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The Associations of Plasma Biomarkers of Inflammation With Histopathologic Lesions, Kidney Disease Progression, and Mortality—The Boston Kidney Biopsy Cohort Study

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Background: Soluble tumor necrosis factor receptor (sTNFR)-1, sTNFR-2, YKL-40, monocyte chemoattractant protein (MCP)-1, and soluble urokinase plasminogen activator receptor (suPAR) have emerged as promising biomarkers of inflammation but have not been evaluated across diverse types of kidney diseases.

Methods: We measured these plasma biomarkers in 523 individuals enrolled into a prospective, observational cohort study of patients undergoing clinically indicated native kidney biopsy at 3 tertiary care hospitals. Two kidney pathologists adjudicated biopsy specimens for semiquantitative scores of histopathology. Proportional hazard models tested associations between biomarkers and risks of kidney disease progression (composite of ≥40% estimated glomerular filtration rate [eGFR] decline or end-stage kidney disease [ESKD]) and death.

Results: Mean eGFR was 56.4±36 ml/min per 1.73 m^2 and the median proteinuria (interquartile range) was 1.6 (0.4, 3.9) g/g creatinine. The most common primary clinicopathologic diagnoses were proliferative glomerulonephritis (29.2%), nonproliferative glomerulopathy (18.1%), advanced glomerulosclerosis (11.3%), and diabetic kidney disease (11.1%). sTNFR-1, sTNFR-2, MCP-1, and suPAR were associated with tubulointerstitial and glomerular lesions. YKL-40 was not associated with any histopathologic lesions after multivariable adjustment. During a median follow-up of 65 months, 182 participants suffered kidney disease progression and 85 participants died. After multivariable adjustment, each doubling of sTNFR-1, sTNFR-2, YKL-40, and MCP-1 was associated with increased risks of kidney disease progression, with hazard ratios ranging from 1.21 to 1.47. Each doubling of sTNFR-2, YKL-40, and MCP-1 was associated with increased risks of death, with hazard ratios ranging from 1.33 to 1.45. suPAR was not significantly associated with kidney disease progression or death.

Conclusions: sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR are associated with underlying histopathologic lesions and adverse clinical outcomes across a diverse set of kidney diseases.

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Chronic kidney disease (CKD) is not a single entity but rather a heterogeneous condition with a wide spectrum of underlying etiologies, pathologic and clinical manifestations, and variable rates of progression. eGFR and albuminuria are the 2 primary clinical indicators used for CKD definition, staging, and prognosis.[1] However, eGFR and albuminuria do not provide specificity regarding the underlying pathobiology and

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are limited in predicting risk of adverse outcomes.[2] Kidney biopsies provide information on histopathologic lesions, which are strong predictors of outcomes in patients with CKD,[3] but kidney biopsies are invasive procedures with risks.[4] Emerging data suggest that inflammatory mechanisms in response to underlying kidney injury may contribute to the development and propagation of histopathologic lesions, which could lead to subsequent kidney disease progression.[5–8] Investigation of the associations of novel biomarkers of inflammation with underlying histopathologic lesions may enable noninvasive identification of individuals at high risk of kidney disease progression.

Prior studies on plasma biomarkers of inflammation in kidney disease and kidney disease progression have largely been restricted to individuals with diabetes or common forms of CKD without biopsy confirmation of cause.[8–13] We measured plasma biomarkers of inflammation (sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR) in samples from the Boston Kidney Biopsy Cohort, a prospective cohort study of individuals undergoing native kidney biopsy. We hypothesized that higher levels of these biomarkers of inflammation are associated with distinct kidney histopathologic lesions and subsequent adverse clinical outcomes.

MATERIALS AND METHODS

Study Population

The Boston Kidney Biopsy Cohort is a prospective, observational cohort study of individuals undergoing native kidney biopsy at 3 tertiary care hospitals in Boston, MA. Details of the study design have been previously described.[3] The study includes adults (≥18 years of age) who underwent a clinically indicated native kidney biopsy between September 2006 and June 2016. Exclusion criteria included the inability to provide written consent, severe anemia, pregnancy, and enrollment in competing studies. Participants provided blood and urine samples on the day of kidney biopsy. We included 523 participants who had available plasma samples for biomarker measurements. The Partners Human Research Committee (the Brigham and Women's Hospital Institutional Review Board) approved the study protocol, which is in accordance with the principles of the Declaration of Helsinki.

Exposures, Blood Collection, and Laboratory Assays

The primary exposures were plasma concentrations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR. Blood samples were collected from participants on the day of biopsy in EDTA-containing vacutainers and were processed, aliquoted, and stored at −80°C within 4 hours of collection. Measurements of biomarkers were made in 523 participants for sTNFR-1 and sTNFR-2, 490 participants for YKL-40, 522 participants for MCP-1, and 498 participants for suPAR. Plasma samples were diluted 5-fold using sample diluent buffer (0.1 mol/l of HEPES, 0.1 mol/l of NaCl, 0.1% TWEEN 20, and 1% BSA; pH 7.4; filter sterilized). Plasma biomarkers were measured using multiplex microbead-based enzyme-linked immunosorbent assays on a Luminex platform. Recombinant proteins (Bio-Techne, Minneapolis, MN) were incubated with microbeads coupled with capture antibodies (Bio-Techne) for 1 hour on an orbital shaker, washed 3 times with 100 μl of phosphate-buffered saline–Tween buffer, and incubated with corresponding secondary antibodies (Bio-Techne) for 1 hour. After incubation, plates were washed with phosphate-buffered saline–Tween buffer and incubated with streptavidin-phycoerythrin for another 15 minutes. Beads were then washed again with phosphate-buffered saline–Tween buffer, and fluorescence was measured on a BioPlex 200 (Bio-Rad) analyzer. Samples were measured in duplicates with coefficients of variation <15% across duplicates.

Evaluation of Histopathology

Methods to evaluate and score histopathologic lesions were previously described.[3] Briefly, kidney biopsies were adjudicated under light microscopy by 2 experienced kidney pathologists who provided semi-quantitative scores of kidney inflammation, fibrosis, vascular sclerosis, and tubular injury (Supplementary Table S1). Of the 13 histopathologic lesions, all were scored during study sessions except for grades of global or segmental glomerulosclerosis, which were taken from the biopsy report, because they were each calculated as a percentage of the total number of glomeruli. We limited statistical analyses on histopathologic lesions to those with adjudicated cases (n=456, 87%) except for analyses of global or segmental glomerulosclerosis because they were taken from the biopsy report. We combined endocapillary glomerular inflammation, extracapillary cellular crescents, focal glomerular necrosis, and fibrocellular crescents into a single dichotomous variable named "glomerular inflammation" because of the relatively low prevalence and limited range of severity for each lesion in this cohort. All participants' charts were reviewed alongside the histopathologic evaluations to provide the final primary clinicopathologic diagnosis.

Clinical Outcomes

The 2 primary outcomes were kidney disease progression and all-cause mortality. Kidney disease progression was defined as ≥40% decline in eGFR or ESKD (dialysis or kidney transplantation). eGFR during

follow-up was obtained from the electronic medical record. The secondary endpoint was progression to ESKD. ESKD status was confirmed by reviewing the electronic medical record and linkage with the United States Renal Data System database.[14] Mortality status was confirmed with the Social Security Death Index. Participants were followed up until the occurrence of death, voluntary study withdrawal, loss to follow-up, or February 1, 2020.

Covariates

We collected participant information at the biopsy visit by self-report or from the electronic medical record, including demographics (age, sex, race), medical history (hypertension, diabetes mellitus, systemic lupus erythematosus, systemic vasculitis, hepatitis B, hepatitis C, malignancy, human immunodeficiency virus non-kidney solid organ transplant), medication lists (angiotensin converting enzyme inhibitor, angiotensin II receptor blocker, mineralocorticoid receptor antagonist, calcium channel blocker, beta-blocker, corticosteroids, other immunosuppressive medications), reason for native kidney biopsy (proteinuria, hematuria, nephrotic syndrome, nephritis syndrome, abnormal eGFR), serum creatinine, and proteinuria. All data were stored at the REDCap electronic data capture tools hosted at Partners Health Care.[15] We used the creatinine-based Chronic Kidney Disease Epidemiology Collaboration equation to calculate the eGFR.[16] We obtained serum creatinine from the electronic medical record on the day of biopsy. In participants for whom this was unavailable, we measured serum creatinine in blood samples collected on the day of biopsy. We obtained spot urine protein-to-creatinine ratio or urine albumin-to-creatinine ratio from the date of kidney biopsy up to 3 months before biopsy from the electronic medical record. If a participant did not have either of these values, we measured urine albumin-to-creatinine ratio from urine collected on the day of kidney biopsy. Serum and urine creatinine were measured using a Jaffe-based method, and urine albumin was measured by an immunoturbidometric method.

Statistical Analysis

Descriptive statistics were summarized as count with percentages for categorical variables and mean $\pm$ standard deviation or median with interquartile range for continuous variables. For skewed data distributions, we performed natural logarithmic transformation as appropriate. Plasma biomarkers were examined as continuous variables (log base 2). We used Spearman correlation coefficients to determine associations between continuous variables and each biomarker. To evaluate associations of plasma biomarkers with histopathologic lesions, we used Wilcoxon rank sum or Kruskal-Wallis tests for 2-group and multiple group comparisons, respectively. To calculate percentage differences of plasma biomarkers by histopathologic lesion, we used multivariable-adjusted linear regression models.

For the outcome of kidney disease progression (time to either $\geq$40% eGFR decline or ESKD), we treated the data as interval censored because the exact date of eGFR decline is not known.[3] We evaluated the association between each plasma biomarker and subsequent kidney disease progression using a nonparametric survival function for interval-censored data with differences assessed by the general log-rank test.[17,18] We modeled the data using Cox proportional hazards regression models that accommodated interval-censored time to event.[19,20] Traditional Cox proportional hazards regression models were used for the outcomes of ESKD and mortality. All proportional hazards models were first fit without adjustment and then stratified by site with multivariable adjustment for covariates, including age, sex, race, baseline eGFR, baseline natural log-transformed proteinuria, and primary clinicopathologic diagnosis. Because levels of biomarkers may differ by the underlying etiology of kidney disease, we adjusted for primary clinicopathologic diagnosis. We tested for statistical interaction between each plasma biomarker and primary clinicopathologic diagnosis (glomerulopathy vs. other diagnoses) for each outcome through multiplicative interaction terms. To test the predictive value of the plasma biomarkers of inflammation, we calculated *c*-statistics for the outcomes of ESKD and death using a base model that was stratified by study site and included age, sex, baseline natural log-transformed proteinuria, primary clinicopathologic diagnosis, and baseline eGFR) and also for the model that further included the 5 plasma biomarkers of inflammation.[21,22] We performed a sensitivity analysis using subdistribution hazards models that acknowledged the competing risk of death.[23] Because medications that suppress the immune system may alter levels of plasma biomarkers of inflammation or the risk of adverse clinical outcomes, we repeated the outcome analysis after further adjustment for the use of immunosuppressive medications or corticosteroids. We confirmed no violations of the proportional hazards assumption through assessment of Schoenfeld residuals. Complete case analysis was used for the analyses because there was less than 5% missing data. All statistical tests were 2-sided, and P values <0.05 were considered significant. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC), and Stata, version 15.0 (StataCorp, College Station, TX).

Table 1. Baseline characteristics of the Boston Kidney Biopsy Cohort ($n = 523$)

Baseline characteristics	
Plasma biomarker concentrations, pg/ml	
sTNFR-1	1012.7 [485.1, 2167.8]
sTNFR-2	6097.6 [3301.5, 12411.6]
YKL-40	53.5 [25.7, 98.6]
MCP-1	135.2 [95.3, 193.1]
suPAR	3044.3 [1809.8, 6493.2]
Clinical characteristics	
Age, yr	52.8 ± 16.6
Female	266 (50.9)
Race	
White	333 (63.7)
Black	104 (19.9)
Other	86 (16.4)
eGFR, ml/min per 1.73 m^2	56.4 ± 36.0
Serum creatinine, mg/dl	1.5 [0.9–2.3]
Proteinuria, g/g creatinine	1.6 [0.4, 3.9]
Reason for biopsy[a]	
Proteinuria	305 (58.3)
Hematuria	127 (24.3)
Nephrotic syndrome	69 (13.2)
Nephritic syndrome	12 (2.3)
Abnormal eGFR	268 (51.2)
Primary clinicopathologic diagnosis[b]	
Proliferative glomerulonephritis	150 (29.2)
Nonproliferative glomerulopathy	93 (18.1)
Advanced glomerulosclerosis	58 (11.3)
Diabetic kidney disease	57 (11.1)
Vascular disease	44 (8.6)
Paraprotein-related disease	42 (8.2)
Tubulointerstitial disease	39 (7.6)
Other	31 (6.0)
Comorbid conditions	
Diabetes mellitus	120 (22.9)
Hypertension	278 (53.2)
Systemic lupus erythematosus	77 (14.7)
Systemic vasculitis	13 (2.5)
Hepatitis B	4 (0.8)
Hepatitis C	10 (1.9)
Malignancy	82 (15.7)
Human immunodeficiency virus	5 (1.0)
Non-kidney solid organ transplant	9 (1.7)
Medications	
ACEi/ARB	242 (46.3)
MRA	12 (2.3)
Calcium channel blockers	137 (26.2)
Beta-blockers	168 (32.1)
Immunosuppression	94 (18.0)
Corticosteroids	97 (18.5)
Immunosuppression or corticosteroids	150 (28.7)
Clinical site	
Site 1	333 (63.7)
Site 2	118 (22.6)
Site 3	72 (13.8)

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; eGFR, estimated glomerular filtration rate; MRA, mineralocorticoid receptor antagonist.
[a]Percentages do not add to 100 as there may have been more than 1 reason for kidney biopsy.
[b]Nine individuals had insufficient tissue to make a clinicopathologic diagnosis; the "other diagnosis" category was composed of participants with minor abnormalities or relatively preserved parenchyma.
Data are presented as mean ± standard deviation, median [interquartile range], and count with frequencies (%) for binary and categorical variables.

RESULTS

Baseline Characteristics

Baseline characteristics of the study cohort and median biomarker levels are shown in Table 1. The mean age was 52.8±16.6 years, 50.9% were women, and 63.7% were white. The most common primary clinicopathologic diagnoses were proliferative glomerulonephritis (29.2%), nonproliferative glomerulopathy (18.1%), advanced glomerulosclerosis (11.3%), and diabetic kidney disease (11.1%). The mean eGFR was 56.4±36.0 ml/min per 1.73 m^2 and the median proteinuria (interquartile range) was 1.6 (0.4, 3.9) g/g creatinine. The median plasma levels (interquartile range) for each biomarker were as follows: sTNFR-1, 1012.7 (485.1, 2167.8) pg/ml; sTNFR-2, 6097.6 (3301.5, 12411.6) pg/ml; YKL-40, 53.5 (25.7, 98.6) ng/ml; MCP-1, 135.2 (95.3, 193.1) pg/ml; and suPAR, 3044.3 (1809.8, 6493.2) pg/ml. Supplementary Table S2 shows the plasma biomarker levels by primary clinicopathologic diagnosis.

Correlations Between eGFR, Proteinuria, and Biomarkers

Table 2 shows associations of plasma biomarkers with eGFR, proteinuria, and each other. Plasma sTNFR-1, sTNFR-2, YKL-40, and suPAR had moderate to strong correlations with eGFR, and plasma MCP-1 had a weak correlation with eGFR. All biomarkers had positive correlations with proteinuria, but the correlation between suPAR and proteinuria was not significant. All plasma biomarkers had positive correlations with one another.

Associations of Plasma Biomarkers With Histopathologic Lesions

Supplementary Figures S1, S2, and S3 show differences in plasma biomarker concentrations across histopathologic lesions in the tubulointerstitial, glomerular, and microvascular compartments, respectively. Figure 1 shows adjusted differences in plasma biomarker concentrations across histopathologic lesions, using multivariable models adjusted for age, sex, race, and eGFR. Plasma sTNFR-1 levels were significantly higher with more severe acute tubular injury, interstitial fibrosis and tubular atrophy (IFTA), mesangial expansion, and the presence of glomerular inflammation compared with less severe lesions, respectively. Plasma sTNFR-2 levels were significantly higher with more severe IFTA, mesangial expansion, and the presence of segmental sclerosis compared with less severe lesions, respectively. Plasma YKL-40 was not significantly associated with any histopathologic lesions. Plasma MCP-1 levels were significantly higher with more severe mesangial expansion and lower with more severe

Table 2. Spearman correlation coefficients between kidney function, proteinuria, and plasma biomarkers

	sTNFR-1	sTNFR-2	YKL-40	MCP-1	suPAR
eGFR	–0.70 (<0.001)	–0.62 (<0.001)	–0.51 (<0.001)	–0.10 (0.02)	–0.46 (<0.001)
Proteinuria	0.28 (<0.001)	0.29 (<0.001)	0.22 (<0.001)	0.11 (0.01)	0.07 (0.10)
sTNFR-1	1	0.86 (<0.001)	0.58 (<0.001)	0.19 (<0.001)	0.45 (<0.001)
sTNFR-2	0.86 (<0.001)	1	0.54 (<0.001)	0.24 (<0.001)	0.41 (<0.001)
YKL-40	0.58 (<0.001)	0.54 (<0.001)	1	0.15 (0.001)	0.24 (<0.001)
MCP-1	0.19 (<0.001)	0.24 (<0.001)	0.15 (0.001)	1	0.13 (0.005)
suPAR	0.45 (<0.001)	0.41 (<0.001)	0.24 (<0.001)	0.13 (0.005)	1

eGFR, estimated glomerular filtration rate; MCP, monocyte chemoattractant protein; sTNFR, soluble tumor necrosis factor receptor, suPAR, soluble urokinase plasminogen activator receptor.
P values are in parentheses.

acute tubular injury compared with less severe lesions, respectively. suPAR levels were significantly higher with the presence of glomerular inflammation compared with the absence of glomerular inflammation.

Associations of Plasma Biomarkers With Kidney Disease Progression

During a median follow-up time of 59 months, 182 participants experienced kidney disease progression, and 124 participants progressed to ESKD. Table 3 shows the unadjusted and multivariable-adjusted associations between each plasma biomarker and kidney disease progression. sTNFR-1, sTNFR-2, YKL-40, and MCP-1 were each associated with increased risks of kidney disease progression, in fully adjusted models including proteinuria and eGFR. Each biomarker was also independently associated with progression to ESKD, but the association between YKL-40 and subsequent ESKD was confounded by eGFR and not significant in adjusted models. The associations of suPAR with kidney disease progression and ESKD were nominally higher but no longer significant after further adjustment for eGFR. There was no evidence of statistical interaction between plasma biomarkers and primary clinicopathologic diagnosis (glomerulopathy vs. other diagnoses) for either kidney disease progression or ESKD (*P* for interaction for kidney disease progression: sTNFR-1, 0.51; sTNFR-2, 0.93; YKL-40, 0.99; MCP-1, 0.50; suPAR, 0.13; *P* for interaction for ESKD: sTNFR-1, 0.64; sTNFR-2, 0.77; YKL-40, 0.34; MCP-1, 0.80; suPAR, 0.06) (Supplementary Table S3). Results from subdistribution hazards models that incorporated the competing risk of death yielded similar results, except that the association between suPAR and progression to ESKD (HR 1.12, 95% confidence interval [CI] 1.01–1.24) was statistically significant (Supplementary Table S4). Further adjustment for immunosuppressive medications did not qualitatively change the results (Supplementary Table S5). We tested the performance of the 5 plasma biomarkers of

Histopathologic Lesions	sTNFR-1	sTNFR-2	YKL-40	MCP-1	suPAR
Glomerular inflammation[a]	26 (4 , 54)	23 (-1 , 52)	-2 (-19 , 18)	-2 (-17 , 14)	37 (4 , 80)
Mesangial expansion[b]	31 (11 , 54)	25 (7 , 47)	1 (-13 , 19)	14 (1 , 29)	3 (-19 , 30)
Segmental sclerosis[a]	20 (0 , 44)	24 (4 , 49)	8 (-10 , 28)	6 (-7 , 21)	-7 (-27 , 19)
Global glomerulosclerosis[c]	2 (-14 , 20)	-3 (-19 ,16)	-2 (-16 , 14)	7 (-6 , 21)	-11 (-29 , 11)
Acute tubular injury[b]	43 (12 , 84)	7 (-16 , 37)	4 (-19 , 32)	-22 (-35 , -6)	26 (-12 , 80)
Inflammation, nonfibrosed interstitium[a]	25 (-1 , 57)	17 (-7 , 48)	9 (-14 , 38)	-1 (-17 , 17)	-10 (-36 , 26)
Inflammation, fibrosed interstium[c]	-1 (-17 , 19)	3 (-14 , 24)	-6 (-21 , 13)	3 (-10 , 17)	-1 (-24 , 28)
Interstitial fibrosis/tubular atrophy[c]	34 (11 , 63)	38 (14 , 66)	9 (-10 , 32)	8 (-7 , 24)	9 (-17 , 43)
Arterial sclerosis[b]	5 (-13 , 26)	9 (-11 , 33)	18 (-1 , 40)	10 (-5 , 27)	-22 (-40 , 1)
Arteriolar sclerosis[b]	-3 (-19 , 16)	-3 (-20 , 18)	3 (-13 , 21)	10 (-4 , 26)	-16 (-34 , 8)

Figure 1. Differences in sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR by histopathologic lesions. Models were fit using log-transformed biomarker as the outcome and each histopathologic lesion as the predictor variable. Percentage differences are derived from linear regression models of log base 2 sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR, respectively. Each individual model was adjusted for age, sex, race, and eGFR. Percentage differences in each biomarker were calculated by raising 2 to the power of the beta-coefficient, subtracting 1, and multiplying by 100 [$(2^{\beta} - 1) \times 100$)] for each respective histopathologic lesion from the linear regression model. eGFR, estimated glomerular filtration rate; MCP, monocyte chemoattractant protein; sTNFR, soluble tumor necrosis factor receptor, suPAR, soluble urokinase plasminogen activator receptor. Shading represents the magnitude of difference for each plasma biomarker by histopathologic lesions.
[a] Reference is absence of lesion.
[b] Reference is none or mild lesion severity.
[c] Reference is 0–25% of cortical volume affected.

Table 3. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with adverse clinical outcomes

	Events	Events per 100 person-years[a]	Model 1, HR (95% CI)	Model 2, HR (95% CI)	Model 3, HR (95% CI)
sTNFR-1					
Kidney disease progression[b]	182	10.1	1.63 (1.47–1.81)	1.58 (1.39–1.78)	1.33 (1.13–1.56)
ESKD	124	5.8	1.87 (1.65–2.11)	1.88 (1.62–2.17)	1.31 (1.07–1.60)
Mortality	85	3.1	1.45 (1.25–1.68)	1.37 (1.15–1.63)	1.17 (0.94–1.46)
sTNFR-2					
Kidney disease progression[b]	182	10.1	1.75 (1.55–1.97)	1.79 (1.55–2.06)	1.47 (1.24–1.75)
ESKD	124	5.8	2.05 (1.75–2.39)	2.17 (1.80–2.61)	1.50 (1.18–1.90)
Mortality	85	3.1	1.62 (1.35–1.95)	1.53 (1.25–1.87)	1.33 (1.04–1.71)
YKL-40					
Kidney disease progression[b]	171	9.9	1.46 (1.30–1.65)	1.41 (1.23–1.62)	1.21 (1.04–1.40)
ESKD	117	5.8	1.58 (1.37–1.84)	1.59 (1.33–1.91)	1.19 (0.99–1.44)
Mortality	77	3.0	1.80 (1.48–2.19)	1.57 (1.26–1.96)	1.45 (1.15–1.82)
MCP-1					
Kidney disease progression[b]	182	10.1	1.24 (1.06–1.45)	1.23 (1.02–1.48)	1.33 (1.09–1.61)
ESKD	124	5.8	1.27 (1.05–1.54)	1.25 (1.00–1.57)	1.47 (1.16–1.88)
Mortality	84	3.1	1.33 (1.05–1.69)	1.32 (1.00–1.74)	1.36 (1.03–1.79)
suPAR					
Kidney disease progression[b]	176	10.6	1.20 (1.12–1.29)	1.17 (1.08–1.27)	1.08 (0.99–1.19)
ESKD	122	6.3	1.26 (1.17–1.37)	1.25 (1.14–1.37)	1.11 (0.99–1.25)
Mortality	82	3.2	1.19 (1.07–1.32)	1.15 (1.02–1.31)	1.08 (0.94–1.24)

CI, confidence interval; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; HR, hazard ratio; MCP, monocyte chemoattractant protein; sTNFR, soluble tumor necrosis factor receptor, suPAR, soluble urokinase plasminogen activator receptor.
Model 1 is unadjusted. Model 2 is stratified by site and adjusted for age, sex, race, natural log transformed proteinuria, and primary clinicopathologic diagnosis. Model 3 is Model 2 and further adjusted for baseline eGFR.
HR per doubling of biomarker.
[a]Approximate events per 100 person-years. For the composite outcome with interval censored data, if an event occurred, the time used is one-half of the interval width plus all of the time before the interval as the approximate exposure time (the exact time an event occurred is not known if a ≥40% decline in eGFR occurred.
[b]Kidney disease progression defined as ≥40% eGFR decline or ESKD.

inflammation for predicting 5-year risk of ESKD in addition to the base model. The *c*-statistic of the base model was 0.85 (95% CI 0.82–0.89). The *c*-statistic of the model after addition of the 5 plasma biomarkers increased to 0.87 (95% CI 0.83–0.89; $P = 0.17$ for comparison with the *c*-statistic of the base model).

Associations of Plasma Biomarkers With All-Cause Mortality

During a median follow-up time of 65 months, 85 participants died. Table 3 shows the unadjusted and multivariable-adjusted associations between each plasma biomarker and subsequent risk of death. sTNFR-2, YKL-40, and MCP-1 were each associated with increased risks of death in adjusted models. sTNFR-1 and suPAR were both associated with increased risk of death, but the associations were confounded by eGFR and not statistically significant in fully adjusted models. There was no evidence of statistical interaction between each plasma biomarker and primary clinicopathologic diagnosis for all-cause mortality (*P* for interaction: sTNFR-1, 0.53; sTNFR-2, 0.86; YKL-40, 0.16; MCP-1, 0.39; suPAR, 0.29) (Supplementary Table S3). Further adjustment for immunosuppressive medications did not qualitatively change the results (Supplementary Table S5). We tested the performance of the 5 plasma biomarkers of inflammation for predicting the 5-year risk of death in addition to the base model. The *c*-statistic of the base model was 0.72 (95% CI 0.65–0.78). The *c*-statistic of the model after addition of the 5 plasma biomarkers increased to 0.76 (95% CI 0.70–0.82; $P < 0.001$ for comparison with *c*-statistic of base model).

DISCUSSION

Inflammation is a key driver of many forms of kidney disease, most obviously in glomerulonephritis and interstitial nephritis, but also in common disorders including diabetic kidney disease and acute kidney injury (AKI).[5–8] In this study involving a diverse set of kidney diseases with biopsy confirmation and long-term clinical follow-up, we found strong associations of biomarkers of inflammation with histopathologic lesions as well as subsequent risks of kidney disease progression and death. The addition of plasma biomarkers of inflammation offered little to modest improvements in risk prediction for the outcomes of ESKD and death as judged by the change in *c*-statistic, respectively. Our findings provide evidence for the importance of inflammation across a wide variety of kidney diseases, with consistent findings for associations in both glomerulopathies and other kidney diseases. Our findings also highlight the potential roles for biomarkers in nephrology to estimate prognosis and noninvasively assess kidney histopathology. Further

discussion of each biomarker illustrates and contextualizes our findings.

Tumor necrosis factor receptors (TNFRs) are activated by TNF-α, which has an essential role in inflammatory processes.[24] TNFR-1 is expressed on nearly every cell in the body and TNFR-2 is primarily expressed in lymphocytes.[24,25] After activation, TNFRs are shed from the cell surface into a soluble form in the bloodstream (sTNFR).[26] Plasma sTNFR-1 and sTNFR-2 have been reported to be associated with tubulointerstitial and glomerular lesions in patients with glomerulopathies[27–29] and glomerular lesions in patients with diabetes mellitus.[30] However, recent work found minimal correlations between circulating sTNFR-1 and sTNFR-2 with glomerular or tubular expression of the corresponding genes and minimal mRNA expression of sTNFR-1 and sTNFR-2 in areas of glomerulosclerosis, IFTA, or lymphocyte infiltration in individuals with established diabetic kidney disease.[8] After multivariable adjustment that included eGFR, we found that both sTNFR-1 and sTNFR-2 are associated with tubulointerstitial and glomerular lesions but follow slightly different patterns of injury. Most of the published literature on sTNFR-1 and sTNFR-2 has focused on diabetes or CKD without biopsy confirmation,[11,12,31,32] with relatively little investigation in individuals with glomerulopathies.[28,29] Our cross-sectional and prospective findings suggest that sTNFR-1 and sTNFR-2 merit further evaluation as biomarkers in glomerular diseases.

YKL-40, also known as chitinase 3–like 1 (CHI3L1), is a glycoprotein produced by macrophages, neutrophils, and other local inflammatory cells.[33] YKL-40 functions as an important mediator of inflammation after ischemia or reperfusion injury by activating phosphoinositide 3-kinase (PI3K)–dependent cell survival signaling via interleukin-13 receptor α2 (IL13Rα2) and stimulating macrophages.[33,34] Through activation of chemokine receptor homologous molecule that is expressed on TH2 lymphocytes (CRTH2), YKL-40 is produced in response to ischemic AKI[33] and promotes pro-fibrotic signaling in settings of sustained tissue injury and maladaptive repair.[34] Prior studies from the cardiovascular literature suggested that YKL-40 is a growth factor for fibroblasts and promotes migration of vascular smooth muscles cells that can lead to tissue remodeling and subsequent vascular disease.[35,36] We found higher levels of YKL-40 in individuals with more severe arterial sclerosis, but these associations were no longer significant after multivariable adjustment. Although a prior study identified an association between higher levels of YKL-40 and mortality in hemodialysis patients,[37] this is the first study, to our knowledge, to demonstrate that higher plasma YKL-40 levels were independently associated with kidney disease progression and mortality in individuals with biopsy-confirmed kidney diseases.

MCP-1, also known as CCL2, is expressed by endothelial cells, macrophages, and fibroblasts and functions as a chemoattractant protein in response to tissue injury.[38,39] Although much of the literature focuses on urinary MCP-1,[39,40] less is known about plasma MCP-1 in the context of kidney disease.[41] We identified significant associations of plasma MCP-1 with acute tubular injury and mesangial expansion. Our finding of an inverse relationship between plasma MCP-1 and acute tubular injury is inconsistent with prior animal and human studies that identified increased expression of MCP-1 in kidney tissue and higher levels of urinary MCP-1 with acute and chronic tubulointerstitial lesions.[42–44] Additional studies in AKI cohorts are warranted to confirm or refute our finding. We also found that plasma MCP-1 was associated with kidney disease progression, ESKD, and death, which has not been reported previously to our knowledge.

suPAR is a marker of inflammation that is primarily expressed by endothelial and immune cells.[45] suPAR has been suggested as a potential cause of focal segmental glomerulosclerosis through activation of podocyte β(3) integrin.[46] While we did not identify significant associations of suPAR with segmental sclerosis, we found that higher levels of suPAR were associated with the presence of glomerular inflammation. Although suPAR levels have been associated with AKI,[47] incident CKD,[10] and kidney disease progression,[13] the associations between suPAR and adverse clinical outcomes were heavily confounded by eGFR and not significant after further adjustment in the current study.

Our cross-sectional findings demonstrated that biomarkers of inflammation track with a number of histopathologic lesions. The reasons for these associations cannot be inferred from this study and may relate to other unmeasured confounding factors. Although the overlap of biomarker levels across histopathologic severity scores was substantial and do not permit accurate noninvasive estimates of specific lesions, multiplexed panels of a comprehensive set of inflammation biomarkers may enhance noninvasive histopathology assessment and represents an important area of investigation. The biomarkers we studied showed associations with future kidney disease progression and death, supporting their potential role as tools to identify high-risk individuals with kidney disease for enrollment in clinical trials.[8,11,48,49] Biomarkers of inflammation could serve as a means of tracking response to therapy,[50,51] particularly for therapies with anti-inflammatory effects[52–56] that are increasingly being developed for a

broad range of kidney disease, including AKI and diabetic kidney disease.

Strengths of our study include the diverse range of kidney diseases, adjudicated histopathologic scores on lesion severity, and prospective design with clinically important outcomes including ESKD and death. Our study has several limitations that warrant consideration as well. We included individuals who underwent clinically indicated native kidney biopsies, which do not represent the majority of cases of CKD and AKI, with a relative over-representation of glomerular diseases. Given the heterogeneity of the cohort, the number of individuals within each clinicopathologic diagnostic category was small, particularly for rare diseases. We did not have data on cause of death, which limited our ability to determine if higher levels of plasma biomarkers of inflammation are associated with specific causes of death. We did not account for therapy administered after the kidney biopsy, which could alter levels of biomarkers or an individual's risk of the proposed outcomes, particularly in individuals requiring immunosuppressive therapy. Although we adjusted for a number of clinical predictors, there may be residual confounding from unmeasured confounders.

In conclusion, we found that plasma biomarkers of inflammation are associated with different patterns of histopathologic injury and are associated with adverse clinical outcomes across a variety of kidney disease etiologies. Our findings suggest that inflammation, a key pathologic driver of a number of kidney diseases, could be assessed noninvasively for estimation of risk, selecting patients for clinical trials, and tracking response to therapy. Future biomarker research in larger prospective cohorts and in randomized controlled trials are needed to identify the optimal biomarkers to improve clinical care and drug development in nephrology.

DISCLOSURE

AS reports personal fees from Horizon Pharma, PLC, AstraZeneca, and CVS Caremark. JVB is cofounder and holds equity in Goldfinch Bio, is coinventor on KIM-1 patents assigned to Partners Healthcare, received grant funding from Boehringer Ingelheim, and has received consulting income related to biomarkers from Biomarin, Aldeyra, Angion, Cadent, PTC, Praxis, Seattle Genomics, and Sarepta. SSW reports personal fees from Public Health Advocacy Institute, CVS, Roth Capital Partners, Kantum Pharma, Mallinckrodt, Wolters Kluewer, GE Health Care, GSK, Mass Medical International, Barron and Budd (vs. Fresenius), JNJ, Venbio, Strataca, Takeda, Cerus, Pfizer, Bunch and James, Harvard Clinical Research Institute (aka Baim), and grants and personal fees from Allena Pharmaceuticals. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

AS, IMS, and SSW were responsible for the concept and design of the study. AS, RP, and SSW adjudicated clinicopathologic diagnoses. IES and HGR were responsible for the adjudication of histopathology. VS and JVB measured the plasma biomarkers. AS, IMS, and SSW were responsible for the statistical analysis. All authors interpreted the data. AS, IMS, and SSW drafted the manuscript. All authors contributed to critical revisions of the manuscript for important intellectual content.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Table S1. Histopathologic scoring system for light microscopy.

Table S2. Absolute plasma biomarker concentrations by primary clinicopathologic diagnosis.

Table S3. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with adverse clinical outcomes by primary clinicopathologic diagnosis.

Table S4. Competing risk analyses for the associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with ESKD.
Table S5. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with adverse clinical outcomes after adjustment for immunosuppressive medications .
Figure S1. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with tubulointerstitial lesions.
Figure S2. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with glomerular lesions.
Figure S3. Associations of sTNFR-1, sTNFR-2, YKL-40, MCP-1, and suPAR with microvascular lesions.

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