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Urinary Markers of Kidney Injury and Kidney Function Decline in HIV-Infected Women

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Abstract

Objective—HIV-infected persons have substantially higher risk of kidney failure than persons without HIV, but serum creatinine levels are insensitive for detecting declining kidney function. We hypothesized that urine markers of kidney injury would be associated with declining kidney function among HIV-infected women.

Methods—In the Women's Interagency HIV Study (WIHS), we measured concentrations of albumin-to-creatinine ratio (ACR), interleukin-18 (IL-18), kidney injury marker-1 (KIM-1), and neutrophil gelatinase-associated lipocalin (NGAL) from stored urine among 908 HIV-infected and 289 uninfected participants. Primary analyses used cystatin C based estimated glomerular filtration rate (CKD-EPI eGFRcys) as the outcome, measured at baseline and two follow-up visits over eight years; secondary analyses used creatinine (CKD-EPI eGFRcr). Each urine biomarker was categorized into tertiles, and kidney decline was modeled with both continuous and dichotomized outcomes.

Results—Compared with the lowest tertiles, the highest tertiles of ACR (−0.15ml/min/1.73m2, p<0.0001), IL-18 (−0.09ml/min/1.73m2, p<0.0001) and KIM-1 (−0.06ml/min/1.73m2, p<0.001) were independently associated with faster eGFRcys decline after multivariate adjustment including all three biomarkers among HIV-infected women. Among these biomarkers, only IL-18 was associated with each dichotomized eGFRcys outcome: ≥3% (Relative Risk 1.40; 95%CI 1.04-1.89); ≥5% (1.88; 1.30-2.71); and ≥10% (2.16; 1.20-3.88) for the highest versus lowest tertile. In alternative models using eGFRcr, the high tertile of KIM-1 had independent associations with 5% (1.71; 1.25-2.33) and 10% (1.78; 1.07-2.96) decline, and the high IL-18 tertile with 10% decline (1.97; 1.00-3.87).

Conclusions—Among HIV-infected women in the WIHS cohort, novel urine markers of kidney injury detect risk for subsequent declines in kidney function.

Keywords

HIV; KIM-1; NGAL; IL-18; albumin-to-creatinine ratio; cystatin C; kidney injury

Introduction

Kidney disease is a major complication of HIV infection. Persons with HIV have substantially elevated risk of end-stage renal disease (ESRD) compared with HIV-uninfected individuals.[1, 2] Elevated creatinine, a standard measure of kidney function, is associated with adverse health outcomes in HIV infection, including mortality, cardiovascular disease (CVD), and heart failure.[3-6] However, creatinine is an imperfect marker of kidney function, due to bias by age, sex, race, muscle mass, and hydration status.[7, 8] In HIV-infected persons, early reductions in kidney function expressed as estimated glomerular filtration rate (eGFR) may not be detected by serum creatinine levels, but appear to be captured by cystatin C.[9, 10] In the Study of Fat Redistribution and Metabolic Change in HIV Infection, reduced kidney function (eGFR <60 mL/min/1.73m^2) was twice as prevalent when defined by cystatin C as by creatinine, and had a much stronger association with mortality risk.[11]

Although cystatin C may detect early reductions in glomerular filtration rate (GFR), substantial kidney injury may occur in HIV-infected persons prior to any discernible effect on kidney filtration. In some patients, kidney injury can be detected before GFR is decreased by measuring the urine albumin-to-creatinine ratio (ACR). One study found a 5-fold odds of albuminuria in HIV-infected individuals compared with HIV-uninfected persons, most of whom had eGFR in the normal range.[12] Both albuminuria and total urinary protein levels have been associated with mortality risk in HIV-infected persons,[5, 6] and albuminuria and

cystatin C have complementary associations with mortality.[11] However, urine albumin is a marker of glomerular injury and thus may not capture renal injury at other sites of the nephron. This is important because both HIV and its treatments may have non-glomerular effects on the kidney. Kidney biopsy studies suggest that the proximal and distal tubules are early targets of HIV.[13-16] Several tubular injury markers have been developed for early detection of acute kidney injury; among these, kidney injury marker-1 (KIM-1), interleukin-18 (IL-18), and neutrophil gelatinase-associated lipocalin (NGAL) have been extensively studied in humans. [17] To our knowledge, these markers have not been evaluated in longitudinal studies of HIV-infected persons.

We designed this study within the Women's Interagency HIV Study (WIHS) to test the hypothesis that markers of tubular injury could forecast the rate of subsequent eGFR decline among women at risk for kidney disease. Our primary hypotheses were that ambulatory women with and without HIV infection would have detectable damage to their kidney tubules, measurable by IL-18, KIM-1, and NGAL levels, and that the level of the injury markers would be associated independently with the rate of subsequent kidney function decline.

Methods

Design

The WIHS is the largest, long-term observational study of the progression of HIV in U.S. women. The WIHS study design and methods have been described previously.[18, 19] In brief, 3,766 women (2,791 HIV-infected and 975 HIV-uninfected) were enrolled in either 1994-1995 (n=2,623) or 2001-2002 (n=1,143) from 6 U.S. sites (Bronx, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, DC). HIV-infected women were recruited to be representative of HIV-infected women in each community. HIV-uninfected women were recruited from similar venues. Participants are interviewed and examined every six months. Serum specimens were stored in a −80°C freezer until biomarker measurement.

The WIHS HIV Kidney Aging study was designed as a nested cohort study to investigate the onset of kidney disease in the setting of HIV, utilizing stored urine and serum specimens. Baseline measures were collected between October 1999 and March 2000, as this was the most recent visit that had collected and stored urine in WIHS. This study included all participants with available serum specimens during this time interval. A total of 1,403 women (1032 HIV-infected and 371 HIV-uninfected) had cystatin C measured at baseline; 1,197 (908 HIV-infected and 289 HIV-uninfected) had stored urine available and at least one follow-up cystatin C measure; these participants were included in the longitudinal analyses of this manuscript. WIHS was approved by the relevant institutional review boards at all study sites. This study of kidney injury was also approved by the University of California, San Francisco, San Francisco Veterans Affairs Medical Center, and Yale committees on human research.

Predictors

Primary predictors in this study were urine ACR, IL-18, KIM-1, and NGAL. All urinary kidney injury biomarkers were measured at the Cincinnati Children's Hospital Medical Center Biomarker Laboratory. Urine albumin and creatinine were measured by immunoturbidimetry and colorimetric enzyme assay, respectively, using a Siemens Dimension Xpand plus HM clinical analyzer (Siemens, Munich, Germany). Urine IL-18 was measured using a commercially available ELISA kit (Medical & Biological Laboratories Co., Nagoya, Japan). The urine KIM-1 ELISA was constructed using commercially available reagents (R & D Systems, Inc., Minneapolis, MN).[20] Urine NGAL was assayed

using a human-specific commercially available ELISA (AntibodyShop, Grusbakken, Denmark).[21] All urine specimens were in continuous storage without prior freeze-thaw. Laboratory personnel were blinded to clinical information about the participants, including HIV status, and specimens were evaluated in random order. Coefficients of variation for the urine measures were: albumin, 5.9%; creatinine, 4.1%; IL-18, 7.2%; KIM-1, 5.2%; and NGAL, 5.4%.

Outcomes

Primary outcomes of this study were derived from cystatin C measures from the three WIHS visits, which were conducted concurrently at the UCLA Clinical Immunology Research Laboratory. Cystatin C was chosen as the outcome because it is less correlated with muscle mass or health status than creatinine, and may thus be less biased in the setting of HIV-infection.[22] Cystatin C was measured by a particle-enhanced immunoturbidometric assay (Gentian), which has been calibrated against the new World Standard Reference material ERM-DA471/IFCC.[23] Intra-assay coefficients of variation, based on 10 replicates, were <2% at serum concentrations of 0.7 and 1.1 mg/L. Inter-assay coefficients of variation were 4.4% and 3.9% at serum concentrations of 0.8 and 2.2 mg/L, respectively. We estimated GFR using the CKD-EPI equation for cystatin C (eGFRcys).[23, 24] Alternative analyses were conducted using serum creatinine estimates of GFR by the CKD-EPI equation. Creatinine measures were conducted at the clinical labs of each WIHS site at the time of collection.

As in our prior work, we truncated eGFR at 120 ml/min/1.73m^2 because the equations have not been validated in persons with very high GFR, and higher GFR estimates are unlikely to be accurate or precise.[10] We analyzed eGFR decline over the eight years of follow-up as both continuous and dichotomous outcomes. The linear outcome was expressed as annual change in eGFR in mL/min/1.73m^2 over the entire 8-year follow-up. We dichotomized eGFR in order to distinguish persons with clinically meaningful rates of declining kidney function: mild (≥3% annual eGFR decline), moderate (≥5% decline), or severe (≥10% decline). We defined each dichotomized outcome by calculating the relative change in eGFR from baseline to each follow-up visit for each participant. We defined the cystatin C analyses as primary *a priori* because the measures were conducted concurrently and were less likely to be biased by health status. Secondary analyses were implemented with the clinical creatinine measures and are presented in Supplementary Tables and Figures.

Adjustment Variables

Other covariates of interest included demographic characteristics, kidney disease risk factors, and HIV-specific risk factors obtained as part of the WIHS semiannual assessment. Specifically, the following characteristics were tested as candidate covariates in all multivariate models: age and race/ethnicity; menopause status, antihypertensive use, diabetes (fasting glucose ≥126mg/dL, self-reported diabetes, self-reported medication, or HbA1c ≥6.5), cigarette smoking (current, former, never); systolic and diastolic blood pressure, LDL and HDL cholesterol, triglycerides, body mass index, and waist circumference. We also tested the following HIV-related characteristics: hepatitis C virus (HCV) infection (defined by detectable HCV RNA), current heroin use, current CD4 cell count, nadir CD4 cell count, history of AIDS diagnosis (including low CD4), current HIV viral load, current HAART use, current NRTI use, current NNRTI use, and current PI use. There was minimal use of tenofovir at the baseline of this study. In a sensitivity analysis, we included tenofovir use during follow-up. Factors forced in the full model included age, race/ethnicity, hypertension and diabetes, current HIV viral load, current CD4 cell count, and HCV infection. All time-varying covariates in the model were time-updated, except for the urine biomarkers, which were collected only at baseline. After forcing the above variables, we used a stepwise backward selection with a significance level of α=0.05 to remove

candidate covariates that were not associated with the outcome. Multiple imputation with the Markov chain Monte Carlo method was used to impute missing covariates, with 5 imputations to yield ~95% relative efficiency.[25] The percentage of observations with missing covariates ranged from less than 1% to 15%.

Statistical Analysis

We began our analyses by comparing the baseline characteristics of HIV-infected and HIV-uninfected participants. We first analyzed the urine injury biomarkers as continuous (log-transformed) predictors of kidney decline, but the assumption of linearity did not hold for all measures. We therefore present results with the biomarkers categorized into tertiles, with the HIV-infected and uninfected participants tertiled separately. We analyzed tertiles of ACR to facilitate comparisons of the effect sizes of ACR with the novel urine markers; however, we also dichotomized ACR at 30 mg/g, the standard clinical cut point.

The association of each injury marker with annual mean change in eGFR was assessed using linear mixed models with random intercepts and slopes.[26] Interaction terms between each injury marker and time were used to determine the rate of change in eGFR. We used generalized estimating equations (GEE) using a Poisson working model to account for clustering from repeated events to determine relative risks for the association of each covariate with the dichotomized kidney outcomes.

To determine whether injury markers were independently associated with kidney outcomes, multivariable models were sequentially adjusted for: 1) demographics, and 2) traditional kidney disease risk factors, HIV-specific risk factors, and ACR. We included ACR because it is an established marker in widespread clinical use. Therefore, any novel marker should be demonstrated to have associations independent of ACR. In our final model, we adjusted for all four injury markers concurrently, allowing them to compete as predictors of each kidney outcome. We also conducted a sensitivity analysis in which we forced in tenofovir use, as a time-dependent covariate, into the final model.

To account for informative loss to follow-up, we also adjusted estimates using an inverse probability weighting approach by modeling the participant's probability of having a non-missing outcome at each visit.[27] The inverse of this probability was then used as a weight applied to persons with known outcome in the multivariable regression analyses of kidney decline. All analyses were conducted using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

In our study, 1,032 HIV-infected women and 371 HIV-uninfected women with cystatin C measured at baseline were included. Over half of women were African-American or reported current smoking; one-fourth had hypertension (Table 1). HCV infection was more common in HIV-infected than HIV-uninfected women. Baseline eGFRcys was significantly lower in HIV-infected compared with uninfected women (median 89 vs. 105 ml/min/ $1.73m^2$, $p<0.0001$). During the approximately 8-year follow-up period, rates of annual eGFRcys decline were -1.18 (95% CI: -1.29, -1.06) in HIV-infected women and -0.97 (-1.16, -0.79) ml/min/$1.73m^2$ in HIV-uninfected women ($p=0.06$ for difference). Among the HV-infected women, eGFRcys decline was faster among those co-infected with HCV (-1.46; -1.79 to -1.14) compared with mono-infected women (-0.92; -1.07 to -0.93 ; $p<0.0001$).

We initially examined the association of tertiles of each biomarker with the linear outcome of rate of change in eGFRcys in HIV-infected women (Figure 1). All four markers were

independently associated with faster eGFRcys decline in both demographic and multivariate-adjusted models. When we controlled for all four biomarkers simultaneously, the highest tertiles of ACR, IL-18, and KIM-1 remained independently associated with kidney function decline, whereas the association of NGAL weakened substantially (Figure 1). The magnitude of association for the highest ACR tertile was larger than that of IL-18 or KIM-1. When we repeated anlyses with eGFRcr as the outcome, the biomarker associations with eGFRcr decline were approximately half the magnitude of the eGFRcys associations (Supplemental Figure 1). The high tertiles of ACR, KIM-1 and NGAL were independently associated with faster eGFRcr decline in single marker models, but only ACR and KIM-1 remained associated in the combined model. Adjustment for tenofovir use during follow-up had no material impact on the associations between the urine biomarkers and kidney function decline.

We next compared biomarker associations with the dichotomized eGFRcys outcomes in HIV-infected women, using similar staged adjusted models (Table 2). In fully adjusted analysis, the highest tertile of ACR was not significantly associated with any of the dichotomized outcomes relative to the lowest tertile. Dichotomized at ACR >30 mg/g, fully adjusted associations were: 1.17 (0.93-1.47), 1.49 (1.14-1.95), and 1.43 (0.83, 2.44) for 3%, 5%, and 10% decline, respectively. The highest tertile of IL-18 was progressively associated with increased risk for each endpoint. The middle tertile of IL-18 was also significantly associated with the 3% and 5% thresholds of kidney function decline. The highest tertile of KIM-1 was significantly associated with a doubling in risk for the 10% annual eGFR decline outcome, but this finding was attenuated by adjustment for IL-18. NGAL showed little association with any of these outcomes; relative risks were: 1.02 (0.79, 1.31), 0.90 (0.65, 1.24), and 0.85 (0.44, 1.63) for 3%, 5%, and 10% decline, respectively. In analyses based on eGFRcr (Supplemental Table 1), significant associations were observed for the high tertile of KIM-1 with the 5% and 10% decline outcomes, and the high tertile of IL-18 was associated with the 10% decline outcome.

We also evaluated associations of biomarker tertiles with kidney function decline in HIV-uninfected women (Figure 2). In the linear analyses, the highest tertiles of IL-18, KIM-1 and NGAL were each individually associated with more rapidly declining kidney function in demographic- and individual multivariate-adjusted analyses, whereas ACR was not associated with decline. When we controlled for all four biomarkers simultaneously, only IL-18 remained independently associated with declining eGFRcys. In analyses using eGFRcr, the high tertiles of ACR and KIM-1 were independently associated with faster kidney function decline (Supplemental Figure 2). For the dichotomized outcomes based on eGFRcys (Table 3), IL-18 was significantly associated with 3% annual decline in fully adjusted models, and showed a weaker, non-significant association with 5% decline. For the 10% annual decline endpoint, the high tertiles of IL-18 and KIM-1 were each associated with substantially elevated risk in individual marker models; but, when combined in the same model, both weakened. An ACR >30 mg/g was also significantly associated with higher risk of the 10% endpoint (2.95, 1.34-6.45), but not with the 3% (1.14, 0.79-1.66) or 5% (1.28, 0.77-2.13) outcomes. In eGFRcr based dichotomized outcomes, the high tertile of KIM-1 had the strongest association with the 5% decline outcome, whereas the high tertile of IL-18 was most strongly associated with 10% decline (Supplemental Table 2). Both associations became statistically non-significant when the biomarkers were adjusted for one another.

Discussion

Although HIV-infected individuals have an elevated risk of CKD and ESRD,[9, 28] ongoing kidney tubular injury has been difficult to quantify, because albuminuria is primarily a

manifestation of glomerular injury and urine proteinuria concentrations are non-specific. We hypothesized that novel markers of kidney injury, which have been developed for the early detection of hospitalized acute kidney injury (AKI), would be detectable in HIV-infected, ambulatory women and would forecast subsequent rates of declining kidney function. We found that both IL-18 and KIM-1 had strong and independent associations with both linear and dichotomized outcomes of kidney decline in HIV-infected women. Of particular interest, the relative effects of ACR and these tubular injury markers had minimal attenuating effect on one another, suggesting that these two categories of injury markers capture distinct patterns of kidney damage.

The findings of this study should be compared with other recent studies in this emerging literature on urine markers of kidney tubular injury. In a study from Tokyo of 424 HIV-infected persons without CKD, Ando and colleagues measured a different set of tubular injury markers—*N*-acetyl-β-D-glucosaminidase, y-glutamyl transpeptidase, β_2-microglobulin and α_1-microglobulin.[29] At 1 year of follow-up, eGFRcr decline was faster among participants with a higher index of tubular damage. In a general population study, the Multi-Ethnic Study of Atherosclerosis (MESA), Peralta and colleagues found that higher urine concentrations of KIM-1, but not NGAL, were associated with rapidly declining kidney function and incident CKD.[30] In contrast, a nested case-control study from the Atherosclerosis Risk in Communities Study (ARIC) identified NGAL, but not KIM-1, as a significant predictor of incident CKD.[31] Relative to these prior manuscripts, our study is the first to combine both HIV-infected and uninfected and to use a cohort design with prolonged follow-up.

Because urine IL-18 and KIM-1 are specific to the proximal tubule of the nephron, they may be particularly useful for detecting HIV-related kidney injury and are biological intermediates in the causal mechanisms of ischemia-reperfusion injury in the kidney. Both biomarkers are present at very low concentrations in healthy patients, and their levels increase by several fold in patients who develop acute kidney injury.[32, 33] IL-18 is produced by proximal tubules, and is activated by *caspase-1*; mice deficient in caspase-1 are protected from acute kidney injury due to impaired IL-18 processing. KIM-1 is also expressed by proximal tubule cells in response to injury, and its ectodomain, shed into the tubule lumen, is detectable in urine.[34] IL-18 and KIM-1 have been studied as early biomarkers in critically ill patients, peri-operative patients, in diabetic nephropathy and polycystic kidney disease, and in acute and chronic kidney disease after kidney transplant.[35-37] Conversely, NGAL is produced predominantly in the distal tubules.[38] The stronger associations of IL-18 and KIM-1 with kidney function decline, relative to NGAL, may suggest that HIV damages the proximal tubules preferentially.

Relative to the mean annual eGFR decline of approximately 1mL/min/1.73m^2, the high tertiles of either IL-18 or KIM-1 were associated with approximately a 7-8% faster rate of annual kidney function decline among the HIV-infected women. . For dichotomized outcomes of rapidly declining kidney function, the high IL-18 tertile identified a group of women with approximately 40%, 90%, and 110% higher adjusted risks for the thresholds of 3%, 5%, and 10% annual decline, respectively. In the general population, rates of kidney decline of 3 ml/min/1.73m^2 per year or 5% annual decline have been shown to have independent associations with death and CVD.[39-41]

Our findings differed somewhat between our primary analyses using cystatin C as the outcome and our secondary analyses using eGFRcr. In the continuous variable model, biomarker associations with eGFRcys were stronger in magnitude than with eGFRcr. IL-18 was the tubular injury marker with the strongest associations with dichotomous eGFRcys outcomes in both HIV-infected and uninfected participants. In analyses of eGFRcr decline,

KIM-1 had stronger overall associations than IL-18. Although the cystatin C and creatinine results differed, we believe that they mutually reinforce the overall finding that urine markers of tubular injury can signal risk for declining kidney function among women with or without HIV-infection.

These findings have potential implications for the clinical care of patients with HIV infection. Current strategies for monitoring the impact of HIV on the kidney rely primarily on surveillance with serum creatinine concentrations, which are an indicator of glomerular filtration rate rather than injury. The effectiveness of antiretroviral therapy for preventing kidney complications could potentially be monitored by specific kidney injury biomarkers, such as ACR, IL-18, and KIM-1. Another application of these biomarkers could be for surveillance of toxicity from antiretroviral medications such as tenofovir.[42] The specimens in this study were collected in 1999-2000, prior to the widespread use of tenofovir. These biomarkers are already used in animal studies to detect drug toxicity.[43] Future studies should evaluate whether an injury marker panel can detect reversible medication-related injury in humans. Finally, kidney injury biomarkers could be used to guide the intensity of risk factor control, such as systolic blood pressure and glycemia targets in order to mitigate ongoing damage to the kidney. Thus, urine biomarkers and plasma filtration markers may be useful to capture the processes of kidney injury and functional decline in the setting of HIV infection.

There are several notable limitations in this study. First, our urine injury markers were measured only at baseline, and were obtained from specimens collected over a decade prior to analysis. However, we believe that any protein degradation would have biased our study toward null results. Second, we used a kidney filtration marker instead of direct GFR measures; however, measured GFR is rarely utilized in clinical research or practice.[44] Our cystatin C measures were conducted concurrently from all three visits, and had excellent precision; however, creatinine measures were conducted in multiple labs and at different time periods. Furthermore, we cannot be certain whether or not our findings apply to HIV-infected or uninfected men, though we have no reason to expect different biology in men. Since tenofovir was not used extensively at the time of our urine collections, we cannot evaluate any effect of tenofovir on kidney injury. Finally, we prioritized measures in the HIV-infected participants, and thus had limited statistical power among the HIV-uninfected.

In conclusion, our study has made novel observations that specific urine biomarkers for tubular injury can forecast subsequent declines in kidney function. Future research should determine whether changes in the biomarkers are accompanied by parallel changes in kidney function, and the specific risk factors for increases in each injury marker. An additional objective should be to determine the role of urine injury biomarkers for predicting kidney function decline in broader samples of the general population. Although our results are promising, the above steps in research are critical to complete prior to the biomarkers being used in clinical medicine.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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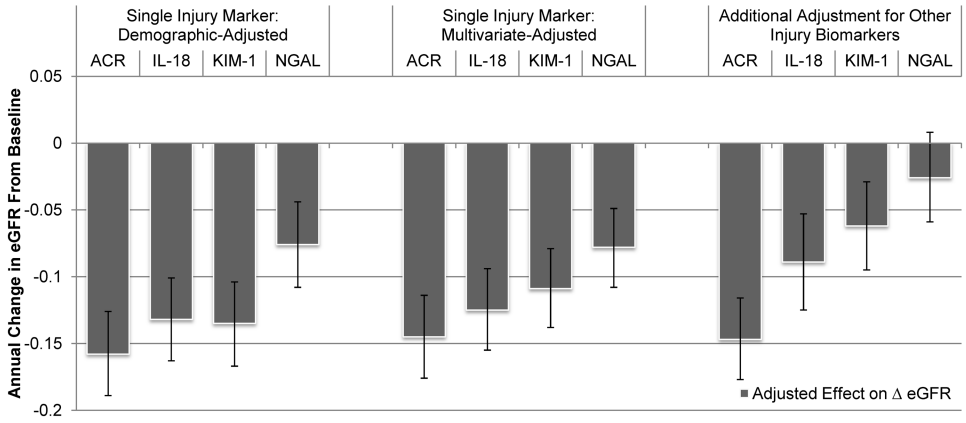

Figure 1. Multivariable-Adjusted Associations of Urine Biomarkers with Annual Change in eGFRcys (mL/min/1.73m^2) in 908 WIHS HIV-Infected Participants

Note: Estimates are highest versus lowest biomarker tertile. Tertile 1 is reference category, and represents those with lowest amount of biomarker; Tertile 3 results shown. Cutoff points for biomarker tertiles are as follows: ACR T1: <7.1 mg/g, T2: 7.1-15.8 mg/g, T3: >15.8 mg/g; IL-18 T1: <81 pg/mL, T2: 81-196 pg/mL, T3: >196 pg/mL; KIM-1 T1: <319 pg/mL, T2: 319-723 pg/mL, T3: >723 pg/mL; NGAL T1: <22 ng/mL, T2: 22-57 ng/mL, T3: >57 ng/mL. Results reported as estimated annual change in eGFR from baseline in mL/min/1.73m^2 (95% confidence interval). Demographic-adjusted model includes single biomarker, age, and ethnicity. Single injury marker multivariable model includes age, ethnicity, traditional kidney risk factors, HIV-related risk factors, and ACR. Additional adjustment for other injury biomarker model includes single injury biomarker multivariable model and all 4 markers.

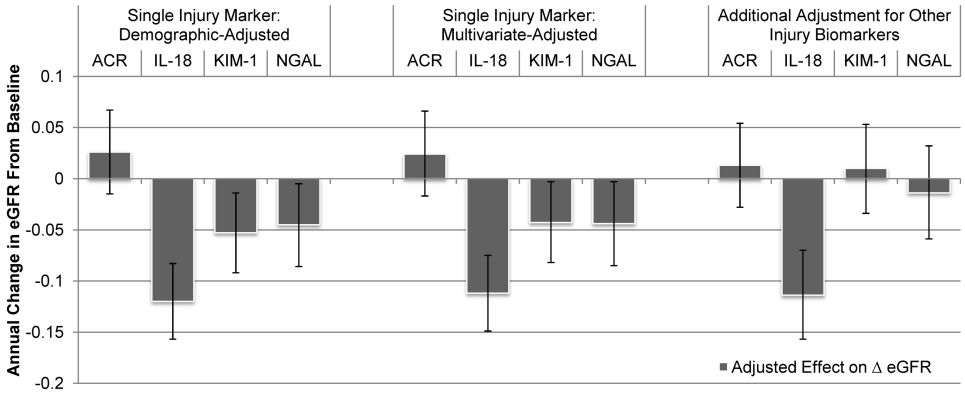

Figure 2. Multivariable-Adjusted Associations of Urine Biomarkers with Annual Change in eGFRcys (mL/min/1.73m^2) in 289 WIHS Uninfected Participants

Tertile 1 is reference category, and represents those with lowest amount of biomarker; Tertile 3 results shown. Cutoff points for biomarker tertiles are as follows: ACR T1: <6.1 mg/g, T2: 6.1-10.0 mg/g, T3: >10.0 mg/g; IL-18 T1: <53 pg/mL, T2: 53-132 pg/mL, T3: >132 pg/mL; KIM-1 T1: <272 pg/mL, T2: 272-645 pg/mL, T3: >645 pg/mL; NGAL T1: <21 ng/mL, T2: 21-53 ng/mL, T3: >53 ng/mL. Results reported as estimated annual change in eGFR from baseline in mL/min/1.73m^2 (95% confidence interval). Demographic-adjusted model includes single biomarker, age, and ethnicity. Single injury marker multivariable model includes age, ethnicity, traditional kidney risk factors, and ACR. Additional adjustment for other injury biomarker model includes single injury biomarker multivariable model and all 4 markers.

Table 1

Baseline Characteristics of HIV-Infected and Uninfected Women in WIHS Cystatin C Study

Characteristic	HIV-Infected (N = 1,032)	HIV-Uninfected (N = 371)	P Value
Baseline Age, years	41 (36-45)	38 (32-44)	<0.0001
<30	65 (6%)	62 (17%)	
30-40	411 (40%)	145 (39%)	
40-50	456 (44%)	130 (35%)	
>50	100 (10%)	34 (9%)	
Race			
African American	610 (59%)	228 (61%)	0.09
Caucasian	198 (19%)	53 (14%)	
Other	224 (22%)	90 (24%)	
History of Coronary Artery Disease	202 (20%)	41 (11%)	<0.0001
Cigarette Smoking			
At Baseline	542 (53%)	221 (60%)	0.063
Past	246 (24%)	73 (20%)	
Never	244 (24%)	77 (21%)	
Diabetes Mellitus	102 (10%)	39 (11%)	0.76
Systolic Blood Pressure, mmHg	118 (108-128)	119 (109-130)	0.079
Diastolic Blood Pressure, mmHg	72 (66-80)	72 (68-80)	0.68
Hypertension	254 (25%)	92 (25%)	0.94
Antihypertensive Use	115 (11%)	38 (10%)	0.70
LDL, mg/dL	101 (78-129)	104 (84-129)	0.13
HDL, mg/dL	44 (35-56)	51 (42-62)	<0.0001
Triglycerides, mg/dL	131 (91-192)	97 (69-142)	<0.0001
Body Mass Index, kg/m^2	27 (23-31)	29 (24-36)	<0.0001
Waist Circumference, cm	88 (80-99)	92 (80-104)	0.0058
Hepatitis C	315 (31%)	67 (18%)	<0.0001
Baseline Heroin Use	48 (5%)	28 (8%)	0.044
Baseline HAART Use	596 (58%)		
Baseline NRTI Use	671 (65%)		
Baseline NNRTI Use	276 (27%)		
Baseline PI Use	411 (40%)		
Baseline CD4	398 (247-581)		
Nadir CD4	214 (113-331)		
History of AIDS *	483 (47%)		
Plasma HIV RNA			
≤80	313 (31%)		
81-1,999	235 (23%)		
2,000-9,999	165 (16%)		
>10,000	313 (31%)		

Characteristic	HIV-Infected (N = 1,032)	HIV-Uninfected (N = 371)	P Value
eGFR Cystatin, ml/min/1.73m^2	89 (75-105)	105 (91-119)	<0.0001

Data are presented as Median (Interquartile Range) or numbers (percent).

* Defined by CD4 count or opportunistic infection.

Abbreviations: Women's Interagency HIV Study (WIHS), Highly Active Antiretroviral Therapy (HAART), nucleoside reverse transcriptase inhibitor (NRTI), nonnucleoside reverse transcriptase inhibitor (NNRTI), protease inhibitor (PI), estimated glomerular filtration rate (eGFR)

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Table 2

Multivariable-Adjusted Associations of Urine Biomarkers with Rapid Decline by eGFRcys in HIV-Infected WIHS Participants

	ACR		IL-18		KIM-1	
	Tertile 2	**Tertile 3**	**Tertile 2**	**Tertile 3**	**Tertile 2**	**Tertile 3**
Cut-Off Points	**7.1-15.7 mg/g**	**>15.7 mg/g**	**81-196 pg/mL**	**>196 pg/mL**	**318-721 pg/mL**	**>721 pg/mL**
3% (N=553)[a]						
Model 1[b]	1.13 (0.84, 1.51)	1.20 (0.89, 1.62)	1.26 (0.96, 1.64)	1.38 (1.07, 1.76)$^{+}$	0.92 (0.70, 1.21)	1.02 (0.80, 1.29)
Model 2[c]	1.09 (0.82, 1.47)	1.13 (0.84, 1.52)	1.23 (0.97, 1.56)	1.32 (1.01, 1.72)$^{+}$	0.92 (0.71, 1.18)	0.97 (0.75, 1.26)
Model 3[d]	1.09 (0.82, 1.45)	1.15 (0.86, 1.53)	1.27 (1.00, 1.63)	1.40 (1.04, 1.89)$^{+}$	0.85 (0.66, 1.10)	0.84 (0.64, 1.11)
5% (N=381)[a]						
Model 1[b]	1.00 (0.66, 1.50)	1.34 (0.90, 1.99)	1.53 (1.09, 2.14)$^{+}$	1.88 (1.36, 2.59)***	0.96 (0.65, 1.41)	1.26 (0.89, 1.78)
Model 2[c]	1.06 (0.73, 1.53)	1.32 (0.91, 1.91)	1.40 (1.03, 1.91)$^{+}$	1.74 (1.25, 2.43)**	0.88 (0.61, 1.27)	1.10 (0.77, 1.57)
Model 3[d]	1.04 (0.73, 1.49)	1.39 (0.99, 1.97)	1.47 (1.06, 2.03)$^{+}$	1.88 (1.30, 2.71)**	0.80 (0.56, 1.13)	0.89 (0.62, 1.29)
10% (N=138)[a]						
Model 1[b]	0.96 (0.45, 2.07)	1.36 (0.65, 2.86)	1.58 (0.88, 2.81)	2.49 (1.34, 4.62)*	1.48 (0.85, 2.57)	2.36 (1.35, 4.10)*
Model 2[c]	1.11 (0.52, 2.34)	1.50 (0.74, 3.03)	1.42 (0.78, 2.58)	2.46 (1.32, 4.57)*	1.31 (0.76, 2.27)	2.05 (1.17, 3.58)$^{+}$
Model 3[d]	1.11 (0.60, 2.04)	1.58 (0.80, 3.14)	1.38 (0.73, 2.64)	2.16 (1.20, 3.88)*	1.23 (0.69, 2.19)	1.62 (0.99, 2.65)

$^{+}$p<0.05,

*p<.01,

**p<.001,

***p<.0001 denotes statistical significance of estimates above.

Results reported as risk of eGFR decline of ≥3%, ≥5%, or ≥10% per year (95% confidence interval).

[a]N=number of participants with at least one outcome.

[b]Model 1: Demographic-adjusted model controls for age, ethnicity, and single biomarker.

[c]Model 2: Multivariable-adjusted full model controls for Model 1 plus traditional kidney risk factors, HIV-related risk factors, and ACR.

[d]Model 3: Multivariable-adjusted full model controls for Model 2 plus all four biomarkers (ACR, IL-18, KIM-1, NGAL).

Estimates are calculated from GEE relative risk models to account for multiple episodes of rapid decline, with inverse probability weighting to account for dropout, and multiple imputation for missing covariates.

Tertile 1 is reference category, and represents those with lowest amount of biomarker; cutoff points for Tertile 1 of each biomarker are as follows: ACR <7.1 mg/g; IL-18 <81 pg/mL; KIM-1 <318 pg/mL.

Table 3

Multivariable-Adjusted Associations of Urine Biomarkers with Rapid Decline by eGFRcys in HIV-Uninfected WIHS Participants

	ACR		IL-18		KIM-1	
	Tertile 2	Tertile 3	Tertile 2	Tertile 3	Tertile 2	Tertile 3
Cut-Off Points	**6.1-10.0 mg/g**	**>10.0 mg/g**	**53-132 pg/mL**	**>132 pg/mL**	**272-645 pg/mL**	**>645 pg/mL**
3% (N=129)[a]						
Model 1[b]	0.55 (0.36, 0.85)*	0.63 (0.41, 0.96)+	2.15 (1.29, 3.60)*	2.08 (1.37, 3.15)**	1.82 (1.14, 2.90)+	1.31 (0.87, 1.98)
Model 2[c]	0.53 (0.35, 0.80)*	0.64 (0.44, 0.94)+	1.81 (1.13, 2.88)+	1.79 (1.16, 2.77)*	1.54 (1.05, 2.25)+	1.15 (0.76, 1.75)
Model 3[d]	0.61 (0.42, 0.87)*	0.75 (0.53, 1.06)	1.82 (1.15, 2.87)+	2.02 (1.29, 3.16)*	1.28 (0.88, 1.87)	0.88 (0.58, 1.34)
5% (N=67)[a]						
Model 1[b]	0.65 (0.33, 1.29)	0.82 (0.42, 1.59)	2.03 (0.91, 4.51)	2.65 (1.33, 5.28)*	2.23 (1.10, 4.55)+	1.77 (0.99, 3.15)
Model 2[c]	0.70 (0.41, 1.22)	0.92 (0.56, 1.53)	1.43 (0.71, 2.87)	1.76 (0.90, 3.44)	1.57 (0.84, 2.94)	1.40 (0.79, 2.49)
Model 3[d]	0.76 (0.44, 1.32)	1.00 (0.60, 1.67)	1.36 (0.67, 2.75)	1.79 (0.88, 3.62)	1.43 (0.77, 2.67)	1.14 (0.63, 2.03)
10% (N=21)[a]						
Model 1[b]	0.86 (0.29, 2.57)	1.44 (0.47, 4.35)	3.95 (0.80, 19.49)	7.32 (1.63, 32.93)*	2.39 (0.46, 12.39)	4.39 (1.06, 18.20)+
Model 2[c]	0.81 (0.27, 2.48)	1.28 (0.46, 3.55)	3.80 (0.79, 18.23)	5.93 (1.34, 26.13)+	2.49 (0.51, 12.12)	4.25 (1.06, 17.09)+
Model 3[d]	1.01 (0.30, 3.37)	1.64 (0.64, 4.19)	3.08 (0.71, 13.33)	3.99 (0.94, 16.93)	1.93 (0.41, 9.13)	2.57 (0.60, 11.10)

+ p<0.05,

* p<.01,

** p<.001,

*** p<.0001 denotes statistical significance of estimates above.

Results reported as risk of eGFR decline of ≥3%, ≥5%, or ≥10% per year (95% confidence interval).

[a] N=number of participants with at least one outcome.

[b] Model 1: Demographic-adjusted model controls for age, ethnicity, and single biomarker.

[c] Model 2: Multivariable-adjusted full model controls for Model 1 plus traditional kidney risk factors, and ACR.

[d] Model 3: Multivariable-adjusted full model controls for Model 2 plus all four biomarkers (ACR, IL-18, KIM-1, NGAL).

Estimates are calculated from GEE relative risk models to account for multiple episodes of rapid decline, with inverse probability weighting to account for dropout, and multiple imputation for missing covariates.

Tertile 1 is reference category, and represents those with lowest amount of biomarker; cutoff points for Tertile 1 of each biomarker are as follows: ACR <6.1 mg/g; IL-18 <53 pg/mL; KIM-1 <272 pg/mL.