Kidney Medicine

Urine Biomarkers of Kidney Tubule Health and Risk of Incident CKD in Persons Without Diabetes: The ARIC, MESA, and REGARDS Studies

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Rationale & Objective: Tubulointerstitial damage is a feature of early chronic kidney disease (CKD), but current clinical tests capture it poorly. Urine biomarkers of tubulointerstitial health may identify risk of CKD.

Study Design: Prospective cohort (Atherosclerosis Risk in Communities [ARIC]) and case-cohort (Multi-Ethnic Study of Atherosclerosis [MESA] and Reasons for Geographic and Racial Differences in Stroke [REGARDS]).

Setting & Participants: Adults with estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2 and without diabetes in the ARIC, REGARDS, and MESA studies.

Exposures: Baseline urine monocyte chemoattractant protein-1 (MCP-1), alpha-1-microglobulin (α1m), kidney injury molecule-1, epidermal growth factor, and chitinase-3-like protein 1.

Outcome: Incident CKD or end-stage kidney disease.

Analytical Approach: Multivariable Cox proportional hazards regression for each cohort; meta-analysis of results from all 3 cohorts.

Results: 872 ARIC participants (444 cases of incident CKD), 636 MESA participants (158 cases), and 924 REGARDS participants (488 cases) were sampled. Across cohorts, mean age ranged from 60 ± 10 to 63 ± 8 years, and baseline eGFR ranged from 88 ± 13 to 91 ± 14 mL/min/1.73 m^2. In ARIC, higher concentrations of urine MCP-1, α1m, and kidney injury molecule-1 were associated with incident CKD. In MESA, higher concentration of urine MCP-1 and lower concentration of epidermal growth factor were each associated with incident CKD. In REGARDS, none of the biomarkers were associated with incident CKD. In meta-analysis of all 3 cohorts, each 2-fold increase α1m concentration was associated with incident CKD (HR, 1.19; 95% CI, 1.08-1.31).

Limitations: Observational design susceptible to confounding; competing risks during long follow-up period; meta-analysis limited to 3 cohorts.

Conclusions: In 3 combined cohorts of adults without prevalent CKD or diabetes, higher urine α1m concentration was independently associated with incident CKD. 4 biomarkers were associated with incident CKD in at least 1 of the cohorts when analyzed individually. Kidney tubule health markers might inform CKD risk independent of eGFR and albuminuria.

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Chronic kidney disease (CKD) affects hundreds of millions of adults worldwide and is a major risk factor for end-stage kidney disease, cardiovascular disease (CVD), and mortality.[1,2] However, diagnosing CKD at actionable stages is challenging; currently, recognition of CKD depends on serum creatinine (SCr) concentration and urine albumin excretion, measures of glomerular filtration and integrity, respectively. Although these markers are independently associated with adverse kidney and non-kidney outcomes, they are insensitive for the detection of early kidney disease and correlate poorly with tubulointerstitial health.[3,4] Biopsy studies have demonstrated that tubulointerstitial pathology is strongly associated with progressive kidney disease—but by the time abnormal SCr and albuminuria are detected, there may be irreversible interstitial fibrosis and tubular atrophy.[5-8]

The need for sensitive and noninvasive measures of tubulointerstitial health fuels the development of new kidney biomarkers. Urine-based biomarkers are promising in light of their easy collection, relationship to the pathologic sites of interest, and consistent associations with important clinical outcomes. Prior work with kidney tubule biomarkers has revealed independent associations with acute kidney injury incidence and progression, CKD progression, CVD, and mortality in several special populations at elevated risk of adverse outcomes.[9-18] However, whether urine biomarkers are informative in populations at lower risk of CKD remains uncertain.

To address this question, we leveraged 3 large cohorts of adults with estimated glomerular filtration rate (eGFR) ≥60 mL/min/1.73 m^2 and without diabetes to measure 5 urine biomarkers of kidney tubule health:

PLAIN-LANGUAGE SUMMARY

This study analyzed 3 cohorts (ARIC, MESA, and REGARDS) of adults without diabetes or prevalent chronic kidney disease (CKD) to determine the associations of 5 urinary biomarkers of kidney tubulointerstitial health with incident CKD, independent of traditional measures of kidney health. Meta-analysis of results from all 3 cohorts suggested that higher baseline levels of urine alpha-1-microglobulin were associated with incident CKD at follow-up. Results from individual cohorts suggested that in addition to alpha-1-microglobulin, monocyte chemoattractant protein-1, kidney injury molecule-1, and epidermal growth factor may also be associated with the development of CKD. These findings underscore the importance of kidney tubule interstitial health in defining risk of CKD independent of creatinine and urine albumin.

monocyte chemoattractant protein-1 (MCP-1), alpha-1-microglobulin (α1m), kidney injury molecule-1 (KIM-1), epidermal growth factor (EGF), and chitinase-3-like protein 1 (YKL-40). These biomarkers represent distinct aspects of tubulointerstitial health, including tubulointerstitial injury and inflammation (MCP-1), proximal tubule reabsorptive capacity (α1m), proximal tubule injury (KIM-1), tubule synthetic function and reparative capacity (EGF), and tubule epithelial cell repair (YKL-40). We hypothesized that baseline urine concentrations of one or more of these kidney tubule biomarkers would be independently associated with incident CKD among adults without diabetes.

METHODS

Study Design and Populations

We selected 3 well-characterized cohorts of community-dwelling adults: Atherosclerosis Risk in Communities (ARIC) study, Multi-Ethnic Study of Atherosclerosis (MESA), and Reasons for Geographic and Racial Differences in Stroke (REGARDS). All participants provided informed consent, and this ancillary study was approved by the institutional review board of Johns Hopkins University. The design and original methods for these cohorts have been previously described.[19-23] In brief, ARIC recruited 15,792 mostly Black and White adults aged 45-64 years from 1987 through 1989 and followed them with exams and telephone surveys. SCr concentration was measured at visit 4 (1996-1998), which was considered baseline for the present analyses, and again at visit 5 (2011-2013), which was used for outcome ascertainment. MESA enrolled 6,814 racially and ethnically diverse adults aged 45-84 years without symptoms or diagnoses of CVD. SCr was measured at baseline (2000-2002), visit 3 (2004-2005), visit 4 (2005-2007), and visit 5 (2010-2011). REGARDS recruited 30,239 Black and White adults aged ≥45 years between 2003 and 2007. Blood and urine specimens were collected at baseline for SCr and urine biomarker measurement. SCr was measured at a second visit between 2013 and 2016. We measured urine biomarkers concurrently with baseline SCr measurements in all cohorts.

All 872 ARIC participants meeting inclusion criteria of baseline eGFR ≥60 mL/min/1.73 m^2, no diagnosis of diabetes, and available SCr at follow-up visit 5 were included in analyses. In addition to excluding participants with diabetes, we excluded participants based on missing covariates, conditioned on V5 attendance and available urinary albumin-creatinine ratio at both time points. There were 444 incident CKD events (see "Outcome Ascertainment" below) over a median follow-up of 14.5 (quartile [Q]1, Q3: 14.1, 15.2) years in ARIC. Participants from MESA and REGARDS were sampled using a case-cohort design, whereby a random subcohort of participants meeting the aforementioned inclusion criteria were sampled for analyses. All additional cases of incident CKD arising from outside the random subcohort were also included in analyses based on the same inclusion criteria. A total of 5,137 participants in MESA met inclusion criteria, from which we randomly sampled a subcohort of 495 individuals. Over a median follow-up of 9.2 (Q1, Q3, 5.5, 9.6) years, there were 158 cases of incident CKD in MESA, with 17 cases arising from within the subcohort and 141 additional cases also included in the analysis. From the overall cohort of 10,299 REGARDS participants with baseline eGFR ≥60 mL/min/1.73 m^2, no diabetes, and a second in-home visit, we randomly sampled a subcohort of 500, of which 493 individuals had adequate urine for these analyses. Over a median follow-up of 9.4 (Q1, Q3, 8.6, 9.9) years, there were a total of 488 incident cases of CKD, with 57 cases arising from the subcohort and 431 cases arising from outside the subcohort included in the analysis.

Kidney Tubule Biomarkers

Frozen urine samples were kept in continuous storage at −80°C. All biomarker assays were performed at a single laboratory (Brigham and Women's Hospital, Boston, MA) using a standard protocol by personnel blinded to clinical outcomes. Urine KIM-1, MCP-1, YKL-40, and EGF were measured on the Luminex 200 platform with a laboratory-developed multiplex assay (Luminex Corporation). Urine α1m was measured on a Siemens BNII nephelometer (Siemens, Inc). All measurements except α1m were made in duplicate, and mean values were used in analyses. If the intra-assay coefficient of variation exceeded 15%, the assay was repeated (Table S1). All models were adjusted for urine creatinine (Ucr) concentration to account for differences in urine tonicity at sample collection.

Covariates

SCr was calibrated to isotope dilution using mass spectrometry and used to calculate eGFR according to the 2009 CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) equation.[24] Urine albumin concentration was measured with the BNII ProSpec (Siemens, Inc). Ucr concentration was measured by the Jaffe method on the Modular P chemistry analyzer (Roche/Hitachi). We adjusted for urine albumin and Ucr concentrations separately in multivariable models, whereas albuminuria was expressed as urinary albumin-creatinine ratio in descriptive statistics.

Sociodemographic and clinical characteristics were obtained in baseline exams for each cohort. Models for all 3 cohorts included the following covariates: age, sex, race, education level, current smoking, body mass index, systolic blood pressure, antihypertensive medication use, Ucr concentration, urine albumin, and eGFR. Models for ARIC also adjusted for history of CVD, which included both coronary heart disease and stroke. Because of the distribution of race across ARIC study sites, we adjusted for combinations of race and study site rather than adjusting for each covariate separately. MESA models did not adjust for history of CVD, coronary heart disease, or stroke because MESA participants had to be free of these diagnoses at baseline for inclusion in the cohort. Models for REGARDS adjusted for coronary heart disease and stroke.

Outcome Ascertainment

We defined the outcome of incident CKD as a decrease to eGFR <60 mL/min/1.73 m^2 and ≥40% decline in eGFR from the baseline value to the value at a subsequent SCr measurement. In the ARIC study, incident CKD was also defined to include participants who received kidney replacement therapy (dialysis or transplant) as identified by linkage to the US Renal Data System registry to capture those who may not have had available eGFR at follow-up.[25] All individuals across the 3 cohorts who satisfied inclusion criteria and met the outcome definition were considered cases and included in analyses.

Statistical Analysis

Using baseline data from each of the 3 cohorts, we tabulated descriptive statistics. Spearman rank correlations between the investigational biomarkers, urine albumin, Ucr, and eGFR were calculated. We modeled the risk of incident CKD for each biomarker separately in each cohort using multivariable Cox proportional hazards regression. Models for MESA and REGARDS accounted for the case-cohort design using the pseudo-likelihood method proposed by Prentice.[26]

For the primary analysis, biomarkers were $\log_2$-transformed to represent each 2-fold higher concentration. An additional analysis modeled urine biomarkers in quartiles to evaluate the functional forms of associations. Values of α1m that were below the lower limit of detection (LLOD) of <5.63 mg/L were set to one-half of the LLOD in ARIC analyses and to 5.62 mg/L in MESA and REGARDS analyses. Two nested models were applied: model 1 adjusted for age, sex, race, education, study site (only in ARIC), body mass index, systolic blood pressure, antihypertensive medication use, prevalent CVD or coronary heart disease and stroke (except for MESA), and Ucr; model 2 additionally adjusted for baseline eGFR and urine albumin. An alternative analysis that indexed urine biomarkers to Ucr, rather than adjusting for Ucr, was also performed and is presented in Tables S2-S4. A 2-tailed P value <0.05 was considered statistically significant.

To estimate the summary effect of each biomarker on risk of incident CKD, we performed a meta-analysis that included all 3 cohorts. The cohort-specific estimates for each biomarker from the fully-adjusted model (model 2) were combined using a fixed effects meta-analysis. The percentage of variation across cohorts due to heterogeneity was expressed as I^2, and between-study variance was expressed as τ^2.

Analyses were performed using IBM SPSS version 26 (IBM Corp), R Core Team (2020) from *R: A language and environment for statistical computing* (R Foundation for Statistical Computing; www.R-project.org), and Stata version 16.1 (StataCorp LLC).

RESULTS

Baseline characteristics of included participants from ARIC, MESA, and REGARDS are presented in Table 1 (Table S9). Compared with participants from ARIC and REGARDS, MESA participants appeared to be younger, had a higher proportion of men with lower body mass index, lower blood pressure, and less antihypertensive medication use. The REGARDS subcohort had the highest proportion of Black participants and lowest proportion of smokers. Mean baseline age was 63 ± 5 years in ARIC, 60 ± 10 years in MESA, and 63 ± 8 years in REGARDS. Mean baseline eGFR was 88 ± 13 mL/min/1.73 m^2 in ARIC, 91 ± 14 mL/min/1.73 m^2 in MESA, and 89 ± 14 mL/min/1.73 m^2 in REGARDS. None of the participants had diabetes, by design.

In ARIC, 63% of α1m and 16% of YKL-40 measurements were below the LLOD. In MESA, 47% of α1m and 6% of YKL-40 measurements were below the LLOD. In REGARDS, 43% of α1m and 3% of YKL-40 measurements were below the LLOD.

Spearman correlations of the urine biomarkers with urinary albumin-creatinine ratio and eGFR in each cohort are presented in Tables S5-S8. The investigational biomarkers were modestly to moderately correlated with each other. The investigational biomarkers were, in general, weakly correlated with urinary albumin-creatinine ratio and eGFR.

Table 1. Baseline Characteristics of Included ARIC, MESA, and REGARDS Participants

	Cohort		
Characteristic	**ARIC**	**MESA**	**REGARDS**
N[a]	872	495	493
Age (y)	63 (5)	60 (10)	63 (8)
Female	499 (57%)	258 (52%)	285 (58%)
Black race	158 (18%)	119 (24%)	147 (30%)
Education			
Less than high school graduate	137 (16%)	73 (15%)	41 (8%)
High School graduate	286 (33%)	82 (17%)	105 (21%)
Some college/vocational school[b]	69 (8%)	123 (25%)	112 (23%)
At least college graduate	380 (44%)	217 (44%)	235 (48%)
Current smokers	111 (13%)	70 (14%)	40 (8%)
Body mass index (kg/m^2)	28.9 (5.4)	27.9 (5.1)	28.4 (5.8)
Hypertensive	400 (46%)	179 (36%)	214 (43%)
SBP (mm Hg)	128 (18)	123 (21)	125 (16)
DBP (mm Hg)	72 (10)	71 (11)	76 (9)
Heart failure[c]	—	0 (0%)	27 (6%)
CAD/CHD/stroke	59 (7%)	0 (0%)	62 (13%)
Antihypertensive medication use	336 (38%)	149 (30%)	177 (36%)
ACEi/ARB use	100 (11%)	43 (9%)	107 (22%)
Diuretic use	133 (15%)	55 (11%)	93 (19%)
eGFR (mL/min/1.73 m^2)	88 (13)	91 (14)	89 (14)
UACR (mg/g)	4 [2, 7]	4.7 [3.1, 9.4]	6.1 [4.2, 9.7]

Note: Data presented as mean (SD), number (%), or median [quartile 1, quartile 3].
Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; ARIC, Atherosclerosis Risk in Communities; CAD, coronary artery disease; CHD, coronary heart disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MESA, Multi-Ethnic Study of Atherosclerosis; REGARDS, Reasons for Geographic and Racial Differences in Stroke; SBP, systolic blood pressure; UACR, urinary albumin-creatinine ratio.
[a]N for ARIC represents size of included cohort; N for MESA and REGARDS represents size of random subcohort.
[b]ARIC included an education category for "vocational school"; MESA and REGARDS included categories for "some college."
[c]ARIC did not record history of heart failure.

Associations of Urine Markers of Tubule Injury and Dysfunction with Incident CKD in Individual Cohorts

Fully-adjusted continuous models demonstrated that higher urine MCP-1, KIM-1, and α1m were each associated with higher risk of incident CKD in ARIC, while urine EGF and YKL-40 were not (Table 2). Analyses by biomarker quartiles were consistent with findings from the continuous $\log_2$ models and suggested a linear relationship of biomarkers with CKD risk (Table 2).

In MESA, fully-adjusted continuous models demonstrated that each 2-fold higher level of urine MCP-1 was associated with incident CKD, whereas each 2-fold higher level of urine EGF was associated with a significantly lower risk of incident CKD (Table 3). Urine α1m, KIM-1, and YKL-40 were not significantly associated with incident CKD in adjusted linear models.

In REGARDS, none of the urine biomarkers was significantly associated with incident CKD in the fully-adjusted linear models (Table 4).

Meta-analysis of ARIC, MESA, and REGARDS

The meta-analyzed summary estimate from the fully-adjusted models across the 3 cohorts demonstrated that higher urine α1m was associated with a significantly greater risk of incident CKD with minimal heterogeneity across cohorts (Table 5). Each 2-fold higher level of α1m was associated with a 19% higher risk of incident CKD (95% confidence interval, 8%-31%) in the continuous model, an association that appeared monotonic across quartiles.

Summary point estimates for MCP-1, KIM-1, EGF, and YKL-40 did not reach statistical significance in meta-analysis. Heterogeneity for MCP-1 was higher across the 3 cohorts with I^2 of 69% (test of homogeneity $P = 0.041$). There was no evidence for associations of KIM-1, EGF, or YKL-40 with incident CKD when data were meta-analyzed across the 3 cohorts.

DISCUSSION

Across 3 cohorts of community-living adults with preserved glomerular filtration rate and without diabetes, we investigated the association of 5 urine biomarkers of kidney tubulointerstitial health with risk of incident CKD. Four of these biomarkers were significantly associated with incident CKD in at least 1 of the 3 cohorts. These associations were independent of baseline eGFR and urine albumin level, highlighting distinct dimensions of kidney tubule health incompletely captured by current methods of kidney function assessment. Of these biomarkers, higher urine α1m had the most consistent association with CKD risk in meta-analysis across all 3 cohorts. These results

Table 2. Association of Urine Biomarkers With Incident CKD in ARIC Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline

MCP-1	**Per 2-Fold Higher**	**Quartile 1: <43.7 pg/mL**	**Quartile 2: 43.7-99.7 pg/mL**	**Quartile 3: 99.8-194.1 pg/mL**	**Quartile 4: >194.1 pg/mL**
Cases/N	438/859	75/181	92/197	120/225	157/269
Model 1	**1.26 (1.14, 1.39)**	1 (ref)	1.16 (0.85, 1.59)	**1.53 (1.11, 2.12)**	**1.94 (1.35, 2.77)**
Model 2	**1.26 (1.14, 1.39)**	1 (ref)	1.16 (0.85, 1.59)	**1.52 (1.10, 2.10)**	**1.92 (1.34, 2.74)**
α1m	**Per 2-Fold Higher**	**Quartile 1:<LLOD**	**Quartile 2: LLOD-7.4 mg/L**	**Quartile 3: 7.5-10.2 mg/L**	**Quartile 4: >10.2 mg/L**
Cases/N	444/872	254/548	59/104	53/98	78/122
Model 1	**1.24 (1.11, 1.39)**	1 (ref)	1.27 (0.93, 1.73)	1.37 (0.99, 1.88)	**1.55 (1.14, 2.12)**
Model 2	**1.20 (1.07, 1.34)**	1 (ref)	1.26 (0.93, 1.71)	1.34 (0.97, 1.85)	**1.43 (1.04, 1.97)**
KIM-1	**Per 2-Fold Higher**	**Quartile 1: <346.6 pg/mL**	**Quartile 2: 346.6-766.4 pg/mL**	**Quartile 3: 766.5-1,421.9 pg/mL**	**Quartile 4: >1,421.9 pg/mL**
Cases/N	444/872	87/194	99/206	104/211	154/261
Model 1	**1.18 (1.07, 1.29)**	1 (ref)	0.96 (0.71, 1.29)	1.18 (0.86, 1.62)	**1.52 (1.06, 2.19)**
Model 2	**1.16 (1.06, 1.27)**	1 (ref)	0.95 (0.71, 1.29)	1.18 (0.86, 1.62)	**1.45 (1.01, 2.08)**
EGF	**Per 2-Fold Higher**	**Quartile 1: <3,443.7 pg/mL**	**Quartile 2: 3,443.7-5,960.6 pg/mL**	**Quartile 3: 5,960.7-9,026.9 pg/mL**	**Quartile 4: >9,026.9 pg/mL**
Cases/N	444/871	105/212	118/225	124/231	97/204
Model 1	0.89 (0.78, 1.01)	1 (ref)	1.01 (0.76, 1.33)	1.11 (0.81, 1.52)	0.90 (0.60, 1.35)
Model 2	0.90 (0.80, 1.03)	1 (ref)	1.00 (0.76, 1.33)	1.14 (0.83, 1.56)	0.93 (0.61, 1.40)
YKL-40	**Per 2-Fold Higher**	**Quartile 1:<127.3 pg/mL**	**Quartile 2: 127.3-235.1 pg/mL**	**Quartile 3: 235.2-389.2 pg/mL**	**Quartile 4: >389.2 pg/mL**
Cases/N	392/752	119/209	90/180	80/170	155/313
Model 1	0.95 (0.88, 1.02)	1 (ref)	0.87 (0.66, 1.15)	0.76 (0.57, 1.01)	**0.78 (0.61, 0.99)**
Model 2	0.94 (0.87, 1.01)	1 (ref)	0.86 (0.65, 1.14)	**0.74 (0.56, 0.99)**	**0.74 (0.58, 0.95)**

Note: Associations presented as hazard ratio (95% confidence intervals). Model 1: adjusted for age, sex, race-center, smoking, body mass index, systolic blood pressure, antihypertensive medication use, prevalent cardiovascular disease, education level, and urine creatinine concentration; model 2 additionally adjusted for baseline eGFR and urine albumin concentration.

Abbreviations: α1m, alpha-1-microglobulin; ARIC, Atherosclerosis Risk in Communities; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; LLOD, lower limit of detection; MCP-1, monocyte chemoattractant protein-1; N, number at risk; ref, reference; YKL-40, chitinase-3-like protein 1.

support our overall hypothesis that urine kidney tubule biomarkers may be useful for elucidating pathways of kidney disease progression, improving early detection of kidney damage, and defining the epidemiology of kidney disease among relatively healthy adults.

The meta-analysis demonstrated that higher urine α1m concentrations were independently associated with incident CKD in a combined sample of over 2,400 adults without diabetes. This association had little heterogeneity across cohorts. Summary estimates for the association of urine MCP-1 with incident CKD were similar to those of α1m, although these meta-analyzed estimates were not statistically significant, and we observed substantially greater heterogeneity for this biomarker across the cohorts. α1m is freely filtered and normally avidly reabsorbed by the proximal tubule; higher urine α1m levels identify decrements in proximal tubule reabsorptive capacity. Urine MCP-1 reflects the infiltration of immune cells into kidney tubules in response to injury and inflammation. Thus, our findings suggest potential roles for proximal tubule reabsorptive dysfunction, in addition to injury and inflammation, in the development of CKD in relatively healthy populations. Prior studies in populations with elevated risk of progressive CKD have demonstrated that many of these biomarkers have strong associations with adverse outcomes.[10,13,27,28] The current study builds upon that work, demonstrating that select urine biomarkers reveal risk of CKD even in populations with lower baseline risk and relatively preserved glomerular filtration rate. Because interstitial fibrosis and tubular atrophy may develop before any apparent declines in glomerular filtration rate,[4] these biomarkers of tubulointerstitial health hold promise to identify very early kidney disease that could be amenable to therapeutic interventions, potentially enabling initiation of therapies that would prevent CKD and associated morbidity.

Higher urine α1m concentrations represent proximal tubule reabsorptive dysfunction and have been independently associated with CKD progression in high-risk populations.[10,29,30] Much like glucose, filtered α1m is normally reabsorbed by tubules and absent from urine, hence the large proportion of undetectable α1m values noted in this study of adults with preserved eGFR. Yet, higher urine α1m was significantly associated with

Table 3. Association of Urine Biomarkers With Incident CKD in MESA Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline

MCP-1	**Per 2-Fold Higher**	**Quartile 1: <102 pg/mL**	**Quartile 2: 102-176 pg/mL**	**Quartile 3: 177-289 pg/mL**	**Quartile 4: >289 pg/mL**
N (total subcohort)	495	124	124	124	123
Cases in subcohort	17	2	6	3	6
Additional cases	142	22	25	34	61
Model 1	**1.53 (1.14, 2.04)**	1 (ref)	1.24 (0.53, 2.92)	1.15 (0.44, 3.01)	2.54 (0.88, 7.32)
Model 2	**1.38 (1.02, 1.86)**	1 (ref)	1.12 (0.47, 2.66)	0.75 (0.28, 2.03)	1.88 (0.64, 5.52)
α1m	**Per 2-Fold Higher**	**Quartile 1:<LLOD**	**Quartile 2: 5.63-7.49 mg/L**	**Quartile 3: 7.50-11.91 mg/L**	**Quartile 4:>11.91 mg/L**
N (total subcohort)	495	251	82	85	77
Cases in subcohort	17	9	3	3	2
Additional cases	142	51	24	29	38
Model 1	**1.35 (1.12, 1.63)**	1 (ref)	1.32 (0.68, 2.54)	1.53 (0.78, 3.01)	**2.42 (1.31, 4.45)**
Model 2	1.17 (0.95, 1.44)	1 (ref)	1.08 (0.55, 2.11)	1.18 (0.60, 2.31)	1.52 (0.79, 2.92)
KIM-1	**Per 2-Fold Higher**	**Quartile 1:<992 pg/mL**	**Quartile 2: 993-1,752 pg/mL**	**Quartile 3: 1,753-2,909 pg/mL**	**Quartile 4:>2,909 pg/mL**
N (total subcohort)	495	124	124	124	123
Cases in subcohort	17	3	7	2	5
Additional cases	141	26	29	34	52
Model 1	1.07 (0.80, 1.45)	1 (ref)	0.89 (0.43, 1.84)	0.91 (0.41, 2.03)	1.40 (0.56, 3.48)
Model 2	0.99 (0.73, 1.34)	1 (ref)	0.81 (0.39, 1.71)	0.78 (0.34, 1.80)	1.04 (0.41, 2.65)
EGF	**Per 2-Fold Higher**	**Quartile 1:<4,987 pg/mL**	**Quartile 2: 4,987-7,367 pg/mL**	**Quartile 3: 7,368-10,607 pg/mL**	**Quartile 4:>10,607 pg/mL**
N (total subcohort)	494	124	123	124	123
Cases in subcohort	17	9	4	3	1
Additional cases	142	42	39	33	28
Model 1	**0.53 (0.33, 0.87)**	1 (ref)	**0.37 (0.17, 0.82)**	**0.41 (0.18, 0.92)**	**0.23 (0.09, 0.62)**
Model 2	**0.57 (0.35, 0.95)**	1 (ref)	0.45 (0.19, 1.06)	0.45 (0.19, 1.07)	**0.27 (0.09, 0.79)**
YKL-40	**Per 2-Fold Higher**	**Quartile 1:<206 pg/mL**	**Quartile 2: 206-380 pg/mL**	**Quartile 3: 381-624 pg/mL**	**Quartile 4:>624 pg/mL**
N (total subcohort)	466	117	116	117	116
Cases in subcohort	15	2	4	6	3
Additional cases	137	35	16	32	54
Model 1	0.94 (0.81, 1.09)	1 (ref)	**0.45 (0.22, 0.94)**	0.70 (0.36, 1.36)	0.74 (0.38, 1.42)
Model 2	0.89 (0.77, 1.03)	1 (ref)	**0.39 (0.18, 0.83)**	0.59 (0.30, 1.16)	0.59 (0.30, 1.15)

Note: Associations presented as hazard ratio (95% confidence intervals). Model 1: adjusted for age, sex, race, education, smoking, body mass index, systolic blood pressure, antihypertensive medication use, and urine creatinine concentration; model 2 additionally adjusted for baseline eGFR and urine albumin concentration.

Abbreviations: α1m, alpha-1-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; LLOD, lower limit of detection; MCP-1, monocyte chemoattractant protein-1; MESA, Multi-Ethnic Study of Atherosclerosis; N, number at risk; ref, reference; YKL-40, chitinase-3-like protein 1.

Table 4. Association of Urine Biomarkers With Incident CKD in REGARDS Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline

MCP-1	**Per 2-Fold Higher**	**Quartile 1: <116 pg/mL**	**Quartile 2: 116-215 pg/mL**	**Quartile 3: 216-329 pg/mL**	**Quartile 4: >329 pg/mL**
N (total subcohort)	492	123	123	123	123
Cases in subcohort	57	13	15	12	17
Additional cases	431	96	121	97	117
Model 1	1.00 (0.80, 1.25)	1 (ref)	0.95 (0.57, 1.59)	0.79 (0.43, 1.44)	0.99 (0.51, 1.93)
Model 2	0.94 (0.75, 1.18)	1 (ref)	0.96 (0.57, 1.61)	0.76 (0.41, 1.38)	0.86 (0.43, 1.71)
α1m	**Per 2-Fold Higher**	**Quartile 1:<LLOD**	**Quartile 2: 5.63-8.21 mg/L**	**Quartile 3: 8.22-12.70 mg/L**	**Quartile 4:>12.70 mg/L**
N (total subcohort)	493	215	98	96	84
Cases in subcohort	57	23	8	10	16
Additional cases	431	185	78	81	87
Model 1	1.18 (0.93, 1.48)	1 (ref)	0.84 (0.53, 1.35)	0.98 (0.60, 1.59)	1.41 (0.86, 2.33)
Model 2	1.12 (0.87, 1.44)	1 (ref)	0.84 (0.53,1.34)	0.98 (0.61, 1.59)	1.30 (0.77, 2.20)
KIM-1	**Per 2-Fold Higher**	**Quartile 1:<1,297 pg/mL**	**Quartile 2: 1,297-2,148 pg/mL**	**Quartile 3: 2,149-3,262 pg/mL**	**Quartile 4:>3,262 pg/mL**
N (total subcohort)	493	124	123	123	123
Cases in subcohort	57	14	12	13	18
Additional cases	431	90	114	118	109
Model 1	0.98 (0.78, 1.22)	1 (ref)	0.92 (0.56, 1.52)	0.84 (0.49, 1.44)	0.72 (0.41, 1.29)
Model 2	0.96 (0.77, 1.21)	1 (ref)	0.97 (0.59, 1.59)	0.80 (0.47, 1.37)	0.71 (0.40, 1.28)
EGF	**Per 2-Fold Higher**	**Quartile 1:<1,621 pg/mL**	**Quartile 2: 1,621-1,994 pg/mL**	**Quartile 3: 1,995-2,810 pg/mL**	**Quartile 4:>2,810 pg/mL**
N (total subcohort)	493	124	123	123	123
Cases in subcohort	57	17	14	13	13
Additional cases	431	102	99	115	115
Model 1	1.17 (0.98, 1.39)	1 (ref)	0.90 (0.57, 1.43)	1.29 (0.81, 2.07)	1.49 (0.92, 2.39)
Model 2	1.15 (0.96, 1.38)	1 (ref)	0.93 (0.58, 1.48)	1.35 (0.84, 2.18)	1.49 (0.91, 2.42)
YKL-40	**Per 2-Fold Higher**	**Quartile 1:<263 pg/mL**	**Quartile 2: 263-424 pg/mL**	**Quartile 3: 425-736 pg/mL**	**Quartile 4:>736 pg/mL**
N (total subcohort)	475	119	119	119	118
Cases in subcohort	55	11	13	20	11
Additional cases	422	94	93	117	118
Model 1	1.03 (0.91, 1.17)	1 (ref)	1.08 (0.69, 1.70)	1.35 (0.86, 2.10)	1.12 (0.70, 1.78)
Model 2	1.02 (0.90, 1.15)	1 (ref)	1.11 (0.71, 1.74)	1.36 (0.86, 2.13)	1.07 (0.67, 1.72)

Note: Associations presented as hazard ratio (95% confidence intervals). Model 1: adjusted for age, sex, race, education, smoking, body mass index, systolic blood pressure, antihypertensive medication use, prevalent coronary heart disease, history of stroke, and urine creatinine concentration; model 2: additionally adjusted for baseline eGFR and urine albumin concentration.

Abbreviations: α1m, alpha-1-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; LLOD, lower limit of detection; MCP-1, monocyte chemoattractant protein-1; N, number at risk; ref, reference; REGARDS, Reasons for Geographic and Racial Differences in Stroke; YKL-40, chitinase-3-like protein 1.

Table 5. Summary Estimates for the Association of Urine Biomarkers With CKD From Meta-analysis of ARIC, MESA, and REGARDS Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline

	Per 2-Fold Higher	Quartile 1	Quartile 2	Quartile 3	Quartile 4	I^2 (%)	Test of Homogeneity (*P* Value)	τ^2
MCP-1	1.18 (0.96, 1.45)	1 (ref)	1.11 (0.86, 1.43)	1.06 (0.60, 1.85)	1.50 (0.85, 2.62)	69	0.04	0.0226
α1m	**1.19 (1.08, 1.31)**	1 (ref)	1.11 (0.86, 1.43)	1.21 (0.94, 1.55)	**1.44 (1.12, 1.84)**	0	0.85	0
KIM-1	1.09 (0.94, 1.20)	1 (ref)	0.96 (0.76, 1.22)	1.04 (0.78, 1.38)	1.09 (0.64, 1.84)	41	0.18	0.0076
EGF	0.92 (0.71, 1.19)	1 (ref)	0.89 (0.64, 1.24)	1.03 (0.65, 1.62)	0.86 (0.43, 1.71)	77	0.013	0.0371
YKL-40	0.95 (0.89, 1.01)	1 (ref)	0.75 (0.52, 1.08)	0.78 (0.62, 0.98)	0.84 (0.57, 1.23)	7	0.34	0.0002

Note: Associations presented as hazard ratio (95% confidence intervals). Estimates from model 2 in each cohort were used for the meta-analysis. I^2 = percentage of variation across the cohorts that is due to heterogeneity. It is computed from Cochran's Q, which is the weighted sum of squared differences between each cohort and the pooled effect across the cohorts. τ^2 = between-study variance.
Abbreviations: α1m, alpha-1-microglobulin; ARIC, Atherosclerosis Risk in Communities; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule-1; MCP-1, monocyte chemoattractant protein-1; MESA, Multi-Ethnic Study of Atherosclerosis; ref, reference; REGARDS, Reasons for Geographic and Racial Differences in Stroke; YKL-40, chitinase-3-like protein 1.

incident CKD across 3 well-characterized cohorts even after adjustment for other risk factors, suggesting that this biomarker remains informative even among lower-risk populations.

Urine MCP-1 is another promising biomarker and was significantly associated with incident CKD in both ARIC and MESA. The absence of associations in REGARDS and a higher degree of heterogeneity observed in meta-analysis for this biomarker make these findings less definitive than those for α1m and require further study. MCP-1, believed to reflect tubulointerstitial inflammation and injury, has been associated with kidney injury and poor outcomes in multiple contexts, particularly among persons with known kidney disease. For example, participants with proteinuric CKD and type 1 diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study had higher concentrations of urine MCP-1 compared to controls with preserved eGFR and normalbuminuria.[31] Higher urine MCP-1 has also been associated with risk of allograft failure among kidney transplant recipients.[11] In persons with type 2 diabetes with preserved kidney function, higher urine MCP-1 concentrations were also associated with subsequent declines in kidney function.[32] In previously hospitalized patients with and without acute kidney injury, higher urine MCP-1 concentrations were associated with greater eGFR decline and higher risk of incident CKD and end-stage kidney disease.[33] These studies demonstrate that tubulointerstitial disease captured by urine MCP-1 can be detected before worsening kidney function in a range of settings. Our study adds to this literature by demonstrating that the same is true among community-dwelling adults without diabetes or clinical kidney disease in 2 of 3 cohorts evaluated.

Our analyses did not identify consistent associations of KIM-1, EGF, or YKL-40 with incident CKD, although evidence from individual cohorts identified some isolated associations. KIM-1, a marker of proximal tubule injury, is perhaps the most well-characterized biomarker in the panel evaluated here. It has been qualified by the US Food and Drug Administration for the detection of kidney injury and the determination of truly normal kidney function in preclinical drug development.[34-36] Previously, higher urine KIM-1 concentration was associated with incident CKD and rapid kidney function decline in a nested case-control study of MESA participants, 18% of whom had baseline diabetes.[37] This study now corroborates that finding in ARIC participants without prevalent diabetes or CKD. EGF is involved in kidney tubule cell repair and regeneration and is viewed as a marker of tubule synthetic function. Unlike the other tubule function and injury biomarkers we studied, lower urine EGF levels have been associated with persistent and progressive declines in kidney function in high-risk groups.[13,38-41] However, associations of urine EGF with kidney outcomes in lower-risk adults more closely resembling the general population have been relatively unexplored. Here, we demonstrated that

lower urine EGF in non-diabetic MESA participants was associated with higher risk of incident CKD, although this was not observed in the other 2 cohorts.

This study benefits from the use of 3 large cohorts with extensive recording of comorbid conditions, known CKD risk factors, and a relatively large number of incident CKD events. An efficient case-cohort design applied to MESA and REGARDS made it feasible to measure several different biomarkers concurrently. All biomarker measurements were performed in a single laboratory with uniform techniques. We used a specific outcome definition, ensuring that incident CKD cases were valid and clinically meaningful.[42] Meta-analysis enabled integration of findings from individual cohorts. However, because the meta-analysis was limited to only 3 studies with important differences in inclusion criteria, the estimates of heterogeneity should be interpreted cautiously. Although all participants analyzed for this study had baseline eGFR >60 mL/min/1.73 m^2 and no diabetes, MESA excluded persons with prevalent CVD. The resulting MESA cohort was slightly younger with a lower proportion of baseline hypertension and lower mean blood pressures, compared with the ARIC and REGARDS participants. Another limitation is the possibility for residual confounding due to the observational study design. Due to relatively long follow-up times, our analyses were susceptible to bias from competing outcomes. For example, participants with abnormal baseline markers of tubule injury and dysfunction may have been more likely to die before developing CKD, thus attenuating the observed associations. Long intervals between SCr measurements made it difficult to identify incident CKD near the time of onset, potentially obscuring associations between biomarkers and the outcome. The incident CKD definition required participants to survive and return to follow-up visits by necessity, thus survival bias may have influenced the results. Because biomarkers were measured only once at baseline, we could not evaluate whether repeat measurements and longitudinal biomarker changes provide additional information. Finally, we could not determine whether the tubulointerstitial pathology underlying urine biomarker levels can be modified by lifestyle changes, treatment of comorbid conditions, or nephroprotective medications.

In conclusion, higher urine α1m measured in community-living individuals without CKD at baseline and diabetes is associated with future development of CKD independent of baseline eGFR, albuminuria, and other risk factors, a finding that was consistent across 3 distinct cohorts. Other biomarkers of kidney tubule health had similar associations in individual cohorts. These findings enhance the understanding of CKD development by demonstrating the early importance of tubule dysfunction in the pathophysiology of kidney disease progression. This insight raises the possibility that kidney tubule biomarkers could identify early tubulointerstitial disease, allowing clinicians to investigate potential causes and institute therapies at actionable stages, before the accumulation of substantial damage and evident glomerular filtration rate loss. This strategy may become increasingly relevant with the advent of drugs proposed to target or prevent kidney fibrosis, which are presumed to be most useful in the earliest stages of kidney disease.[43-45] In support of this goal, future studies should continue to evaluate kidney tubule biomarkers in younger and healthier populations, document changes over time, and determine whether the damage represented by urine biomarkers is therapeutically actionable.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Baseline Characteristics of the MESA Subcohort and Additional ESKD Cases.

Table S2: Association of Urine Biomarkers Indexed to Urine Creatinine Concentration With Incident CKD in ARIC Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline.

Table S3: Association of Urine Biomarkers Indexed to Urine Creatinine Concentration With Incident CKD in MESA Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline.

Table S4: Association of Urine Biomarkers Indexed to Urine Creatinine Concentration With Incident CKD in REGARDS Participants With eGFR ≥60 mL/min/1.73 m^2 and Without Diabetes at Baseline.

Table S5: Spearman Correlations of Urine Biomarkers, UACR, and eGFR in ARIC.

Table S6: Spearman Correlations of Urine Biomarkers, UACR, and eGFR in the MESA Random Subcohort.

Table S7: Spearman Correlations of Urine Biomarkers, UACR, and eGFR in the REGARDS Random Subcohort.

Table S8: Coefficients of Variation (CV) for Investigational Urine Biomarkers Measured in Duplicate.

Table S9. Baseline Characteristics of the REGARDS Subcohort and Additional ESKD Cases.

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