Associations of Urine Biomarkers with Kidney Function Decline in HIV-Infected and Uninfected Men

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Keywords

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Abstract

Background: HIV-infected (HIV+) persons are at increased risk of chronic kidney disease, but serum creatinine does not detect early losses in kidney function. We hypothesized that urine biomarkers of kidney damage would be associated with subsequent changes in kidney function in a contemporary cohort of HIV+ and HIV-uninfected (HIV-) men. **Methods:** In the Multicenter AIDS Cohort Study, we measured baseline urine concentrations of 5 biomarkers from 2009 to 2011 in 860 HIV+ and 337 HIV- men: albumin, alpha-1-microglobulin ($\alpha 1m$), interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), and procollagen type III N-terminal propep-

tide (PIINP). We evaluated associations of urine biomarker concentrations with annual changes in estimated glomerular filtration rate (eGFR) using multivariable linear mixed models adjusted for demographics, traditional kidney disease risk factors, HIV-related risk factors, and baseline eGFR.

Results: Over a median follow-up of 4.8 years, the average annual eGFR decline was 1.42 mL/min/1.73 m²/year in HIV+ men and 1.22 mL/min/1.73 m²/year in HIV- men. Among HIV+ men, the highest vs. lowest tertiles of albumin (–1.78 mL/min/1.73 m²/year, 95% CI –3.47 to –0.09) and $\alpha 1m$ (–2.43 mL/min/1.73 m²/year, 95% CI –4.14 to –0.73) were each associated with faster annual eGFR declines after multivariable adjustment. Among HIV- men, the highest vs. lowest tertile of $\alpha 1m$ (–2.49 mL/min/1.73 m²/year, 95% CI –4.48 to –0.50) was independently associated with faster annual eGFR decline. Urine IL-18, KIM-1, and PIINP showed no independent associations with eGFR decline, regardless of HIV

serostatus. **Conclusions:** Among HIV+ men, higher urine albumin and $\alpha 1m$ are associated with subsequent declines in kidney function, independent of eGFR. © 2019 S. Karger AG, Basel

Introduction

In the era of combination antiretroviral therapy, HIV infection has become a chronic condition in which age-related comorbidities have become more common and may occur at an earlier age [1, 2]. Compared to uninfected individuals, HIV-infected individuals are at higher risk of developing proteinuria, chronic kidney disease (CKD), and end-stage kidney disease [3–5]. In addition, HIV-infected individuals are at increased risk of cardiovascular disease, heart failure, and mortality, which are all endpoints strongly associated with CKD [6].

Clinical practice guidelines by the HIV Medicine Association of the Infectious Diseases Society of America recommend monitoring for kidney disease with serum creatinine and urine albumin, but both tests have limitations [7]. Serum creatinine, which is used to calculate an estimated glomerular filtration rate (eGFR), lacks sensitivity for detecting early reductions in kidney function in HIV-infected individuals [4, 8]. In addition, substantial kidney injury can occur prior to a measurable reduction in kidney function. Urine albumin is the most common clinically used kidney injury marker and reflects endothelial damage to the glomerulus. However, kidney tubular epithelial cells are a long-term reservoir site for HIV infection, and kidney biopsy studies have shown that tubular injury and interstitial fibrosis are strong predictors of CKD progression in HIV-infected and uninfected populations [9–14].

Urine alpha-1-microglobulin ($\alpha 1m$), a marker of proximal tubular dysfunction, interleukin-18 (IL-18), kidney injury molecule-1 (KIM-1), markers of proximal tubular injury, and procollagen type III N-terminal propeptide (PIIINP), a marker of renal interstitial fibrosis, are promising urine biomarkers of kidney tubule health. We previously found that HIV-infected women have significantly higher urine $\alpha 1m$, IL-18, KIM-1, and PIIINP levels compared to uninfected women [15–17]. Furthermore, we observed among HIV-infected women that higher urine albumin, $\alpha 1m$, IL-18, and KIM-1 levels were each associated with faster eGFR decline over 8 years of follow-up [18, 19]. Whether or not levels of these urine biomarkers are associated with kidney function decline in a more contemporary cohort, including HIV-infected men, are unclear.

In this longitudinal study of HIV-infected and uninfected men, we evaluated associations of levels of urine biomarkers of glomerular injury (albuminuria), tubular dysfunction ($\alpha 1m$), tubular injury (IL-18 and KIM-1), and interstitial fibrosis (PIIINP) with subsequent changes in kidney function. We hypothesized that more extensive kidney damage, as measured by higher urine biomarker concentrations, would be independently associated with faster eGFR decline.

Materials and Methods

Study Design

The Multicenter AIDS Cohort Study (MACS) is one of the largest cohort studies of HIV-infected individuals in the United States. The design and methods have been described previously [20]. In brief, 6,972 men who have sex with men with HIV infection or at high risk for HIV infection volunteered for enrollment in 1984–1985, 1987–1990, and 2001–2003 from 4 sites: Baltimore, Chicago, Los Angeles, and Pittsburgh. MACS participants attend semiannual visits for follow-up evaluations that include a standardized questionnaire, a physical examination, and collection of laboratory specimens.

The MACS Kidney Study was designed as a nested cohort to investigate the development and progression of kidney disease among HIV-infected and uninfected men. This longitudinal study included all 883 HIV-infected men with urine samples collected between October 1, 2009, and September 30, 2011, and a random sample of 350 uninfected men with available urine specimens from this time period. For the present study, we excluded 36 individuals who did not have a follow-up serum creatinine measurement, leaving a final analytic sample of 1197 (860 HIV-infected and 337 HIV-uninfected).

Predictors

We measured urine concentrations of the following biomarkers: albumin, $\alpha 1m$, IL-18, KIM-1, and PIIINP. All biomarkers were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory. Details regarding the selected commercial assays and inter-assay coefficients of variation are shown in online supplemental Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000502898). Urine specimens were refrigerated immediately after collection and centrifuged at 5,000 g to remove cellular debris. The supernatant was aliquoted into 1-mL vials and then stored at -80°C until biomarker measurement without prior freeze-thaw. Laboratory personnel performing the biomarker assays were blinded to participants' clinical information.

Outcomes

The primary outcome was annual mean change in eGFR, expressed in $\text{mL}/\text{min}/1.73\text{ m}^2$ per year. We calculated eGFR using the 2009 CKD Epidemiology Collaboration creatinine equation [21]. Serum creatinine was measured semi-annually at the clinical labs of each MACS site using the modified Jaffe method, traceable to isotope dilution mass spectrometry. Our secondary outcomes included: (1) incident CKD, defined as an eGFR $<60\text{ mL}/\text{min}/1.73\text{ m}^2$ on 2 consecutive visits separated by at least 3 months with at least

1 mL/min/1.73 m² per year decline among persons with baseline eGFR >60 mL/min/1.73 m² and (2) annual eGFR decline ≥5% of the baseline eGFR.

Covariates

Demographics, kidney disease risk factors, and HIV-related parameters were assessed at the baseline visit, except where noted below, and included in all multivariable models. Demographic variables included age and race/ethnicity. Metabolic health variables included diabetes mellitus (defined as fasting glucose ≥126 mg/dL, hemoglobin A_{1c} ≥6.5%, or self-reported history of diabetes and diabetes medication use) and body mass index. Cardiovascular health variables included systolic and diastolic blood pressure, hypertension (defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or self-reported history of hypertension and antihypertensive medication use), self-reported cardiovascular disease, and levels of low- and high-density lipoprotein cholesterol. Behavioral health risk factors included cigarette smoking status (current, past, or never), current heroin use, hepatitis C virus (HCV) infection (confirmed by detectable plasma HCV RNA following a positive HCV antibody result). HIV infection characteristics included CD4 T-lymphocyte count/mm³ (nadir, baseline, and time-updated), plasma HIV-1 RNA level (peak, baseline, and time-updated), history of clinical AIDS diagnosis, current TDF use, and other highly active antiretroviral therapy (HAART) use.

Statistical Analysis

The percentage of missing observations for each baseline covariate ranged from <1 to 28%. While all participants had eGFR measured at baseline, the percentage of missing eGFR increased over time, from 11% at year 1 to 53% at year 5. Because 24% of urine α1m values were below the detectable limit of the assay (0.53 mg/dL), we used a left-censored Tobit regression model to impute undetectable α1m values. For all other variables, we used pattern mixture models with the neighboring-case missing values method to impute missing data, using 100 imputations to ensure high relative efficiency and reliable estimation [22–24]. This method is well-suited to handle longitudinal data with dropout patterns that may not represent data that are missing at random. Imputation models included eGFR as well as all exposures and covariates listed above.

We compared demographic and baseline characteristics by HIV serostatus using χ² and Mann-Whitney U tests for categorical and continuous variables, respectively. We analyzed levels of urine biomarkers as continuous variables (log-transformed to achieve normality of right-skewness) and as categorical variables divided into tertiles. We also analyzed urine albumin as a dichotomous variable using the standard clinical cut-point (albumin-to-creatinine ratio [ACR] ≤30 vs. >30 mg/g). We modeled the associations of each urine biomarker with annual mean change in eGFR using linear mixed effect models. All models were constructed separately by HIV serostatus. We accounted for serial within-subject correlations using a first-order autoregressive moving average covariance structure and controlled for baseline eGFR and follow-up time so that coefficients would be interpretable as annual changes in eGFR attributable to each covariate [25]. We estimated adjusted risk ratios for each two-fold higher level of each baseline urine biomarker with incident CKD and annual eGFR decline ≥5% using Poisson regression with a robust variance estimator [26]. We adjusted for urine creatinine in all models to correct for tonicity.

To determine whether levels of individual urine biomarkers were independently associated with study outcomes, multivariable models were adjusted for demographics, traditional kidney disease risk factors, and HIV-related risk factors (as listed above in the Covariates section). We also modeled all biomarkers in combination and evaluated all pairwise and 3-way biomarker combinations in order to identify markers that were simultaneously statistically significant. We performed interaction testing to evaluate whether biomarker level associations with change in eGFR differed by TDF use. Because HIV infection may have direct effects on kidney tubular health and with eGFR, we also performed interaction testing to evaluate whether biomarker level associations with change in eGFR differed by HIV RNA levels. Based on observed findings, we additionally performed interaction testing to evaluate whether urine albumin associations varied by levels of α1m.

All analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, USA).

Results

At baseline, the median age was 52 years among the 860 HIV-infected men and 54 years among the 337 uninfected men (Table 1). Compared to uninfected men, HIV-infected men had a higher baseline prevalence of eGFR <60 mL/min/1.73 m² (9 vs. 4%, *p* < 0.001) and ACR >30 mg/g (17 vs. 8%, *p* < 0.001). Among the HIV-infected men, 85% were using HAART and 66% were using TDF; the median CD4 lymphocyte count was 576 cells/mm³, and 78% had undetectable HIV RNA levels. Over a median follow-up period of 4.8 years (interquartile range 4.4–5.3), the average rate of annual eGFR decline was 1.42 mL/min/1.73 m² per year (95% CI 1.31–1.53) in HIV-infected men and 1.22 mL/min/1.73 m² per year (95% CI 0.74–1.70) in uninfected men.

In unadjusted analyses among the HIV-infected men, higher baseline urine albumin and α1m concentrations were associated with faster annual eGFR declines (Table 2). There was minimal attenuation of these associations after multivariable adjustment for traditional and HIV-related kidney disease risk factors. When biomarker levels were analyzed as continuous variables, each two-fold higher level of urine albumin at baseline was associated with a 0.58 mL/min/1.73 m² per year faster rate of eGFR decline in multivariable-adjusted models. Compared to participants with baseline ACR ≤30 mg/g, those with ACR >30 mg/g had faster eGFR declines. In tertile analyses, the highest vs. lowest tertiles of baseline urine albumin and α1m were each associated with faster eGFR declines in multivariable-adjusted models. By contrast, IL-18, KIM-1, and PIIINP were not significantly associated with eGFR decline in unadjusted or adjusted analyses. When we modeled levels of all 5 urine biomarkers simultaneously,

Table 1. Baseline characteristics by HIV status

	HIV+ (n = 860)	HIV- (n = 337)	p value
Age, years	52 (46–58)	54 (49–62)	<0.001
Race			
African American	264 (31)	94 (28)	0.15
Caucasian	526 (61)	224 (66)	
Other	70 (8)	19 (6)	
Diabetes mellitus	107 (16)	43 (15)	0.75
BMI, kg/m ²	26 (24–30)	27 (24–32)	0.015
Hypertension	360 (46)	150 (47)	0.75
History of cardiovascular disease	56 (7)	17 (5)	0.34
Systolic blood pressure, mm Hg	126 (116–135)	128 (117–136)	0.14
Diastolic blood pressure, mm Hg	78 (71–84)	78 (71–84)	0.64
LDL, mg/dL	108 (88–132)	115 (92–137)	0.011
HDL, mg/dL	45 (38–55)	50 (41–60)	<0.001
Smoking			
Current	250 (30)	76 (23)	0.05
Past	378 (45)	170 (52)	
Never	210 (25)	82 (25)	
HCV infection	83 (10)	30 (9)	0.69
Current heroin use	11 (1)	4 (1)	0.90
eGFR, mL/min/1.73 m ²	91 (76–103)	89 (78–100)	0.71
eGFR <60 mL/min/1.73 m ²	74 (9)	12 (4)	0.002
ACR >30 mg/g	142 (17)	28 (8)	<0.001
HAART use	723 (85)		
TDF use	555 (66)		
NRTI use	728 (85)		
NNRTI use	401 (47)		
PI use	383 (45)		
Current CD4, cells/mm ³	576 (411–740)		
Nadir CD4, cells/mm ³	287 (177–408)		
History of AIDS	123 (14)		
Current HIV RNA >40 copies/mL	188 (22)		
Peak HIV RNA >100,000 copies/mL	331 (40)		

Data are presented as median (IQR) or numbers (%). *p* values are from χ^2 tests for categorical variables and Mann-Whitney U tests for continuous variables.

AIDS, acquired immunodeficiency syndrome; eGFR, estimated glomerular filtration rate; HAART, highly active antiretroviral therapy; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; BMI, body mass index; HCV, hepatitis C virus; ACR, albumin-to-creatinine ratio.

higher urine albumin remained associated with faster eGFR decline (-0.61 mL/min/1.73 m² annually per two-fold higher urine albumin, 95% CI -1.13 to -0.09), whereas the associations of levels of other markers, including $\alpha 1$ m, with eGFR were attenuated and had weaker associations that did not reach statistical significance.

We next modeled associations of levels of urine biomarkers with annual changes in eGFR among HIV-uninfected men. In unadjusted analyses, higher baseline urine albumin and $\alpha 1$ m concentrations were each associated with faster annual eGFR declines (Table 3). After multi-

variable adjustment, ACR >30 vs. ≤ 30 mg/g and the highest vs. lowest tertile of $\alpha 1$ m remained significantly associated with faster eGFR declines. When modeled as a continuous variable, each two-fold higher $\alpha 1$ m at baseline also remained independently associated with faster eGFR declines. In models including levels of all 5 urine biomarkers simultaneously, only $\alpha 1$ m remained associated with faster eGFR decline (-0.86 , 95% CI -1.58 to -0.15 mL/min/1.73 m² annually per two-fold higher $\alpha 1$ m).

To explore effect modification between kidney tubule dysfunction and glomerular injury, we evaluated whether

Table 2. Association of urine biomarkers with annual eGFR change in HIV+ MACS participants

Biomarker	Annual eGFR change, mL/min/1.73 m ² /year (95% CI)	Unadjusted difference, mL/min/1.73 m ² /year (95% CI)	Adjusted difference*, mL/min/1.73 m ² /year (95% CI)
<i>Albumin</i>			
Continuous**		−0.63 (−1.12 to −0.15)	−0.58 (−1.03 to −0.12)
T1: <0.6 mg/dL	−0.64 (−1.57 to 0.29)	Reference	Reference
T2: 0.6–1.6 mg/dL	−0.73 (−1.57 to 0.11)	−0.09 (−1.49 to 1.31)	−0.17 (−1.54 to 1.21)
T3: >1.6 mg/dL	−2.87 (−4.00 to −1.73)	−2.23 (−3.97 to −0.48)	−1.78 (−3.47 to −0.09)
ACR ≤30 mg/g	−0.88 (−1.32 to −0.44)	Reference	Reference
ACR >30 mg/g	−4.02 (−5.83 to −2.21)	−3.14 (−5.20 to −1.08)	−3.00 (−4.88 to −1.11)
<i>α1m</i>			
Continuous		−0.47 (−0.91 to −0.02)	−0.40 (−0.87 to 0.06)
T1: <0.9 mg/dL	−0.32 (−1.33 to 0.69)	Reference	Reference
T2: 0.9–2.4 mg/dL	−0.86 (−1.73 to 0.01)	−0.54 (−2.02 to 0.95)	−0.40 (−1.87 to 1.07)
T3: >2.4 mg/dL	−3.10 (−4.04 to −2.16)	−2.78 (−4.40 to −1.15)	−2.43 (−4.14 to −0.73)
<i>IL-18</i>			
Continuous		0.07 (−0.26 to 0.41)	0.19 (−0.15 to 0.52)
T1: <22.9 pg/mL	−1.18 (−2.11 to −0.26)	Reference	Reference
T2: 22.9–51.2 pg/mL	−1.45 (−2.31 to −0.58)	−0.26 (−1.68 to 1.16)	0.10 (−1.29 to 1.49)
T3: >51.2 pg/mL	−1.64 (−2.72 to −0.57)	−0.46 (−2.14 to 1.22)	0.31 (−1.35 to 1.97)
<i>KIM-1</i>			
Continuous		0.03 (−0.43 to 0.49)	0.13 (−0.33 to 0.59)
T1: <0.49 ng/mL	−1.20 (−2.18 to −0.21)	Reference	Reference
T2: 0.49–1.02 ng/mL	−2.03 (−2.83 to −1.24)	−0.84 (−2.25 to 0.58)	−0.35 (−1.74 to 1.04)
T3: >1.02 ng/mL	−1.04 (−2.08 to −0.00)	0.15 (−1.54 to 1.84)	0.38 (−1.28 to 2.03)
<i>PIIINP</i>			
Continuous		−0.45 (−1.09 to 0.18)	−0.49 (−1.10 to 0.12)
T1: <4.70 ng/mL	−0.71 (−1.77 to 0.35)	Reference	Reference
T2: 4.70–10.09 ng/mL	−1.60 (−2.46 to −0.73)	−0.88 (−2.41 to 0.64)	−0.79 (−2.28 to 0.69)
T3: >10.09 ng/mL	−1.97 (−2.96 to −0.97)	−1.25 (−3.01 to 0.50)	−1.43 (−3.15 to 0.30)

Biomarkers modeled individually, not jointly.

* Adjusted for age, race/ethnicity, urine creatinine, baseline eGFR, diabetes mellitus, systolic and diastolic blood pressure, hypertension, cardiovascular disease, low- and high-density lipoprotein cholesterol levels, triglyceride level, body mass index, waist circumference, cigarette smoking status, serum albumin, current heroin use, HCV infection, current CD4 lymphocyte count, plasma HIV-1 RNA level, history of clinical AIDS diagnosis, TDF use, and highly active antiretroviral therapy use.

** Continuous predictors are modeled per two-fold higher level of biomarker at baseline.

ACR, albumin-to-creatinine ratio; α1m, alpha-1-microglobulin; eGFR, estimated glomerular filtration rate; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; PIIINP, procollagen type III N-terminal propeptide; MACS, Multicenter AIDS Cohort Study; HCV, hepatitis C virus.

urine levels of α1m modified the association of urine albumin with changes in eGFR. Among men who had simultaneously elevated levels of urine albumin and α1m (Fig. 1, online suppl. Table 2), rates of eGFR decline were substantially faster compared to those with lower levels (*p* value for interaction: 0.09 in HIV-infected and 0.03 in HIV-uninfected). Among HIV-infected men, the adjusted difference in annual eGFR decline was −3.02 (95% CI −4.81 to −1.23) mL/min/1.73 m² per year faster among those with levels in the highest tertile of both markers (*n* =

159) relative to participants with lower levels of both markers (*n* = 446). In HIV-uninfected men, the adjusted difference in annual eGFR decline was −2.09 (95% CI −4.38 to 0.20) mL/min/1.73 m² per year faster among those with levels in the highest tertiles of both markers (*n* = 61) relative to participants with lower levels of both markers (*n* = 174).

Because both tenofovir exposure and HIV viremia can impact kidney tubular health, we then evaluated whether TDF use and detectable HIV RNA levels at baseline mod-

Table 3. Association of urine biomarkers with annual eGFR change in HIV– MACS participants

Biomarker	Annual eGFR change, mL/min/1.73 m ² /year (95% CI)	Unadjusted difference, mL/min/1.73 m ² /year (95% CI)	Adjusted difference*, mL/min/1.73 m ² /year (95% CI)
<i>Albumin</i>			
Continuous**		–0.47 (–0.90 to –0.03)	–0.22 (–0.61 to 0.18)
T1: <0.4 mg/dL	–0.24 (–1.61 to 1.13)	Reference	Reference
T2: 0.4–0.9 mg/dL	–1.07 (–2.16 to 0.01)	–0.83 (–2.80 to 1.13)	–0.22 (–2.14 to 1.71)
T3: >0.9 mg/dL	–2.35 (–3.78 to –0.92)	–2.11 (–4.50 to 0.28)	–0.85 (–3.12 to 1.42)
ACR ≤ 30 mg/g	–0.82 (–1.34 to –0.30)	Reference	Reference
ACR > 30 mg/g	–5.54 (–8.67 to –2.40)	–4.72 (–8.03 to –1.40)	–3.13 (–6.19 to –0.08)
<i>α1m</i>			
Continuous		–1.15 (–1.73 to –0.56)	–0.74 (–1.34 to –0.14)
T1: <0.5 mg/dL	0.69 (–0.53 to 1.92)	Reference	Reference
T2: 0.5–0.9 mg/dL	–1.17 (–2.40 to 0.05)	–1.87 (–3.85 to 0.12)	–0.76 (–2.79 to 1.26)
T3: > 0.9 mg/dL	–3.17 (–4.40 to –1.95)	–3.87 (–5.86 to –1.88)	–2.49 (–4.48 to –0.50)
<i>IL-18</i>			
Continuous		–0.20 (–0.59 to 0.18)	–0.04 (–0.42 to 0.34)
T1: <15.4 pg/mL	–0.40 (–1.75 to 0.96)	Reference	Reference
T2: 15.4–33.6 pg/mL	–1.57 (–2.66 to –0.47)	–1.17 (–3.11 to 0.76)	–0.52 (–2.39 to 1.35)
T3: > 33.6 pg/mL	–1.69 (–2.99 to –0.38)	–1.29 (–3.52 to 0.94)	–0.04 (–2.28 to 2.21)
<i>KIM-1</i>			
Continuous		–0.19 (–0.75 to 0.37)	0.02 (–0.55 to 0.59)
T1: <0.32 ng/mL	–0.87 (–2.20 to 0.46)	Reference	Reference
T2: 0.32–0.85 ng/mL	–1.10 (–2.17 to –0.03)	–0.23 (–2.16 to 1.69)	–0.31 (–2.08 to 1.46)
T3: >0.85 ng/mL	–1.68 (–2.98 to –0.38)	–0.81 (–2.99 to 1.37)	–0.01 (–2.13 to 2.11)
<i>PIIINP</i>			
Continuous		0.18 (–0.60 to 0.96)	0.08 (–0.67 to 0.84)
T1: <3.75 ng/mL	–0.98 (–2.35 to 0.39)	Reference	Reference
T2: 3.75–7.99 ng/mL	–1.38 (–2.49 to –0.27)	–0.40 (–2.38 to 1.59)	–0.96 (–2.91 to 0.98)
T3: >7.99 ng/mL	–1.29 (–2.54 to –0.04)	–0.31 (–2.48 to 1.86)	–0.72 (–2.85 to 1.42)

Biomarkers modeled individually, not jointly.

* Models adjust for age, race/ethnicity, urine creatinine, baseline eGFR, diabetes mellitus, systolic and diastolic blood pressure, hypertension, cardiovascular disease, low- and high-density lipoprotein cholesterol levels, triglyceride level, body mass index, waist circumference, cigarette smoking status, serum albumin, current heroin use, HCV infection.

** Continuous predictors are modeled per two-fold higher level of biomarker at baseline.

ACR, albumin-to-creatinine ratio; α1m, alpha-1-microglobulin; eGFR, estimated glomerular filtration rate; IL-18, interleukin-18; KIM-1, kidney injury molecule-1; PIIINP, procollagen type III N-terminal propeptide; HCV, hepatitis C virus.

ified the associations of the urine biomarkers with changes in eGFR in HIV-infected participants. Each two-fold higher urine albumin and IL-18 at baseline were associated with faster eGFR declines among non-TDF users compared to TDF users, whereas associations of urine α1m, KIM-1, and PIIINP with eGFR decline did not differ by TDF use (online suppl. Table 3). Higher urine biomarker levels were associated with faster eGFR declines in men with detectable HIV RNA levels compared to men with undetectable HIV RNA, although differences reached statistical significance only for IL-18 ($p = 0.023$). Each two-fold higher IL-18 at baseline was associated

with -0.39 mL/min/1.73 m² per year (95% CI -1.02 to 0.24) faster eGFR decline among men with detectable HIV RNA levels and $+0.42$ mL/min/1.73 m² per year (95% CI 0.05 – 0.79) slower eGFR decline among those with undetectable HIV RNA.

Among HIV-infected participants, 98 (12%) developed incident CKD and 155 (18%) had an annual eGFR decline $\geq 5\%$ over the approximately 5 years of follow-up. In unadjusted analyses, higher baseline urine albumin and α1m concentrations were individually associated with risks of both incident CKD and $\geq 5\%$ annual eGFR decline (Table 4). However, in multivariable models that

adjusted for baseline eGFR, the associations were attenuated and none remained statistically significant.

Among HIV-uninfected participants, 23 (6.8%) developed incident CKD and 22 (6.8%) had an annual eGFR decline $\geq 5\%$ during follow-up. In unadjusted analyses, only higher levels of urine albumin were associated with risk of incident CKD (risk ratio per two-fold higher urine albumin: 1.34, 95% CI 1.00–1.79), but this association was substantially attenuated and not statistically significant after multivariable adjustment including adjustment for baseline eGFR. None of the urine biomarkers were significantly associated with risk of $\geq 5\%$ annual eGFR decline among HIV-uninfected participants; unadjusted point estimates were similar to those observed in the HIV-infected participants.

Discussion

As the burden of chronic, noninfectious comorbidities accumulates in an aging HIV population, early and accurate identification of kidney disease has become increasingly important. In this large ambulatory cohort, higher urine albumin and $\alpha 1m$ concentrations at baseline were individually associated with faster kidney function decline in HIV-infected and uninfected men, independent of baseline eGFR and kidney disease risk factors. When we adjusted for all biomarkers simultaneously, associations with kidney function decline remained significant for urine albumin in HIV-infected individuals and for urine $\alpha 1m$ in HIV-uninfected individuals. Participants in the highest tertiles for both urine albumin and $\alpha 1m$ had substantially faster kidney function decline, as compared with participants in the lowest tertiles for each marker. By contrast, urine IL-18, KIM-1, and PIIINP did not show statistically significant associations with eGFR changes in either HIV-infected or uninfected participants.

The associations of higher urine albumin and $\alpha 1m$ levels with kidney function decline among HIV-infected men are consistent with previous studies [18, 19, 27]. By contrast, the null associations with incident CKD and rapid kidney function decline may have been due to loss of information from categorizing eGFR, reduced power, or the relatively preserved kidney function of MACS participants at baseline. In the Women's Interagency HIV Study (WIHS), a large, multicenter cohort of HIV-infected women, higher urine albumin levels were associated with faster eGFR decline, and higher urine $\alpha 1m$ levels were associated with risk of incident CKD, independent

Fig. 1. Annual change in eGFR across tertiles of urine albumin and $\alpha 1m$ in HIV-infected and uninfected MACS participants. Bars represent unadjusted estimated annual change in eGFR in mL/min/1.73 m², with 95% CIs displayed. Estimates are compared across the highest versus lower tertiles of urine albumin and $\alpha 1m$ and are derived from linear mixed effect models. $\alpha 1m$, alpha-1-microglobulin; eGFR, estimated glomerular filtration rate; T3, highest tertile; <T3, first or second tertile; Ualb, urine albumin.

of albuminuria and baseline eGFR [18, 19]. Urine albumin primarily reflects glomerular injury and is an established risk factor for CKD progression [28]. Our results support current HIV guidelines that recommend monitoring for kidney damage with albuminuria [7] and suggest further investigation into potential use of urine $\alpha 1m$ for risk stratification in future studies. In contrast to albumin, $\alpha 1m$ is a freely filtered, low-molecular-weight protein that is fully reabsorbed by the proximal tubule; elevated levels in urine indicate dysfunction of the proximal tubule [29]. Our data suggest that albuminuria and urine $\alpha 1m$ may provide complementary information about subsequent risk of declining kidney function, but further work is needed to validate these findings.

Similar to several previous studies in the general population, our study found no significant associations of urine IL-18, KIM-1, or PIIINP levels with eGFR decline [30–33]. IL-18 is a proinflammatory cytokine released by proximal tubular cells in response to injury or inflammation, while KIM-1 is upregulated and overexpressed by dedifferentiated proximal tubular cells after injury [34, 35]. Urine PIIINP reflects the presence of type III collagen deposits in the renal interstitium during fibrosis, and higher urine PIIINP concentrations correlate with increased severity of fibrosis on kidney biopsy [36, 37]. We hypothesized that higher urine IL-18, KIM-1, and PIIINP

Table 4. Association of urine biomarkers with rapid eGFR decline and incident CKD in HIV+ MACS participants

	Unadjusted RR (95% CI)	Multivariable- adjusted RR (95% CI)*	Multivariable-adjusted + eGFR RR (95% CI)*
5% annual eGFR decline (<i>n</i> = 155/860)			
Albumin	1.16 (1.03–1.30)	1.12 (1.00–1.27)	1.11 (0.98–1.25)
α1m	1.13 (1.01–1.28)	1.12 (0.99–1.26)	1.09 (0.97–1.23)
IL-18	1.17 (0.96–1.43)	1.10 (0.92–1.33)	1.10 (0.92–1.32)
KIM-1	1.03 (0.86–1.23)	1.00 (0.84–1.19)	1.00 (0.85–1.19)
PIIINP	1.05 (0.89–1.25)	1.03 (0.87–1.21)	1.00 (0.85–1.17)
Incident CKD (<i>n</i> = 98/786)			
Albumin	1.18 (1.04–1.34)	1.16 (0.99–1.35)	1.08 (0.96–1.22)
α1m	1.23 (1.08–1.40)	1.17 (1.01–1.35)	1.09 (0.93–1.27)
IL-18	1.11 (0.91–1.35)	1.06 (0.87–1.30)	1.11 (0.90–1.37)
KIM-1	1.11 (0.92–1.35)	1.00 (0.81–1.23)	1.03 (0.85–1.26)
PIIINP	1.17 (0.96–1.43)	1.10 (0.90–1.35)	1.04 (0.88–1.24)

Biomarkers are modeled per two-fold higher level at baseline and are modeled individually, not jointly.

*Models adjust for age, race/ethnicity, urine creatinine, diabetes mellitus, systolic and diastolic blood pressure, hypertension, cardiovascular disease, low- and high-density lipoprotein cholesterol levels, triglyceride level, body mass index, waist circumference, cigarette smoking status, serum albumin, current heroin use, HCV infection, current CD4 lymphocyte count, plasma HIV-1 RNA level, history of clinical AIDS diagnosis, TDF use, and highly active antiretroviral therapy use.

α1m, alpha-1-microglobulin; eGFR, estimated glomerular filtration rate; IL-18, interleukin-18; RR, risk ratio; KIM-1, kidney injury molecule-1; PIIINP, procollagen type III N-terminal propeptide; HCV, hepatitis C virus; CKD, chronic kidney disease; MACS, Multi-center AIDS Cohort Study.

levels would be associated with kidney function decline because tubulointerstitial injury and fibrosis are strong predictors of CKD progression [9, 10, 13, 14]. Contrary to our findings, higher urine IL-18 and KIM-1 levels among HIV-infected and uninfected participants of the WIHS were independently associated with larger eGFR declines [18]. Additionally, a cohort study of middle-aged, ethnically diverse men and women also showed higher levels of KIM-1 were associated with an increased risk of incident CKD [38]. Among ambulatory older adults, higher urine PIIINP levels have been associated with the risk of CKD progression [39]. The discrepancy between our findings and previous work in HIV-infected individuals could be explained by differences in the study population. By comparison to the WIHS, our study consisted of men who were older, had higher prevalence of diabetes mellitus and hypertension, lower prevalence of smoking and hepatitis C, and higher prevalence of HIV viral suppression. Additional studies will be required to determine the optimal subset of urinary biomarkers for prognostication and monitoring of kidney health among HIV-infected individuals.

Strengths of our study include the use of a multicenter, diverse, contemporary cohort representative of HIV-infected men in the United States with a follow-up period

of approximately 5 years, assessment of levels of multiple biomarkers of kidney damage, and inclusion of a similar group of HIV-uninfected men. Our study also has several limitations. First, we were unable to estimate GFR using serum cystatin C levels, which may be a more sensitive indicator of early losses in kidney function [4]. Second, urine biomarkers were measured once at baseline, so we were unable to evaluate changes in biomarker levels over time. Repeated biomarkers may produce stronger associations with kidney-related outcomes that are several years after the exposure. Third, our observational study may not have accounted for all potential confounders given the numerous risk factors for kidney disease, although these models were comprehensively constructed with numerous potential confounders. Third, our results are not generalizable to HIV-infected women or to HIV-infected individuals not on antiretroviral therapy.

In summary, we observed that higher levels of urine albumin and α1m, a marker of kidney tubule dysfunction, were associated with faster declines in kidney function in a cohort of HIV-infected and uninfected men. Further studies are needed to determine the utility of monitoring levels of biomarkers of kidney tubular health, in addition to eGFR and albuminuria, among persons living with HIV.

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Statement of Ethics

The Institutional Review Boards of participating institutions approved the study protocol (IRB #10-00827), and informed consent was obtained from all study participants. The University of California, San Francisco and San Francisco Veterans Affairs Medical Center committees on human research approved this study.

Disclosure Statement

None declared. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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Author Contributions

S.B.A., R.S., M.M.E., M.G.S., and V.K.J. are responsible for the research idea and study design. D.K.N. and F.J.P. are responsible for data acquisition. S.B.A., R.S., M.M.E., M.G.S., V.K.J., D.K.N., F.J.P., M.D.W., K.H., M.R.B., C.R.P., and J.H.I. are responsible for data analysis and interpretation. R.S. is responsible for statistical analysis. V.K.J. is responsible for supervision and mentorship.

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