Nephron

Clinical Practice: Research Article

Nephron
DOI: 10.1159/000534578

Received: May 17, 2023
Accepted: October 7, 2023
Published online: December 1, 2023

Urine Uromodulin, Kidney Tubulointerstitial Fibrosis, and Furosemide Response

Alexander L. Bullen[a, b] Sucheta Vaingankar[b] Magdalena Madero[c] Salvador Lopez Gil[d] Etienne Macedo[b] Joachim H. Ix[a, b] Dena E. Rifkin[a, b] Pranav S. Garimella[b]

[a]Nephrology Section, Veterans Affairs San Diego Healthcare System, La Jolla, CA, USA; [b]Division of Nephrology-Hypertension, Department of Medicine, University of California San Diego, San Diego, CA, USA; [c]Nephrology Department, National Institute Cardiology Ignacio Chávez, Mexico City, Mexico; [d]Nephrology Department, Centro Médico ABC Observatorio, Colonia Las Americas, Álvaro Obregón, Mexico City, Mexico

Keywords
Biomarker · Uromodulin · Fibrosis · Chronic kidney disease · Furosemide stress test

Abstract
Background: Interstitial fibrosis and tubular atrophy (IFTA) are common findings on biopsy in chronic kidney disease (CKD) and are strongly predictive of kidney failure. IFTA is poorly correlated with estimated glomerular filtration rate (eGFR) and albuminuria, the most common measures of kidney function. Thus, IFTA is prognostically important, yet its presence and severity are invisible to the clinician except when kidney biopsies are obtained. ***Objectives:*** The objective of this study was to investigate (1) the cross-sectional association between urine uromodulin (uUMOD) and IFTA and (2) to determine whether uUMOD levels were associated with diuretic response after a furosemide stress test. ***Methods:*** We performed logistic regression to evaluate the association between uUMOD and fibrosis. We used linear regression models to assess the association of uUMOD with diuretic response. ***Results:*** Among 52 participants, the mean age was 42 ± 16 years, 48% were women, 23% had diabetes, and the median eGFR was 56 mL/min/1.73 m^2. The mean uUMOD concentration was 5.1 (8.4) μg/mL. Each halving of uUMOD was associated with 1.74 higher odds (95% CI: 1.10, 2.75) of grade 2 or 3 fibrosis. However, this association was no longer significant after adjusting for baseline eGFR and albuminuria. Each halving of uUMOD was associated with a decreased response to furosemide. This association was also no longer significant after adjusting for baseline eGFR and albuminuria. ***Conclusion:*** In a population of individuals with a wide range of kidney function undergoing clinically indicated kidney biopsies, we did not find an association between uUMOD and interstitial fibrosis or response to loop diuretics after adjusting for eGFR and albuminuria.

Introduction

Interstitial fibrosis and tubular atrophy (IFTA) are common findings on biopsy in chronic kidney disease (CKD) [1]. Despite IFTA being poorly correlated with

karger@karger.com
www.karger.com/nef

Correspondence to:
Pranav S. Garimella, pgarimella@health.ucsd.edu

estimated glomerular filtration rate (eGFR) and albuminuria [2], it is very strongly predictive of progression to kidney failure [3]. Thus, IFTA is common and prognostically important, yet its presence and severity are invisible to the clinician except in instances where a kidney biopsy is obtained. The diuretic response to a single dose of furosemide (the furosemide stress test, FST [4]) is a useful prognostic tool to non-invasively evaluate tubular function and has been associated with degree of IFTA [5]. Several biomarkers of tubule function that correlate with IFTA have emerged as potential candidates to improve our understanding of global kidney function in addition to glomerular filtration [6]. Urine uromodulin (uUMOD) is one such biomarker that is shown to be associated with kidney function decline and additionally implicated in the role in sodium reabsorption and diuretic response [7, 8].

uUMOD is the most abundant urine protein in healthy adults [9]. Higher levels of uUMOD may serve as a surrogate for kidney tubule functional capacity. We have previously demonstrated that lower levels of uUMOD are associated with higher risk of acute kidney injury (AKI) and CKD progression, independent of eGFR and urine albumin-to-creatinine ratio (uACR). The relationship uUMOD with IFTA and its association with the FST are relatively unknown. Thus, the objectives of this study were to investigate (1) the cross-sectional association between uUMOD and IFTA in persons undergoing a kidney biopsy for clinical indications and (2) to determine whether uUMOD levels were associated with diuretic response after an FST. We hypothesized that lower uUMOD would identify individuals with greater severity of IFTA on kidney biopsy and that it would be associated with a lower urine output in response to furosemide.

Methods

We evaluated a cohort of 52 participants older than 18 years of age who underwent native and allograft kidneys biopsies for clinical indications and had available urine sample for biomarker assay. The details of this study have been previously described [5]. For the FST, subjects who had not received diuretics in the previous 7 days received an intravenous dose of furosemide of 1 mg/kg. Those that had received diuretics previously were given a dose of 1.5 mg/kg. The doses of furosemide used were in accordance to the FST protocol previously published [10]. Strict urine output quantification was done for the first 6 h. The following day, participants underwent a kidney biopsy. All participants provided written informed consent, and the study was approved by the Institutional Review Board of the parent institution. A renal pathologist was blinded to the clinical diagnosis and evaluated the kidney biopsies. Fibrosis percentage was assessed by an optic lector machine (morphometry) using the Analyzer Olympus BX51 Microscope, image Software Image-PN-Plus 6, and Camera: VF Evolution C (half Cybernetics). uUMOD was measured at the beginning of the study in duplicate using the Meso Scale Discovery R-PLEX Human Uromodulin kits. The analytic range was 244–1,000,000 pg/mL. Urine creatinine was also measured at the beginning of the study, concurrently with uUMOD.

Given skewed distributions, we log-base-0.5 transformed uUMOD to facilitate interpretation of parameter estimates as "per halving" of uUMOD. Due to our sample size, for the primary outcome, interstitial fibrosis score was categorized into two groups according to fibrosis percentage: <25% (grade 1) and ≥25% (grade 2 or 3). We utilized logistic regression to evaluate the association between uUMOD and fibrosis. We used linear regression models to evaluate the association of uUMOD with urine output. Models were adjusted for the following potential confounders that were selected a priori based on biological plausibility: age, sex, urine creatinine, obesity, diabetes mellitus, hypertension, eGFR, and urine albumin. We performed similar analyses with p-cresol sulfate, another proximal tubule marker, for comparison.

All analyses were conducted using Stata/MP Version 15.1 (StataCorp LCC, College Station, TX, USA). p values <0.05 were considered statistically significant for all analyses.

Results

All 52 study participants were Hispanic, the mean (±SD) age was 42 ± 16 years, 48% were women, 23% had diabetes, and the median eGFR (IQR) was 56 mL/min/1.73 m^2 (32, 88). The mean (±SD) uUMOD was 5.1 (8.4) μg/mL. Participants in the lowest tertile (Table 1) had a greater prevalence of hypertension and diabetes mellitus and lower eGFR. Degree of fibrosis was also greatest in the lowest tertile of uUMOD in unadjusted analysis. Participants in the highest tertile of uUMOD had the highest urine output in response to FST (1.6 [1.3, 2.2] L).

Relationship between uUMOD and Moderate or Severe Interstitial Fibrosis

In primary analyses, each halving of uUMOD was associated with 1.71 higher odds of grade 2 or 3 fibrosis (OR: 1.71, 95% CI: 1.10, 2.67). This association remained statistically significant after adjusting for DM (OR: 2.02, 95% CI: 1.18, 3.44). However, after further adjusting for eGFR and urine albumin, the association was no longer statistically significant (OR: 1.36, 95% CI: 0.75, 2.44), as shown in Table 2. The results were similar when stratified by transplant status (Table 3).

Relationship between uUMOD and Urine Output in Response FST

Table 4 shows the association between uUMOD and urine output. Each halving of uUMOD was associated with lower 2-h cumulative urine output (−81 [−144, −18],

Table 1. Baseline characteristics of study participants based on median uUMOD concentration

Variables	Tertile 1	Tertile 2	Tertile 3
Range, μg/mL	<1.84	1.85–3.7	>3.8
N	19	16	17
Age, years (SD)	41 (17)	42 (16)	42 (15)
Female sex, *n* (%)	7 (39)	10 (59)	8 (47)
Obesity, *n* (%)	2 (11)	1 (6)	0
Diabetes mellitus, *n* (%)	6 (33.3)	5 (29.4)	1 (6)
Hypertension, *n* (%)	9 (50)	6 (35)	7 (41)
ACEi use, *n* (%)	2 (11.1)	4 (23.5)	2 (11.8)
ARB use, *n* (%)	4 (22.2)	5 (29.4)	8 (47.1)
Loop diuretic use, *n* (%)	12 (67)	10 (59)	8 (47)
Thiazide use, *n* (%)	2 (11)	1 (6)	1 (6)
Potassium-sparing diuretics, *n* (%)	3 (17)	2 (12)	4 (23.5)
NSAID use, *n* (%)	1 (6)	0	0
Native kidney	8 (44.4)	11 (64.7)	14 (82.4)
Fibrosis, *n* (%)			
1	5 (27.8)	11 (64.7)	12 (70.6)
2	6 (33.3)	4 (23.5)	3 (17.7)
3	7 (38.9)	2 (11.8)	2 (11.8)
Median fibrosis (morphometry) (IQR)	43 (22, 61)	18 (11, 39)	9 (8, 25)
Reason for biopsy, *n* (%)			
Acute allograft dysfunction	10 (55.6)	5 (31.2)	2 (11.8)
Lupus nephritis	2 (11.1)	2 (12.5)	2 (11.8)
Nephrotic syndrome	0	4 (25)	6 (35.3)
Non-nephrotic proteinuria	1 (6)	0	2 (11.8)
Proteinuria and renal dysfunction	2 (11.1)	2 (12.5)	1 (6)
Other	3 (15.8)	3 (18.9)	4 (23.5)
Missing	1 (6)	0	0
Furosemide dose, mean (SD)	84 (25)	81 (25)	92 (24)
Median total urine output, liters (IQR)	1.3 (0.9, 1.7)	1.2 (1, 1.5)	1.6 (1.3, 2.2)
Median uACR (IQR)	0.41 (0.15, 0.91)	0.28 (0.11, 1.10)	0.46 (0.28, 0.93)
Median eGFR, mL/min/1.73 m^2 (IQR)	32 (14, 53)	55 (40, 87)	87 (74, 121)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; NSAID, nonsteroidal anti-inflammatory drug; uACR, urine albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate.

Table 2. Association of uUMOD concentration (per halving) with moderate or severe fibrosis (grade 2 or 3)

Events (*n* = 24/52)	OR (95% CI)	*p* value
Model 1	1.74 (1.10, 2.75)	0.02
Model 2	1.75 (1.10, 2.78)	0.02
Model 3	1.30 (0.79, 2.17)	0.30

Model 1: adjusted for age, sex, urine creatinine. Model 2: model 1 + diabetes mellitus. Model 3: model 2 + baseline eGFR and urine albumin.

Table 3. Association of uUMOD concentration (per halving) with moderate or severe fibrosis (grade 2 or 3) by transplant status

Events (n = 24/33)	OR (95% CI)	*p* value
Native		
Model 1	2.67 (1.15, 6.23)	0.02
Model 2	2.73 (1.14, 6.51)	0.02
Model 3	3.58 (0.99, 12.95)	0.05
Transplant		
Events (n = 10/19)		
Model 1	0.77 (0.30, 1.95)	0.58
Model 2	0.79 (0.31, 2.02)	0.63
Model 3	0.44 (0.09, 2.17)	0.32

Model 1: adjusted for age, sex, urine creatinine. Model 2: model 1 + diabetes mellitus. Model 3: model 2 + baseline eGFR and urine albumin.

p = 0.01). However, these associations were no longer significant after adjusting for other covariates including eGFR and urine albumin (−55 [−125, 14], p = 0.11).

Table 4. Association of uUMOD concentration (per halving) with urine output response to furosemide stress test

	β coefficient (95% CI)	Standard error	*p* value
Model 1	−81 (−144, −18)	31.4	0.01
Model 2	−72 (−134, −10)	30.9	0.02
Model 3	−55 (−125, 14)	34.3	0.11

Model 1: adjusted for age, sex, urine creatinine. Model 2: model 1 + furosemide dose. Model 3: model 2 + baseline eGFR and urine albumin.

Similarly, when we evaluated the association stratified by kidney transplant status, the association between uUMOD and diuretic response was not statistically significant until the addition of baseline eGFR and urine albumin, as shown in Table 5.

For further evaluation, we also evaluated p-cresol sulfate, another proximal tubule function marker (Table 6), but did not find a significant association in any of the models with diuretic response.

Table 5. Association of uUMOD concentration (per halving) with urine output response to furosemide stress test by transplant status

	β coefficient (95% CI)	Standard error	*p* value
Native			
Model 1	−77 (−144, −18)	34.84	0.04
Model 2	−74 (−146, −2)	35.05	0.04
Model 3	−54 (−127, 20)	35.8	0.15
Transplant			
Model 1	−228 (−398, −58)	79.31	0.01
Model 2	−205 (−356, −53)	70.12	0.01
Model 3	−144 (−301, 13)	71.23	0.07

Model 1: adjusted for age, sex, urine creatinine. Model 2: model 1 + furosemide dose. Model 3: model 2 + baseline eGFR and urine albumin.

Table 6. Association of fractional excretion of p-cresol sulfate with urine output response to furosemide stress test

	β coefficient (95% CI)	Standard error	*p* value
Model 1	−6 (−125, 113)	58.93	0.92
Model 2	3 (−113, 120)	57.97	0.95
Model 3	3 (−108, 113)	54.84	0.96

Model 1: adjusted for age, sex, urine creatinine. Model 2: model 1 + furosemide dose. Model 3: model 2 + baseline eGFR and urine albumin.

Discussion

In this study, among persons with CKD undergoing kidney biopsies for clinical indications, lower uUMOD was associated with a higher degree of IFTA but was not statistically significant after taking into consideration eGFR and albuminuria. uUMOD or Tamm-Horsfall protein is a 95 kDa glycoprotein produced by the thick ascending limb of the loop of Henle and early distal convoluted tubule [11]. Multiple studies have found an association between higher uUMOD levels and higher eGFR, suggesting that uromodulin may be a marker of greater kidney functional reserve [7, 9, 12]. Uromodulin excretion has positively and linearly been associated with renal length and volume measured via ultrasound [8]. We have previously demonstrated that lower uUMOD concentrations are associated with CKD progression, independent of eGFR and uACR [7]. One study of 19 persons with glomerulonephritis showed that greater tubule damage on biopsy was associated with lower uUMOD levels [13]. Melchinger and colleagues evaluated the association between uUMOD and IFTA among participants with native kidneys. The authors found that per each doubling of uUMOD, there was 2.5% lower severity of IFTA, independent of baseline eGFR and albuminuria [14]. In this study, we wanted to not only evaluate the association between uUMOD and IFTA in a cohort of Hispanic participants with native and transplanted kidneys but also study the functional role of uUMOD in response to a diuretic challenge.

Uromodulin regulates the activity of the sodium-potassium chloride transporter and renal outer medullary potassium channel, two primary ion transporters involved in NaCl reabsorption by the TAL segment. Thus, we hypothesized that higher uUMOD may associate with greater urine output in response to the FST. While this association was observed in the unadjusted model, it was attenuated after multivariable adjustment. It is plausible that we did not find a significant association with IFTA and urine output due to our relatively small sample size.

Our study has several strengths. First, our prospective study evaluated the association between uUMOD and interstitial fibrosis, the final common pathway of CKD. Having a biomarker that could potentially determine the degree of fibrosis would be of significant clinical utility. Second, we evaluated participants with native kidneys as well as transplants.

Our study also has limitations. First, we had a small number of participants which limited the power of study. Second, the FST was designed to evaluate the severity of AKI which was not the goal of our study. Rather, the original study evaluated the association of kidney histology with urine output after a protocolized FST.

In summary, in a population of individuals across a wide range of kidney function undergoing clinically indicated kidney biopsies, we did not find an association between uUMOD and interstitial fibrosis or response to loop diuretics after adjusting for eGFR and albuminuria.

Statement of Ethics

All methods were carried out in accordance with relevant guidelines and regulations. The research was approved by the Institutional Research Ethics Committee from the Instituto Nacional de Cardiologia and registered with the number 15-920. Written informed consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

A.L.B. is supported by the Department of Veterans Affairs Mentored Career Development Award IK2BX004986 and a pilot grant from the University of Alabama at Birmingham and UC San Diego-O'Brien Center for Acute Kidney Injury Research (P30 DK079337). PSG is supported by National Institutes of Health (NIH) grant K23DK114556. Dr. Joachim H. Ix was supported by a midcareer mentoring award from the NIDDK (K24DK110427). The parent study was funded by the Nephrology Division of the Instituto Nacional de Cardiología, Juan Badiano No. 1, 14080-Tlalpan, Mexico City, Mexico.

Author Contributions

Alexander L. Bullen and Pranav S. Garimella conceptualized the study and were responsible for methodology. Alexander L. Bullen was responsible for formal analysis. Alexander L. Bullen wrote the original draft. Pranav S. Garimella, Sucheta Vaingankar, Magdalena Madero, Salvador Lopez Gil, Etienne Macedo, Joachim H. Ix, and Dena E. Rifkin reviewed and edited the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

References

1 Bohle A, Müller GA, Wehrmann M, Mackensen-Haen S, Xiao JC. Pathogenesis of chronic renal failure in the primary glomerulopathies, renal vasculopathies, and chronic interstitial nephritides. Kidney Int Suppl. 1996;54:S2–9.
2 Rule AD, Amer H, Cornell LD, Taler SJ, Cosio FG, Kremers WK, et al. The association between age and nephrosclerosis on renal biopsy among healthy adults. Ann Intern Med. 2010;152(9):561–7.
3 Hewitson TD. Renal tubulointerstitial fibrosis: common but never simple. Am J Physiol Renal Physiol. 2009;296(6):F1239–44.
4 Nelson RG, Grams ME, Ballew SH, Sang Y, Azizi F, Chadban SJ, et al. Development of risk prediction equations for incident chronic kidney disease. JAMA. 2019;322(21):2104–14.
5 Rivero J, Rodríguez F, Soto V, Macedo E, Chawla LS, Mehta RL, et al. Furosemide stress test and interstitial fibrosis in kidney biopsies in chronic kidney disease. BMC Nephrol. 2020;21(1):87.
6 Bullen AL, Katz R, Jotwani V, Garimella PS, Lee AK, Estrella MM, et al. Biomarkers of kidney tubule health, CKD progression, and acute kidney injury in SPRINT (systolic blood pressure intervention trial) participants. Am J Kidney Dis. 2021;78(3):361–8.e1.
7 Garimella PS, Katz R, Ix JH, Fried LF, Kritchevsky SB, Devarajan P, et al. Association of urinary uromodulin with kidney function decline and mortality: the health ABC study. Clin Nephrol. 2017;87(6):278–86.
8 Pruijm M, Ponte B, Ackermann D, Paccaud F, Guessous I, Ehret G, et al. Associations of urinary uromodulin with clinical characteristics and markers of tubular function in the general population. Clin J Am Soc Nephrol. 2016;11(1):70–80.
9 Garimella PS, Sarnak MJ. Uromodulin in kidney health and disease. Curr Opin Nephrol Hypertens. 2017;26(2):136–42.
10 Chawla LS, Davison DL, Brasha-Mitchell E, Koyner JL, Arthur JM, Shaw AD, et al. Development and standardization of a furosemide stress test to predict the severity of acute kidney injury. Crit Care. 2013;17(5):R207.
11 Lhotta K. Uromodulin and chronic kidney disease. Kidney Blood Press Res. 2010;33(5):393–8.
12 Garimella PS, Biggs ML, Katz R, Ix JH, Bennett MR, Devarajan P, et al. Urinary uromodulin, kidney function, and cardiovascular disease in elderly adults. Kidney Int. 2015;88(5):1126–34.
13 Thornley C, Dawnay A, Cattell WR. Human Tamm-Horsfall glycoprotein: urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. Clin Sci. 1985;68(5):529–35.
14 Melchinger H, Calderon-Gutierrez F, Obeid W, Xu L, Shaw MM, Luciano RL, et al. Urine uromodulin as a biomarker of kidney tubulointerstitial fibrosis. Clin J Am Soc Nephrol. 2022;17(9):1284–92.