Urine uromodulin as a biomarker of kidney tubulointerstitial fibrosis

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Supplemental table 1. Classification of histological features

Histological feature	Definition
Interstitial fibrosis tubular atrophy	Continuous (% of biopsy tissue affected)* and categories (<10%, 10-25%, >25%)
Glomerulosclerosis	Continuous (% of total glomeruli with global glomerulosclerosis) and categories (<10%, 10-25%, >25%)
Infiltrate	Categories: <10%: minimal, none; 10-25%: present, patchy, focal, active; >25%: diffuse
Eosinophils	Categories (yes/no)
Arterionephrosclerosis	Categories: <10%: absent, none, minimal; 10-25%: mild, present; >25%: moderate, severe
Acute tubular injury	Categories; No: absent, not reported; Yes: acute, diffuse, focal, mild, moderate, patchy, present, severe, yes.
Tubulitis	Categories (yes, no)
Crescents	Categories (yes, no)

IF/TA was classified as 0 if reported as none.

Supplemental Table 2. Degree of missingness of key covariates

Characteristic	Missing
Demographics	
Age, years	0
Female	0
Black race	5
Diabetes	3
Hypertension	0
Cirrhosis	3
Chronic kidney disease	28
Body mass index (BMI), kg/m2	1
Medication use	
PPI use	2
NSAID use	2
Antibiotic use	6
Prebiopsy Laboratory Features	
Creatinine, mg/dl	23
eGFR, ml/min	28
Protein to creatinine ratio, mg/mg	83
Albumin to creatinine ratio, mg/mg	0
Features at Biopsy	
AKI or AKD	0
Dialysis	1
Creatinine, mg/dl	0
Blood Urea Nitrogen, mg/dl	0
Bicarbonate, serum	0
Hemoglobin, g/dl	4
Kidney size, inches	107
Histology	
IF/TA	27
Global glomerulosclerosis	33
Infiltrate	27
Arterionephrosclerosis	62
Eosinophils	0
ATI	0
Tubulitis	0
Crescents	3

PPI = proton pump inhibitor, NSAID = non-steroidal anti-inflammatory drug, eGFR = estimated glomerular filtration rate, AKI = acute kidney injury, ADK = acute kidney disease, IF/TA = interstitial fibrosis/tubular atrophy, ATI = acute tubular injury.

Supplemental Table 3. Correlation of histological features with urine uromodulin level

Characteristic	Correlation coefficient	P-value
Baseline features		
Creatinine	-0.42	6.11E-16
eGFR	0.37	3.59E-12
Albuminuria	-0.06	0.27
Proteinuria	-0.06	0.3
Features at biopsy		
Serum Creatinine	-0.43	1.54E-17
Blood urea nitrogen	-0.24	4.99E-06
Bicarbonate, serum	0.2	9.41E-05
Anion Gap	-0.18	6.62E-04
Kidney size	0.18	4.70E-03
Hemoglobin	0.14	0.02
Histological features		
IF/TA	-0.31	6.87E-09
Glomerulosclerosis	-0.21	1.05E-04
Eosinophils	-0.15	3.94E-03
Infiltrate	-0.15	6.36E-03
Arterio-nephrosclerosis	-0.13	0.02
ATI	0.07	0.16
Tubulitis	-0.07	0.21
Crescents	-0.03	0.55

Spearman correlation coefficient. eGFR = estimated glomerular filtration rate, IF/TA = interstitial fibrosis/tubular atrophy, ATI = acute tubular injury.

Supplemental Table 4. Correlation of urine uromodulin level with other kidney biomarkers

Biomarker	Rho	P-value	Bonferroni corrected
Albumin	0.06	0.27	1
KIM-1	0.19	0.0004	0.01
NGAL	-0.22	<0.0001	0.001
IL-6	-0.28	<0.0001	<0.0001
TNF-a	-0.36	<0.0001	<0.0001
IL-9	-0.11	0.04	1
MCP-1	0.04	0.44	1
YKL-40	-0.21	<0.0001	0.002

Spearman correlation coefficient

Supplemental Table 5. Univariable and multivariable association of uromodulin level with histological features

Characteristic	Uromodulin level (log2 transformed)	
	Univariable	Multivariable
IF/TA, per 1%	**-7.19 (-9.30, -5.09)**	**-3.47 (-5.68, -1.25)**
Glomerulosclerosis, per 1%	**-6.39 (-8.85, -3.93)**	**-3.26 (-5.97, -0.55)**
Infiltrate, present vs. absent	**-0.06 (-0.10, -0.02)**	-0.02 (-0.06, 0.03)
Eosinophils, present vs. absent	**-0.06 (-0.09, -0.02)**	-0.02 (-0.07, 0.03)
Arterionephrosclerosis, present vs. absent	**-0.05 (-0.09, -0.01)**	-0.02 (-0.06, 0.03)
Tubulitis, present vs. absent	-0.04 (-0.08, 0.00)	-0.02 (-0.07, 0.02)
Tubular injury, present vs. absent	0.03 (-0.01, 0.06)	0.04 (0.00, 0.09)
Crescents, present vs. absent	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)

Linear regression analysis for outcome of histological features and exposure as log$_2$uromodulin level.

Values represent unit difference in outcome per 2-fold difference in urine uromodulin level.

Significant associations in bold.

Multivariable model controls for baseline (pre-biopsy) eGFR, urine albumin to creatinine ratio and serum creatinine at biopsy
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Supplemental Table 6. Association of urine uromodulin with tubulointerstitial and glomerular fibrosis after controlling for histological diagnosis

Histological feature	Difference in histological feature per unit difference in uromodulin
Interstitial fibrosis (% of kidney tissue)[1]	
Per 2-fold difference	**-2.0 (-4.0, -0.02)**
T1 (0.14-0.81 mcg/g)	Ref.
T2 (0.82-1.65 mcg/g)	2.4 (-2.6, 7.43)
T3 (1.67-12.24 mcg/g)	**-6.1 (-11.4, -0.77)**
Glomerulosclerosis (% of glomeruli)[2]	
Per 2-fold difference	-1.0 (-3.5, 1.52)
T1 (0.14-0.81 mcg/g)	Ref.
T2 (0.82-1.65 mcg/g)	-1.9 (-8.2, 4.31)
T3 (1.67-12.24 mcg/g)	2.3 (-4.4, 8.94)

Analysis: Linear regression analysis with outcome as histological feature (interstitial fibrosis or glomerulosclerosis) and exposure as uromodulin level (log$_2$ or tertile). Values represent unit differences in outcome (% of total biopsy tissue for IF/TA or % of glomeruli that have global sclerosis for glomerulosclerosis) for each unit (2-fold or tertile) difference of urine uromodulin level. Significant associations in bold.
Urine uromodulin to creatinine ratio
Controls for baseline eGFR, baseline urine albumin to creatinine ratio and serum creatinine at biopsy; histological diagnosis; glomerulosclerosis[1] and interstitial fibrosis[2].

Supplemental Table 7. Association of uromodulin with tubulointerstitial and glomerular fibrosis without indexing to urine creatinine

Histological feature	Difference in histological feature per unit difference in uromodulin		
	Model 1	Model 2	Model 3
Interstitial fibrosis (% of kidney tissue)[1]			
Per 2-fold difference	**-7.6 (-9.4, -5.8)**	**-4.5 (-6.5, -2.4)**	**-3.2 (-5.1, -1.3)**
T1 (0.14-0.81 mcg/g)	Ref.	Ref.	Ref.
T2 (0.82-1.65 mcg/g)	**-11.1 (-16.9, -5.3)**	**-6.5 (-12.3, -0.8)**	**-6.5 (-11.8, -1.1)**
T3 (1.67-12.24 mcg/g)	**-21.5 (-27.3, -15.6)**	**-10.8 (-17.2, -4.3)**	**-8.4 (-14.3, -2.5)**
Glomerulosclerosis (% of glomeruli)[2]			
Per 2-fold difference	**-5.3 (-7.4, -3.2)**	**-2.9 (-5.3, -0.3)**	-0.6 (-2.8, 1.8)
T1 (0.14-0.81 mcg/g)	Ref.	Ref.	Ref.
T2 (0.82-1.65 mcg/g)	-2.1 (-9.0, 4.8)	1.8 (-5.3, 8.8)	4.2 (-2.2, 10.6)
T3 (1.67-12.24 mcg/g)	**-15.4 (-22.2, -8.7)**	-5.3 (-13.0, 2.5)	0.2 (-6.9, 7.4)

Analysis: Linear regression analysis with outcome as histological feature (interstitial fibrosis or glomerulosclerosis) and exposure as uromodulin level (log_2 or tertile). Values represent unit differences in outcome (% of total biopsy tissue for IF/TA or % of glomeruli that have global sclerosis for glomerulosclerosis) for each unit (2-fold or tertile) difference of urine uromodulin level. Significant associations in bold.
Absolute uromodulin level (without indexing to creatinine)
Model 1, univariable; model 2 controls for baseline eGFR, urine albumin to creatinine ratio and serum creatinine at biopsy; model 3, additionally controls for glomerulosclerosis[1] and interstitial fibrosis[2]

Supplemental Table 8. Association of uromodulin with tubulointerstitial and glomerular fibrosis with multiple imputations to account for missing data

Histological feature	Difference in histological feature per 2-fold difference in uromodulin
Interstitial fibrosis (% of kidney tissue)[1]	**-3.4 (-6.3, -0.55)**
Glomerulosclerosis (% of glomeruli)[2]	-2.2 (-5.8, 1.5)

Analysis: Linear regression analysis with outcome as histological feature (interstitial fibrosis or glomerulosclerosis) and exposure as uromodulin level (log_2 or tertile). Values represent unit differences in outcome (% of total biopsy tissue for IF/TA or % of glomeruli that have global sclerosis for glomerulosclerosis) for each unit (2-fold or tertile) difference of urine uromodulin level. Significant associations in bold.

Controls for baseline eGFR, urine albumin to creatinine ratio and serum creatinine at biopsy, and either glomerulosclerosis[1] or interstitial fibrosis[2]

Supplemental Table 9. Comparison of uromodulin staining on various mouse kidney biopsy models

Comparison	Mean Difference (95% CI)	P Value
Fibrotic vs. Repaired	-0.097 (-0.140, -0.053)	<0.0001
Fibrotic vs. Normal	-0.048 (-0.003, -0.094)	0.04
Normal vs. Repaired	-0.048 (-0.094, -0.003)	0.04

Difference noted in micrometer^2 of kidney tissue staining for uromodulin

Supplemental Figure 1. STARD flow diagram

392 participants enrolled between 1/2015-6/2018

28 Excluded
- 22 without urine samples
- 6 did not undergo a kidney biopsy

364 included in analysis