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Urine Biomarkers of Kidney Tubule Health and Incident CKD Stage 3 in Women Living With HIV: A Repeated Measures Study

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Rationale & Objective: Single measurements of urinary biomarkers reflecting kidney tubule health are associated with chronic kidney disease (CKD) risk in HIV infection, but the prognostic value of repeat measurements over time is unknown.

Study Design: Cohort study.

Setting & Participants: 647 women living with HIV infection enrolled in the Women's Interagency Health Study.

Exposures: 14 urinary biomarkers of kidney tubule health measured at 2 visits over a 3-year period.

Outcome: Incident CKD, defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m^2 at two 6-month visits and an average eGFR decline ≥ 3% per year.

Analytical Approach: We used multivariable generalized estimating equations adjusting for CKD risk factors to evaluate baseline, time-updated, and change-over-time biomarker associations with incident CKD. We compared CKD discrimination between models with and without a parsimoniously selected set of biomarkers.

Results: During a median 7 years of follow-up, 9.7% (63/647) developed CKD. In multivariable-adjusted analyses, 3 of 14 baseline biomarkers associated with incident CKD. In contrast, 10 of 14 time-updated biomarkers and 9 of 14 biomarkers modeled as change over time associated with incident CKD. Urinary epidermal growth factor (EGF), α_1-microglobulin (A1M), and albumin were selected using penalized regression methods. In the time-updated model, lower urinary EGF (risk ratio [RR] per 2-fold higher time-updated biomarker levels, 0.69; 95% CI, 0.58-0.81), higher urinary A1M (RR, 1.47; 95% CI, 1.25-1.73), and higher urinary albumin excretion (RR, 1.21; 95% CI, 1.03-1.42) were jointly associated with increased risk for CKD. Compared with a base model (C statistic, 0.75), CKD discrimination improved after adding urinary EGF, A1M, and albumin values across baseline (C = 0.81), time-updated (C = 0.83), and change-over-time (C = 0.83) models (P < 0.01 for all).

Limitations: Observational design, incident CKD definition limited to eGFR.

Conclusions: Repeat urinary biomarker measurements for kidney tubule health have stronger associations with incident CKD compared with baseline measurements and moderately improve CKD discrimination in women living with HIV infection.

Visual Abstract included

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People living with HIV (PLWH) are experiencing dramatically greater life expectancies in parallel with a growing burden of age-related comorbid conditions, including chronic kidney disease (CKD).[1-4] CKD is more common and occurs at younger ages in PLWH compared with the general population.[5,6] In addition, CKD in PLWH has strong associations with end-stage kidney disease, cardiovascular disease, heart failure, and mortality.[7,8] As PLWH continue to survive to older ages, earlier identification and prevention of CKD will become increasingly important.

Conventional measures of kidney health have several limitations when used to detect and diagnose kidney disease in PLWH. Serum creatinine serves as a surrogate for overall kidney function by enabling estimation of glomerular filtration rate, but it lacks sensitivity for detecting early reductions in kidney function.[9,10] In addition, substantial kidney injury can occur before measurable changes in serum creatinine.[11] Urinary albumin complements serum creatinine as a biomarker reflecting glomerular damage but may fail to detect the tubulointerstitial pathology often seen in HIV-associated nephropathy, antiretroviral nephrotoxicity, and CKD.[12-14] Furthermore, urinary albumin is rarely measured in PLWH.

Novel urinary biomarkers reflecting kidney tubule health have shown promise in characterizing kidney disease in PLWH. We previously demonstrated that levels of several kidney tubule biomarkers, measured at a single baseline time point, are higher in PLWH compared with uninfected individuals and are independently associated with longitudinal kidney function decline and mortality.[15-20] PLWH often have regular longitudinal follow-up, which makes repeat urinary biomarker measurements feasible. However, it is unknown whether urinary biomarker measurements repeated over time have additional prognostic information above and beyond their baseline value in the evaluation of CKD risk.

PLAIN-LANGUAGE SUMMARY

Chronic kidney disease (CKD) occurs frequently and early in People living with HIV (PLWH). Novel urinary biomarkers reflecting kidney tubule injury, dysfunction, inflammation, and repair have shown promise in capturing CKD risk in PLWH, but the value of repeating biomarker measurements over time is unknown. We measured 14 kidney tubule biomarkers at 2 visits among 647 women living with HIV infection and evaluated their ability to predict the development of CKD. Compared with kidney tubule biomarkers measured at baseline, repeat biomarker measurements have stronger associations with CKD risk and moderately improved the ability to predict CKD. These findings suggest that clinical monitoring with kidney tubule biomarkers may be the next step forward in the early detection and prevention of CKD in PLWH.

In this study, we measured 14 urinary biomarkers at 2 visits across a 3-year period and evaluated baseline, time-updated, and change-over-time associations with incident CKD among women living with HIV in the Women's Interagency HIV Study (WIHS) cohort. The mechanisms of kidney damage and representative urinary biomarkers included glomerular injury (urinary albumin); tubular injury (kidney injury molecule 1 [KIM-1], interleukin 18 [IL-18], neutrophil gelatinase–associated lipocalin, osteopontin, and clusterin); proximal tubular dysfunction(α_1-microglobulin [A1M], β_2-microglobulin, cystatin C, and trefoil factor 3); inflammation (monocyte chemoattractant protein 1); tubular fibrosis and repair (epidermal growth factor [EGF] and chitinase 3-like protein 1); and loop of Henle function (uromodulin).

We hypothesized that time-updated and change-over-time biomarker measurements would have stronger associations with incident CKD compared with baseline measurements, and that models including a parsimoniously selected set of urinary biomarkers would have greater CKD risk discrimination than models without urinary biomarkers.

METHODS

Study Design

The WIHS design and methods have been described previously.[21] In brief, 3,766 HIV-infected and uninfected women of similar backgrounds were enrolled in 1994 to 1995 and 2001 to 2002 from 6 sites (Bronx/Manhattan, Brooklyn, Chicago, Los Angeles, San Francisco, and Washington, DC). The WIHS protocol comprises a baseline visit and follow-up visits every 6 months; each visit includes an interviewer-administered questionnaire, physical examination, and collection of laboratory specimens.

Among women living with HIV in WIHS, we designed a nested study to investigate the trajectory of kidney injury and function. We included 647 women living with HIV who had available urine and serum specimens collected twice and had an estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 m^2 at the time of the first specimen collection. The first urine specimen was collected between October 2009 and March 2011, and the second urine specimen was collected a median of 2.5 (interquartile range [IQR], 2.4-2.5) years later. Follow-up started at the first urine specimen collection and was truncated in April 2017. There were few losses to follow-up (35/647; 5.4%).

WIHS was approved by the institutional review boards of all participating institutions, and informed consent was obtained from all study participants. This study was also approved by the Committee on Human Research of the University of California San Francisco.

Urinary Biomarker Measurements

Urine specimens were in continuous storage at −80 °C until biomarker measurement. All urinary biomarkers were measured at the University of California San Francisco Kidney Health Research Collaboration Biomarker Laboratory. Baseline and follow-up urinary biomarkers were measured on the same plates to minimize assay drift. Urinary creatinine was measured using an enzymatic assay (Roche Diagnostics) and urinary A1M was measured using a nephelometric assay (Siemens BN II Nephelometer). All other urinary biomarkers were measured in duplicate using multiplex immunoassays (Meso Scale Discovery). All biomarker intra-assay coefficients of variation were <10% and are shown in Table S1. Laboratory personnel performing the biomarker assays were blinded to participants' clinical information.

Outcome

The primary outcome was incident CKD, defined as eGFR < 60 mL/min/1.73 m^2 measured at 2 consecutive 6-month visits and an average annual eGFR decline ≥ 3% per year. Serum creatinine was measured in local laboratories for each study site with assays using the modified Jaffé method traceable to isotope-dilution mass spectrometry. We calculated creatinine-based eGFR using the corresponding CKD Epidemiology Collaboration estimating equation.[22]

Covariates

Demographics, traditional kidney disease risk factors, and HIV-related characteristics were assessed at each examination. The following covariates were included in all models: age, race/ethnicity, diabetes (defined using

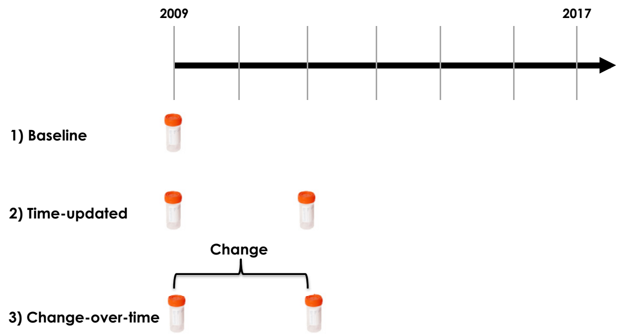

Figure 1. Modeling approaches to repeat urinary biomarker associations with incident chronic kidney disease (CKD). Each study participant had baseline and follow-up urinary biomarker measurements. In baseline models, only baseline biomarker levels were used. In time-updated models, baseline biomarker levels were used until updated by follow-up biomarker levels. In change-over-time models, baseline biomarker levels were used until updated by the absolute change between baseline and follow-up levels. Change-over-time models included a separate term for the baseline biomarker level.

confirmatory criteria for fasting glucose ≥ 126 mg/dL, self-reported diabetes, self-reported diabetes medication use, or glycated hemoglobin ≥ 6.5%), 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 history of cardiovascular disease, statin use, low- and high-density lipoprotein cholesterol levels, body mass index, cigarette smoking status (current, past, or never), serum albumin level, self-reported current intravenous drug use, hepatitis C virus (HCV) infection (confirmed by detectable HCV RNA following a positive HCV antibody result), current and nadir CD4 lymphocyte count, current and peak plasma HIV-1 RNA level, history of clinical AIDS diagnosis, duration of HIV infection, and duration of antiretroviral therapy (tenofovir disoproxil fumarate [TDF], ritonavir, or highly active antiretroviral therapy, defined in accordance with US Department of Health and Human Services treatment guidelines [www.aidsinfo.gov]). Undetectable HIV viral load was defined as plasma HIV-1 RNA < 80 copies/mL. The percentage of participants with missing covariate data was <5%.

Statistical Analysis

We summarized clinical characteristics at the baseline and follow-up urinary biomarker collection visits for all participants. For each of the 14 urinary biomarkers, we modeled associations with incident CKD with 3 approaches: (1) using the baseline concentration, (2) time-updating the baseline concentration with the follow-up concentration, and (3) using the absolute change-over-time concentration with adjustment for the baseline concentration as a separate predictor (Fig 1). The inclusion of separate terms for change-over-time and baseline concentrations allows for distinguishing within- and between-person changes in biomarker levels and their associations with CKD risk.

For each approach, we obtained relative risks per 2-fold higher value of the biomarkers using modified Poisson regression combined with generalized estimating equations to account for repeat measurements within participants. We analyzed urinary biomarker levels as continuous variables (log-transformed due to a right-skewed distribution). All models controlled for urinary creatinine to account for variations in urine concentrations.

To determine whether individual biomarkers were independently associated with incident CKD, multivariable models additionally adjusted for urinary albumin, demographics, traditional CKD risk factors, and HIV-related CKD risk factors (as listed in the Covariates section). Time-updated and change-over-time analyses included covariates from both baseline and follow-up visits. In our primary analyses, we did not adjust for eGFR because it is used to define the outcome. As sensitivity analysis, we additionally adjusted for baseline eGFR in multivariable models.

We next modeled all 14 urinary biomarkers in combination and used 5 different penalized regression methods to identify a parsimonious set of biomarkers that was jointly associated with incident CKD. Penalized regression can perform simultaneous coefficient estimation and variable selection in the setting of high-dimensional data. We used the following methods: (1) least absolute shrinkage and selection operator (LASSO), (2) adaptive LASSO, (3) elastic net, (4) smoothly clipped absolute deviation, and (5) minimax concave penalty. We used cross-validation to determine the number of included predictors and the degree of shrinkage to avoid overfitting. In addition, we estimated marginal false discovery rates to assess whether urinary biomarkers selected by penalized regression were reliable.[23]

We performed this process modeling all baseline urinary biomarker measurements and covariates in combination and then separately modeling all biomarker and covariate data at the follow-up biomarker collection visit. We retained biomarkers that appeared to show consistent selection across penalized regression and marginal false discovery rate results. Because our previous work showed that changes in several urinary biomarkers vary by HIV viral load, we also evaluated whether the selected biomarker associations with incident CKD varied between participants with detectable versus undetectable baseline HIV viral load.

To determine whether the selected urinary biomarkers improved CKD discrimination, we estimated differences in C statistics calculated from a base model without urinary biomarkers and models including the selected urinary biomarkers individually and in

Table 1. Baseline Clinical Characteristics of Women Living With HIV in the WIHS Urinary Biomarker Substudy

Baseline Parameter	WIHS Biomarker Substudy (N = 647)
Age, y	45 [40-51]
Race	
African American	432 (67%)
Other	100 (15%)
White	115 (18%)
Hispanic	133 (21%)
Smoking	
Current	249 (38%)
Past	210 (32%)
Never	188 (29%)
Diabetes	130 (20%)
Hypertension	229 (35%)
LDL cholesterol, mg/dL	93 [76-118]
Statin use	93 (14%)
BMI, kg/m^2	29 [25-34]
Current ART	
HAART use	487 (75%)
NRTI use	483 (75%)
NNRTI use	202 (31%)
PI use	274 (42%)
TDF use	396 (61%)
Current CD4, cells/mm^3	518 [343-730]
Lifetime nadir CD4, cells/mm^3	213 [113-307]
History of AIDS	233 (36%)
Plasma HIV RNA < 80 copies/mL	386 (60%)
Peak HIV RNA > 10,000 copies/mL	510 (79%)
Hepatitis C virus infection	124 (19%)
Heroin use	8 (1%)
Urinary ACR, mg/g	4.6 [2.1-11.2]
eGFR, mL/min/1.73 m^2	104 [89-117]

Note: Data are presented as median [interquartile range] or number (percent). Abbreviations: ACR, albumin-creatinine ratio; ART, antiretroviral therapy; BMI, body mass index; eGFR, estimated glomerular filtration rate; HAART, highly active antiretroviral therapy; LDL, low-density lipoprotein; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir disoproxil fumarate; WIHS, Women's Interagency Health Study.

combination. The base model included all demographics, traditional CKD risk factors, and HIV-related CKD risk factors outlined in the Covariates section. We also compared a base model with urinary albumin to models with the selected urinary biomarkers in combination. The C statistics were derived from logistic regression models estimating the odds of any CKD during follow-up and using the biomarker measurements at baseline, follow-up, and the change in biomarkers for the baseline model, time-updated model, and change-from-baseline model, respectively. We performed internal validation of estimated C statistics with cross-validation.

Penalized regression analyses were conducted using the R package *ncvreg* (R Development Core Team, Vienna, Austria). All other analyses were performed using the SAS system, version 9.4 (SAS Institute, Inc).

RESULTS

Median duration of follow-up was 7.0 (IQR, 6.8-7.1) years. Median age at baseline was 45 (IQR, 40-51) years, 67% were African American, and 20% had diabetes. Median duration of HIV infection was 14 (IQR, 8-15) years, 75% were receiving highly active antiretroviral therapy, median CD4 count was 518 cells/mm^3, and 60% had undetectable viral levels (Table 1). Compared with women living with HIV in the WIHS cohort not included in our study, participants were on average younger, more were African American, fewer were Hispanic, fewer had hypertension, and participants had higher CD4 counts and eGFR (Table S2).

Median eGFR and urinary albumin-creatinine ratio at baseline were 104 (IQR, 89-117) mL/min/1.73 m^2 and 4.6 (IQR, 2.1-11.2) mg/g, respectively. A total of 9.7% (63/647) of participants developed CKD, and median eGFR at the time of CKD diagnosis was 53 (IQR, 46-57) mL/min/1.73 m^2. Incident CKD occurred a median 3.7 (IQR, 3.0-5.6) and 1.5 (IQR, 0.9-3.2) years after the baseline and follow-up urinary biomarker measurements, respectively. The average annual eGFR decline was faster among participants who developed CKD compared with those who did not (5.23 vs 1.88 mL/min/1.73 m^2 per year; $P < 0.01$). Participants who developed CKD also had significantly lower urinary EGF and significantly higher concentrations of 10 other biomarkers at the baseline and follow-up biomarker measurements, as well as significantly greater increases in urinary A1M (Table S3; Fig S1).

We first modeled associations of baseline biomarkers with risk for incident CKD. In models that were adjusted for urinary creatinine, 11 of the 14 urinary biomarkers were individually associated with incident CKD (Table 2). Most of these associations were attenuated and no longer significant after multivariable adjustment for urinary albumin and traditional and HIV-related CKD risk factors. Only lower baseline urinary EGF, higher A1M, and higher albumin remained independently associated with increased risk for CKD.

When modeling time-updated biomarker associations, the same 11 urinary biomarkers had significant associations with incident CKD in analyses adjusting for urinary creatinine (Table 2). Although these associations were attenuated after multivariable adjustment, 10 of the 14 time-updated biomarker associations remained independently associated with increased risk for CKD (Fig 2).

When modeling change-over-time biomarker associations, 11 of the 14 urinary biomarkers were significantly associated with risk for incident CKD in analyses that were adjusted for urinary creatinine and the baseline biomarker concentration (Table 3). Although the strength of these associations modestly attenuated after multivariable adjustment, longitudinal changes in 9 of 14 biomarkers remained independently associated with incident CKD. Overall, the multivariable-adjusted change-over-time biomarker associations were stronger than the baseline biomarker associations (Fig 2).

Table 2. Baseline and Time-Updated Associations of Individual Urinary Biomarkers With Risk for Incident CKD Among Women Living With HIV in WIHS

	Baseline Biomarkers			**Time-Updated Biomarkers**		
Urinary Biomarker (per 2-fold higher level)	**Unadjusted RR (95% CI)**	**Multivariable Adjusted[a] RR (95% CI)**	**Multivariable Adjusted + Baseline eGFR[a] RR (95% CI)**	**Unadjusted RR (95% CI)**	**Multivariable Adjusted[a] RR (95% CI)**	**Multivariable Adjusted + Baseline eGFR[a] RR (95% CI)**
KIM-1	1.65 (1.31-2.09)[b]	1.04 (0.81-1.32)	0.99 (0.81-1.19)	1.94 (1.63-2.31)[b]	1.27 (1.09-1.48)[b]	1.22 (1.06-1.41)[b]
IL-18	1.34 (1.16-1.54)[b]	1.16 (0.92-1.47)	1.29 (1.06-1.57)[b]	1.42 (1.22-1.65)[b]	1.18 (1.00-1.40)	1.20 (1.05-1.36)[b]
NGAL	1.32 (1.17-1.49)[b]	1.03 (0.88-1.21)	0.98 (0.84-1.14)	1.51 (1.34-1.71)[b]	1.22 (1.10-1.34)[b]	1.11 (1.02-1.21)[b]
Clusterin	1.09 (0.90-1.32)	1.00 (0.94-1.06)	0.96 (0.91-1.01)	1.26 (0.92-1.73)	1.04 (0.97-1.12)	1.02 (0.95-1.09)
Osteopontin	1.05 (0.80-1.37)	0.92 (0.74-1.14)	0.88 (0.70-1.11)	1.15 (0.88-1.51)	1.00 (0.84-1.19)	0.99 (0.82-1.19)
A1M	1.90 (1.58-2.28)[b]	1.49 (1.13-1.97)[b]	1.28 (1.01-1.62)[b]	1.97 (1.72-2.25)[b]	1.65 (1.39-1.94)[b]	1.45 (1.26-1.68)[b]
B2M	1.37 (1.20-1.58)[b]	1.15 (1.00-1.32)	1.04 (0.92-1.18)	1.47 (1.32-1.64)[b]	1.32 (1.19-1.46)[b]	1.19 (1.07-1.31)[b]
Cystatin C	1.41 (1.18-1.69)[b]	1.00 (0.84-1.20)	0.90 (0.76-1.07)	1.54 (1.42-1.66)[b]	1.32 (1.20-1.45)[b]	1.20 (1.09-1.33)[b]
TFF3	1.20 (1.03-1.39)[b]	1.04 (0.92-1.17)	0.99 (0.89-1.11)	1.30 (1.14-1.48)[b]	1.17 (1.08-1.28)[b]	1.11 (1.02-1.21)[b]
MCP-1	1.41 (1.14-1.74)[b]	0.93 (0.74-1.16)	1.02 (0.80-1.29)	1.84 (1.55-2.19)[b]	1.37 (1.16-1.62)[b]	1.33 (1.15-1.54)[b]
EGF	0.61 (0.50-0.75)[b]	0.64 (0.49-0.83)[b]	0.85 (0.63-1.15)	0.49 (0.40-0.59)[b]	0.57 (0.48-0.68)[b]	0.69 (0.59-0.79)[b]
YKL-40	1.19 (1.04-1.37)[b]	0.90 (0.80-1.02)	0.87 (0.76-0.99)[b]	1.44 (1.34-1.53)[b]	1.17 (1.07-1.28)[b]	1.10 (1.02-1.18)[b]
Uromodulin	1.23 (0.95-1.60)	1.23 (0.83-1.80)	1.15 (0.79-1.67)	0.90 (0.71-1.14)	0.86 (0.67-1.11)	0.85 (0.67-1.08)
Albumin	1.70 (1.50-1.93)[b]	1.51 (1.25-1.82)[b]	1.50 (1.23-1.82)[b]	1.65 (1.44-1.88)[b]	1.46 (1.26-1.70)[b]	1.36 (1.18-1.57)[b]

Note: All estimates adjust for urinary creatinine. Biomarkers are modeled individually, not jointly.
Abbreviations: A1M, α_1-microglobulin; B2M, β_2-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase–associated lipocalin; RR, risk ratio; TFF3, trefoil factor 3; WIHS, Women's Interagency Health Study; YKL-40, chitinase 3-like protein 1.
[a]Adjusted models additionally control for demographics, traditional kidney risk factors, and HIV-related risk factors. Models without urinary albumin as the main predictor additionally adjusted for urinary albumin.
[b]$P < 0.05$.

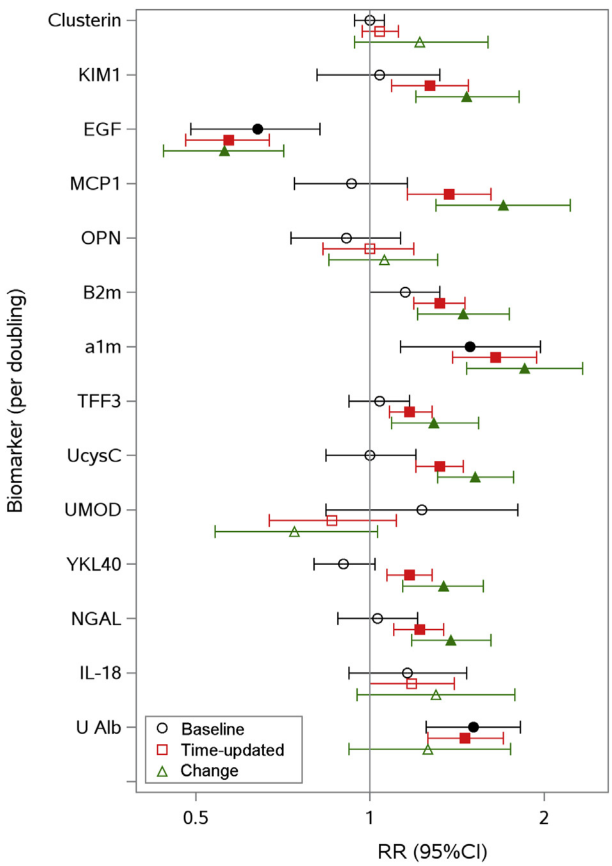

Figure 2. Multivariable-adjusted baseline, time-updated, and change-over-time associations of individual urinary biomarkers with risk for incident chronic kidney disease (CKD) among women living with HIV in the Women's Interagency Health Study. Associations of baseline (black circles), time-updated (red squares), and change-over-time (green triangles) urinary biomarkers with risk for incident CKD with 95% CIs displayed. Associations estimated from generalized estimating equation models that adjusted for demographics, traditional and HIV-related kidney disease risk factors, urinary creatinine, and urinary albumin. Biomarkers are modeled individually, not jointly. Filled symbols denote statistically significant associations. Abbreviations: a1m, α_1-microglobulin; B2m, β_2-microglobulin; EGF, epidermal growth factor; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase–associated lipocalin; OPN, osteopontin; TFF3, trefoil factor 3; U Alb, urinary albumin; UcysC, urinary cystatin C; UMOD, uromodulin; YKL40, chitinase 3-like protein 1.

Next, we used 5 penalized regression methods to select a parsimonious urinary biomarker set that independently associated with risk for incident CKD. In multivariable-adjusted models, urinary EGF, A1M, and albumin were consistently selected by all 5 penalty functions and also had the lowest marginal false discovery rates. In baseline and time-updated multivariable-adjusted models, lower urinary EGF, higher urinary A1M, and higher urinary albumin were jointly associated with increased risk for CKD (Table 4). In the change-over-time model, smaller changes in EGF and greater changes in A1M were jointly associated with increased risk for CKD, whereas change in urinary albumin showed little association with risk for CKD. Joint associations of urinary EGF, A1M, and urinary albumin with incident CKD did not vary by HIV viremia status across models ($P \geq 0.20$ for all biomarker × HIV viremia interaction tests).

There were small to moderate improvements in discrimination of CKD risk with each addition of urinary EGF, A1M, and albumin to a base model that included demographics, traditional CKD risk factors, and HIV-related CKD risk factors (Table 5). The model without urinary biomarkers had the lowest C statistic: 0.75 (95% CI, 0.68-0.82), while time-updated and change-over-time models that included urinary EGF, A1M, and albumin had the highest C statistics: 0.83 (95% CI, 0.77-0.88). Across baseline, time-updated, and change-over-time approaches, the combined addition of urinary EGF, A1M, and albumin moderately improved C statistics compared with base models without urinary biomarkers, as well as base models including urinary albumin.

In sensitivity analyses that additionally adjusted multivariable models for baseline eGFR, associations were mildly attenuated but overall similar (Tables 2 and 3; Fig S2). Across penalized regression methods, urinary IL-18 showed consistent selection among baseline biomarkers, A1M was selected among follow-up biomarkers, and albumin was selected among both baseline and follow-up biomarkers. The combined addition of urinary IL-18, A1M, and albumin moderately improved C statistics compared with base models without urinary biomarkers (Table S4).

DISCUSSION

In this cohort of women living with HIV, we found that multiple urinary biomarkers of kidney tubule health were associated with incident CKD independent of traditional and HIV-related CKD risk factors and urinary albumin. In addition, repeat urinary biomarker measurements and their changes had stronger associations with CKD risk compared with baseline measurements alone. When modeling urinary biomarkers in combination, lower EGF, higher A1M, and higher albumin levels consistently associated with increased risk for incident CKD and moderately improved CKD risk discrimination. Together, these findings suggest that repeat urinary biomarkers of kidney tubule health may have utility in the early detection of kidney disease in PLWH.

Our findings are consistent with previous studies in the HIV-infected population, which show that single urinary biomarker measurements reflecting kidney tubule health are associated with longitudinal kidney function decline. WIHS studies before the widespread use of TDF–based combination antiretroviral therapy showed that higher baseline urinary albumin, A1M, IL-18, and KIM-1 levels

Table 3. Change-Over-Time Associations of Individual Urinary Biomarkers With Risk for Incident CKD Among Women Living With HIV in WIHS

Urinary Biomarker (per 2-fold higher change from baseline)	Unadjusted RR (95% CI)	Multivariable Adjusted[a] RR (95% CI)	Multivariable Adjusted + Baseline eGFR[a] RR (95% CI)
KIM-1	1.89 (1.47-2.42)[b]	1.47 (1.20-1.81)[b]	1.46 (1.19-1.78)[b]
IL-18	1.48 (0.98-2.24)	1.30 (0.95-1.78)	1.19 (0.88-1.61)
NGAL	1.52 (1.31-1.78)[b]	1.38 (1.18-1.62)[b]	1.27 (1.06-1.53)[b]
Clusterin	1.33 (0.98-1.83)	1.22 (0.94-1.60)	1.22 (0.92-1.63)
Osteopontin	1.19 (0.89-1.61)	1.06 (0.85-1.31)	1.06 (0.80-1.40)
A1M	1.80 (1.41-2.29)[b]	1.85 (1.47-2.33)[b]	1.64 (1.30-2.07)[b]
B2M	1.47 (1.21-1.79)[b]	1.45 (1.21-1.74)[b]	1.30 (1.07-1.58)[b]
Cystatin C	1.56 (1.41-1.72)[b]	1.52 (1.31-1.77)[b]	1.45 (1.22-1.72)[b]
TFF3	1.32 (1.11-1.57)[b]	1.29 (1.09-1.54)[b]	1.19 (0.99-1.43)
MCP-1	1.86 (1.53-2.27)[b]	1.70 (1.30-2.22)[b]	1.64 (1.24-2.17)[b]
EGF	0.46 (0.35-0.59)[b]	0.56 (0.44-0.71)[b]	0.62 (0.49-0.78)[b]
YKL-40	1.47 (1.37-1.57)[b]	1.34 (1.14-1.57)[b]	1.29 (1.08-1.54)[b]
Uromodulin	0.75 (0.57-0.99)[b]	0.74 (0.54-1.03)	0.72 (0.52-1.00)
Albumin	1.33 (1.01-1.75)[b]	1.26 (0.92-1.75)	1.18 (0.88-1.58)

Note: All estimates adjust for urinary creatinine and the baseline biomarker level. Biomarkers are modeled individually, not jointly.
Abbreviations: A1M, α_1-microglobulin; B2M, β_2-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor; eGFR, estimated glomerular filtration rate; IL-18, interleukin 18; KIM-1, kidney injury molecule 1; MCP-1, monocyte chemoattractant protein 1; NGAL, neutrophil gelatinase–associated lipocalin; RR, risk ratio; TFF3, trefoil factor 3; WIHS, Women's Interagency Health Study; YKL-40, chitinase 3-like protein 1.
[a]Adjusted models additionally control for demographics, traditional kidney risk factors, and HIV-related risk factors. Models without urinary albumin as the main predictor additionally adjusted for urinary albumin.
[b]$P < 0.05$.

were independently associated with faster eGFR decline.[15,17] In a contemporary multicenter cohort of men living with HIV in the United States, higher baseline urinary albumin and A1M also associated with faster eGFR decline, although not with incident CKD.[20] In general population cohorts, higher urinary KIM-1, neutrophil gelatinase–associated lipocalin, and monocyte chemoattractant protein 1 levels, and lower urinary EGF levels, have also been shown to be associated with risk for incident CKD.[24-27]

The major finding that repeat urinary biomarker measurements have stronger associations with CKD compared with baseline measurements suggests that there is added prognostic value in repeating measurements of urinary biomarkers of kidney tubule health among PLWH in the ambulatory setting. To our knowledge, this is one of the first studies to evaluate associations of repeat kidney tubule biomarker measurements with risk for incident CKD in any population. Our findings may have several complementary explanations. First, dynamic biomarker changes may capture subclinical progressive kidney tubule damage that leads to tubulointerstitial fibrosis, which is a hallmark of all forms of CKD.[14] Second, repeat biomarker measurements may better reflect the evolving burden, duration, and severity of CKD risk factors. Third, urinary biomarker associations with CKD may diminish over time and need updating to remain reflective of the ongoing injury within the kidneys. An exception to the overall pattern that we observed was change in urinary albumin, which had a weakened non-significant association with incident CKD. This may have been due to the low baseline urinary albumin concentrations and small changes between baseline and follow-up measurements.

Another key finding from our study is that we ultimately narrowed our biomarker panel using several penalized regression methods to urinary EGF, A1M, and albumin, which improved CKD risk discrimination beyond a base model with traditional and HIV-related CKD risk factors, as well as beyond the addition of

Table 4. Baseline, Time-Updated, and Change-Over-Time Associations of Parsimoniously Selected Urinary Biomarkers With Risk for Incident CKD Among Women Living With HIV in WIHS

Urinary Biomarker (per 2-fold higher level)	Multivariable Adjusted[a] RR (95% CI)		
	Baseline	Time-Updated	Change-Over-Time
EGF	0.75 (0.55-1.01)	0.69 (0.58-0.81)	0.70 (0.57-0.87)
A1M	1.41 (1.05-1.90)	1.47 (1.25-1.73)	1.61 (1.28-2.02)
Albumin	1.41 (1.13-1.78)	1.21 (1.03-1.42)	0.92 (0.71-1.19)

Abbreviations: A1M, α_1-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor; RR, risk ratio; WIHS, Women's Interagency Health Study.
[a]Urinary EGF, A1M, and albumin are modeled jointly. Models additionally adjust for urinary creatinine, demographics, traditional CKD risk factors, and HIV-related CKD risk factors.

Table 5. C Statistics for Discrimination of Incident CKD With and Without Inclusion of Urinary EGF, A1M, and Albumin

	Baseline		Time-Updated		Change-Over-Time	
Model	**C Statistic (95% CI)**	**P[a]**	**C Statistic (95% CI)**	**P[a]**	**C Statistic (95% CI)**	**P[a]**
Base model	0.75 (0.68-0.82)	Reference	0.75 (0.68-0.82)	Reference	0.75 (0.68-0.82)	Reference
+ Urinary EGF	0.76 (0.70-0.83)	0.009	0.77 (0.70-0.83)	0.04	0.77 (0.70-0.83)	0.18
+ Urinary A1M	0.78 (0.72-0.85)	0.03	0.81 (0.75-0.87)	0.006	0.81 (0.75-0.87)	0.006
+ Urinary albumin	0.77 (0.70-0.84)	0.10	0.79 (0.73-0.86)	0.02	0.79 (0.73-0.85)	0.06
+ Urinary EGF, A1M, and albumin	0.81 (0.75-0.86)[b]	0.001	0.83 (0.77-0.88)[b]	0.002	0.83 (0.77-0.88)[b]	0.002

Note: C statistics and 95% CI calculated using logistic regression with cross-validated predicted probabilities. Base model includes all demographic characteristics, traditional CKD risk factors, and HIV-related CKD risk factors covariates.

Abbreviations: A1M, α_1-microglobulin; CKD, chronic kidney disease; EGF, epidermal growth factor.

[a]*P* values compare C statistics between base model with versus without urinary biomarkers.

[b]C statistic different from base model + urinary albumin, $P < 0.05$.

urinary albumin alone. In sensitivity analyses that additionally adjusted for baseline eGFR, we narrowed our biomarker panel to urinary IL-18, A1M, and albumin. The selection of these measures and their combined discriminative ability may be explained by each biomarker reflecting a distinct axis of kidney health. Urinary albumin is an established marker of glomerular damage and function that reflects glomerular capillary wall permeability to macromolecules. In contrast, A1M is a freely filtered low-molecular-weight protein that is avidly reabsorbed by the proximal tubule in persons with healthy kidneys. Thus, higher urinary concentrations indicate proximal tubule injury or dysfunction.[28] Urinary EGF is a biomarker of renal repair hypothesized to mediate recovery following injury by enhancing the dedifferentiation and proliferation of surviving kidney tubule epithelial cells.[29,30] Conversely, sustained kidney tubule EGF receptor activation has been implicated in the development of CKD by inducing interstitial fibrosis.[31,32] IL-18 is a proinflammatory cytokine released into urine by proximal tubular cells in response to injury or inflammation.[33]

Our findings highlight the utility of combining biomarkers, which capture distinct pathophysiologic mechanisms and together provide a more comprehensive assessment of kidney health than any single biomarker. However, although the improved CKD risk discrimination is an important proof of concept demonstrating the prognostic value of kidney tubule health measures, our analysis was not designed to develop and validate a new CKD risk prediction model. In addition, the clinical importance of a modest improvement in CKD risk discrimination with kidney tubule biomarkers is uncertain. Additional cohorts of PLWH can enrich this field further by demonstrating whether alternate panels of candidate biomarkers have greater prognostic utility for CKD risk in different populations.

Strengths of our study include a contemporary and diverse cohort of women living with HIV, repeat urinary biomarker measurements, adjustment for multiple traditional and HIV-related CKD risk factors, and use of a large curated panel of urinary biomarkers that localize to different regions of the nephron. We were also able to analyze baseline and follow-up biomarker measurements on the same plates with low intra-assay coefficients of variation.

Our study also has several limitations. First, our biomarker measurements were performed at 2 study visits, which limited the characterization of longitudinal changes in kidney tubule health in greater detail. More frequent biomarker measurements during the follow-up period may have stronger time-updated associations with incident CKD. Second, urinary albumin was not frequently measured between biomarker collection visits, which limited our definition of incident CKD to longitudinal changes in eGFR only. Third, despite extensive adjustment for multiple traditional and HIV-related CKD risk factors, our study may not have accounted for all potential confounders. Fourth, although we studied several urinary biomarkers, we did not include formal adjustments for multiple comparisons. We hypothesized a priori that the intercorrelated biomarkers associate with CKD in a mutually reinforcing, biologically coherent pattern that should not be viewed exclusively as a series of independent tests. To reduce the possibility of false discovery, we used several penalized regression methods and estimated false discovery rates to produce parsimonious models in the setting of multiple biomarkers. However, chance findings are still possible. Fifth, we were unable to correlate urinary biomarker levels with pathology on kidney biopsies. Finally, our results may not be generalizable to individuals with significant albuminuria, men living with HIV, or kidney disease in individuals without HIV.

In summary, repeat urinary biomarker measurements of kidney tubular health are independently associated with incident CKD in women living with HIV. From a panel of 14 urinary biomarkers, EGF, A1M, and albumin appear to provide the most information about CKD risk. Our findings support the biomarker assays as having adequate precision to detect relevant biological changes across several years and suggest that repeating ambulatory urinary measures of kidney tubule health may be the next step forward in monitoring kidney health in people living with HIV infection. Additional studies are needed to determine whether longitudinal patterns in kidney tubule health can predict varying eGFR trajectories, distinguish between the multifactorial causes of kidney disease in people living with HIV infection, and inform clinical decisions in the prevention of CKD.

SUPPLEMENTARY MATERIAL

Supplementary Material (PDF)

Figure S1: Box plots of relative percentage change in urinary biomarker concentrations among women living with HIV in WIHS stratified by incident CKD status

Figure S2: Multivariable-adjusted baseline, time-updated, and change-over-time associations of individual urinary biomarkers with risk for incident CKD among women living with HIV in WIHS, controlling for baseline eGFR

Table S1: Biomarker intra-assay coefficients of variation

Table S2: Comparison of baseline demographic and clinical characteristics between women living with HIV in WIHS included versus excluded in the urinary biomarker substudy

Table S3: Baseline and follow-up urinary biomarker concentrations among women living with HIV in WIHS stratified by incident CKD status

Table S4: C statistics for discrimination of incident CKD with and without inclusion of urinary IL-18, A1M, and albumin, controlling for baseline eGFR

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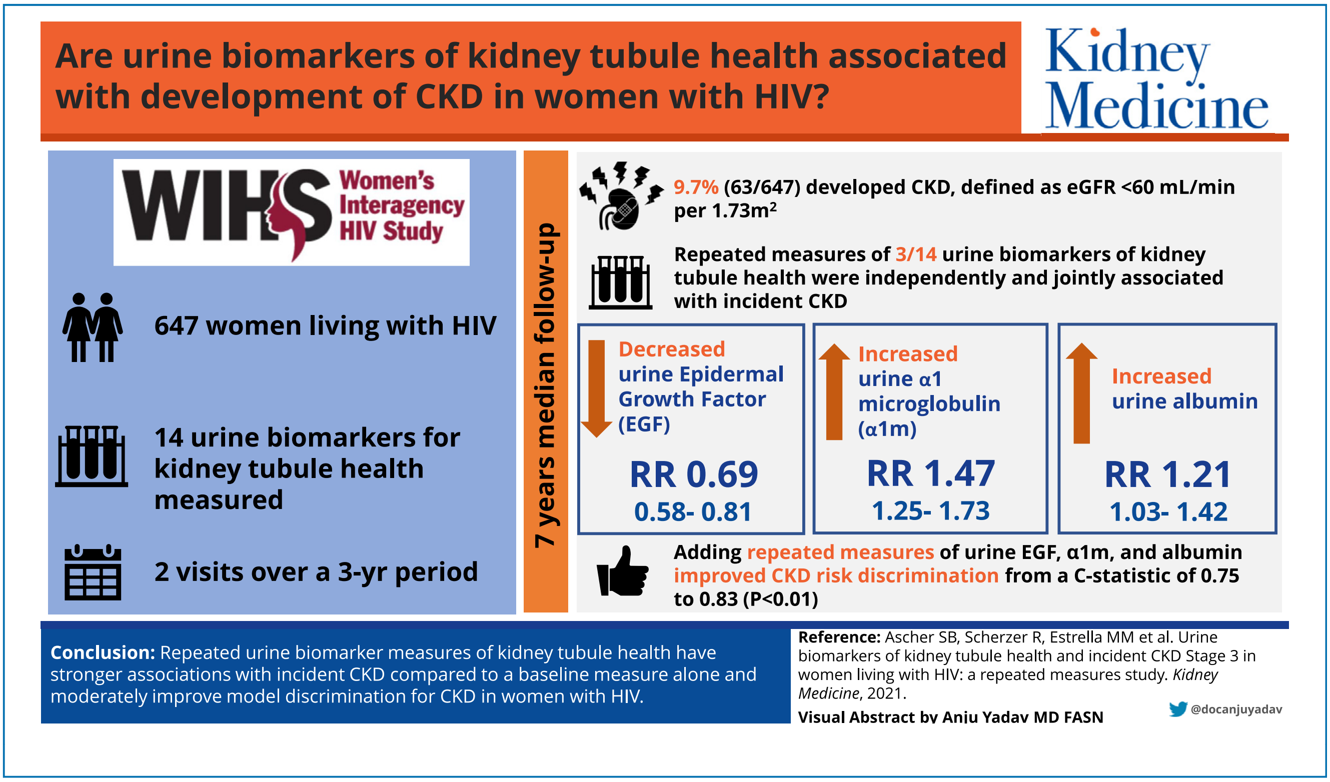

Are urine biomarkers of kidney tubule health associated with development of CKD in women with HIV?
Kidney Medicine
WIHS Women's Interagency HIV Study
647 women living with HIV
14 urine biomarkers for kidney tubule health measured
2 visits over a 3-yr period
7 years median follow-up
9.7% (63/647) developed CKD, defined as eGFR <60 mL/min per 1.73m²
Repeated measures of 3/14 urine biomarkers of kidney tubule health were independently and jointly associated with incident CKD
Decreased urine Epidermal Growth Factor (EGF)
RR 0.69
0.58- 0.81
Increased urine α1 microglobulin (α1m)
RR 1.47
1.25- 1.73
Increased urine albumin
RR 1.21
1.03- 1.42
Adding repeated measures of urine EGF, α1m, and albumin improved CKD risk discrimination from a C-statistic of 0.75 to 0.83 (P<0.01)
Conclusion: Repeated urine biomarker measures of kidney tubule health have stronger associations with incident CKD compared to a baseline measure alone and moderately improve model discrimination for CKD in women with HIV.
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