ASSOCIATIONS OF URINARY UROMODULIN WITH CLINICAL CHARACTERISTICS AND MARKERS OF TUBULAR FUNCTION IN THE GENERAL POPULATION

*Menno Pruijm, *Belen Ponte, Daniel Ackermann, Fred Paccaud, Idris Guessous, Georg Ehret, Antoinette Pechère-Bertschi, Bruno Vogt, Markus Mohaupt, Pierre-Yves Martin, Sonia Youhanna, Nadine Nagele, Peter Vollenweider, Gérard Waeber, Michel Burnier, Olivier Devuyst, Murielle Bochud

SUPPLEMENTAL MATERIAL

METHODS

Renal ultrasound:

In SKIPOGH, renal grey-scale B mode ultrasound was performed according to a standardized procedure, as published previously (1). The longitudinal dimensions of each kidney were measured in a sagittal plane visually estimated to represent the largest longitudinal diameter. The width and transverse diameter were measured in a transverse plane perpendicular to the longitudinal axis of the kidney. The mean of three separate measurements of each parameter was reported. Renal volume was calculated as 0.523 x length x width x transverse diameter (2). Subjects with renal cysts were excluded from further analysis, since in these subjects, renal length and volume cannot be reliably assessed and cannot be considered as representative markers of renal parenchyma (3, 4).In each center, all ultrasounds were performed by the same experienced operator. As different operators performed the ultrasound examinations, a concordance study of 20 participants was undertaken in which two operators examined the kidneys blinded to the other's results, as published previously (1). The following results were found for the reproducibility of the ultrasound data: Lin's correlation coefficients for right and left kidney length were respectively 0.90 and 0.82, and intra-class correlation coefficients were 0.91 and 0.82 (all: $p<0.005$).

The accuracy of ultrasonic kidney volume measurement was assessed in twenty subjects in Lausanne who underwent on the same day renal magnetic resonance imaging (MRI) and renal ultrasound in an affiliated study, as published previously (1). Renal MRI-based dimensions were assessed by an independent, experienced radiologist who was blinded for the ultrasound results. MRI-based kidney volume was calculated using the same formula as renal ultrasound-measured kidney volume. Lin's correlation coefficients for right and left kidney volume between renal MRI and ultrasound were and 0.79 and 0.79 (both: $p<0.005$). The Lin's correlation coefficients for right and left kidney length between MRI and ultrasound were 0.86 and 0.89, respectively (both: $p<0.005$).

Statistical methods:

SKIPOGH: two-level multivariable mixed linear regressions, adjusting for center and family, were used to take into account the multi-centric and family-based nature of the study. In order to explore which variables were associated with 24hUER, we first performed unadjusted linear regression analyses with urinary uromodulin excretion as the dependent variable and age, sex, body height and body weight, smoking, diabetes hypertension, any diuretic use, 24h urinary volume and creatinine excretion as the independent ones. Then, all the variables of interest were adjusted for the other covariates, except urinary creatinine, in model 1. In model 2, the 24h urinary creatinine excretion was added as a covariate. We then assessed whether kidney function and kidney mass were associated with 24h UER. 24h UER was the dependant variable and we analyzed separately its association with estimated glomerular filtration rate (eGFR CKD-EPI), 24h urinary creatinine clearance (uGFR), ultrasound-assessed kidney length and kidney volume. Models 1 were adjusted for age, sex, body height and weight, as well as for the classical CKD risk factors diabetes, hypertension, active smoking and urinary volume. Results of the linear regression analyses are presented as beta-coefficients (β) and their 95% confidence intervals.

The associations between 24h urinary electrolytes and osmolality (dependent variables) with 24h UER (independent variable) were adjusted for age, sex, body height and weight, eGFR, and 24h urinary volume.

Graphical associations between 24h urinary uromodulin excretion (24h UER) and measured GFR, renal length and renal volume as markers of renal mass, and osmolar excretion were created by generating adjusted residuals from mixed linear models, including only age and sex as covariates (but taking into account the family and center effects) and then scaling them by adding the mean of the dependent variable.

CoLaus: Unadjusted and multivariable linear regression analyses were performed to assess the association between urinary uromodulin concentration (dependent variable) and eGFR (independent variable). The same adjustments were made as for SKIPOGH in model 1 (except urinary volume); in model 2, urinary creatinine concentration was added as a covariate. Stratified analyses were also conducted for CKD-EPI< and ≥90ml/min/1.73m^2 as we observed a similar non-linear relationship graphically.

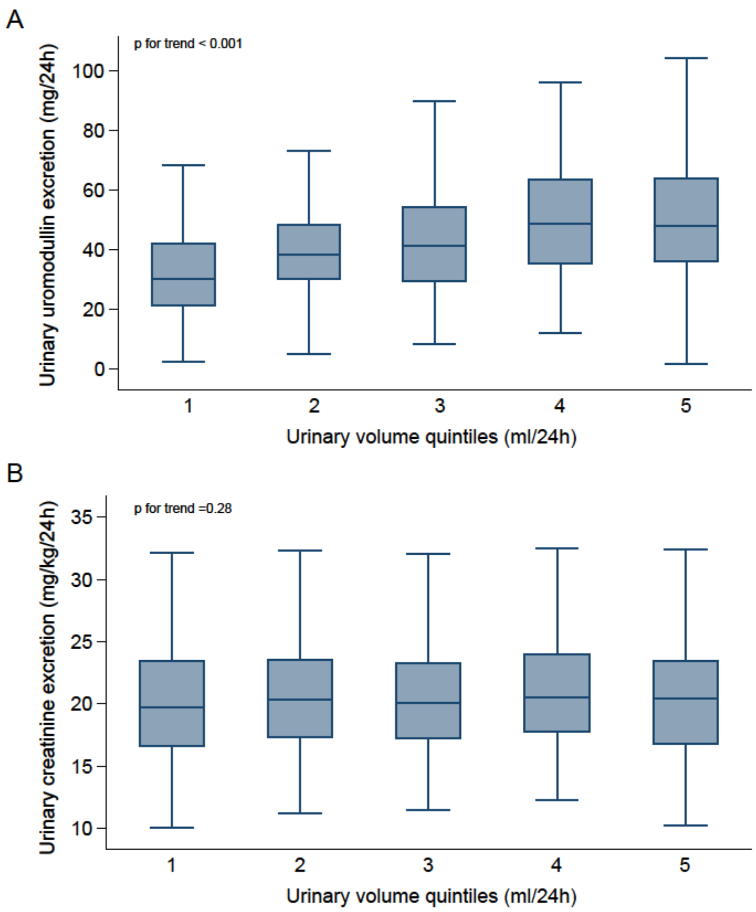
For the electrolytes analyses, electrolytes concentrations, taken one-at-a-time, were the dependent variable and uromodulin concentration the independent variable of interest, while adjusting for the same covariates as SKIPOGH in model 1 (except urinary volume) and adding urinary creatinine concentration in model 2.

RESULTS

Determinants of 24h urinary uromodulin excretion (24hUER):

We found a positive association between 24h urine volume and uromodulin excretion, as illustrated by the increase of 24h uromodulin excretion over quintiles of 24h urine volume, as shown in the supplementary figure 1A (p trend<0.001).

Supplementary Figure 1:



One might argue that those with larger 24h urine volumes had a more complete 24h urine collection than those with smaller volumes. However, the 24h creatinine excretion (in mg/kg body weight, as a proxy of completeness of urine collection) did not increase over quintiles of 24h urine volume (p trend=0.28, see supplementary figure 1B and the table below).

Table 1:

Quintile	median urine volume (ml)	24h creatinine excretion (mg/kg body weight)	Inter quartile range
1	900	19.7	6.9
2	1282	20.4	6.3
3	1600	20.1	6.1
4	2002	20.5	6.2
5	2753	20.4	6.6

Therefore, the positive associations between uromodulin and volume are not explained by more complete 24h urine collections in those with higher daily volume and uromodulin excretions.

Associations between urinary uromodulin and markers of glomerular filtration

In order to assess whether standardisation for BSA played a role, additional analyses for CKD-EPI unadjusted for BSA, cystatin C and eGFR according to the Cockroft formula were performed.

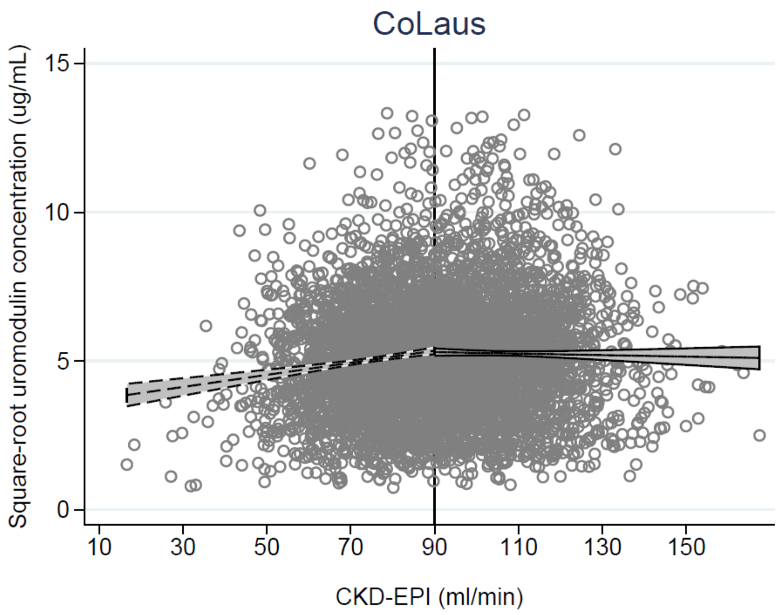
Results of crude and multivariable regression analyses are shown in the following Table*:*

Table 2:

Independent variable of interest, study	**Unadjusted**		**Model 2**	
Dependent variables	**β (95%CI)**	***P* value**	**β (95%CI)**	***P* value**
Uromodulin excretion (mg/24h), SKIPOGH				
CKD-EPI (ml/min/1.73m^2) (n=817)	0.01(0.01; 0.02)	<0.001	0.01 (0.005; 023)	0.002
<90 ml/min/1.73m^2 (n=241)	0.04 (0.02;0.06)	<0.001	0.04 (0.02; 0.06)	<0.001
≥90 ml/min/1.73m^2 (n=576)	0.002 (-0.01; 0.01)	0.7	0.007 (-0.01; 0.02)	0.3
CKD-EPI (ml/min) (n=810)	0.01 (0.001; 0.02)	<0.001	0.01 (0.005; 0.022)	0.002
<90 ml/min (n=198)	0.05 (0.02; 0.008)	0.002	0.02 (0.006;0.04)	0.008
>90ml/min (n=612)	0.01 (0.002; 0.03)	0.02	0.01 (0.002;0.03)	0.02
CKD-EPI$_{\text{cystatine C}}$ (n=723)	0.02 (0.01;0.03)	<0.001	0.018 (0.008;0.03)	0.001
<90 ml/min (n=60)	0.06(0.03;0.09)	<0.001	0.06 (0.03:0.09)	<0.001
>90ml/min (n=663)	0.02 (0.007;0.03)	0.001	0.01 (-0.003; 0.02)	0.12
Cockroft (n=816)	0.008 (0.005;0.01)	<0.001	0.01 (0.004; 0.02)	0.001
<90 ml/min (n=228)	0.025 (0.01;0.04)	0.001	0.03 (0.01;0.05)	0.002
>90 ml/min (n=588)	0.005(0.0001;0.01)	0.045	0.009 (0.001;0.02)	0.02

In CoLaus, we observed similar results when using CKD-EPI expressed in ml/min (see Supplementary Figure 2). Cystatin C was not measured in CoLaus.

Supplementary Figure 2:



Thus, similar results were obtained when using CKD-EPI, Cockroft or Cystatin C based-formula's as marker of eGFR: associations were stronger for values <90 ml/min than those ≥90 ml/min.

References:

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3. Ponte B, Pruijm M, Ackermann D, Vuistiner P, Eisenberger U, Guessous I, Rousson V, Mohaupt MG, Alwan H, Ehret G, Pechere-Bertschi A, Paccaud F, Staessen JA, Vogt B, Burnier M, Martin PY,Bochud M: Reference values and factors associated with renal resistive index in a family-based population study. *Hypertension* 63**:**136-42, 2014
4. Ponte B, Pruijm M, Ackermann D, Vuistiner P, Guessous I, Ehret G, Alwan H, Youhanna S, Paccaud F, Mohaupt M, Pechere-Bertschi A, Vogt B, Burnier M, Martin PY, Devuyst O,Bochud M: Copeptin is associated with kidney length, renal function, and prevalence of simple cysts in a population-based study. *J Am Soc Nephrol* 26**:**1415-25, 2015