Urinary Biomarkers and Risk of ESRD in the Atherosclerosis Risk in Communities Study

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Abstract

Background and objectives Liver fatty acid binding protein (L-FABP), kidney injury molecule 1 (KIM-1), *N*-acetyl-β-D-glucosaminidase (NAG), and neutrophil gelatinase–associated lipocalin (NGAL) are urinary markers of tubular injury that may also be markers of chronic kidney damage. We evaluated the association of these markers with incident ESRD in a community-based sample from the Atherosclerosis Risk in Communities Study.

Design, setting, participants, & measurements This was a matched case-control study of 135 patients with ESRD and 186 controls who were matched on sex, race, kidney function, and diabetes status at baseline (Atherosclerosis Risk in Communities Study visit 4, 1996–1998). Urinary KIM-1 indexed to creatinine (Cr), NAG/Cr, NGAL/Cr, and L-FABP/Cr were measured in stored spot urine samples from the baseline examination. Associations of KIM-1/Cr, NAG/Cr, and NGAL/Cr with patients with incident ESRD through 2008 were modeled continuously and categorically (quartiles) using conditional logistic regression. L-FABP/Cr was modeled only categorically because of a large number of measurements below the lower limit of detection for the assay (2.4 ng/ml).

Results No significant associations were observed for NAG/Cr, NGAL/Cr, or L-FABP/Cr with ESRD. Those in the highest category for KIM-1/Cr had a higher risk of ESRD compared with those with undetectable biomarker levels (reference group) in unadjusted models (odds ratio, 2.24; 95% confidence interval, 1.97 to 4.69; P=0.03) or adjustment for age (odds ratio, 2.23; 95% confidence interval, 1.06 to 4.67; P=0.03). This association was attenuated with additional adjustment for baseline kidney function (odds ratio, 2.02; 95% confidence interval, 0.95 to 4.31; P=0.07 after additional adjustment for eGFR and natural log of the urinary albumin-to-creatinine ratio). No association between KIM-1/Cr and ESRD was found when KIM-1/Cr was analyzed as a continuous variable.

Conclusions Elevated urinary KIM-1/Cr may be associated with a higher risk of incident ESRD, but it does not add to risk prediction after accounting for traditional markers of kidney function in this population.

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Due to the number of contributing authors, the affiliations are provided in the Supplemental Material.

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Introduction

CKD is common in the United States and associated with higher risk of mortality, cardiovascular disease, and progression to ESRD (1–4). The few well established biomarkers for risk stratification in CKD (*e.g.*, serum creatinine and cystatin C and urinary albumin) may not adequately capture risk, particularly at early stages of disease. Identifying new biomarkers associated with CKD progression has the potential to enhance identification of high-risk patients and improve outcomes in CKD.

Several urinary markers of tubular injury associated with AKI may also have clinical utility as markers of chronic kidney damage and are under investigation as markers of CKD and CKD progression (5–8). As part of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) CKD Biomarkers Consortium, we sought to determine whether levels of urinary biomarkers liver fatty acid binding protein (L-FABP), kidney injury molecule 1 (KIM-1), *N*-acetyl-β-D-glucosaminidase (NAG), and neutrophil gelatinase–associated lipocalin (NGAL) are associated with incident ESRD in a nested case-control study of adults from the Atherosclerosis Risk in Communities (ARIC) Study.

Materials and Methods

Study Population

The ARIC Study is a prospective, community–based study of 15,792 adults ages 45–64 years old recruited from four United States communities (Forsyth County, North Carolina; Minneapolis, Minnesota; Washington County, Maryland; and Jackson, Mississippi) and examined in 1987–1989 (9). Follow-up visits occurred in 1990–1992 (visit 2), 1993–1995 (visit 3), 1996–1998 (visit 4), and 2011–2013 (visit 5). Visit 4 was selected as the baseline visit for this study, because spot urine samples were collected and stored at −70°C. Participants who were free of ESRD at visit 4 and had available serum

creatinine–based eGFR, diabetes status, and urinary albumin-to-creatinine ratio (UACR) measurements were eligible for this case-control study. Black participants at centers in Minneapolis, Minnesota and Washington County, Maryland were excluded because of small numbers; in Jackson, Mississippi, only black participants were recruited. The Institutional Review Boards at all ARIC Study sites reviewed and approved the study protocol, including banking of spot urine samples for future studies.

Matching Factor and Covariate Assessment

Kidney function and participant characteristics selected as matching factors were assessed at the ARIC Study visit 4. Both blood and spot urine samples were collected from participants during the clinic visit. Serum creatinine was measured using a modified Jaffe method and calibrated to the assay performed at the Cleveland Clinic. eGFR was subsequently calculated using the 2009 Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation (10). UACR (milligrams per gram) was determined from urinary albumin and creatinine measured in the spot urine samples. Diabetes was defined as the presence of a fasting blood glucose ≥126 mg/dl, the presence of a nonfasting blood glucose ≥200 mg/dl, a self-reported history of diabetes, or a self-reported use of medication for diabetes.

Case Definition and Selection of Patients and Controls

Cases included incident ESRD events after visit 4 identified through hospitalization surveillance through December 31, 2008. Incident ESRD cases were defined as (*1*) hospitalizations with International Classification of Diseases, Ninth Revision, Clinical Modification codes specified for kidney transplant or ESRD or a procedural code indicating dialysis (excluding those with a concomitant code for AKI) and (*2*) deaths with corresponding International Classification of Diseases, Tenth Revision, Clinical Modification codes specified for ESRD or kidney failure, excluding those with AKI codes unless there was a concomitant history of CKD (11–14). Controls were frequency matched to patients on the basis of the following criteria at visit 4: (*1*) eGFR category on the basis of the CKD-EPI Equation (<45, 45–59, 60–74, 75–89, 90–105, or ≥105 ml/min per 1.73 m^2), (*2*) UACR category (<30, 30–299, or ≥300 mg/g), (*4*) diabetes status, (*4*) sex, and (*5*) race. Overall, 185 patients with incident ESRD and 260 controls were identified, of whom 141 patients with incident ESRD and 209 controls had urine samples available and measurements for all four biomarkers and urinary creatinine. For the final analysis, participants in matching factor strata that included only patients or only controls (resulting in uninformative strata) were excluded, resulting in a final sample size of 135 patients and 186 controls.

Exposure Assessment

The CKD Biomarkers Consortium was established in 2008 by the NIDDK to advance the field of CKD biomarker discovery and validation. Urinary biomarkers were assayed at performance laboratories designated by the Consortium using the visit 4 spot urine samples that had undergone one freeze-thaw cycle. Urinary albumin was quantified by an immunoturbidometric method, creatinine was measured by a kinetic colorimetric assay on a Roche Automated Analyzer (Indianapolis, IN), and L-FABP was measured by a two–site sandwich ELISA assay (CMIC, Tokyo, Japan) at the University of Pennsylvania. At Brigham and Women's Hospital, KIM-1 was measured by a microbead–based sandwich ELISA on a Bioplex-200 Platform (Bio-Rad, Hercules, CA), and NAG was measured by an enzymatic assay (Roche). NGAL was measured by a noncompetitive sandwich assay with chemiluminescent signal detection on an ARCHITECT Platform (Abbott Diagnostics, Abbott Park, IL) at University College in Dublin, Ireland.

Statistical Analyses

Baseline characteristics of patients and controls were compared using *t* tests for continuous and chi-squared tests for categorical characteristics. Urinary biomarkers were indexed to urinary creatinine in our primary analyses for consistency with other analyses of urinary biomarkers in the CKD Biomarkers Consortium (15). KIM-1 indexed to creatinine (KIM-1/Cr), NAG/Cr, and NGAL/Cr were evaluated categorically as quartiles and continuously with a $\log_{10}$ transformation to account for their skewed distributions. Given the high percentage of participants with L-FABP measurements that were undetectable (0 ng/ml; 29.0%) or measurements >0 ng/ml but below the assay limit of detection (LOD; 2.4 ng/ml) as determined by the performance laboratory during assay validation (21.5%), L-FABP was evaluated categorically in regression models: (*1*) undetectable, (*2*) detectable but below the LOD, (*3*) above the LOD for L-FABP but below the median measurement of L-FABP/Cr, or (*4*) above the LOD for L-FABP and at or above the median of measurements of L-FABP/Cr.

Associations of continuous log–transformed KIM-1/Cr, NAG/Cr, NGAL/Cr, and L-FABP/Cr (log[L-FABP/Cr+1] to account for zero values) with continuous participant characteristics were evaluated using Pearson correlation coefficients. We used conditional logistic regression to estimate the incidence odds ratio (OR) of ESRD while accounting for the matching factors. Model adjustment beyond accounting for matching factors included (*1*) unadjusted, (*2*) adjustment for baseline age, and (*3*) sequential adjustment for eGFR (modeled as a linear spline with a knot at 60 ml/min per 1.73 m^2) and natural log–transformed UACR to account for potential residual confounding within baseline kidney function categories. We determined *P* trend across quartile, with biomarker quartile modeled as a linear term. In sensitivity analyses, we evaluated the association of 1/urinary Cr with ESRD using conditional logistic regression to determine whether potential associations observed with biomarkers indexed to urinary creatinine reflected associations with creatinine and not the biomarkers of interest. We also examined ESRD associations of biomarkers without normalization to urinary creatinine in conditional logistic regression models. A *P* value <0.05 was considered statistically significant, and analyses were performed using Stata SE, version 12.1.

Results

Study Sample Characteristics

Baseline characteristics of the study population by case status are presented in Table 1. Patients and controls were of similar age at baseline and by design, did not differ for the matching factors sex, race, and diabetes status. Despite

Table 1. Baseline characteristics by ESRD case status

Characteristic	ESRD Cases	Controls	*P* Value
N	135	186	
Age (yr)	64.9 (5.5)	64.7 (5.7)	0.77
Sex, *N* (%) women	59 (43.7)	81 (43.6)	0.98
Race, *N* (%) black	56 (41.5)	67 (36.0)	0.32
Diabetes, *N* (%)	85 (63.0)	97 (52.2)	0.05
Fasting blood glucose	144 (63)	136 (71)	0.29
eGFR (ml/min per 1.73 m^2)	58.7 (29.6)	71.0 (23.7)	<0.001
eGFR (ml/min per 1.73 m^2) group, *N* (%)			0.002
≤44	51 (37.8)	31 (16.7)	
45–59	22 (16.3)	41 (22.0)	
60–74	15 (11.1)	30 (16.1)	
75–89	26 (19.3)	42 (22.5)	
90–104	12 (8.9)	22 (11.8)	
≥105	9 (6.5)	2 (10.8)	
ACR (mg/g), median (interquartile range)	73 (9–705)	13 (3–97)	<0.001
ACR (mg/g) group			<0.001
<30	53 (39.3)	101 (54.3)	
30–299	36 (26.7)	57 (30.7)	
≥300	46 (34.1)	28 (15.1)	
Normalized urinary biomarkers			
L-FABP/Cr			<0.001
Undetectable (0 ng/ml)	33 (24.4)	60 (32.3)	
Below LOD for L-FABP (>0–2.4 ng/ml)	19 (14.1)	50 (26.9)	
Above LOD and below median detectable (<6.67 ng/mg)	30 (22.2)	49 (26.3)	
Above LOD and above median detectable (≥6.67 ng/mg)	53 (39.3)	27 (14.5)	
KIM-1/Cr (pg/mg)	914 (536–1851)	665 (381–1047)	<0.001
NAG/Cr (milliunits/mg)	5.6 (3.1–9.9)	3.9 (2.5–6.2)	0.002
NGAL/Cr (ng/mg)	30.2 (10.7–86.8)	14.8 (8.5–40.2)	<0.001
Raw urinary biomarkers			
L-FABP			<0.001
Undetectable (0 ng/ml)	33 (24.4)	60 (32.6)	
Below LOD (>0–2.4 ng/ml)	19 (14.1)	50 (26.9)	
Above LOD and below median detectable (≥2.4 to <7.59 ng/ml)	31 (23.0)	48 (25.8)	
Above LOD and above median detectable (≥7.59 ng/ml)	52 (38.5)	28 (15.0)	
KIM-1 (pg/ml)	821 (365–1542)	573 (299–1233)	0.03
NAG (milliunits/ml)	4.3 (2.3–8.2)	3.9 (2.1–6.5)	0.07
NGAL (ng/ml)	24.6 (8.8–77.9)	15.1 (7.5–36.0)	0.001

Continuous characteristics are presented as mean (SD) or median (25th–75th percentile). Categorical characteristics are presented as *N* (%). ACR, albumin-to-creatinine ratio; L-FABP, urinary liver fatty acid binding protein; Cr, indexed to urinary creatinine; LOD, limit of detection; KIM-1, urinary kidney injury molecule 1; NAG, urinary *N*-acetyl-β-D-glucosaminidase; NGAL, urinary neutrophil gelatinase–associated lipocalin.

matching on eGFR and albumin-to-creatinine ratio (ACR) groups, patients had lower baseline eGFR and higher baseline ACR than controls. Patients were less likely to have undetectable L-FABP (23.2% versus 32.8%) and more likely than controls to have L-FABP above the LOD (62.2% versus 40.6%). Median KIM-1/Cr, NAG/Cr, and NGAL/Cr levels were significantly higher in patients than in controls. KIM-1/Cr, NAG/Cr, and NGAL/Cr correlated negatively with eGFR and positively with UACR. Weak but significant correlations were observed for NAG/Cr with fasting blood glucose and NGAL/Cr with systolic BP and body mass index (Table 2).

Association of Urinary Biomarkers with Incident ESRD

For the primary analysis, KIM-1, NAG, and NGAL were indexed to urinary creatinine in conditional logistic regression models (Table 3). NAG/Cr and NGAL/Cr were not associated with ESRD when modeled categorically or continuously (Table 3). KIM-1/Cr was not associated with ESRD when modeled continuously, but the highest quartile of KIM-1/Cr was associated with a >2-fold increased odds of incident ESRD when compared with the lowest quartile in unadjusted models (quartile 4 versus quartile 1: hazard ratio, 2.25; 95% confidence interval [95% CI], 1.08 to 4.70; *P*=0.03), which was unchanged with adjustment for age (*P*=0.03) (Table 3). This association was attenuated with additional adjustment for baseline kidney function (OR, 2.14; 95% CI, 1.01 to 4.53; *P*=0.05 in a model adjusted for baseline eGFR; OR, 2.02; 95% CI, 0.95 to 4.31; *P*=0.07 after additional adjustment for lnUACR).

Compared with participants with an undetectable L-FABP level, the highest L-FABP/Cr category (≥6.67 ng/mg) was associated with a nonsignificant 2.2 increased odds of incident ESRD compared with undetectable biomarker levels before and after adjustment for age (OR, 2.22; 95% CI, 0.92 to

Table 2. Partial Pearson correlations of $\log_{10}$–transformed urinary biomarkers with participant characteristics accounting for urinary creatinine normalization (n=321)

Biomarker	Age	SBP	eGFR	lnUACR	FBG	BMI
L-FABP/Cr	0.05	0.24[a]	−0.56[a]	0.68[a]	0.07	0.06
KIM-1/Cr	0.18[b]	0.17[b]	−0.33[a]	0.40[a]	0.05	−0.03
NAG/Cr	0.09	0.16[b]	−0.33[a]	0.45[a]	0.17[b]	0.04
NGAL/Cr	0.04	0.25[a]	−0.41[a]	0.48[a]	0.005	0.11

SBP, systolic BP; lnUACR, natural log of the urinary albumin-to-creatinine ratio; FBG, fasting blood glucose; BMI, body mass index; L-FABP/Cr, urinary liver fatty acid binding protein indexed to urinary creatinine; KIM-1/Cr, urinary kidney injury molecule 1 indexed to creatinine; NAG/Cr, urinary *N*-acetyl-β-D-glucosaminidase indexed to creatinine; NGAL/Cr, urinary neutrophil gelatinase–associated lipocalin indexed to creatinine.
[a]$P<0.001$.
[b]$P<0.01$.

5.33; P=0.08 and OR, 2.23; 95% CI, 0.93 to 5.35; P=0.07, respectively) (Table 3). This association was further attenuated with sequential adjustment for eGFR and lnUACR (Table 3).

In sensitivity analyses with markers not indexed to urinary creatinine, no significant associations or trends were observed for KIM-1, NAG, NGAL, or L-FABP with incident ESRD (Table 4). Urinary creatinine alone was not significantly associated with incident ESRD in models accounting only for matching factors (OR, 1.15 per SD in ln[1/Cr]; 95% CI, 0.89 to 1.48; P=0.28), age adjustment (OR, 1.15 per SD in ln[1/Cr]; 95% CI, 0.89 to 1.48; P=0.28), and additionally, continuous eGFR and lnUACR (OR, 1.06 per SD in ln[1/Cr]; 95% CI, 0.82 to 1.38; P=0.65).

Discussion

In this analysis, we describe associations of urinary markers of renal tubular injury with ESRD in a community-based sample of white and black participants in the United States from the ARIC Study. We observed that elevated urinary KIM-1/Cr but not NAG/Cr, NGAL/Cr, or L-FABP/Cr was associated with a higher risk of incident ESRD in univariate analysis. However, this association lost statistical significance in fully adjusted models accounting for both baseline kidney function (eGFR) and kidney damage (ACR), although 95% CIs were relatively large. Furthermore, neither urinary KIM-1/Cr on a continuous scale nor absolute concentrations of urinary KIM-1 was associated with a higher risk of incident ESRD.

A growing body of work has described these urinary markers (in particular, NGAL and KIM-1) as potential risk factors for CKD and ESRD, although findings have been inconsistent across cohorts, with substantial variability in how biomarkers were assessed and modeled across studies. For example, earlier work in the ARIC Study showed that urinary NGAL but not KIM-1 was associated with higher risk of stage 3 CKD (6), whereas in the Multi-Ethnic Study of Atherosclerosis, higher urinary KIM-1 but not NGAL was associated with increased incident stage 3 CKD and rapid eGFR decline (5). Similar to the prior study in the ARIC Study, in Pima Indians with type 2 diabetes, NGAL/Cr but not KIM-1/Cr was associated with increased ESRD risk over 13.5 years median follow-up (15). In the Chronic Renal Insufficiency Cohort (CRIC), higher absolute NGAL levels were associated with a higher risk of progressive CKD, defined as incident ESRD or halving of eGFR; L-FABP, NAG, and KIM-1 were not measured in that report (8).

Although several studies have reported associations of L-FABP with renal outcomes in adults with diabetes (16,17), associations of L-FABP with clinical outcomes have not been widely studied among patients without diabetes. Characteristics of L-FABP in our study sample suggest that there are differences in the analysis and interpretation compared with prior work in persons with diabetes, because a substantial proportion of our study population had L-FABP values that were below the lower LOD established in our performance laboratory, requiring us to model L-FABP categorically rather than continuously. We cannot formally determine if the high proportion of samples below the lower LOD is because of the age of the biospecimens used for this analysis or the low levels of the analyte in this nondiabetic population with CKD.

Finally, few studies have investigated the association of urinary NAG with ESRD events, but our study is consistent with prior reports in the Pima Indians and adults with autosomal dominant polycystic kidney disease, where urinary NAG was not associated with CKD progression or eGFR decline after adjustment for traditional risk factors for CKD progression (15,18).

Our findings highlight methodologic issues related to studies of biomarkers and CKD progression that may explain some of the variability in findings in studies in the current literature. First, as recently shown by Yang *et al.* (19) using data from the CRIC Study, both the starting eGFR of the study population and the definition of CKD progression used (*e.g.*, eGFR slope versus halving of eGFR versus ESRD) can change the associations of risk factors with CKD progression. For example, those with more elevated baseline eGFR are at higher risk for halving of eGFR, whereas those with lower baseline eGFR are at higher risk for ESRD. Thus, differences in the baseline renal function of the cohort of interest and the selection of renal end points may change the association of risk factors with CKD progression. Unfortunately, both baseline eGFR and renal end points are often study design features that cannot be altered. For example, baseline eGFRs in this case-control study were 58.7 and 71.0 ml/min per 1.73 m^2 in patients and controls, respectively, compared with 149 ml/min in the Pima Indians and 42.4 ml/min per 1.73 m^2 in the CRIC Study (two of the other populations analyzed as part of the CKD Biomarkers

Table 3. Odds ratios and 95% confidence intervals for incident ESRD for urinary biomarkers modeled categorically and continuously indexed to urinary creatinine (*n*=321)

	Unadjusted[a]		Adjusted for Age		Adjusted for Age and eGFR		Adjusted for Age, eGFR, and lnUACR	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
L-FABP/Cr (ng/mg)								
Undetectable (0 ng/ml)	Reference		Reference		Reference		Reference	
Detectable; below LOD	0.74 (0.35 to 1.53)	0.41	0.74 (0.35 to 1.54)	0.42	0.72 (0.34 to 1.51)	0.38	0.77 (0.36 to 1.62)	0.49
≥0.97 to <6.67	0.99 (0.48 to 2.03)	0.97	0.99 (0.48 to 2.04)	0.97	0.93 (0.45 to 1.92)	0.85	0.99 (0.48 to 2.07)	0.99
≥6.67	2.22 (0.92 to 5.33)	0.08	2.23 (0.93 to 5.35)	0.07	1.87 (0.77 to 4.58)	0.17	1.75 (0.71 to 4.30)	0.22
P trend		0.15		0.15		0.29		0.32
KIM-1/Cr (pg/mg)								
Q1≤432.30	Reference		Reference		Reference		Reference	
Q2>432.30 to ≤789.69	1.25 (0.63 to 2.50)	0.53	1.25 (0.62 to 2.50)	0.53	1.28 (0.64 to 2.56)	0.49	1.23 (0.61 to 2.47)	0.57
Q3>789.69 to ≤1214.91	1.23 (0.60 to 2.51)	0.57	1.22 (0.60 to 2.50)	0.58	1.21 (0.59 to 2.50)	0.61	1.26 (0.61 to 2.62)	0.53
Q4>1214.91	2.25 (1.08 to 4.70)	0.03	2.23 (1.07 to 4.68)	0.03	2.14 (1.01 to 4.53)	0.05	2.02 (0.95 to 4.31)	0.07
P trend		0.04		0.05		0.07		0.08
Continuous (log scale; per SD)	1.30 (0.98 to 1.71)	0.07	1.29 (0.98 to 1.71)	0.07	1.27 (0.95 to 1.68)	0.10	1.24 (0.93 to 1.66)	0.14
NAG/Cr (milliunits/mg)								
Q1≤2.69	Reference		Reference		Reference		Reference	
Q2>2.69 to ≤4.55	1.52 (0.75 to 3.08)	0.24	1.52 (0.75 to 3.09)	0.24	1.44 (0.71 to 2.94)	0.31	1.44 (0.70 to 2.98)	0.32
Q3>4.55 to ≤7.50	1.41 (0.69 to 2.90)	0.35	1.41 (0.68 to 2.89)	0.35	1.35 (0.65 to 2.78)	0.42	1.31 (0.63 to 2.72)	0.47
Q4>7.50	2.05 (0.91 to 4.61)	0.08	2.05 (0.91 to 4.62)	0.08	1.89 (0.82 to 4.37)	0.14	1.70 (0.73 to 3.97)	0.22
P trend		0.11		0.12		0.19		0.29
Continuous (log scale; per SD)	1.08 (0.82 to 1.42)	0.57	1.08 (0.82 to 1.42)	0.56	1.03 (0.77 to 1.36)	0.85	0.99 (0.75 to 1.31)	0.93
NGAL/Cr (ng/mg)								
Q1≤9.38	Reference		Reference		Reference		Reference	
Q2>9.38 to ≤18.71	0.75 (0.37 to 1.50)	0.42	0.75 (0.37 to 1.51)	0.42	0.72 (0.36 to 1.45)	0.36	0.67 (0.33 to 1.35)	0.26
Q3>18.71 to ≤50.75	2.05 (0.98 to 4.28)	0.06	2.04 (0.97 to 4.27)	0.06	2.02 (0.96 to 4.26)	0.06	1.83 (0.87 to 3.88)	0.11
Q4>50.75	1.57 (0.67 to 3.64)	0.30	1.56 (0.67 to 3.63)	0.30	1.24 (0.52 to 2.96)	0.63	1.00 (0.40 to 2.45)	0.99
P trend		0.10		0.10		0.23		0.46
Continuous (log scale; per SD)	1.31 (0.95 to 1.81)	0.10	1.31 (0.94 to 1.81)	0.11	1.18 (0.83 to 1.66)	0.36	1.09 (0.77 to 1.55)	0.63

lnUACR, natural log of the urinary albumin-to-creatinine ratio; OR, odds ratio; 95% CI, 95% confidence interval; L-FABP/Cr, urinary liver fatty acid binding protein indexed to urinary creatinine; LOD, limit of detection; KIM-1/Cr, urinary kidney injury molecule 1 indexed to creatinine; Q, quartile; NAG/Cr, urinary *N*-acetyl-β-D-glucosaminidase indexed to creatinine; NGAL/Cr, urinary neutrophil gelatinase–associated lipocalin indexed to creatinine.

[a]Matching factors incorporated into conditional logistic regression model with no additional covariates.

Table 4. Odds ratios and 95% confidence intervals for incident ESRD for urinary biomarkers modeled categorically and continuously not indexed to urinary creatinine (*n*=321)

	Unadjusted*		Adjusted for Age		Adjusted for Age and eGFR		Adjusted for Age, eGFR, and lnUACR	
	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
L-FABP (ng/ml)								
Undetectable (0)	Reference		Reference		Reference		Reference	
>0 to <2.4	0.75 (0.36 to 1.56)	0.43	0.75 (0.36 to 1.56)	0.44	0.73 (0.35 to 1.54)	0.41	0.78 (0.37 to 1.64)	0.51
≥2.4 to <7.62	1.03 (0.50 to 2.09)	0.94	1.03 (0.50 to 2.09)	0.94	0.95 (0.47 to 1.95)	0.90	1.02 (0.49 to 2.09)	0.97
≥7.62	2.04 (0.88 to 4.73)	0.10	2.06 (0.89 to 4.77)	0.09	1.77 (0.75 to 4.18)	0.19	1.66 (0.70 to 3.94)	0.25
P trend		0.14		0.14		0.27		0.31
KIM-1 (pg/ml)								
≤354.45	Reference		Reference		Reference		Reference	
>354.45 to ≤624.42	0.98 (0.50 to 1.91)	0.96	0.99 (0.51 to 1.92)	0.97	0.94 (0.48 to 1.84)	0.80	0.94 (0.48 to 1.86)	0.86
>624.42 to ≤1363.16	1.33 (0.68 to 2.61)	0.41	1.33 (0.68 to 2.60)	0.41	1.33 (0.68 to 2.63)	0.41	1.47 (0.74 to 2.93)	0.27
>1363.16	1.22 (0.61 to 2.44)	0.58	1.21 (0.60 to 2.43)	0.60	1.12 (0.55 to 2.29)	0.75	1.16 (0.56 to 2.40)	0.68
P trend		0.43		0.45		0.55		0.45
Continuous (log scale; per SD)	1.10 (0.85 to 1.41)	0.47	1.09 (0.85 to 1.41)	0.48	1.09 (0.84 to 1.40)	0.52	1.12 (0.86 to 1.45)	0.40
NAG (milliunits/ml)								
≤2.19	Reference		Reference		Reference		Reference	
>2.19 to ≤3.96	1.09 (0.53 to 2.24)	0.81	1.09 (0.53 to 2.24)	0.82	1.10 (0.54 to 2.25)	0.80	1.27 (0.61 to 2.64)	0.53
>3.96 to ≤6.93	0.75 (0.36 to 1.55)	0.43	0.75 (0.36 to 1.55)	0.44	0.68 (0.32 to 1.90)	0.30	0.76 (0.36 to 1.62)	0.48
>6.93	0.90 (0.42 to 1.95)	0.80	0.91 (0.42 to 1.95)	0.80	0.88 (0.41 to 1.90)	0.74	0.91 (0.95 to 1.99)	0.82
P trend		0.55		0.56		0.46		0.51
Continuous (log scale; per SD)	0.96 (0.74 to 1.24)	0.77	0.96 (0.74 to 1.24)	0.77	0.93 (0.71 to 1.21)	0.58	0.95 (0.73 to 1.23)	0.68
NGAL (ng/ml)								
≤8.1	Reference		Reference		Reference		Reference	
>8.1 to ≤18.9	0.75 (0.37 to 1.51)	0.42	0.75 (0.37 to 1.51)	0.42	0.73 (0.37 to 1.48)	0.39	0.78 (0.38 to 1.57)	0.48
>18.9 to ≤42.2	0.86 (0.42 to 1.75)	0.68	0.86 (0.42 to 1.75)	0.67	0.83 (0.40 to 1.70)	0.61	0.78 (0.38 to 1.64)	0.52
42.2 ng/ml	1.49 (0.66 to 3.38)	0.34	1.48 (0.65 to 3.36)	0.35	1.26 (0.55 to 2.88)	0.59	1.23 (0.53 to 2.85)	0.63
P trend		0.41		0.41		0.66		0.77
Continuous (log scale; per SD)	1.16 (0.85 to 1.59)	0.34	1.16 (0.85 to 1.59)	0.35	1.05 (0.76 to 1.46)	0.75	1.03 (0.74 to 1.43)	0.86

lnUACR, natural log of the urinary albumin-to-creatinine ratio; OR, odds ratio; 95% CI, 95% confidence interval; L-FABP, urinary liver fatty acid binding protein; KIM-1/Cr, urinary kidney injury molecule 1; NAG, urinary *N*-acetyl-*β*-D-glucosaminidase; NGAL, urinary neutrophil gelatinase–associated lipocalin.

[a]Matching factors incorporated into conditional logistic regression model with no additional covariates.

Consortium) (8,15). Second, a lack of standardization regarding biomarker measurement and normalization across studies may affect the association of biomarkers with CKD progression. Here, we reported the associations with biomarkers both indexed and not indexed to urinary creatinine. The rationale for indexing biomarkers to urine creatinine is to account for differences in urine concentration (20). Some studies have suggested that lower urinary creatinine concentration itself may be associated with CKD progression, perhaps because low urine creatinine concentration reflects impaired urine concentrating ability and thus, a dimension of CKD beyond eGFR, such as lower lean body mass (20–22). Importantly, we did not find an association of 1/urinary Cr with ESRD, suggesting that the indexed results do not simply reflect the association of 1/urinary Cr with the outcome.

In contrast to our prior study in the Pima Indians, the associations of tubular injury biomarkers with ESRD in the ARIC Study population were modest and not statistically significant when adjusted for baseline kidney function. Follow-up times, sample storage, and freeze-thaw cycles were similar in the two cohorts. Biomarker assays were performed using the same reagents in a single-performance laboratory for each biomarker. Racial differences and differences in comorbidities may be responsible, in part, for these findings in addition to the differences in baseline eGFR and outcome ascertainment. Specifically, Pima Indians have an extraordinarily high rate of type 2 diabetes and CKD/ESRD caused by diabetic nephropathy, whereas the ARIC Study includes white and black individuals, many of whom do not have diabetes. In the Pima Indians, ESRD was defined by the initiation of RRT (or death from diabetic kidney disease if the participant refused dialysis). By contrast, in the ARIC Study, ESRD was defined on the basis of hospitalization coding data.

Strengths of our study include a well defined and characterized population with the ability to match patients to controls because of the large size of the parent ARIC Study cohort, the detailed characterization of the study population, and the availability of banked samples stored long term under controlled conditions. Potential limitations include differences in baseline eGFR and albuminuria between patients and controls despite matching, which are not surprising given the strength of the association of these factors with CKD progression and ESRD. These variables were included in multivariable adjusted models to account for potential residual confounding within categories of baseline kidney function. The use of diagnostic codes from acute hospitalizations to define ESRD is also a potential limitation. However, this definition has been used previously in the ARIC Study to identify individuals with ESRD (11–13), with similar findings to other studies that have used definitions of ESRD on the basis of United States Renal Data System linkage data. Although our case-control design allows for efficient biomarker measurement across a large cohort, because ESRD was a relatively rare event in the population, we have limited power to detect differences, and a type II error (false negative) cannot be excluded. Finally, although these samples were stored at −80°C, they were banked in 1996–1998, and the ARIC Study protocol did not require centrifugation. KIM-1, NGAL, NAG, and L-FABP have been stable in a number of storage studies performed to date, but overall duration of storage has been substantially shorter. We did compare UACR measured when the samples were collected and at the time of these novel biomarker measurements; there was excellent agreement between these measurements (data not shown).

In summary, although KIM-1 indexed to urinary creatinine was associated with incident ESRD in univariate analysis in this matched case-control study, we found no independent association between it or other tubular injury biomarkers and incident ESRD after accounting for baseline kidney function. At present, studies conducted by our Consortium of urinary tubular injury biomarkers do not suggest that there are consistent, independent associations of these biomarkers with CKD progression. Additional studies are needed to determine if there are subpopulations in which these biomarkers may be of utility.

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Disclosures

J.V.B. is a coinventor on kidney injury molecule 1 patents, which have been licensed by Partners Healthcare to a number of companies, and has received royalty income from Partners Healthcare. K.D.L. had reagents donated for previous biomarker studies by Abbott and CMIC.

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