage. Lower urine osmolality levels were found in more advanced CKD stages (P = 0.03, across CKD stages). *P < 0.05 vs stage 1 (Mann-Whitney U test). Boxplots represent median, interquartile ranges and their 5 – 95 percentiles.

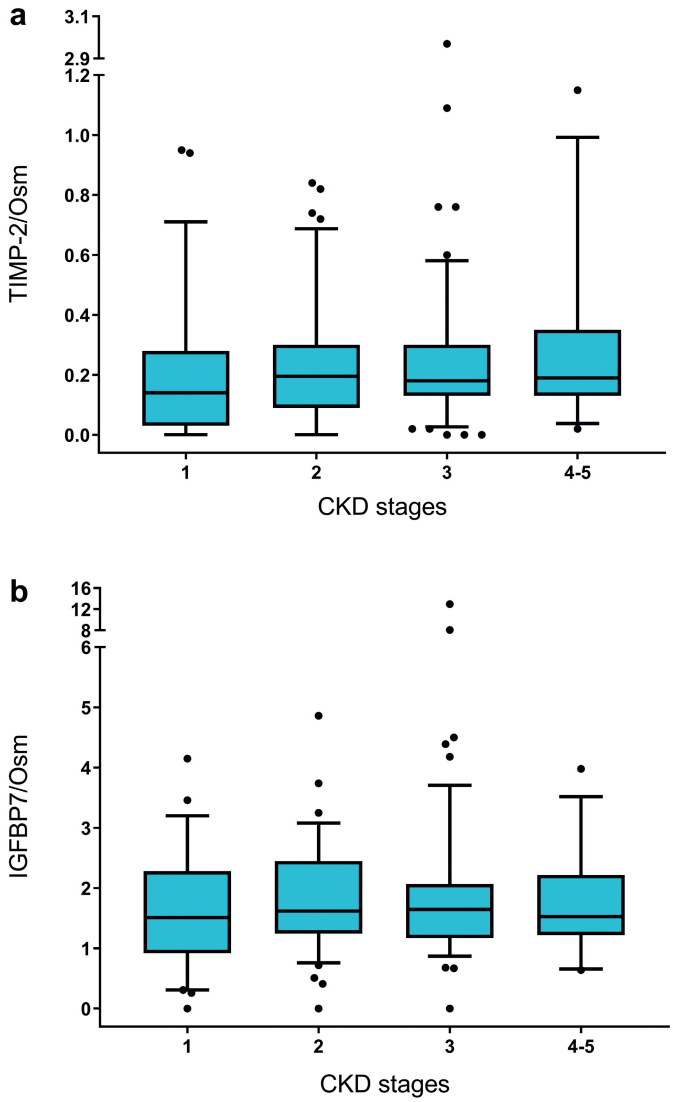

Figure 4: Urine tissue inhibitor of metalloproteinases-2 (TIMP-2)/osmolality (Osm) and urine insulin-like growth factor-binding protein 7 (IGFBP7)/Osm in autosomal dominant polycystic kidney disease (ADPKD) according to chronic kidney disease (CKD) stage. TIMP-2/Osm (a, P = 0.11) and IGFBP7/Osm (b, P = 0.57) did not differ significantly across CKD stages. Boxplots represent median, interquartile ranges and their 5 – 95 percentiles.

In addition to the comparison of biomarkers across the CKD stages, we analysed the association of both urinary markers with eGFR as a continuous variable. In a multivariable regression model with only conventional markers, age, urine osmolality and urine protein were significantly associated with eGFR ($R^2 = 0.48$, $P < 0.001$). Adding urinary TIMP-2 ($R^2 = 0.48$) or IGFBP7 ($R^2 = 0.49$) or TIMP-2 x IGFBP7 ($R^2 = 0.49$) did not improve the model ($P > 0.05$ for the addition of these biomarkers).

Correlation of urinary TIMP-2 and IGFBP7 with htTKV

For 95 ADPKD patients, measurements of htTKV were available. This subgroup was stratified into 5 subclasses (1A – 1E) according to the Mayo classification system, which is based on htTKV and age. Patients who were classified as atypical (Mayo class 2, n = 7) were excluded from analyses. In this subgroup, patients had a median eGFR of 52.1 (IQR 41.4 – 60.7) ml/min/1.73m². There was a large spread in htTKV, with values ranging from 314 to 4481 (median 1044) ml/m. Osmolality corrected TIMP-2 ($P = 0.16$) and IGFBP7 ($P = 0.41$), and creatinine corrected TIMP-2 ($P = 0.09$) and IGFBP7 ($P = 0.50$) did not differ across the Mayo subclasses 1A-B (n = 15), 1C (n = 39), 1D (n = 23) or 1E (n = 18) (**Figures 5A** and **5B**). Furthermore, a multivariable linear regression model including htTKV as continuous variable and all potential confounders (age, sex, eGFR, urine creatinine, urine osmolality and urine protein) showed no association between htTKV and TIMP-2 or IGFBP7 or their product ($R^2 = 0.13$, $R^2 = 0.13$, and $R^2 = 0.12$, respectively, all $P > 0.05$). This also applied for the model built only on the clinical risk markers ($R^2 = 0.11$, $P = 0.09$).

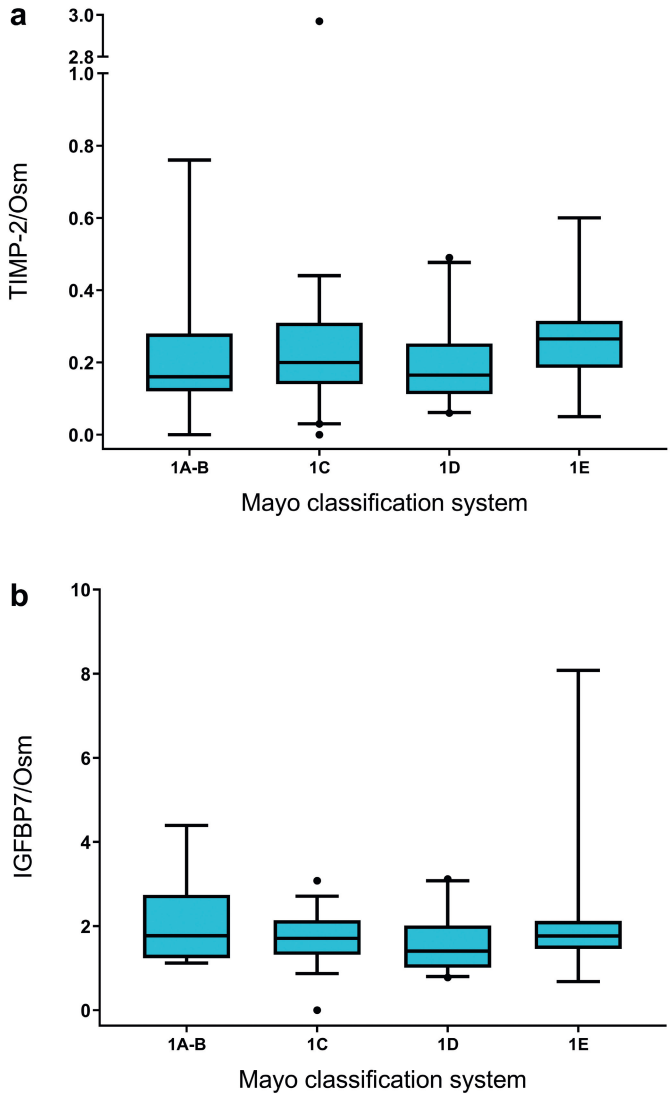

Figure 5: Urine tissue inhibitor of metalloproteinases-2 (TIMP-2)/osmolality (Osm) and urine insulin-like growth factor-binding protein 7 (IGFBP7)/Osm in autosomal dominant polycystic kidney disease (ADPKD) according to total kidney volume (TKV). TIMP-2/Osm (P = 0.16) and IGFBP7/Osm (P = 0.41) did not differ across strata for TKV. Kidney volume was classified according to the Mayo classification system, based on age and height-adjusted total kidney volume. Boxplots represent median, interquartile ranges and their 5 – 95 percentiles.

Correlation of urinary TIMP-2 and IGFBP7 with albuminuria

Additionally, we analyzed the association of biomarker levels and albuminuria, seeing that this may influence biomarker levels. Patients were stratified according to their urine albumin-creatinine ratio (ACR) into three categories of albuminuria: < 3 mg/mmol (n = 112), 3 – 30 mg/mmol (n = 95) and > 30 mg/mmol (n = 8). Creatinine corrected IGFBP7 (P = 0.26), and osmolality corrected IGFBP7 (P = 0.92) were comparable across all groups. Patients with albuminuria had higher levels of urinary TIMP-2 levels corrected for creatinine (P < 0.001) or osmolality (P = 0.004). The median TIMP-2/Osm was 0.2 (IQR 0.1 – 0.3) pmol/mOsm for patients with a normal to mildly increased ACR, 0.2 (IQR 0.2 – 0.4) pmol/mOsm for those with moderate albuminuria, and 0.4 (IQR 0.2 – 0.5) pmol/mOsm in patients with severe albuminuria (P = 0.004, across groups).

Correlation of urinary TIMP-2 and IGFBP7 with types of gene mutation

Genetic analysis was available for 196 patients and showed pathogenic mutations in the PKD1 gene (n = 132, 67.3%), PKD2 gene (n = 39, 19.9%), in both genes (n = 9, 4.6%) or in none of these two genes (n = 16, 8.2%). In this subgroup, urine biomarker levels did not differ between patients with a PKD1 or PKD2 mutation (P = 0.30 for TIMP-2, and P = 0.76 for IGFBP7). Creatinine corrected TIMP-2 (P = 0.23) and IGFBP7 (P = 0.93), and osmolality corrected TIMP-2 (P = 0.15) and IGFBP7 (P = 0.51) levels were also comparable between the groups. In patients with a PKD1 mutation, the median TIMP-2/Osm level was 0.2 (IQR 0.1 – 0.3) pmol/mOsm, and the median IGFBP7/Osm level was 1.7 (1.3 – 2.2) pmol/mOsm. In patients with a PKD2 mutation the median TIMP-2/Osm level was 0.2 (IQR 0.1 – 0.3) pmol/mOsm, and the median IGFBP7/Osm level 1.7 (1.3 – 2.2) pmol/mOsm. Additionally, no differences were found between the two genetic mutation groups in the association of both biomarkers with eGFR in multivariable regression analyses adjusted for all potential confounders (P for interaction = 0.35 for TIMP2, and P for interaction = 0.09 for IGFBP7).

Discussion

This study evaluated urinary TIMP-2 and IGFBP7 as potential biomarkers for disease severity in patients with ADPKD. Levels of TIMP-2 and IGFBP7, corrected for urine creatinine or osmolality, remained stable regardless of renal dysfunction or kidney volume. The advent of renoprotective treatment requires early risk stratification in ADPKD. Because the disease course of ADPKD is highly variable, it is important to select patients at high risk of rapid disease progression for timely treatment, and to identify low-risk patients to avoid unnecessary treatment. Conventional markers for disease severity in ADPKD including GFR indexed for age and htTKV both have their limitations. Therefore, there is an unmet clinical need for alternative markers that associate with disease severity and progression in the early stages of ADPKD.

Urinary TIMP-2 and IGFBP7 have been validated as biomarkers to predict the onset of AKI.[9] A urine test, based on the product of TIMP-2 and IGFBP7, has been approved by the US Food and Drug Administration for predicting early-stage AKI. Urinary TIMP-2 has also separately shown to be of predictive value in renal transplant recipients for the occurrence and duration of graft dysfunction.[20] Because ADPKD is characterized by tubular dysfunction, we measured these urinary tubular damage markers in a CKD cohort with ADPKD and different disease stages. The advantage of urinary markers is that their measurement is relatively easy and inexpensive. Because we were particularly interested in the distal tubular marker TIMP-2, we measured this marker separately in addition to IGFBP7 and their product. We hypothesized that urinary TIMP-2 levels increased in patients with ADPKD in response to multiple repetitive acute ischemic events due to cyst growth and compression of surrounding tissue. In contrast to our hypothesis, normalized levels of TIMP-2 as well as IGFBP7 were lower in patients with ADPKD as compared with healthy controls, and no differences between the rate of disease severity (eGFR, htTKV, type of gene mutation) were found in ADPKD. This makes it less likely that these biomarkers can predict progression of disease. However, this point remains uncertain as the design of our study was cross-sectional and this would require a follow-up study with sequential measurements of biomarkers and eGFR over time.

The mechanisms underlying increased urinary TIMP-2 and IGFBP7 were recently determined in mice with acute renal injury. In AKI, elevated urinary excretion is caused by increased glomerular permeability for these proteins, impaired proximal tubular reabsorption, and release of these proteins from tubular cells.[21] A number of reasons may explain the findings in our study. The lower biomarker levels in ADPKD as compared with controls may be explained by an impaired glomerular filtration rate. As ADPKD progresses, lower filtration may be offset by impaired proximal tubular reabsorption and increased release of these proteins from tubular cells. The net-result is that there is no significant change in these biomarkers as ADPKD progresses. In an ischemia-reperfusion injury model secretion of IGFBP7 and TIMP-2 in tubular cells is suppressed for 24h, followed by abundant secretion after 6h of reperfusion with normalized secretion after 24h of reperfusion.[10] In ADPKD, the injury is of a different kind and the intensity may be too limited compared with AKI to cause an increase in these markers. Furthermore, in ADPKD the type of injury may differ in that it is irreversible, with fibrosis in more advanced stages of ADPKD. Lastly, the injury may be intermittent in which case an increase in biomarker levels may elude detection.

Our data are in line with the study by Kashani et al., which focused on AKI, but also included some patients with CKD (n = 65).[9] In the graphs provided in that paper, the product of urinary TIMP-2 and IGFBP7 did not differ between patients with CKD and controls. Another

small study has investigated urinary IGFBP7 in type 2 diabetic patients (n = 46) (mean eGFR 74.9 ± 23.6 ml/min/1.73m²). In contrast to our study, urinary levels of IGFBP7 were significantly higher in patients with macroalbuminuria than in those with normoalbuminuria or microalbuminuria and correlated with eGFR.[22] This may be related to the specific pathophysiology of diabetic nephropathy, which is characterized by glomerular dysfunction.

The study has a number of strengths. First, the study population was of sufficient size and was well distributed across the stages of CKD to allow a firm conclusion. Second, the biomarkers were analyzed in relation to the conventional markers of disease severity, assessed as eGFR, htTKV, albuminuria, and type of gene mutation. In the earlier stages of disease, htTKV is a better marker of progression of ADPKD than eGFR. Third, TIMP-2 and IGFBP7 levels were corrected for urinary creatinine as well as urinary osmolality. Most studies have normalized urinary biomarkers to creatinine only, which is influenced by muscle mass and variability in filtration and secretion.[23] Our data confirmed the well-known fact that urine osmolality is reduced in ADPKD patients with more advanced CKD. However, also after correcting for urinary osmolality biomarker levels were similar at various stages of CKD, in accordance with the results after correcting for urinary creatinine.

This study also has limitations. First, the urine samples were stored at -80°C for prolonged periods and had gone through two freeze-thaw cycle. Schuh et al evaluated the effects of repeated freeze-thaw cycles and prolonged storage for 5 years on urinary NGAL, KIM-1 and IL-18. There was a minimal decrease of 1% to 3% from baseline values after 3 freeze-thaw cycles and -80°C storage for 5 years.[24] Such differences are not clinically relevant. There are no data published so far regarding long-term storage of urinary TIMP-2 and IGFBP7. In our study, we did not find an association between storage time and biomarker levels (data not shown). Second, healthy control samples were obtained from various sources. For some controls, actual data on kidney function was missing, but they all were considered to have normal renal function based on their medical history. The aim was to investigate urinary markers to monitor disease severity in patients with ADPKD and not necessarily to differentiate patients from controls. Therefore, the focus was on the group of patients with ADPKD, for which data on eGFR was complete. Last, htTKV was not available for a substantial proportion of patients, which reduces the power of the analysis to detect differences. Nevertheless, the subgroup was of sufficient size to draw a firm conclusion.

In conclusion, in patients with ADPKD, we did not find differences in urinary TIMP-2 and IGFBP7 levels according to disease severity including eGFR, htTKV and/or type of genetic mutation.

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