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## Relationships Between Renal Parameters and Serum and Urine Markers of Inflammation in Those With and Without HIV Infection

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### Abstract

We sought to determine the relationships among intrarenal and systemic inflammation <sup>and</sup> renal disease in HIV. We compared paired serum and urinary levels (normalized to urine creatinine) of monocyte chemoattractant protein-1 (MCP-1), regulated on activation normal T cell expressed and secreted (RANTES), interferon- $\gamma$ -induced protein-10 (IP-10), interleukin-8 (IL-8), and  $\beta_2$ -microglobulin (B2M) between two groups of HIV-infected subjects not receiving antiretroviral therapy (ART) [A: not expecting to initiate ART immediately due to having CD4 cell counts  $\geq 350/\mu\text{l}$ ,  $N=26$ ; B: about to initiate ART,  $N=19$ ], a group of HIV-infected subjects receiving virologically suppressive antiretroviral therapy [C,  $N=30$ ], and a group of HIV-uninfected, healthy volunteers [D,  $N=45$ ]. We then correlated these inflammatory biomarker levels with urine protein/creatinine ratios (uPCR), urine albumin/creatinine ratios (uACR), and estimated glomerular filtration rates (eGFR). Urine inflammatory biomarker levels were highest in Group B. When combining all four study groups, statistically significant positive correlations included uPCR with urine IL-8, urine MCP-1, urine IP-10, and serum IP-10 and uACR with urine IL-8, urine B2M, serum IP-10, and serum B2M. eGFR was statistically significantly negatively correlated with serum MCP-1 and serum B2M. Paired serum and urine levels of IP-10 and B2M (but not IL-8, RANTES, or MCP-1) were significantly correlated with each other in the overall group. The levels of urine inflammatory markers tested differed by HIV status and use of virologically suppressive ART. These urine and serum inflammatory markers were differentially correlated with uPCR, uACR, and eGFR, suggesting that different intrarenal and systemic inflammatory pathways may contribute to different measures of nephropathy.

### Introduction

**K**IDNEY DISEASE REMAINS A MAJOR COMPLICATION of human immunodeficiency virus (HIV) infection. Compared to HIV-uninfected individuals, HIV-infected persons have significantly greater risks of proteinuria,<sup>1</sup> albuminuria,<sup>2</sup> and lower estimated glomerular filtration rates (eGFR).<sup>3</sup> In addition, proteinuria,<sup>4</sup> albuminuria,<sup>5,6</sup> and impaired renal function prior to antiretroviral therapy (ART) are independent predictors of both progression to AIDS<sup>4</sup> and overall mortality.<sup>4,7,8</sup> As such, HIV-related renal disease may be part of a systemic inflammatory or immunological process.

We previously found in a study of HIV-infected subjects who were not receiving ART that dipstick proteinuria  $\geq 1+$  was significantly associated with greater T cell activation as measured by percentages of peripheral blood CD8<sup>+</sup>

CD38<sup>+</sup>HLA-DR<sup>+</sup> cells.<sup>9</sup> In a recent pilot study of HIV-infected patients not on ART, we investigated the contribution of intrarenal inflammation to renal injury by assessing the relationships between urinary inflammatory biomarkers and quantitative proteinuria and albuminuria.<sup>10</sup> We found that urinary levels of monocyte chemoattractant protein-1 (MCP-1) and regulated on activation normal T cell expressed and secreted (RANTES), but not interleukin-8 (IL-8) or interferon- $\gamma$ -induced protein-10 (IP-10), were significantly associated with both proteinuria and albuminuria.<sup>10</sup> In addition, proteomic analysis from this pilot study suggested that  $\beta_2$ -microglobulin (B2M) was universally detected in the urine samples analyzed.

To further characterize the systemic and intrarenal inflammatory profiles and their relationships with kidney disease in HIV, we measured paired urinary and serum levels of

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MCP-1, RANTES, IL-8, IP-10, and B2M along with quantitative proteinuria, albuminuria, and renal function in HIV-infected persons not receiving antiretroviral therapy, HIV-infected persons receiving virologically suppressive ART, and HIV-uninfected controls.

## Materials and Methods

### Study participants

This study included 75 HIV-seropositive patients and 45 HIV-seronegative subjects who completed other local studies by our group (ClinicalTrials.gov NCT00796822, NCT00864916, NCT00919724, and NCT01270802) and who consented to have their sera and urine samples stored and available for future analysis. Samples from all participants from these previously conducted trials were used for the current analysis. Participants were recruited from the HIV outpatient clinics associated with the Indiana University Health medical system and all were over the age of 18 years.

The 75 HIV-positive subjects were classified into three groups based on antiretroviral history, CD4 count, and plasma HIV RNA levels. Groups A and B were HIV-infected patients who enrolled into trials assessing the utility of pentoxifylline to reduce inflammation and improve endothelial function as measured by flow-mediated dilation of the brachial artery. The samples assayed in the current analysis were from the baseline visits of these two trials. Group A included 26 HIV-seropositive patients who had a CD4 cell count  $\geq 350/\mu\text{l}$ , were not considered nonadherent to ART, and were not known to be long-term nonprogressors, had no receipt of ART within 6 months of sample acquisition, and were not expected to initiate ART for at least 3 months during the trial period.<sup>11</sup> Group B included 19 HIV-seropositive patients who had no receipt of ART within 6 months of sample acquisition but were about to initiate ART per their HIV provider; there was no CD4 count eligibility criterion.

Group C included 30 HIV-seropositive patients who had received tenofovir/emtricitabine/efavirenz (TDF/FTC/EFV) as their initial ART regimen for at least 1 year and were virologically suppressed (defined as having an HIV-1 RNA level  $< 50$  copies/ml) at study screening.<sup>12</sup> Group D included 45 HIV-seronegative persons without medical comorbidities and matched with subjects in Group A and Group B based on age ( $\pm 10$  years), sex, and smoking status (current vs. not current); the choice of these matching characteristics was based on demographic factors known to be associated with HIV-related endothelial function measured using the flow-mediated dilation technique. Major exclusion criteria in all groups included known cardiovascular disease, diabetes mellitus, uncontrolled hypertension, thyroid abnormalities, systemic inflammatory disease other than hepatitis B or C coinfection, pregnancy or breastfeeding during the study, creatinine clearance  $< 50$  ml/min, hemoglobin  $< 9.0$  g/dl, alanine (ALT) or aspartate (AST) aminotransferase  $> 3$  times the upper limit of normal, total bilirubin  $> 2.5$  times the upper limit of normal, or ongoing fever or active infection/malignancy requiring treatment during the study visit.

### Study design

We performed a cross-sectional analysis to compare paired serum and urinary levels of IL-8, RANTES, MCP-1, IP-10,

and B2M between the four study groups. Of note, we chose not to combine Groups A and B as these participants were considered sufficiently dissimilar due to their enrollment characteristics. We measured the serum levels of these markers in order to determine if serum levels compared similarly and correlated with their respective urine levels. These inflammatory markers were then analyzed in relation to spot urine protein/creatinine ratio (uPCR), spot urine albumin/creatinine ratio (uACR), and eGFR calculated using the 2012 CKD-EPI creatinine-cystatin C combined equation.<sup>13</sup> All urine and serum samples were obtained after an 8-h fast on the morning of the study visit. The study was approved by the Indiana University institutional review board. All participants provided written, informed consents for both participation in the parent trials and for use of their specimens for future use.

### Biomarker measurements

We measured urine IL-8, RANTES, MCP-1, IP-10, and B2M using a custom enzyme-linked immunosorbent assay (ELISA) multiplex (EMD Millipore, Billerica, MA).<sup>14</sup> These urine chemokines were normalized to urinary creatinine to account for variances in concentrations. Serum MCP-1 and IP-10 and cystatin C (calibrated to the IDMS standard) were measured using individual ELISA assays. Serum RANTES, B2M, and IL-8 were separately measured using another Millipore multiplex kit. Urine protein and urine creatinine were measured using a spectrophotometric assay and using the Jaffe kinetic reaction with picric acid, respectively. Urine albumin was measured using an immunoprecipitin reaction. All samples were run in duplicate.

### Statistical analysis

Categorical variables were summarized as frequencies (percentages) and were compared using either Pearson's chi-square tests or Fisher's exact tests depending on the expected cell frequencies. Since the continuous variables did not have normal distributions, nonparametric statistics were used. Medians (quartile 1, quartile 3) were used for descriptive statistics, and rank-based methods were used for comparisons. Kruskal-Wallis one-way analysis of variance and Wilcoxon rank sum test were used for unadjusted comparisons between each study group. Proportional odds ordinal logistic models were used to adjust for demographic factors for comparisons of each HIV-infected study group (Groups A, B, C) with the uninfected control group (Group D). Spearman's rank-based correlation coefficients were calculated to assess for relationships between each inflammatory marker with each renal parameter and between each paired serum and urine inflammatory marker in the overall cohort and within each study group. The Holm-Bonferroni method was used to adjust for multiple testing in all analyses. Adjusted two-sided  $p$  values  $< 0.05$  were considered as significant. All analyses were performed in R [R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. www.R-project.org/].

## Results

### Study cohort characteristics

The characteristics of each group are shown in Table 1. The overall median age was 37 years with no significant age

◀T1

TABLE 1. CHARACTERISTICS OF THE STUDY GROUPS

Baseline characteristics	HIV-infected			HIV-uninfected	
	A (n=26)	B (n=19)	C (n=30)	D (n=45)	Overall (n=120)
Age (years)	36.7 (28.6, 43.2)	34.2 (28.5, 42.3)	38.3 (30.2, 44.5)	33.9 (25.7, 45.1)	36.9 (29.0, 43.0)
Gender					
Female	7 (27%)	3 (16%)	3 (10%)	11 (24%)	24 (20%)
Male	19 (73%)	16 (84%)	27 (90%)	34 (76%)	96 (80%)
Race					
Black	16 (62%)	12 (63%)	18 (60%)	14 (31%)	60 (50%)
Nonblack	10 (38%)	7 (37%)	12 (40%)	31 (69%)	60 (50%)
Ethnicity					
Hispanic or Latino	1 (4%)	0 (0%)	0 (0%)	1 (2%)	2 (2%)
Not Hispanic or Latino	25 (96%)	19 (100%)	30 (100%)	44 (98%)	118 (98%)
Smoking status					
Nonsmoker	15 (58%)	8 (42%)	12 (40%)	23 (51%)	58 (48%)
Smoker	11 (42%)	11 (58%)	18 (60%)	22 (49%)	62 (52%)
CD4 count (cells/ $\mu$ l)					
> 350	585 (433, 639)	186.5 (51.75, 375)	541 (423, 960)	880 (723.5, 1163.5)	593 (414, 880)
> 350	22 (88%)	6 (33%)	25 (86%)	39 (100%)	92 (83%)
HIV-1 RNA (log <sub>10</sub> copies/ml)					
<50 copies/ml	4.17 (3.69, 4.61)	5.03 (4.46, 5.61)	1.32 (1.32, 1.32)	NA	3.64 (1.30, 4.63) <sup>a</sup>
<50 copies/ml	1 (4%)	0 (0%)	28 (97%)	0	29 (40%) <sup>a</sup>
uPCR	0.06 (0.05, 0.08)	0.09 (0.06, 0.18)	0.08 (0.05, 0.11)	0.04 (0.04, 0.06)	0.06 (0.04, 0.10)
>0.3	1 (4%)	4 (21%)	0 (0%)	0 (0%)	5 (4%)
>0.2	2 (8%)	4 (21%)	3 (10%)	1 (2%)	10 (8%)
uACR	0.0041 (0.0026, 0.0083)	0.0114 (0.0045, 0.0304)	0.0038 (0.0025, 0.0074)	0.0035 (0.0025, 0.0053)	0.0039 (0.0029, 0.0082)
>0.03	3 (12%)	5 (26%)	1 (3%)	0 (0%)	9 (8%)
Estimated GFR (ml/min/1.73 <sup>2</sup> )					
<60	97.01 (87.6, 108.01)	90.3 (75.35, 100.28)	102.58 (90.18, 114.46)	98.62 (86.03, 108.87)	97.43 (84.86, 110.69)
<60	1 (4%)	2 (11%)	1 (3%)	0 (0%)	4 (3%)
<90	8 (31%)	9 (47%)	7 (24%)	15 (34%)	39 (33%)
Serum IL-8 (pg/ml)	2.6 (1.3, 4.1)	3.4 (2.6, 3.9)	1.8 (1.3, 3.3)	2.2 (1.5, 3.1)	2.4 (1.5, 3.5)
Serum RANTES (pg/ml)	28,581 (7,229, 102,655)	9,800 (166, 23,211)	20,475 (22, 57,118)	7,528 (79, 35,410)	14,336 (228, 49,823)
Serum MCP-1 (pg/ml)	201 (170, 281)	301 (220, 441)	200 (161, 250)	185 (155, 236)	200 (166, 277)
Serum IP-10 (pg/ml)	423 (234, 542)	524 (435, 778)	169 (147, 268)	124 (95, 181)	196 (133, 428)
Serum B2M (mcg/ml)	3,205,500 (2,198,900, 3,656,775)	4,005,500 (3,415,750, 6,319,250)	1,528,100 (1,079,515, 1,882,200)	1,878,900 (1,603,750, 2,229,750)	2,128,700 (1,663,150, 3,401,450)
Urine IL-8/Cr ( $\times 10^{-9}$ )	15.6 (3.2, 33.2)	11.5 (5.7, 32.2)	4.1 (2.0, 14.1)	5.1 (3.0, 11.1)	6.4 (2.6, 17.9)
Urine RANTES/Cr ( $\times 10^{-9}$ )	8.5 (5.2, 14.3)	13.0 (9.8, 17.7)	8.1 (5.9, 11.8)	9.5 (7.1, 14.9)	9.7 (6.7, 14.9)
Urine MCP-1/Cr ( $\times 10^{-7}$ )	3.4 (2.6, 5.8)	5.4 (4.0, 6.1)	4.1 (2.5, 5.4)	3.4 (2.5, 4.9)	3.9 (2.6, 5.5)
Urine IP-10/Cr ( $\times 10^{-8}$ )	9.3 (6.2, 21.8)	13.8 (8.3, 25.7)	1.1 (0.16, 3.0)	3.7 (2.7, 5.5)	4.1 (2.6, 11.1)
Urine B2M/Cr	41.7 (29.4, 63.7)	36.7 (20.8, 46.9)	9.2 (7.3, 12.8)	18.3 (12.7, 25.2)	19.5 (11.1, 36.7)

<sup>a</sup>For the HIV-infected groups only (Groups A, B, C).

Note: Categorical variables presented as count (percentage) and continuous variables presented as median (quartile 1, quartile 3). Group A, HIV-infected, antiretroviral-naive, CD4 cell counts >350/ $\mu$ l; Group B, HIV-infected, antiretroviral-naive, initiating antiretroviral therapy; Group C, HIV-infected, virologically suppressed on tenofovir/emtricitabine/efavirenz; Group D, HIV-uninfected, healthy controls.

cr, creatinine; GFR, glomerular filtration rate; IL-8, interleukin-8; RANTES, regulated on activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon- $\gamma$ -induced protein 10; B2M,  $\beta_2$ -microglobulin; NA, not applicable; uPCR, urine protein to creatinine ratio; uACR, urine albumin to creatinine ratio.

differences among the four groups. More than 70% were male in each study group. The majority of participants were non-Hispanic. Among the HIV-infected groups (Groups A, B, C), more than 60% were black, whereas only 31% of the HIV-uninfected subjects in Group D were black. Active smokers comprised nearly half of the overall group of 120 participants. As expected, the median CD4 cell count was lower and the median HIV-1 RNA log<sub>10</sub> c/ml level was higher in Group B compared to both Groups A and C. The frequencies of participants with uPCR >0.2 and uACR >0.03, which are considered abnormal pathological levels, were similar to those found previously in HIV,<sup>15,16</sup> with the highest rates found in Group B. Only 3% of the overall group had eGFR <60 ml/min/1.73<sup>2</sup>, although nearly a third had an eGFR <90 ml/min/1.73<sup>2</sup>, which are similar to rates of reduced eGFR in HIV found previously.<sup>17</sup> Of note, Group B had the highest frequencies of reduced eGFR using both cutoffs.

#### Comparisons of inflammatory markers between groups

The levels of each urine and serum inflammatory marker are shown in Table 1. The statistically significant differences between groups (corrected for multiple testing) are shown in Fig. 1. Except for urine IL-8/Cr and urine MCP-1/Cr, for which no statistically significant differences were found between any of the pairwise group comparisons, we found at least one statistically significant difference in pairwise comparisons for the other eight inflammatory marker measurements. There did not appear to be similar patterns across groups when comparing serum and urine biomarker levels for IL-8, RANTES, or MCP-1. However, there did appear to be similar patterns between serum and urine IP-10 and B2M levels, with the highest levels in Group B followed by Group A. Significantly lower levels of serum and urine levels of IP-10 and B2M were found in Groups C and D compared to the untreated HIV-1-infected Groups A and B. Of note, given the marked differences in black race representation between the HIV-infected groups and the uninfected group, we specifically visually examined the distributions of each inflammatory marker by race and did not discern any obvious differences.

To account for differences in age, race, sex, and smoking status between groups, we then constructed adjusted models accounting for these demographic variables to assess for differences in each inflammatory marker between Group D as the HIV-uninfected, healthy control reference group with each of the three HIV-infected groups (Table 2). In these adjusted comparisons, Group B was found to have significantly higher levels of urine IL-8, urine RANTES, and urine MCP-1 compared to Group D. Urine IL-10 and B2M levels were significantly higher in Groups A and B, while signifi-

cantly lower in Group C, when compared to group D. Group B had significantly greater serum IL-8 and serum MCP-1 levels compared to Group D. Serum RANTES levels remained significantly higher in Group A compared to Group D. Serum IP-10 levels in each of the HIV-infected groups were significantly higher compared to the uninfected Group D. Groups A and B had significantly higher levels of serum B2M, whereas Group C had significantly lower levels, when compared to Group D.

#### Correlations between serum and urinary levels of each inflammatory marker

Table 3 shows correlations between paired serum and urinary levels of each inflammatory marker in all four study groups and in the combined group. Statistically significant positive correlations were found between serum and urinary levels of IP-10 and B2M when all four study groups were combined. When correlations were analyzed separately in each study group, serum and urinary levels of IP-10 were significantly positively correlated in Group A and in Group B. Serum and urine MCP-1 levels were significantly negatively correlated in Group A. There were no significant correlations between any paired serum and urine inflammatory markers in Groups C and D.

#### Correlations between inflammatory markers and renal parameters

Table 4 shows the correlations between each inflammatory marker and uPCR, uACR, and eGFR in the entire study cohort of 120 participants. uPCR was positively correlated with urine IL-8/Cr, urine MCP-1/Cr, urine IP-10/Cr, and serum IP-10. uACR was positively correlated with urine IL-8, urine B2M, serum IP-10, and serum B2M. eGFR was negatively correlated with both serum MCP-1 and serum B2M. When each study group was analyzed separately (data not shown), however, only urine B2M was significantly positively correlated with uACR in Group A ( $\rho=0.64$ ;  $p=0.016$ ), and serum MCP-1 was negatively correlated with eGFR in Group D ( $\rho=-0.51$ ;  $p=0.01$ ). There were no other significant correlations within each study group after adjusting for multiple comparisons.

#### Discussion

To our knowledge, this is the first study investigating urinary inflammatory markers and their potential contributions to nephropathy in HIV infection. We expanded upon our previous pilot study<sup>10</sup> by measuring paired serum and urine markers in HIV-infected patients not receiving (Groups A and B) and receiving ART (Group C) as well as in healthy uninfected volunteers (Group D). As seen in Fig. 1, we found

F1 ▶

T2 ▶

◀ T3

◀ T4

**FIG. 1.** Pairwise comparisons of serum and urine levels of interleukin-8 (IL-8), regulated on activation normal T cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), interferon- $\gamma$ -induced protein-10 (IP-10), and  $\beta_2$ -microglobulin (B2M) between the four study groups. Only  $p$ -values <0.05 after Holm-Bonferroni corrections are shown. The boxplots denote quartile 3 (top line of box), median (middle line within the box), and quartile 1 (bottom line of box) for each distribution of inflammatory marker. The two outermost horizontal lines represent the maximum and minimum values if no outliers were detected. Outlier values were drawn as open circles for data points smaller than [quartile 1–1.5 times the interquartile range (drawn as the lowest horizontal line)] or larger than [quartile 3 + 1.5 times the interquartile range (drawn as the highest horizontal line)].

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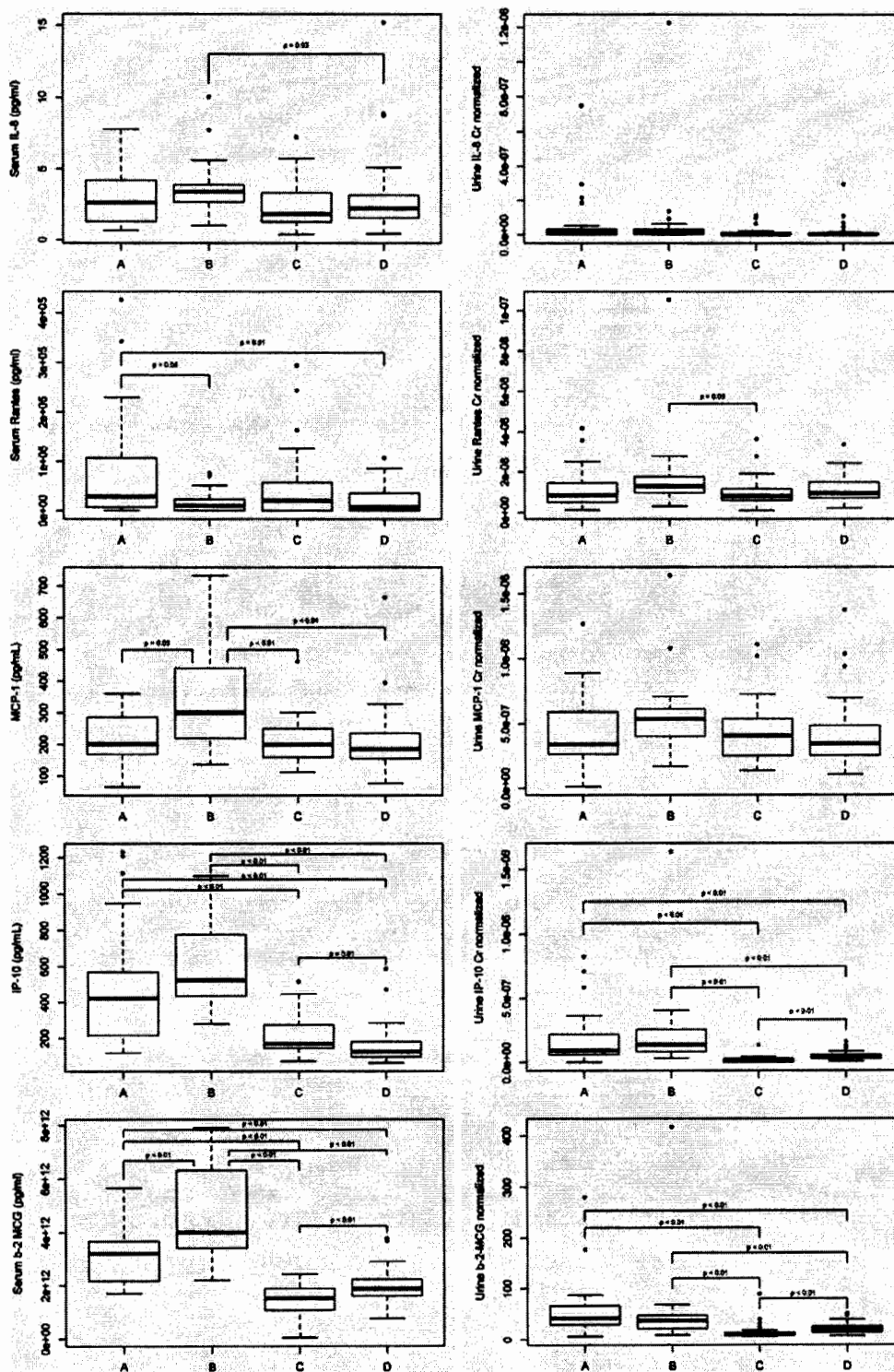


TABLE 2. COMPARISONS OF EACH HIV-INFECTED GROUP (GROUPS A, B, C) WITH THE UNINFECTED CONTROL GROUP FOR EACH INFLAMMATORY MARKER AFTER ADJUSTMENT FOR DEMOGRAPHIC CHARACTERISTICS

Group	Urine IL-8/Cr		Urine RANTES/Cr		Urine MCP-1/Cr		Urine IP-10/Cr		Urine B2M/Cr	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Group A	0.7178	0.1187	-0.5663	0.231	0.0039	0.9931	<b>2.1304</b>	<b>0.0001</b>	<b>2.5113</b>	<b>0.0001</b>
Group B	<b>1.2072</b>	<b>0.0167</b>	<b>1.0986</b>	<b>0.0207</b>	<b>1.086</b>	<b>0.0288</b>	<b>2.7256</b>	<b>0.0001</b>	<b>2.1692</b>	<b>0.0001</b>
Group C	-0.0594	0.8897	-0.2577	0.5753	0.0937	0.8394	<b>-1.7248</b>	<b>0.0004</b>	<b>-1.4235</b>	<b>0.0025</b>

Group	Serum IL-8		Serum RANTES		Serum MCP-1		Serum IP-10		Serum B2M	
	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value	Coefficient	p-value
Group A	0.2279	0.6188	<b>1.3117</b>	<b>0.0035</b>	0.5752	0.194	<b>3.9128</b>	<b>0.0001</b>	<b>2.7593</b>	<b>0.0001</b>
Group B	<b>1.0667</b>	<b>0.0252</b>	-0.2258	0.6358	<b>2.2372</b>	<b>0.0001</b>	<b>5.0374</b>	<b>0.0001</b>	<b>4.873</b>	<b>0.0001</b>
Group C	-0.4025	0.3808	0.3289	0.4842	0.1958	0.6446	<b>1.4772</b>	<b>0.0011</b>	<b>-1.2114</b>	<b>0.0123</b>

Note: Each group was compared to Group D as the reference group. All comparisons were adjusted for age, smoking status, race, and gender. Bolded data are those with  $p$ -values  $< 0.05$ . Group A, HIV-infected, antiretroviral-naive, CD4 cell counts  $> 350/\mu\text{l}$ ; Group B, HIV-infected, antiretroviral-naive, initiating antiretroviral therapy; Group C, HIV-infected, virologically suppressed on tenofovir/emtricitabine/efavirenz; Group D, HIV-uninfected, healthy controls.

Cr, creatinine; IL-8, interleukin-8; RANTES, regulated on activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon- $\gamma$ -induced protein 10; B2M,  $\beta_2$ -microglobulin.

differing patterns of urinary inflammatory biomarker levels across these study groups. Moreover, some urinary markers did (IP-10, B2M) whereas others did not (IL-8, RANTES, MCP-1) mirror patterns of their paired serum levels across the four study groups. In addition, there were varying relationships between these urinary inflammatory markers with markers of renal injury (proteinuria and albuminuria) and renal function (eGFR).

Because of imbalances in demographic characteristics among the study groups, we also adjusted for race, sex, age, and smoking status to further assess if the inflammatory markers in the HIV-infected groups were significantly different from the uninfected controls. The differences seen in pairwise group comparisons for the serum biomarkers were confirmed in these multivariable analyses, as shown in Table 2. After multivariable adjustment, significantly higher urinary levels of IL-8, RANTES, and MCP-1 (in addition to urinary IP-10 and B2M) were now found in Group B compared to Group D. As such, it appears that untreated HIV-infected patients with lower CD4 cell counts have greater levels of renal inflammation, as assessed using all five urine bio-

markers in the current study, compared to healthy controls. This may reflect greater overall inflammation and, in particular, intrarenal immune activation<sup>18</sup> found in those with greater immunosuppression. Previous studies have suggested that HIV infection is associated with tubular or glomerular defects, therefore the urinary excretion of various proteins, including B2M, might be increased.<sup>19-21</sup> Surprisingly, the urinary levels of IP-10 and B2M were higher in healthy volunteers than in HIV-infected patients virally suppressed with TDF/FTC/EFV. Previous observational studies suggest that the urinary B2M level would be increased in patients receiving tenofovir-based regimens due to tenofovir-induced tubulopathy.<sup>20</sup> It is possible that some unmeasured confounding led to these unexpected results.

As shown in Table 3, urine IP-10 and B2M levels (but not IL-8, RANTES, or MCP-1) were significantly correlated with their serum counterparts in the entire study cohort, with paired IP-10 levels strongly correlated, in particular, in the untreated HIV-infected Groups A and B. The reasons for these findings are unclear. All five biomarkers studied here are low-molecular-weight proteins with similar weights ranging

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TABLE 3. CORRELATIONS BETWEEN PAIRED SERUM AND URINE INFLAMMATORY MARKERS IN EACH STUDY GROUP AND OVERALL

Inflammatory marker	HIV-infected			HIV-uninfected	
	A (n=26)	B (n=19)	C (n=30)	D (n=45)	Overall (n=120)
IL-8	0.02 (0.9102)	0.26 (0.2761)	0.05 (0.7945)	0.06 (0.6974)	0.09 (0.3559)
RANTES	0.3 (0.1312)	0.28 (0.2374)	0.12 (0.5486)	0.28 (0.0681)	0.17 (0.0691)
MCP-1	<b>-0.6 (0.0013)</b>	0.33 (0.1726)	0.03 (0.8833)	0.21 (0.166)	0.09 (0.3408)
IP-10	<b>0.45 (0.0213)</b>	<b>0.78 (0.0001)</b>	0.24 (0.2121)	0.07 (0.6518)	<b>0.54 (&lt;0.0001)</b>
B2M	-0.19 (0.3533)	0.25 (0.3094)	0.06 (0.7781)	-0.26 (0.0936)	<b>0.44 (&lt;0.0001)</b>

Note: Data presented as correlation coefficient ( $p$ -value). Bolded data are those with  $p$ -values  $< 0.05$ . Group A, HIV-infected, antiretroviral-naive, CD4 cell counts  $> 350/\mu\text{l}$ ; Group B, HIV-infected, antiretroviral-naive, initiating antiretroviral therapy; Group C, HIV-infected, virologically suppressed on tenofovir/emtricitabine/efavirenz; Group D, HIV-uninfected, healthy controls.

IL-8, interleukin-8; RANTES, regulated on activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon- $\gamma$ -induced protein 10; B2M,  $\beta_2$ -microglobulin.



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TABLE 4. CORRELATIONS BETWEEN INFLAMMATORY MAKERS AND RENAL PARAMETERS IN THE OVERALL GROUP (N = 120)

Inflammatory marker	uPCR	uACR	Estimated GFR
Urine IL-8/Cr	<b>0.31 (0.0176)</b>	<b>0.33 (0.0073)</b>	0.06 (1)
Urine RANTES/Cr	0.27 (0.0652)	0.27 (0.0669)	-0.03 (1)
Urine MCP-1/Cr	<b>0.42 (&lt;0.0001)</b>	0.26 (0.0796)	-0.04 (1)
Urine IP-10/Cr	<b>0.31 (0.0205)</b>	0.23 (0.2466)	-0.19 (0.4835)
Urine B2M/Cr	0.18 (0.6288)	<b>0.41 (&lt;0.0001)</b>	-0.09 (1)
Serum IL-8 (pg/ml)	0.22 (0.307)	0.14 (1)	-0.09 (1)
Serum RANTES (pg/ml)	0.12 (1)	0.06 (1)	0.12 (1)
Serum MCP-1 (pg/ml)	0.19 (0.4519)	0.12 (1)	<b>-0.49 (&lt;0.0001)</b>
Serum IP-10 (pg/ml)	<b>0.39 (&lt;0.0003)</b>	<b>0.32 (0.0092)</b>	-0.21 (0.3186)
Serum B2M (mcg/ml)	0.25 (0.1208)	<b>0.3 (0.0205)</b>	<b>-0.34 (0.0065)</b>

Note: Data presented as correlation coefficient (*p*-value). Bolded data are those with *p*-values < 0.05 after adjustment for multiple testing.

Cr, creatinine; GFR, glomerular filtration rate; IL-8, interleukin-8; RANTES, regulated on activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1; IP-10, interferon- $\gamma$ -induced protein 10; B2M,  $\beta_2$ -microglobulin; uPCR, urine protein to creatinine ratio; uACR; urine albumin to creatinine ratio.

from ~8 kDa to 13 kDa. So if glomerular filtration of their respective circulating serum levels was responsible for the correlations between serum and urine levels, then we would have expected significant correlations between paired serum and urine levels with all five biomarkers. The absence of correlations for some of the biomarkers suggests that there may be compartmentalization between the circulating and intrarenal markers of inflammation studied here.

The most clinically relevant analyses were those focused on the correlations between inflammatory biomarkers and markers of renal disease. In the overall cohort, we found that serum IP-10 levels and urinary levels of IL-8, MCP-1, and IP-10 were each significantly correlated with proteinuria. In contrast, urinary IL-8 and B2M and serum B2M and IP-10 were significantly correlated with albuminuria in the overall cohort. Given that proteinuria in HIV is mostly comprised of nonalbuminuric globulin proteins,<sup>15,22</sup> which could reflect greater systemic immune activation or tubulopathy, whereas albuminuria primarily reflects glomerular disease, we expected to find different correlation patterns between these renal injury markers and the inflammatory markers assessed. IL-8 levels are associated with greater neutrophil migration and T cell activation but do not reflect monocyte activation. MCP-1, in contrast, reflects greater monocyte and T cell activation and migration.

IP-10 is an interferon-induced protein that signals monocyte and T cell migration. Interestingly, interferons may induce a renal focal segmental glomerulosclerosis that is histologically similar to HIV-associated nephropathy (HIVAN).<sup>23</sup> In addition, the apolipoprotein L1 (ApoL1) gene, whose polymorphisms predispose to HIVAN,<sup>24,25</sup> itself may be induced by interferon- $\gamma$ .<sup>26</sup> Thus, IP-10 may have particular significance as a marker of HIV-related renal diseases. B2M

is a general immune activation marker. In contrast to our pilot study, RANTES, a marker of T cell activation and migration, was not associated with either proteinuria or albuminuria in the current analyses. Thus, our results support both monocyte and neutrophil signals of activation and migration as potential contributors to HIV-related proteinuria and albuminuria. However, these findings should be interpreted cautiously. Of note, the only within group correlation in relation to renal injury was found between urinary B2M and uACR in Group A; we do not have an obvious reason for the lack of a similar correlation in the Group B HIV-infected participants with greater immunodeficiency.

We also noted in the overall cohort that urinary inflammation markers were not associated with renal function, although serum MCP-1 and B2M were statistically significantly and negatively correlated with eGFR. However, the relationship between MCP-1 and eGFR in the overall cohort may have been driven solely by the uninfected controls (Group D) and thus were not pertinent to HIV-infected patients. It should be noted that the significant correlations found had rho values < 0.5, suggesting that the contribution of inflammation, as measured using the markers in this study, to renal disease in HIV may be modest. Our study is limited in that we did not have histological data in this study to assess whether the chemokines measured were directly related to an actual cellular influx of monocytes or neutrophils. Biopsy studies done previously from patients with HIVAN demonstrate a dense infiltrate of monocytes with increased production of MCP-1, RANTES, IL-8, and IP-10.<sup>18</sup> To our knowledge, similar histological data are lacking in patients on ART.

Additional limitations of our study should be noted. The sample sizes of the individual study groups may have been too small to detect significant differences between groups in some of the biomarker levels and to detect significant correlations between inflammatory biomarkers and renal disease markers within study groups. Although the proportions of participants with abnormal uPCR and uACR levels were similar to those reported previously, the absolute numbers of such participants were relatively small. The cross-sectional study design does not allow us to make inferences regarding causality between inflammation and nephropathy in HIV. The results of our study as they pertain to ART-treated HIV-infected participants (Group C) are limited only to those receiving tenofovir/emtricitabine/efavirenz; it is not known if the results would differ for other treatment regimens. We also studied only five biomarkers; future studies will need to assess whether HIV-related nephropathy may be associated with other markers of inflammation or immune activation. Finally, the imbalance in black race representation between the HIV-infected and uninfected study groups may have confounded the results.

### Conclusions

To our knowledge, this is the first study of its kind to assess intrarenal and systemic inflammatory markers in various groups of HIV-infected patients receiving and not receiving ART vs. uninfected controls. We found that intrarenal inflammation, as assessed in our study, is greater in untreated HIV-infected patients, especially those with low CD4 counts, when compared to both HIV-infected patients receiving

virologically suppressive ART and to uninfected controls. Proteinuria and albuminuria in the overall study cohort were variably associated with different inflammatory marker profiles, possibly reflecting different inflammatory pathways. However, we did not find strong relationships between inflammatory markers and renal markers in those with HIV, whether treated with ART or not. Future studies using anti-inflammatory therapies and/or antiretroviral therapy will be needed to determine whether renal inflammation is causally related to both HIVAN as well as the <sup>other</sup> various nephropathies found in those infected with HIV.

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AU1: Disclosure statement accurate? If not, amend as needed.

*Yes.*