Original Paper

Urinary Angiotensinogen and Renin Excretion are Associated with Chronic Kidney Disease

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Key Words

Chronic kidney disease • Biomarkers • Angiotensinogen • Renin-angiotensin system • Epidemiology

Abstract

Background/Aims: Several studies sought to identify new biomarkers for chronic kidney disease (CKD). As the renal renin-angiotensin system is activated in CKD, urinary angiotensinogen or renin excretion may be suitable candidates. We tested whether urinary angiotensinogen or renin excretion is elevated in CKD and whether these parameters are associated with estimated glomerular filtration rate (eGFR). We further tested whether urinary angiotensinogen or renin excretion may convey additional information beyond that provided by albuminuria. **Methods:** We measured urinary and plasma angiotensinogen, renin, albumin and creatinine in 177 CKD patients from the *Greifswald Approach to Individualized Medicine* project and in 283 healthy controls from the *Study of Health in Pomerania*. The urinary excretion of specific proteins is given as protein-to-creatinine ratio. Receiver operating characteristic (ROC) curves, spearman correlation coefficients and linear regression models were calculated. **Results:** Urinary angiotensinogen [2,511 (196-31,909) vs. 18.6 (8.3-44.0) pmol/g, $*P < 0.01$] and renin excretion [0.311 (0.135-1.155) vs. 0.069 (0.045-0.148) pmol/g, $*P < 0.01$] were significantly higher in CKD patients than in healthy controls. The area under the ROC curve was significantly larger when urinary angiotensinogen, renin and albumin excretion were combined than with urinary albumin excretion alone. Urinary angiotensinogen (β -coefficient -2.405, standard error 0.117, $P < 0.01$) and renin excretion (β -coefficient -0.793, standard error 0.061, $P < 0.01$) were inversely associated with eGFR. Adjustment for albuminuria, age, sex, systolic blood pressure and body mass index did not significantly affect the results. **Conclusion:** Urinary angiotensinogen and

renin excretion are elevated in CKD patients. Both parameters are negatively associated with eGFR and these associations are independent of urinary albumin excretion. In CKD patients urinary angiotensinogen and renin excretion may convey additional information beyond that provided by albuminuria.

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Introduction

Chronic kidney disease (CKD) is a major global health burden [1-3]. The prevalence of CKD is about 5-10% in European countries [4-8] and may be even higher with estimates around 13% in the USA [9]. The course of the disease is variable with some patients remaining stable for a long time and others progressing rapidly to end-stage renal disease (ESRD) [1]. CKD is associated with significant cardiovascular morbidity and mortality [10-12]. The clinical diagnosis of CKD is mainly based on glomerular filtration rate (GFR) and urinary albumin excretion [13].

In order to identify those CKD patients who will rapidly progress to ESRD or who are at a particular high risk for cardiovascular disease (CVD), several studies have been launched to find new biomarkers [14]. An established biomarker in CKD must be associated with disease progression and/or the risk of CVD. Potential candidates should also be associated with the severity of CKD and convey additional information beyond that provided by albuminuria [15].

Based on experimental [16-18] and clinical evidence [19-24] that the local renal renin-angiotensin system (RAS) is activated in CKD, several groups have explored the possibility that urinary angiotensinogen excretion may qualify as a suitable candidate for a biomarker for the disease [25-29]. All of these studies [25-29] found that urinary angiotensinogen excretion was negatively associated with GFR. Two studies [25, 28] reported that the negative correlation between urinary angiotensinogen excretion and GFR persisted after statistical adjustment for urinary albumin excretion, suggesting that the appearance of angiotensinogen in the urine reflects local renal angiotensinogen production rather than passage of the protein through a defective glomerular filter. Interestingly, another clinical study [30] suggested that urinary renin excretion may be better suited to reflect the intrarenal RAS activity than urinary angiotensinogen excretion. This notion was in part based on findings that urinary angiotensinogen excretion but not urinary renin excretion correlated positively with urinary albumin excretion [30].

Here, we tested whether urinary angiotensinogen or renin excretion are elevated in CKD patients compared to healthy controls and whether urinary angiotensinogen or renin excretion are associated with estimated GFR (eGFR) in a population of healthy subjects and CKD patients covering a broad range of normal to pathologically decreased eGFR. We further tested whether urinary angiotensinogen or renin excretion are associated with urinary albumin excretion or with the plasma levels of the respective proteins. Finally, we examined whether the negative associations between urinary angiotensinogen or renin excretion and eGFR depend on urinary albumin excretion.

Materials and Methods

Study population

The study was conducted in CKD patients from the *Greifswald Approach to Individualized Medicine* (GANI_MED) project and in subjects without renal disease from the *Study of Health in Pomerania* (SHIP). GANI_MED is currently conducted at the Universitätsmedizin Greifswald (University Hospital Greifswald), Germany, and comprises several clinical patient cohorts ($n > 5,000$ patients) with an emphasis on renal, metabolic and cardiovascular diseases. The project includes the establishment of standardized protocols for

the assessment of medical history, clinical data and laboratory biomarkers as well as for the collection and storage of biosamples. A central data management structure has been implemented to capture and integrate all relevant clinical data for research purposes. Project design and cohort profile have been described in detail elsewhere [31]. SHIP is a population-based survey conducted in West Pomerania including the city of Greifswald. In the baseline examination (SHIP-0) between 1997 and 2001, a total of 4,308 men and women participated (response rate 69%). Out of the 4,308 participants, 3,300 (25-86 years) were re-examined in a follow-up survey (SHIP-1) between 2002 and 2006. All SHIP data presented in the present work are from SHIP-1. Study design and cohort profile have been described in detail elsewhere [32, 33]. The investigations in GANI_MED and SHIP were carried out in accordance with the Declaration of Helsinki, including written informed consent of all participants. The present study was approved by the institutional review board of the University of Greifswald, Germany.

The study population was recruited in three steps. First, all patients that (1.) had been enrolled in the cohort *Renal and Cardiovascular Diseases* of the GANI_MED project by 31 December 2013, (2.) suffered from CKD, (3.) had provided blood and urine samples for laboratory analyses, and (4.) had consented to storage and further analyses of these samples were considered ($n=184$). CKD was defined as eGFR below 60 ml/min/1.73m² consistent with the definition of CKD $\geq$ stage 3 proposed by the National Kidney Foundation Disease Outcomes Quality Initiative [34]. From this cohort, we excluded all patients with insufficient plasma or urine sample volumes ($n=3$), resulting in a remaining sample of 181 patients.

Second, we considered the entire SHIP-1 cohort ($n=3,300$) to select healthy controls. From this cohort, we excluded all subjects (1.) without consent to storage of plasma or urine samples or with insufficient plasma or urine sample volumes ($n=169$), (2.) with an eGFR below 60 ml/min/1.73m² ($n=309$) or missing serum creatinine concentration ($n=8$), (3.) with urinary albumin-to-creatinine ratio >2.5 mg/mmol in men and >3.5 mg/mmol in women ($n=440$) or missing urinary albumin or creatinine concentrations ($n=5$), (4.) with intake of angiotensin I-converting enzyme inhibitors (ACEIs), angiotensin II receptor blockers (ARBs), diuretics, or direct renin inhibitors (DRIs) ($n=499$), (5.) with missing data on plasma renin concentration ($n=11$), or (6.) with missing data on body mass index (BMI) ($n=2$), resulting in a remaining sample of 1,857 subjects that were free of renal diseases. This sample was screened for age (± 5 years) and sex-matched controls that would balance our CKD patients using the Greedy Matching Algorithm [35] implemented in a SAS Makro. The matching procedure resulted in 177 patient/control pairs that were used for the case-control study.

Third, we recruited additional subjects from the SHIP-1 cohort with an eGFR between 40-60 ml/min/1.73 m² for our regression analyses. These subjects were selected according to the following criteria: (1.) sufficient plasma and urine sample volumes, (2.) eGFR between 40-60 ml/min/1.73m², (2.) no intake of ACEIs, ARBs, diuretics, or DRIs, (3.) data on plasma renin concentration available, (4.) data on serum creatinine concentration available, (5.) data on urinary creatinine and albumin concentrations available, and (6.) data on BMI available. These criteria were satisfied by a total of 106 subjects. There was no overlap with the control group as identified by the matching procedure (see second step). The reason for adding these subjects to our study population for the regression analyses was that our initial recruiting procedure was designed to result in a subgroup with low eGFR (CKD patients, only 14 of which had an eGFR above 40 ml/min/1.73m²) and another subgroup with high eGFR (healthy controls, all of which had an eGFR above 60 ml/min/1.73m²). We therefore had to include further subjects with an eGFR in the range between 40-60 ml/min/1.73m² in order to avoid missing potential non-linear associations.

The three recruiting steps resulted in a final study population of 460 subjects (177 from GANI_MED and 283 from SHIP-1) covering a broad range of eGFR values for the association study. As drugs interfering with the RAS may have potentially affected our results, we reanalyzed our data excluding all patients taking ACEIs, ARBs, diuretics, or DRIs. The sample size for this sub-analysis was $n=308$.

Biochemical measurements

Plasma and urinary angiotensinogen concentrations were measured with a commercially available solid-phase enzyme-linked immunosorbent assay kit (Human Total Angiotensinogen Assay Kit, Immunobiological Laboratories Co., Ltd., Gunma, Japan) according to the manufacturer's instructions. Briefly, samples were centrifuged (urine: 20 min at 4°C, 1,000 *g*, plasma: 6 min at 4°C, 3,000 *g*) and diluted (urine: 1:2 to 1:2,000; plasma: 1:5,000 and 1:10,000) with enzyme immune assay buffer. An immobilized

rabbit anti-human angiotensinogen antibody was used to bind urinary or plasma angiotensinogen to the microtiter plate. Horseradish peroxidase (HRP)-conjugated mouse anti-human angiotensinogen antibody and tetramethylbenzidine were used to detect the amount of bound angiotensinogen. HRP catalyzes the conversion of tetramethylbenzidine to diimin that can be read photometrically at 450 nm. Plasma and urinary angiotensinogen concentrations were determined by nonlinear regression and standard curve.

Plasma and urinary renin concentrations were measured with a commercially available radioimmunoassay (Renin III Generation, Cisbio Bioassays, Bedford, MA, USA) according to the manufacturer's instructions. Urine and plasma samples were used without any further treatment. An immobilized monoclonal mouse anti-human renin antibody was used to bind renin of the samples to the wall of the tubes. A ¹²⁵I-iodine-conjugated mouse anti-human renin antibody was added to detect the amount of bound renin. After three hours of incubation, radioactivity was measured with a gamma counter (Gamma Counter LB 211, Berthold Technologies, Bad Wildbad, Germany). Plasma and urinary renin concentrations were determined by linear regression and standard curve.

During the course of the study, standard laboratory methodology for measurements of serum and urinary creatinine concentrations at our institution switched from a modified kinetic Jaffé reaction (Siemens Dimension RxL, Siemens Healthcare Diagnostics, Eschborn, Germany) to an enzymatic method (Dimension VISTA, Siemens Healthcare Diagnostics, Eschborn, Germany). In order to avoid a potential bias due to the use of two different methods, we reanalyzed a total of 360 samples using both methods. As a result of this comparative analysis, the following formulas were introduced to align the data obtained with the Jaffé method to those obtained with the enzymatic method: (1) $[\text{creatinine}]_{\text{plasma}} = 0.915 \times [\text{creatinine}]_{\text{Jaffé}} - 3.61$; (2) $[\text{creatinine}]_{\text{urine}} = 0.996 \times [\text{creatinine}]_{\text{Jaffé}} - 0.242$. eGFR was calculated according to the four-variable Modification of Diet in Renal Disease formula [36]. Urinary albumin concentration was measured with a nephelometric assay (BN ProSpec Analyzer, Dade Behring, Deerfield, IL, USA, in SHIP-1 and Dimension VISTA, Siemens Healthcare Diagnostics, Eschborn, Germany, in GANI_MED).

Statistical analyses

Data are expressed as proportions or median (interquartile range). Urinary albumin, renin or angiotensinogen concentrations below detection limits were set equal to the respective detection limit. Urinary excretions of specific proteins are given as protein-to-creatinine ratios. Protein concentrations and protein-to-creatinine ratios were log-transformed to obtain normally distributed values. Differences between CKD patients and controls were evaluated by chi-square or Kruskal-Wallis tests. Receiver operating characteristic (ROC) analyses, based on logistic regression models, were performed to assess the power of urinary angiotensinogen or renin excretion to distinguish between patients and controls. Further, we assessed whether the combination of urinary angiotensinogen, renin and albumin excretion would increase the area under the ROC curve and thus improve the prediction of CKD compared to the urinary albumin excretion alone. Associations between urinary angiotensinogen or renin excretion and eGFR, urinary albumin excretion, or plasma angiotensinogen or renin concentrations, respectively, were assessed by Spearman's correlation. Associations between urinary angiotensinogen or renin excretion and eGFR were assessed with multivariable linear regression. The regression models are presented unadjusted and adjusted for urinary albumin excretion alone or for the combination of urinary albumin excretion, age, sex, systolic blood pressure and BMI. Statistical significance was accepted at $P < 0.05$. Statistical analyses were performed with SAS (Version 9.3 SAS Institute Inc., Cary, USA) and Prism 5.01 (©1992-2007 GraphPad Software Inc., La Jolla, CA, USA). Graphs were generated with Prism 5.01 and SAS.

Results

The study population for the case-control study consisted of 177 CKD patients from GANI_MED and 177 healthy controls from SHIP-1. In each group there were 118 men and 59 women. As expected, eGFR was significantly lower in CKD patients than in healthy controls (Table 1). The study population for the association study consisted of all subjects that had been enrolled in the case-control study plus 106 persons with intermediate eGFR values (Table 1).

Table 1. General characteristics of the study population

	Case-control study*		P	Association study#
	Controls (n=177)	CKD (n=177)		Subjects (n=460)
Men (%)	66.7	66.7	1.00	57.2
Age (years)	70 (58-77)	70 (58-77)	0.78	69 (58-77)
Diabetes (%)	10.2	47.5	<0.01	23.5
BMI (kg/m ²)	27 (24-29)	29 (25-34)	<0.01	28 (25-31)
SBP (mmHg)	135 (126-148)	132 (121-144)	0.03	134 (123-147)
DBP (mmHg)	81 (75-88)	73 (68-80)	<0.01	78 (72-85)
eGFR (ml/min/1.73 m ²)	94 (85-104)	17 (10-34)	<0.01	66 (27-88)
Medication with				
ACEIs (%)	0.0	33.9	<0.01	13.0
ARBs (%)	0.0	27.1	<0.01	10.4
Diuretics (%)	0.0	74.6	<0.01	28.7
DRIs (%)	0.0	1.1	0.16	0.4

Data are given as median (interquartile range) or proportion. Group differences were tested with chi-square or Kruskal-Wallis tests; ACEIs - angiotensin I-converting enzyme inhibitors; ARBs - angiotensin II receptor blockers; BMI - body mass index; CKD - chronic kidney disease; DBP - diastolic blood pressure; DRIs - direct renin inhibitors; eGFR - estimated glomerular filtration rate; SBP - systolic blood pressure

Table 2. Urinary excretions of angiotensinogen, renin and albumin as well as plasma concentrations of angiotensinogen, renin and creatinine in 177 healthy controls and 177 patients with chronic kidney disease (CKD)

	Controls (n=177)	CKD (n=177)	P
Urinary excretion			
AGT/Crea (pmol/g)	18.6 (8.3-44.0)	2,511(196-31,909)	<0.01
Renin/Crea (pmol/g)	0.069(0.045-0.148)	0.311(0.135-1.155)	<0.01
Alb/Crea (mg/g)	8.0(5.1-12.9)	251.3(44.7-825.5)	<0.01
Plasma concentration			
AGT (nmol/l)	763(640-913)	679(509-836)	<0.01
Renin (fmol/l)	203(124-303)	495(246-1,270)	<0.01
Crea (mg/dl)	0.8(0.7-0.9)	3.4(1.9-6.0)	<0.01

Data are given as median (interquartile range); AGT - angiotensinogen; AGT/Crea - angiotensinogen-to-creatinine ratio; Alb - albumin; Alb/Crea - albumin-to-creatinine ratio; Crea - creatinine; Renin/Crea - renin-to-creatinine ratio

Fig. 1. Urinary angiotensinogen (AGT) excretion (A) and urinary renin excretion (B) in 177 healthy controls and 177 patients with chronic kidney disease (CKD). Results are given as natural logarithms of the urinary angiotensinogen-to-creatinine ratio and the urinary renin-to-creatinine ratio, respectively. *P<0.01.

CKD patients showed significantly higher urinary angiotensinogen excretion than healthy controls (Table 2 and Figure 1A), although plasma angiotensinogen concentration was significantly lower in patients than in controls (Table 2). CKD patients also showed

Fig. 2. Receiver operating characteristic curves for urinary angiotensinogen (given as angiotensinogen-to-creatinine ratio, AGT/Crea) and renin excretion (given as renin-to-creatinine ratio, Renin/Crea) (A) as well as for urinary albumin excretion (given as albumin-to-creatinine ratio, Alb/Crea) and the full model including urinary albumin, angiotensinogen and renin excretions based on a logistic regression model (B). AUC - area under the curve; $n=177$ patients with chronic kidney disease, $n=177$ controls.

Fig. 3. Scatter-plots with regression lines for urinary angiotensinogen (AGT) excretion and estimated glomerular filtration rate (eGFR) (A) as well as for urinary renin excretion and eGFR (B). Data are expressed as natural logarithms of the urinary angiotensinogen-to-creatinine ratio, the urinary renin-to-creatinine ratio and eGFR. $n=460$.

significantly higher urinary renin excretion than healthy controls (Table 2 and Figure 1B), but plasma renin concentration was also significantly higher in patients than in controls (Table 2).

In the ROC analyses, both urinary angiotensinogen and renin excretion had considerable discriminatory power to differentiate between CKD patients and controls, with urinary angiotensinogen excretion outperforming urinary renin excretion in this respect (Figure 2A). The area under the ROC curve was significantly larger when urinary angiotensinogen, renin and albumin excretion were combined than with urinary albumin excretion alone (Figure 2B).

There were statistically significant negative correlations between urinary angiotensinogen or renin excretion and eGFR (Figure 3). Furthermore, there were statistically significant positive correlations between urinary angiotensinogen or renin excretion and urinary albumin excretion (angiotensinogen: $r=0.789$, $P<0.01$; renin: $r=0.555$, $P<0.01$) (data not shown). Both, urinary angiotensinogen and renin excretion, correlated only weakly with the plasma concentrations of the respective proteins (Figure 4). This correlation was nominally negative for angiotensinogen (Figure 4).

The negative associations between urinary angiotensinogen or renin excretion and eGFR were confirmed in linear regression models and persisted after adjustment for

Fig. 4. Scatterplots with regression lines for urinary angiotensinogen (AGT) excretion and plasma angiotensinogen concentration (A) as well as for urinary renin excretion and plasma renin concentration (B). Data are expressed as natural logarithms of the urinary angiotensinogen-to-creatinine ratio, plasma angiotensinogen concentration, the urinary renin-to-creatinine ratio and plasma renin concentration. $n=460$.

Table 3. Associations between the urinary angiotensinogen-to-creatinine ratio and eGFR as well as between the urinary renin-to-creatinine ratio and eGFR. Results from multivariable linear regression analyses without adjustment as well as with adjustments for urinary albumin-to-creatinine ratio alone or for urinary albumin-to-creatinine ratio, age, sex, systolic blood pressure and body mass index in 460 subjects

	ln[AGT/Crea (pmol/g)]			ln[Renin/Crea (pmol/g)]		
	β	S.E.	P	β	S.E.	P
without adjustment						
ln[eGFR (ml/min/1.73 m ²)]	-2.405	0.117	<0.01	-0.793	0.061	<0.01
adjusted for urinary albumin excretion						
ln[eGFR (ml/min/1.73 m ²)]	-0.963	0.132	<0.01	-0.400	0.081	<0.01
adjusted for urinary albumin excretion, age, sex, SBP, BMI						
ln[eGFR (ml/min/1.73 m ²)]	-1.076	0.138	<0.01	-0.389	0.086	<0.01

AGT - angiotensinogen; β - regression coefficient; BMI - body mass index; Crea - creatinine; eGFR - estimated glomerular filtration rate; SBP - systolic blood pressure; S.E. - standard error

urinary albumin excretion and after adjustment for urinary albumin excretion, age, sex, systolic blood pressure and BMI (Table 3). Furthermore, excluding all patients taking ACEIs, ARBs, diuretics, or DRIs did not significantly affect our results (angiotensinogen: $\beta=-1.929$, S.E.=0.188, $P<0.01$; renin: $\beta=-0.671$, S.E.=0.103, $P<0.01$).

Discussion

In the present study, CKD patients had higher urinary angiotensinogen and renin excretion than healthy controls. Furthermore, both, urinary angiotensinogen and renin excretion were negatively associated with eGFR and positively with urinary albumin excretion. While it is well known that CKD patients show increased urinary angiotensinogen excretion and that the latter correlates negatively with eGFR [25-29], there is currently little information on urinary renin excretion in CKD patients and a possible relation of urinary renin excretion to eGFR, respectively [30].

In a recent study [30], van den Heuvel et al. reported increased urinary renin excretion in diabetic patients compared to non-diabetic controls. The urine/plasma concentration

ratio of renin did not correlate with eGFR [30]. Based on their findings that patients receiving ACEIs or ARBs showed decreased urinary renin levels compared to patients that did not receive these drugs and that this effect was independent of plasma renin concentration, these authors suggested that urinary renin may closely reflect the activity of the intrarenal RAS rather than being plasma derived [30]. Since urinary angiotensinogen did not respond to the presumed effects of RAS blockade on the activity of the intrarenal RAS, the authors further concluded that urinary renin may be better suited to monitor the activity of the intrarenal RAS than urinary angiotensinogen.

In contrast to our study, van den Heuvel et al. [30] found no correlation between urinary renin and eGFR. There are several explanations for this discrepancy including (1.) a smaller range of eGFR values with few patients having an eGFR of less than 60 ml/min/1.73 m², (2.) fewer subjects ($n=101$ vs. $n=460$), and (3.) the use of a different parameter for urinary renin excretion (urine/plasma concentration ratio of renin vs. urinary renin-to-creatinine ratio) in the previous study [30].

Since angiotensinogen and renin have similar molecular weight (approximately 50 and 37 kDa, respectively), which is somewhat lower than that of albumin (approximately 66 kDa), they may pass the renal glomerular filter to a certain extent. In fact, the appearance of significant amounts of albumin in the urine is one of the major hallmarks of a damaged glomerular filter in CKD. As albuminuria is well established in diagnosing and staging CKD, any potential new biomarker for CKD needs to convey additional information beyond that provided by albuminuria. Since urinary albumin is mainly plasma-derived and reflects the damage to the glomerular filter, we sought to determine whether urinary angiotensinogen or renin would provide additional information. To this end, we (1.) tested whether urinary angiotensinogen or renin excretion correlated with urinary albumin excretion, (2.) investigated whether urinary angiotensinogen or renin excretion were affected by the plasma concentrations of the respective proteins, and (3.) reanalyzed our data concerning the associations between urinary angiotensinogen or renin excretion and eGFR after statistical adjustment for urinary albumin excretion.

In our hands, there were statistically significant positive correlations between both, urinary angiotensinogen or renin excretion and urinary albumin excretion. While angiotensinogen or renin may have taken the same route through the glomerular filter as albumin, these findings are compatible with the alternative explanation that urinary angiotensinogen or renin may be predominantly kidney-derived and reflect an activated renal RAS with deteriorating renal function. In order to allow an adequate explanation, these findings must therefore be seen in the context of our further results (see below). Our findings concerning the correlation between urinary angiotensinogen and albumin excretion are in agreement with several other studies [25-29] which obtained similar results. Information on the relation between urinary renin and albumin excretion is sparse. Van den Heuvel et al. [30] found no correlation between urine/plasma concentration ratio of renin and albumin in diabetic and non-diabetic patients, supporting the notion that urinary renin may be predominantly kidney-derived and may not reflect the passage of renin through a defective glomerular filter.

If angiotensinogen or renin were to reach the urine predominantly as plasma-derived proteins through a defective glomerular filter, their urinary excretion should positively correlate with their plasma concentration. In contrast to this requirement, CKD patients showed increased urinary angiotensinogen excretion despite decreased plasma angiotensinogen concentrations in our case-control study. Furthermore, we found a weak negative correlation between urinary angiotensinogen excretion and plasma angiotensinogen concentration and a similarly weak positive correlation between urinary renin excretion and plasma renin concentration in the overall study population. Together, our data on angiotensinogen strongly argue against the hypothesis that urinary angiotensinogen is predominantly plasma-derived. Our findings are supported by results from other groups [25-29] who failed to detect a significant correlation between urinary angiotensinogen

excretion and plasma angiotensinogen concentration [25, 27-29], while the former correlated positively with renal angiotensinogen mRNA abundance [26]. As for renin, the correlation between its urinary excretion and its plasma concentration was nominally positive, but the weak correlation coefficient suggests that this statistical association may not be biologically relevant. Literature data on this issue are again sparse precluding relevant comparisons with other work.

To further exploit our measurements regarding possible hints as to the source of urinary angiotensinogen or renin, we reanalyzed our data on the relation between urinary angiotensinogen or renin excretion and eGFR after statistical adjustment for urinary albumin excretion. The results show that the association between urinary angiotensinogen or renin excretion and eGFR remained statistically significant with little impact on effect strengths after adjustment for urinary albumin excretion. The fact that the negative associations between urinary angiotensinogen or renin excretion and eGFR were independent from urinary albumin excretion suggests that both, urinary angiotensinogen and renin excretion, may convey useful information that goes beyond the information provided by urinary albumin excretion. The ROC analyses showed that both, urinary angiotensinogen and renin excretion, had considerable discriminatory power to differentiate between CKD patients and healthy controls and that the combination of urinary angiotensinogen, renin and albumin excretion resulted in higher discriminatory power than urinary albumin excretion alone, supporting the suggestion that the urinary angiotensinogen and renin excretion may convey useful information that goes beyond the information provided by urinary albumin excretion alone.

As ACEIs, ARBs, diuretics, and DRIs may affect both, the systemic and the local renal RAS, we reanalyzed our data excluding all patients taking one or more of these drugs. Interestingly, this measure did not affect our results, suggesting that the negative associations between urinary angiotensinogen or renin excretion and eGFR may reflect the extent of kidney disease rather than effects of RAS-related drugs in CKD patients. As factors like age, sex, systolic blood pressure and BMI are associated with the progression of CKD, we reanalyzed our data adjusting for these variables. This measure did not affect our results, suggesting that the negative associations between urinary angiotensinogen or renin excretion and eGFR were independent of age, sex, systolic blood pressure and BMI.

Our study has several strengths and limitations. A particular strength of our study is the broad and continuous range of eGFR from less than 10 to well above 100 ml/min/1.73m² in our study population, which we achieved by including renal patients from a large clinical study (GANI_MED) together with well-matched and thoroughly characterized healthy individuals from a population-based study (SHIP) that was performed in the same region and by the staff of the same university hospital (Universitätsmedizin Greifswald). A further strength is the standardized data collection procedure performed by specially trained and certified examiners in both major studies (GANI_MED and SHIP). Unfortunately, the epidemiological study design did not allow us to perform repeated blood and urine samplings including 24-h urinary samples and measurements. A further limitation arises from the cross-sectional study design, which does not allow analyzing causality between the measures. Thus, it remains to be determined in further studies whether urinary angiotensinogen or urinary renin excretion can be useful biomarkers to help predict CKD progression or the risk of CVD in renal patients.

Conclusion

In conclusion, the results of our study demonstrate negative associations between urinary angiotensinogen or renin excretion and eGFR which are independent from urinary albumin excretion. Both, urinary angiotensinogen and renin excretion may convey additional information beyond that provided by albuminuria. The findings of our study support the suitability of urinary angiotensinogen and renin excretion as biomarkers in CKD.

Disclosure Statement

The authors of this manuscript state that they do not have any conflict of interests and nothing to disclose.

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