BRIEF REPORT

Neither Proteinuria Nor Albuminuria Is Associated With Endothelial Dysfunction in HIV-Infected Patients Without Diabetes or Hypertension

Samir K. Gupta,[1] Changyu Shen,[2] Kieren J. Mather,[3] Rajiv Agarwal,[4] and Michael P. Dubé[1,a]

[1]Division of Infectious Diseases, [2]Division of Biostatistics, [3]Division of Endocrinology and Metabolism, and [4]Division of Nephrology, Indiana University School of Medicine, Indianapolis

It is unknown whether systemic endothelial dysfunction underlies the association between nephropathy and cardiovascular disease (CVD) in persons infected with human immunodeficiency virus (HIV). Spot urine protein to creatinine ratio, spot urine albumin to creatinine ratio, creatinine clearance, estimated glomerular filtration rate, and flow-mediated dilation (FMD) of the brachial artery were evaluated in 123 study participants infected with HIV (58 receiving antiretroviral therapy [ART] and 65 not receiving ART) with no history of diabetes or hypertension. None of the renal markers, modeled as either continuous or categorical variables, correlated with FMD. Contrary to expectations, endothelial dysfunction may not be the link between nephropathy and CVD in HIV.

Proteinuria and albuminuria, which are markers of renal glomerular dysfunction, are well-known predictors of cardiovascular disease (CVD) in the general population [1]. The development of systemic endothelial dysfunction, especially when measured by flow-mediated dilation (FMD) of the brachial artery, predicts future cardiovascular events [2]. Thus, systemic endothelial dysfunction has been suggested as the mechanistic link between glomerular diseases and CVDs [3].

Proteinuria, albuminuria, and endothelial dysfunction are commonly found in the human immunodeficiency virus (HIV)–infected population [4, 5]. Given that proteinuria has recently been found to be associated with CVD in individuals infected with HIV [6], it is plausible that these markers of glomerular injury may be associated with systemic endothelial dysfunction in this population. However, in a previously published pilot study of 34 subjects performed by our group, we did not find that either proteinuria or albuminuria was associated with FMD in HIV-infected patients [7]. Given the small size of this initial study, it was important to corroborate these findings in a larger study that included both patients receiving and not receiving antiretroviral therapy (ART). As a secondary objective, we also evaluated the relationships between renal function, as measured by estimated creatinine clearance rate (CrCl) and by estimated glomerular filtration rate (eGFR), and systemic endothelial dysfunction. Because our aim was to assess the relationships between markers of HIV-related glomerular injury and endothelial dysfunction, we excluded from these studies patients with diabetes or hypertension as potential confounding conditions.

Methods

Study Design and Subject Population. We performed a cross-sectional analysis of a convenience sample of 123 HIV-infected patients referred to our research clinic for participation in various endothelial function studies, including those from our previously published pilot study [7]. We enhanced inclusion of those not yet on ART by performing their study procedures just before ART was initiated by their providers. The choice of ART medications was selected by individual practitioners in routine clinical care. These studies were approved by the Indiana University Institutional Review Board, and all volunteers provided written informed consent.

Participants were at least 18 years of age with documented HIV infection. Major exclusion criteria included fasting serum glucose >126 mg/dL at screening or known history of diabetes mellitus, active untreated infection (opportunistic or otherwise), known CVD, known hypertension or uncontrolled elevated blood pressure, current use of lipid-lowering medications or chronic systemic glucocorticoids, fever during the study visits, and pregnancy.

Study Procedures. The participants were studied as outpatients at the Indiana Clinical Research Center after a fast of at least 8 hours. Fasting blood samples were obtained for HIV

Received 4 June 2011; accepted 23 August 2011.

[a]Present Affiliation: Division of Infectious Diseases, Keck School of Medicine, University of Southern California, Los Angeles

Presented in part: 16th Conference on Retroviruses and Opportunistic Infections (CROI 2009), Montreal, Canada, 8–11 February 2009. Abstract 746.

Correspondence: Samir K. Gupta, MD, Division of Infectious Diseases, Indiana University School of Medicine, Emerson Hall, Suite 421, 545 Barnhill Dr, Indianapolis, IN 46202 (sgupta1@iupui.edu).

The Journal of Infectious Diseases 2011;204:1946–50

0022-1899 (print)/1537-6613 (online)/2011/20412-0018$14.00
DOI: 10.1093/infdis/jir668

disease measures (CD4 cell count and HIV-1 RNA level), serum creatinine, and metabolic variables. Urine was obtained for measurement of albumin, protein, and creatinine. Both blood and urine samples were collected on the morning of the day of ultrasound imaging.

Urine protein and urine creatinine were measured using a spectrophotometric assay (Beckman Coulter) and using the Jaffe kinetic reaction with picric acid (Roche Diagnostics), respectively. Urine albumin was measured using an immunoprecipitin reaction (reagents from DiaSorin). Serum creatinine was measured using the Jaffe kinetic reaction but was not calibrated to the Cleveland Clinic standard. Renal function was estimated using both the Cockcroft-Gault and the 4-variable Modification of Diet in Renal Disease equations. Urine protein-creatinine ratios and albumin-creatinine ratios were then calculated, with a protein-creatinine ratio >0.2 g/g and albumin-creatinine ratio >30 mg/g considered abnormal. Insulin resistance was estimated using the homeostasis model assessment of insulin resistance.

FMD and nitroglycerin-mediated dilation of the brachial artery were performed according to established guidelines [8]. All ultrasound procedures were performed by a single registered vascular technician, and all vascular measurements were made by a single investigator (SKG) using AccessPoint 2004 software (Freeland Systems). The intraclass correlations for reproducibility for baseline diameter and FMD measured twice in 12 healthy volunteers in our laboratory under these conditions were 0.97 and 0.73, respectively.

Statistical Analysis. Continuous variables are presented as median (interquartile range) unless otherwise specified, and categorical variables are presented as frequencies and percentages. Baseline characteristics between those receiving and not receiving ART were compared using Wilcoxon rank-sum test or by χ^2 test for continuous and categorical data, respectively. Correlations were evaluated by Spearman rank correlation coefficient. The detectable correlation between albuminuria and FMD in our convenience sample of 123 subjects at 80% power and a 5% type I error was 0.25.

Multiple linear regressions were constructed with FMD as the dependent variable and proteinuria, albuminuria, and glomerular function as independent variables; other potential confounding variables found to be associated with FMD in univariable analyses with a P value <.05 were included in these models. Two-sided P values <.05 were considered statistically significant. All analyses were performed using SAS version 9.2 (SAS Software).

Results

Cohort Characteristics. A total of 123 study participants, of whom 58 were receiving ART and 65 were not receiving ART at the time of the study visit, were included in these analyses and are described in Table 1. The percentages of patients with spot urine protein-creatinine ratios >0.2 g/g in the total cohort, in those receiving ART, and in those not receiving ART were 11%, 9%, and 13%, respectively. The percentages of those with spot urine albumin-creatinine ratios > 30 mg/g in the total cohort, in those receiving ART, and in those not receiving ART were 12%, 12%, and 11%, respectively. The percentages of participants in the entire cohort with albumin-creatinine ratios ranging from 0 to 4.9 mg/g, 5–9.9 mg/g, 10–19.9 mg/g, and 20–29.9 mg/g were 58%, 21%, 6%, and 4%, respectively. Only 3 study participants had eGFR <60 mL/min/1.73^2.

Associations Between Renal Parameters and Endothelial Function. As shown in Table 2, we assessed the univariable correlations between urine protein-creatinine ratio, urine albumin-creatinine ratio, CrCl, eGFR, and the other clinical variables listed in Table 1 with FMD in the overall cohort and in the subgroups receiving and not receiving ART. None of the 4 renal parameters of interest were associated with FMD in univariable analyses when assessed as either continuous or categorical variables (CrCl and eGFR were not assessed as categorical variables due to the small number of study participants with lower renal function). The relationships between FMD and body mass index (β = .096; P = .16) and current smoking (β = −.10; P = .9) were also not significant; none of the other clinical variables assessed in Table 1 were significantly associated with FMD.

We then constructed 4 multivariable regression models with each of the 4 renal parameters as the primary independent variable and FMD as the dependent variable, adjusting for baseline diameter, male sex, and nitroglycerin-mediated dilation. Again, none of the 4 renal parameters were associated with FMD. In these models, only baseline diameter was independently associated with FMD. Additional models with protein-creatinine ratio and albumin-creatinine ratio defined categorically as >0.2 g/g and >30 mg/g, respectively, again did not show that proteinuria or albuminuria was associated with FMD.

Associations Between Antiretrovirals and Endothelial Function. We also assessed the relationships between FMD and exposure to specific antiretrovirals and antiretroviral combinations because these may be potential confounding variables for the primary relationships of interest, namely, those between renal markers and FMD. We compared participants receiving tenofovir (n = 30) against those not receiving any ART and against those not receiving tenofovir. Similar comparisons were performed with participants receiving abacavir (n = 11), tenofovir with efavirenz (n = 8), abacavir with efavirenz (n = 3), tenofovir with protease inhibitors (n = 20), and abacavir with protease inhibitors (n = 7). None of these antiretrovirals or combinations were associated with FMD in these comparisons (data not shown; all P > .2). In particular, the regression coefficient for abacavir with FMD was not significant (β = −0.07; P = .96). Thus, we did not include specific ART in the multivariable models assessing the relationships between renal markers and FMD.

Table 1. Characteristics of the Overall Study Cohort by Antiretroviral Therapy Status[a]

Variable	Total (N = 123)	Receiving ART (n = 58)	Not receiving ART (n = 65)	*P* value
Age, years	40.2 (34.7–46.2)	42.6 (37.4–47.5)	38.9 (29.5–44.7)	.008
Male sex, no. (%)	93 (76)	40 (69)	53 (83)	.09
Black race, no. (%)	47 (39)	22 (38)	25 (39)	1.00
Current smoker, no. (%)	60 (53)	23 (41)	31 (53)	.20
Systolic blood pressure, mm Hg	117 (109–127)	118 (108–126)	117 (110–129)	.66
Body mass index, kg/m^2	25.1 (22.1–28.4)	24.8 (21.7–28.8)	25.1 (22.1–27.9)	.80
CD4 cell count/μL	428 (280–708)	469 (362–770)	385 (257–623)	.02
HIV-1 RNA level <400 copies/mL, no. (%)	55 (47)	46 (84)	9 (14)	<.001
HOMA-IR	2.36 (1.76–3.29)	2.29 (1.59–3.21)	2.45 (1.93–3.54)	.21
Serum triglycerides, mg/dL	107 (79–177)	112 (82–179)	101 (79–170)	.39
Serum total cholesterol, mg/dL	164 (145–196)	177 (160–221)	155 (123–179)	<.0001
Serum HDL cholesterol, mg/dL	43 (34–52)	48 (35, 57)	40 (29, 47)	.008
Serum LDL cholesterol, mg/dL	97 (81–121)	103 (90–131)	93 (64–111)	.009
Serum non-HDL cholesterol, mg/dL	121 (104–145)	138 (111–158)	115 (81–139)	.001
Serum creatinine, mg/dL	0.90 (0.80–1.00)	0.80 (0.70–1.00)	0.90 (0.80–1.00)	.15
Creatinine clearance, mL/min	118.0 (95.6–138.8)	117.0 (92.5–139.0)	118.0 (99.0–138.5)	.70
Creatinine clearance, <60 mL/min, no. (%)	3 (3)	2 (4)	1 (2)	1.00
Estimated glomerular filtration rate, mL/min/1.73^2	102.3 (90.3–119.5)	102.3 (87.0–119.4)	102.3 (93.2–119.6)	.50
Estimated glomerular filtration rate, <60 mL/min/1.73^2	3 (3)	1 (2)	2 (4)	.60
Urine protein-creatinine ratio, g/g	0.076 (0.055–0.114)	0.078 (0.056–0.123)	0.075 (0.050–0.096)	.62
Urine protein-creatinine ratio, >0.2 g/g	12 (11)	5 (9)	7 (13)	.55
Urine albumin-creatinine ratio, mg/g	3.7 (2.3–8.2)	3.8 (2.2–8.7)	3.7 (2.3–7.3)	.83
Urine albumin-creatinine ratio, >30 mg/g	13 (12)	7 (12)	6 (11)	1.00
Baseline brachial artery diameter, cm	0.40 (0.36–0.45)	0.40 (0.33–0.45)	0.41 (0.37–0.45)	.39
Flow-mediated dilation, %	5.04 (2.85–7.20)	5.47 (2.82–7.20)	4.87 (2.89–7.20)	.65
Nitroglycerin-mediated dilation, %	15.76 (12.42–22.27)	15.00 (11.68–21.03)	16.16 (13.34–23.66)	.23

Abbreviations: ART, antiretroviral therapy; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein.

[a] Data are presented as median (interquartile range) unless otherwise specified. *P* values are for the comparisons between those receiving and not receiving ART.

Discussion

In this large study of HIV-infected individuals, we found no associations between markers of renal disease and systemic endothelial function despite having the power to detect a low proportion of variability (R^2) of albuminuria attributable to FMD of 0.0625. Smaller magnitude correlations are likely not

Table 2. Univariable Regression Coefficients (Standard Errors) of Renal Parameters and Other Clinical Variables for Flow-Mediated Dilution in the Overall Study Cohort and in Subgroups Receiving and Not Receiving Antiretroviral Therapy

	Total cohort		Receiving ART		Not receiving ART	
Variable	FMD β (SE)	*P* value	FMD β (SE)	*P* value	FMD β (SE)	*P* value
Baseline diameter, cm	−23.59 (5.31)	<.0001	−19.65 (7.22)	.009	−28.06 (7.97)	.0009
Male sex	−2.52 (0.81)	.002	−2.92 (1.01)	.006	−1.97 (1.35)	.15
NTGMD	0.14 (0.05)	.004	0.13 (0.07)	.05	0.14 (0.06)	.02
Urine PCR	−0.92 (0.97)	.34	−0.74 (1.26)	.56	−1.15 (1.53)	.45
Urine PCR >0.2 g/g	−2.11 (1.24)	.09	1.80 (1.97)	.36	−2.26 (1.37)	.18
Urine ACR	−1.40 (1.42)	.33	−0.84 (1.81)	.65	−2.23 (2.32)	.34
Urine ACR >30 mg/g	−0.48 (1.21)	.69	−1.50 (1.64)	.36	0.55 (1.79)	.76
CrCl, mL/min	0.001 (0.01)	.90	0.02 (0.01)	.20	−0.01 (0.01)	.32
eGFR, mL/min/1.73^2	−0.01 (0.01)	.36	−0.01 (0.01)	.58	−0.02 (0.02)	.48

Abbreviations: ACR, albumin-creatinine ratio; ART, antiretroviral therapy; CrCl, creatinine clearance; eGFR, estimated glomerular filtration rate; FMD, flow-mediated dilution; NTGMD, nitroglycerin-mediated dilution; PCR, protein-creatinine ratio; SE, standard error.

clinically relevant, and therefore our sample size was adequate to demonstrate the presence or absence of meaningful associations. These results corroborated our earlier findings [7] by showing that proteinuria and albuminuria are not associated with FMD of the brachial artery in nondiabetic, nonhypertensive persons infected with HIV. In addition, we found that CrCl and eGFR in this cohort with relatively preserved renal function were also not associated with FMD. More important, our study was large enough to evaluate sizable numbers of participants both receiving and not receiving ART and found that use or nonuse of ART did not lead to differential findings. Although this study was not sufficiently powered to detect associations between individual ART drugs and FMD, we found no evidence that the use of certain antiretrovirals or antiretroviral combinations were likely to be confounding variables in these analyses.

It has been hypothesized that global endothelial dysfunction would impair large conduit arteries, including brachial and coronary arteries, as well as microvascular arterial beds, such as renal glomeruli. Such a systemic derangement might explain the well-established associations between nephropathy and incident CVD [3]. Systemic endothelial dysfunction measured by FMD has been linked to markers of renal disease in some populations [9]. However, in an analysis of 2256 participants in the large Framingham Heart Study Offspring cohort, neither eGFR <60 mL/min/1.73^2 nor the presence of microalbuminuria was independently associated with lower FMD [10]. Thus, the current evaluation was warranted in subjects with HIV infection.

The renal characteristics of our study cohort are reflective of the general HIV population. The percentages of those with an albumin-creatinine ratio >30 mg/g (12%) and a protein-creatinine ratio >0.2 g/g (11%) in the current study are similar to those reported previously [5, 11]. The 3% of participants in our study with CrCl or eGFR <60 mL/min/1.73^2 is also similar to previous reports [12]. Thus, the lack of significant associations with endothelial function was likely not due to unusual renal characteristics of our study group. However, because the renal markers were measured only once, we cannot exclude the possibility of misclassification bias. We certainly cannot exclude the possibility that HIV-infected patients with more pronounced renal insufficiency would have more impaired endothelial function. We also acknowledge that markers of renal disease may be associated with endothelial dysfunction in HIV-infected patients who also have diabetes or hypertension. In addition, it is possible that small vessel or capillary dysfunction may be associated with renal disease markers even if larger conduit artery dysfunction, which is measured by FMD, is not.

Because systemic endothelial function does not appear to be associated with proteinuria or albuminuria in HIV, the positive relationships between nephropathy and CVD in HIV [6] may be due to alternative mechanisms. It is possible that these conditions are linked by HIV replication itself as suggested recently by findings in the Strategies for Management of Antiretroviral Therapy trial [13], in which untreated HIV infection was associated with higher rates of both end-stage renal failure and CVD events. In addition, we have previously shown that CD8 immune activation is associated with proteinuria in untreated HIV infection [14]. Because immune activation has also recently been linked with carotid artery lesions [15], it would be reasonable to speculate that immune activation could serve as an underlying mechanism between renal disease and CVD in HIV. Because this study did not include circulating markers of inflammation or endothelial dysfunction, such as interleukin-6, soluble vascular cell adhesion molecule 1, or D-dimer, further study is needed to investigate these possibilities to determine how best to reduce the rates of both renal disease and CVD in the HIV-infected population.

Notes

Acknowledgments. We thank Martha Greenwald, NP, for exemplary research assistance and Jeffrey Waltz, RVT, for performing the ultrasonography. We also acknowledge Pamela Volmer and Dr Kenneth Ryder for performing the quantitative urine studies. Finally, we thank the study participants for their generous time and effort.

Financial support. This work was supported by the National Heart, Lung, and Blood Institute (K23 HL073682 and R01HL072711) and the Indiana Clinical Translational Sciences Institute funded in part by the National Center for Research Resources (RR025761).

Potential conflicts of interest. S. K. G. has received grant funding from Janssen (Tibotec) Therapeutics, Merck & Co, and Gilead Sciences. K. J. M. has received grant funding from Merck & Co. M. P. D. has received grant funding from GlaxoSmithKline and consultancies from Serono. All other authors: no conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Chronic Kidney Disease Prognosis Consortium, Matsushita K, van der Velde M, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet **2010**; 375:2073–81.
2. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. Circulation **2009**; 120:502–9.
3. Stehouwer CD, Fischer HR, van Kuijk AW, Polak BC, Donker AJ. Endothelial dysfunction precedes development of microalbuminuria in IDDM. Diabetes **1995**; 44:561–4.
4. Solages A, Vita JA, Thornton DJ, et al. Endothelial function in HIV-infected persons. Clin Infect Dis **2006**; 42:1325–32.
5. Szczech LA, Grunfeld C, Scherzer R, et al. Microalbuminuria in HIV infection. AIDS **2007**; 21:1003–9.
6. Choi AI, Li Y, Deeks SG, Grunfeld C, Volberding PA, Shlipak MG. Association between kidney function and albuminuria with cardiovascular events in HIV-infected persons. Circulation **2010**; 121:651–8.
7. Gupta SK, Mather KJ, Agarwal R, Saha CK, Considine RV, Dube MP. Proteinuria and endothelial dysfunction in stable HIV-infected patients. A pilot study. J Acquir Immune Defic Syndr **2007**; 45:596–8.
8. Corretti MC, Anderson TJ, Benjamin EJ, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol **2002**; 39:257–65.

9. Jawa A, Nachimuthu S, Pendergrass M, Asnani S, Fonseca V. Impaired vascular reactivity in African-American patients with type 2 diabetes mellitus and microalbuminuria or proteinuria despite angiotensin-converting enzyme inhibitor therapy. J Clin Endocrinol Metab **2006**; 91:31–5.
10. Foster MC, Keyes MJ, Larson MG, et al. Relations of measures of endothelial function and kidney disease: the Framingham Heart Study. Am J Kidney Dis **2008**; 52:859–67.
11. Gupta SK, Smurzynski M, Franceschini N, Bosch RJ, Szczech LA, Kalayjian RC. The effects of HIV type-1 viral suppression and non-viral factors on quantitative proteinuria in the highly active antiretroviral therapy era. Antivir Ther **2009**; 14:543–9.
12. Mocroft A, Kirk O, Reiss P, et al. Estimated glomerular filtration rate, chronic kidney disease and antiretroviral drug use in HIV-positive patients. AIDS **2010**; 24:1667–78.
13. El-Sadr WM, Lundgren JD, Neaton JD, et al. CD4+ count-guided interruption of antiretroviral treatment. N Engl J Med **2006**; 355: 2283–96.
14. Gupta SK, Komarow L, Gulick RM, et al. Proteinuria, creatinine clearance, and immune activation in antiretroviral-naive HIV-infected subjects. J Infect Dis **2009**; 200:614–8.
15. Kaplan RC, Sinclair E, Landay AL, et al. T cell activation and senescence predict subclinical carotid artery disease in HIV-infected women. J Infect Dis **2011**; 203:452–63.