Complement Component 3 Is Associated with Metabolic Comorbidities in Older HIV-Positive Adults

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Abstract

Our objective was to evaluate the association of plasma inflammatory biomarkers with MetS in an older population of treated HIV-infected (HIV⁺) as compared to age-matched HIV-negative (HIV⁻) adults. This was done in a retrospective observational study. Plasma concentrations of complement component 3 (C3), cystatin C, fibroblast growth factor 1, interleukin 6, oxidized LDL, soluble RAGE, soluble CD163, soluble CD14, and osteopontin were measured in 79 HIV⁺ participants on combination antiretroviral treatment (cART) with a suppressed HIV viral load and 47 HIV⁻ participants with a median age of 59 (range 50 to 79). Outcomes were individual MetS components (hypertension, type II diabetes, dyslipidemia, and obesity) and MetS. Covariates were screened for inclusion in multivariable models. Odds ratios are reported per 50 mg/dl increase in C3. In the HIV⁺ group, higher C3 levels were associated with MetS (OR 3.19, $p=0.004$), obesity (OR 2.02, $p=0.01$), type II diabetes (OR 1.93, $p=0.02$), and at a trend level with dyslipidemia (OR 1.87, $p=0.07$) and hypertension (OR 1.66, $p=0.09$). C3 levels were significantly higher in HIV⁺ participants with MetS compared to those without MetS ($p=0.002$). C3 was higher among HIV⁺ patients with three or four MetS components as compared to those with one or two ($p=0.04$) and those with none ($p=0.002$). No associations were found between C3 and the outcomes for HIV⁻ participants. C3 is strongly associated with both MetS and MetS components in an older HIV⁺ sample on cART compared to HIV⁻ controls. C3 warrants further investigation as a marker of cardiometabolic risk among persons aging with HIV.

Introduction

DESPITE THE REDUCTION in HIV-related mortality with the use of combined antiretroviral therapy (cART), metabolic abnormalities in the treated HIV-positive (HIV⁺) population are increasing in prevalence.¹ Components of the metabolic syndrome (MetS), including dyslipidemia, hypertension, insulin resistance, and central obesity, have all been observed in this aging population, though the underlying causes of HIV-associated cardiometabolic disease have not been firmly established.^{2–4} Hypotheses include cART-related toxicities and persisting effects of HIV disease such as immune dysregulation and chronic low-level inflammation.^{3,5–7}

In this study we aimed to better understand the link between chronic inflammation and MetS in older, treated HIV⁺ individuals compared to age-matched HIV negative (HIV⁻)

controls. We measured nine inflammatory biomarkers chosen to reflect a broad range of inflammatory mechanisms in peripheral blood of 79 HIV⁺ and 47 HIV⁻ participants and analyzed their association with MetS and individual MetS component conditions (hypertension, type II diabetes, dyslipidemia, and obesity). We hypothesized that MetS and its components are associated with distinct inflammatory profiles in HIV⁺ versus HIV⁻ populations and that such biomarker profiles may be useful tools to assess cardiometabolic disease risk in the treated HIV⁺ population.

Materials and Methods

Participants

The present study included 79 HIV⁺ and 47 HIV⁻ community-dwelling adults from the California HIV/AIDS

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Research Program Successfully Aging Seniors with HIV (SASH) study administered by the UCSD HIV Neurobehavioral Research Program (HNRP). Inclusion criteria for SASH were an age of at least 50 years, receiving stable cART (for HIV⁺), and plasma HIV RNA less than 48 copies/ml (for HIV⁺). Study participants were recruited from ongoing studies being conducted at the HNRP focused on HIV and cognition, wherein participants with a known history of non-HIV-related neurologic disorders or any conditions known to be associated with impaired cognitive functioning were excluded (e.g., severe TBI). The study was approved by the UCSD Institutional Review Board and all participants provided written informed consent.

Biomarker quantification

All inflammatory biomarker assays were measured by immunoassay in duplicate in EDTA plasma derived from peripheral blood samples collected by routine phlebotomy. Commercial immunoassay suppliers were R&D Systems [soluble CD163 (sCD163), soluble CD14 (sCD14), soluble receptors for advanced glycation end-products (RAGE), and cystatin C; Minneapolis, MN], Immuno-Biological Laboratories-America (osteopontin; Minneapolis, MN) Meso Scale Discovery [interleukin (IL)-6, fibroblast growth factor (FGF)-1; Rockville, MD], Assaypro (complement C3; St. Charles, MO), and ALPCO (oxidized LDL; Salem, NH). Measurements were repeated if the coefficient of variation was greater than 20% or if the measurement was greater than four standard deviations from the mean. A total of at least 10% of samples in each assay was repeated to further assess batch consistency.

Outcomes

Primary outcomes were MetS, dyslipidemia, type II diabetes, hypertension, and obesity. Obesity was defined as a body mass index (BMI) greater than or equal to 30 kg/m². Dyslipidemia, type II diabetes, and hypertension were defined by the presence of both self-reported diagnosis and current, specific drug treatment for the condition. In a small number of patients, dyslipidemia, type II diabetes, and hypertension were defined by self-reported diagnosis only (hypertension: six patients; type II diabetes: five patients; dyslipidemia: seven patients). The primary definition of MetS was the International Diabetes Federation (IDF) 2006 definition, requiring increased central adiposity (or BMI greater than or equal to 30 kg/m²) plus at least two of the following factors: treatment for raised triglycerides or reduced HDL cholesterol, treatment of previously diagnosed hypertension, and previously diagnosed type II diabetes.⁸ Due to the prevalence of differing MetS definitions, we also tested our results using two versions of the Adult Treatment Panel (ATP) III criteria.⁹ The first, termed “standard ATP III,” used the ATP criteria for waist circumference (>102 cm for men, >88 cm for women, measured in a subset of 53 participants) and replaced the ATP quantitative criteria of raised triglycerides/low HDL cholesterol, blood pressure, and fasting glucose with our outcomes of dyslipidemia, hypertension, and type II diabetes, respectively. At least three of these four factors (waist circumference, dyslipidemia, hypertension, or type II diabetes) constituted a diagnosis of MetS. The second definition, termed “modified ATP III,”

was identical to Standard ATP III but replaced minimum waist circumference with our outcome of obesity.

Covariates

Candidate covariates for multivariable models predicting the outcomes included demographic factors, noncardiometabolic comorbidities, and cART variables for HIV⁺ participants. All multivariable logistic regression models predicting comorbidities included age, sex, ethnicity (white vs. non-white), smoking (ever and current), and cART drug classes for HIV⁺ participants [present exposure to protease inhibitors (PIs) or nonnucleoside reverse transcriptase inhibitors (NNRTIs)]. Other covariates were included if they showed an association with the comorbidity or biomarker at the $\alpha=0.10$ level. Screened covariates included AIDS diagnosis, estimated HIV infection duration, current cART regimen duration, duration of exposure to all cART drugs, self-reported nadir CD4⁺ T cell count, current CD4⁺ T cell count, lifetime history of drug or alcohol abuse, HCV serostatus, and chronic pulmonary disease.

Statistical analysis

For each biomarker, outliers greater than seven standard deviations above the mean were removed (one removed for cystatin C, one for IL-6). Correlations were performed with the nonparametric Spearman method. C3, IL-6, and cystatin C were log₁₀ transformed to reduce skewness for comparison across MetS and comorbidity burden groups (one-tailed *t*-test and one-way ANOVA with Tukey's post hoc test, respectively). Univariable and multivariable logistic regression on untransformed biomarker data were used to test the association of biomarkers with the outcomes (dyslipidemia, type II diabetes, hypertension, obesity, and MetS). Receiver operating characteristic (ROC) curve analysis was performed on untransformed biomarker data. Covariate screens were conducted with univariable logistic regression, Spearman correlation, or Fisher's exact test, as appropriate. Unit odds ratios are calculated for an increase of 50 mg/dl (C3), 1 pg/ml (IL-6), or 0.1 ng/ml (cystatin C). Comparison of demographic and biomarker data across HIV⁺ and HIV⁻ groups was performed with Fisher's exact test or two-tailed *t*-test, as appropriate. All statistical tests were performed with JMP 11.0.0 (SAS, 2013).

Results

Participants

HIV⁺ participants in this study were predominately men (68/79, 86%) and white (64/79, 81%) with a median age of 59 years (IQR = 9) (Table 1). The median estimated duration of HIV infection was 19.8 years, the median duration of exposure to all cART drugs was 133 months (11.1 years), and the mean nadir and current CD4⁺ T cell counts were 188 cells/mm³ and 639 cells/mm³, respectively (Table 2). HIV⁺ and HIV⁻ participants had a similar prevalence of lifetime substance abuse, and there was no difference in current or lifetime alcohol, cannabis, cocaine, methamphetamine, or opioid use between the groups ($p>0.05$ for all). Of the primary outcomes in the HIV⁺ group, obesity was observed in 16 (20.2%) participants, type II diabetes in 20 (25.3%), hypertension in 40 (50.6%), dyslipidemia in 48 (60.8%), and

TABLE 1. DEMOGRAPHICS AND BIOMARKERS

	HIV ⁺	HIV ⁻	p value
All subjects (N)	79	47	
Demographics and comorbidities			
Age, median (IQR, N)	59 (9, 79)	59 (10, 47)	0.34
Sex, male	68/79 (86%)	27/47 (57%)	<0.001
Ethnicity			
white	64/79 (81%)	30/47 (64%)	0.04
black	10/79 (12.7%)	11/47 (23%)	0.14
hispanic	2/79 (2.5%)	4/47 (9%)	0.19
other	3/79 (3.8%)	2/47 (4%)	0.99
Hepatitis C virus	19/79 (24.1%)	7/47 (15%)	0.26
Chronic pulmonary disease	19/79 (24.1%)	5/45 (9%)	0.09
Smoking, current	28/79 (35.4%)	13/47 (28%)	0.43
Smoking, ever	32/79 (40.5%)	19/47 (40%)	0.99
Lifetime substance abuse or dependence	54/78 (69.2%)	25/46 (54%)	0.12
Biomarkers			
Complement C3, mean (SD, N) (mg/dl)	118 (48, 79)	115 (56, 47)	0.43
Cystatin C, mean (SD, N) (ng/ml)	0.75 (0.18, 78)	0.64 (0.19, 47)	<0.001
FGF-1, mean (SD, N) (pg/ml)	7.42 (5.42, 79)	8.46 (7.20, 47)	0.48
IL-6, mean (SD, N) (pg/ml)	1.31 (1.32, 78)	1.05 (0.85, 47)	0.07
Oxidized LDL, mean (SD, N) (ng/ml)	71.5 (115, 58)	73.7 (151, 18)	0.51
RAGE, mean (SD, N) (pg/ml)	1372 (629, 78)	1230 (451, 47)	0.25
sCD163, mean (SD, N) (ng/ml)	1473 (1045, 79)	1146 (737, 45)	0.01
sCD14, mean (SD, N) (pg/ml)	2264 (751, 79)	1745 (627, 45)	<0.001
Osteopontin, mean (SD, N) (ng/ml)	408 (223, 79)	324 (168, 45)	0.05

FGF-1, fibroblast growth factor 1; RAGE, receptor for advanced glycation endproducts.

MetS in 10 (12.8%) (Table 3). Compared to HIV⁺ participants, the HIV⁻ population included fewer males (57% vs. 86%, $p<0.001$) and fewer cases of dyslipidemia (34% vs. 60.8%, $p=0.006$) but a similar prevalence of MetS ($p=0.79$). HIV⁺ participants had higher triglycerides (201 vs. 160 mg/dl, $p=0.05$), lower HDL cholesterol (46 vs. 57 mg/dl, $p=0.04$), and lower total cholesterol (180 vs. 197 mg/dl, $p=0.04$), but similar systolic BP, diastolic BP, BMI, waist circumference, random serum glucose, and LDL cholesterol compared to HIV⁻ participants ($p>0.05$ for all, Table 3). Compared to HIV⁺ participants, HIV⁻ participants showed lower plasma cystatin C ($p<0.001$), lower plasma sCD163 ($p=0.01$), and lower sCD14 ($p<0.001$) but similar C3 ($p=0.43$) (Table 1)

Correlations

Correlations were performed between C3 and BMI, systolic BP, diastolic BP, waist circumference, total cholesterol, LDL, HDL, triglycerides, and random plasma glucose. In the HIV⁺ group, C3 was correlated with total cholesterol ($r=0.36$, 95% CI 0.15–0.54, $p=0.001$), LDL cholesterol ($r=0.30$, 95% CI 0.07–0.50, $p=0.01$), and at a trend level with glucose ($r=0.21$, 95% CI –0.01–0.42, $p=0.06$) and triglycerides ($r=0.19$, 95% CI –0.03–0.41, $p=0.08$) but not systolic BP, diastolic BP, or waist circumference ($p>0.05$). In the HIV⁻ group, C3 was correlated with BMI ($r=0.32$, 95% CI 0.03–0.56, $p=0.03$) and triglycerides ($r=0.37$, 95% CI 0.08–0.60, $p=0.01$) but not with systolic BP, diastolic BP, waist circumference, total cholesterol, LDL, HDL, or random plasma glucose ($p>0.05$ for all).

Finally, correlations were performed among inflammatory biomarkers in the HIV⁺ group. Statistically significant correlations were found between sCD14 and either IL-6 ($r=0.11$, $p=0.04$) or OPN ($r=0.30$, $p=0.03$), and between cystatin C and either RAGE ($r=0.19$, $p=0.02$), sCD14 ($r=0.15$, $p=0.045$), or OPN ($r=0.18$, $p=0.03$).

Univariable analyses

For each biomarker, univariable logistic regression was used to determine the association with MetS, hypertension, dyslipidemia, type II diabetes, and obesity. In the HIV⁺ group, a higher C3 was associated with type II diabetes (OR 1.72, 95% CI 1.03–2.97, $p=0.04$), dyslipidemia (OR 2.02, 95% CI 1.17–3.86, $p=0.02$), obesity (OR 2.00, 95% CI 1.16–3.61, $p=0.02$), and MetS (OR 2.79, 95% CI 1.47–5.92, $p=0.003$). In addition, a higher IL-6 was associated with MetS (OR 1.61, 95% CI 1.07–2.72, $p=0.04$) and showed a

TABLE 2. HIV⁺ GROUP CHARACTERISTICS

HIV disease factors	
AIDS	53/79 (67%)
Estimated duration of HIV infection, median (IQR, N) (years)	19.8 (13.2, 78)
Duration of current cART regimen, median (IQR, N) (months)	33.1 (50.0, 78)
Duration of exposure to all cART drugs, median (IQR, N) (months)	133 (123, 78)
Nadir CD4 ⁺ T cell count, mean (SD, N) (cells/mm ³)	188 (191, 78)
Current CD4 ⁺ T cell count, mean (SD, N) (cells/mm ³)	639 (339, 76)
cART regimen	
Protease inhibitor	41/79 (51.9%)
NNRTI	39/79 (49.3%)

NNRTI, nonnucleoside reverse transcriptase inhibitor.

TABLE 3. CARDIOMETABOLIC OUTCOMES AND CONTINUOUS MEASURES

	<i>HIV</i> ⁺	<i>HIV</i> ⁻	<i>p</i> value
Primary outcomes			
Metabolic syndrome	10/78 (12.8%)	7/45 (15.5%)	0.79
Obesity	16/78 (20.5%)	13/45 (29%)	0.38
Type II diabetes	20/79 (25.3%)	8/47 (17%)	0.38
Hypertension	40/79 (50.6%)	20/47 (43%)	0.46
Dyslipidemia	48/79 (60.8%)	16/47 (34%)	0.006
Cardiometabolic measures			
Systolic BP, mean (SD) (mmHg)	133 (21)	136 (20)	0.6
Diastolic BP, mean (SD) (mmHg)	76 (10)	77 (13)	0.5
BMI, mean (SD) (kg/m ²)	27 (5.6)	28 (5.0)	0.7
Waist circumference, mean (SD) (cm)	101 (15)	99 (19)	0.7
Random serum glucose, mean (SD) (mg/dl)	109 (45)	112 (54)	0.7
Total cholesterol, mean (SD) (mg/dl)	180 (37)	197 (46)	0.04
HDL cholesterol, mean (SD) (mg/dl)	46 (17)	57 (24)	0.007
LDL cholesterol, mean (SD) (mg/dl)	96 (31)	108 (38)	0.08
Triglycerides, mean (SD) (mg/dl)	201 (121)	160 (84)	0.05

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

trend-level association with hypertension (OR 1.79, 95% CI 1.08–3.69, $p=0.06$) and obesity (OR 1.49, 95% CI 1.02–2.44, $p=0.06$). Other biomarkers were not associated with any of the five outcomes. In the *HIV*⁻ group, C3 was not associated with diabetes, hypertension, dyslipidemia, obesity, or MetS (IDF 2006) ($p>0.05$ for all) and higher IL-6 was associated with obesity (OR 2.61, 1.18–8.28, $p=0.04$).

IL-6 and C3 were each higher in *HIV*⁺ participants with MetS compared to those without ($\log_{10}$ IL-6: 0.26 vs. -0.034,

Cohen's $d=1.13$, $p=0.005$; $\log_{10}$ C3: 0.19 vs. 0.019, Cohen's $d=1.08$, $p=0.008$) (Fig. 1). *HIV*⁺ participants were divided into three groups based on the number of comorbid metabolic conditions (dyslipidemia, type II diabetes, hypertension, or obesity): no metabolic comorbidities (14 participants), one or two comorbidities (48), and three or four comorbidities (16). C3 values differed between groups (overall $p=0.004$) and increased with the number of comorbidities ($\log_{10}$ C3: 0: -0.045; 1–2: 0.036; 3–4: 0.15) (Fig. 1). Pairwise comparison

FIG. 1. Comparison of C3 among metabolic syndrome (MetS) and comorbidity groups. (A) Comparison of $\log_{10}$ C3 values among *HIV*⁺ participants using three clinical definitions of MetS. $\log_{10}$ C3 values are higher in MetS cases with each definition (IDF 2006: $p=0.008$; Standard ATP III: $p=0.048$; Modified ATP III: $p=0.009$; one-tailed t -test). (B) Comparison of $\log_{10}$ C3 values for MetS versus no MetS in *HIV*⁻ participants. No differences were identified ($p>0.05$ for all comparisons). (C) Comparison of $\log_{10}$ C3 values across *HIV*⁺ participants with 0, 1–2, or 3–4 MetS component comorbidities (hypertension, dyslipidemia, obesity, type II diabetes). Individuals with 3–4 comorbidities showed higher $\log_{10}$ C3 than those with either 1–2 ($p=0.039$) or 0 ($p=0.002$) comorbidities (one-way ANOVA followed by Tukey's post hoc test). (D) Comparison of $\log_{10}$ C3 values across *HIV*⁻ participants with 0, 1–2, or 3–4 MetS component comorbidities. No differences were found ($p>0.05$ for all comparisons). * $p<0.05$; ** $p<0.01$.

identified differences between those with three or four comorbidities and either the reference group (no comorbidities, $p=0.002$) or those with one or two comorbidities ($p=0.04$). IL-6 values tended to differ between groups ($p=0.06$) and pairwise comparison reached a trend level (three or four comorbidities vs. no comorbidities: $p=0.07$; three or four comorbidities vs. one or two comorbidities: $p=0.09$). In the HIV⁻ group, C3 levels showed no difference between MetS versus no MetS by any definition ($p>0.05$) and did not differ between comorbidity burden groups ($p>0.05$; Fig. 1).

HIV⁺: multivariable analyses

The associations of IL-6 and C3 with the outcomes among HIV⁺ participants were further investigated in multivariable models adjusting for age, sex, ethnicity, cART drug classes, smoking, and additional covariates that passed the univariable screen (see Materials and Methods). Among screened covariates, current CD4⁺ T cell count ($p=0.046$) was associated with IL-6 and no associations were found for C3. For outcomes, both nadir ($p=0.03$) and current ($p=0.005$) CD4⁺ T cell counts were associated with hypertension. Diagnosis of AIDS ($p=0.06$), HCV serostatus ($p=0.07$), and total duration of exposure to cART drugs ($p=0.06$) were associated with dyslipidemia. None of the screened covariates was associated with type II diabetes, obesity, or MetS.

In the multivariable models C3 remained significantly associated with MetS (OR 3.19, 95% CI 1.54–7.98, $p=0.004$), type II diabetes (OR 1.93, 95% CI 1.1–3.57, $p=0.02$), obesity (OR 2.02, 95% CI 1.14–3.75, $p=0.01$), and at a trend level with dyslipidemia (OR 1.87, 95% CI 0.98–4.03, $p=0.07$) and hypertension (OR 1.66, 95% CI 0.94–129, $p=0.09$) (Table 4). In the model for dyslipidemia, the outcome was associated with white ethnicity (OR 10.7, 95% CI 2.39–68, $p=0.004$), PI use (OR 8.01, 95% CI 1.24–81, $p=0.04$), and NNRTI use (OR 8.01, 95% CI 1.28–83, $p=0.04$). In separate models, IL-6 was associated at a trend level with MetS (OR 1.42, 95% CI 0.93–2.24, $p=0.09$) and obesity (OR 1.49, 95% CI 0.98–2.55, $p=0.09$) but not with hypertension ($p=0.14$). ROC curve analysis identified a threshold C3 concentration of 163.5 mg/dl for diagnosis of MetS (AUC=0.71, 95% CI 0.54–0.88, $p=0.02$) with a sensitivity of 57.1% and specificity of 90.6%.

Alternate MetS definitions

We tested the robustness of the association of C3 and MetS in HIV⁺ participants using two alternate definitions of MetS (see Materials and Methods). Using our Standard ATP III criteria in a subset of participants with waist circumference data ($n=53$, see Materials and Methods), C3 was associated with MetS in univariable analysis (OR 2.02, 95% CI 1.06–4.27, $p=0.04$) and at a trend level in multivariable analysis (OR 1.92, 95% CI 0.91–4.29, $p=0.08$). Using our Modified ATP III criteria, replacing minimum waist circumference with obesity ($n=78$, see Materials and Methods), C3 was associated with MetS in univariable analysis (OR 1.98 95% CI 1.04–4.11, $p=0.04$) and at a trend level in multivariable analyses (OR 2.06, 95% CI 0.99–4.66, $p=0.06$). Higher C3 concentrations were also associated with MetS (versus no MetS) by either Standard ATP III criteria ($p=0.048$) or Modified ATP III criteria ($p=0.009$) (Fig. 1).

HIV⁻: univariable and multivariable analyses

In the covariate screen for the HIV⁻ group, hypertension showed a marginal association with HCV infection ($p=0.09$). Univariable logistic regression in this group showed that higher cystatin C was associated with hypertension (OR 1.55, 95% CI 1.08–2.46, $p=0.03$), obesity (OR 1.66, 95% CI 1.14–2.69, $p=0.02$), and MetS (IDF 2006; OR 1.74, 95% CI 1.16–2.93, $p=0.01$). Higher RAGE was associated with lower odds of hypertension (OR 0.85, 95% CI 0.72–0.98, $p=0.04$), and higher sCD163 was associated with MetS (IDF 2006; OR 1.13, 95% CI 1.02–1.27, $p=0.02$).

In multivariable logistic regression, cystatin C remained associated with obesity (OR 1.81, 95% CI 1.19–3.21, $p=0.02$), MetS (IDF 2006; OR 1.89, 95% CI 1.18–3.49, $p=0.02$), and at a trend level with hypertension (OR 1.62, 95% CI 1.02–2.85, $p=0.06$). In the model for hypertension, HCV infection was associated with the outcome (OR = 13.8, 95% CI 1.48–223, $p=0.02$). Cystatin C was higher in HIV⁻ participants with MetS versus no MetS by all three definitions (IDF 2006: -0.01 vs. -0.23 , Cohen's $d=0.11$, $p=0.02$; Standard ATP III: -0.12 vs. -0.23 , Cohen's $d=0.16$, $p=0.02$; Modified ATP III: -0.15 vs. -0.26 , Cohen's $d=0.11$, $p=0.01$) (Fig. 2). Cystatin C increased with the number of metabolic comorbidities ($\log_{10}$ cystatin C, 0 comorbidities: -0.26 ; 1–2:

TABLE 4. RESULTS OF MULTIVARIABLE LOGISTIC REGRESSION MODELS FOR HIV⁺

Outcome	Covariate ^a	OR ^b	95% CI	p-value
Metabolic syndrome (IDF 2006)	C3	3.19	1.54–7.98	0.004
Type II diabetes	C3	1.93	1.10–3.57	0.02
	Age	1.12	1.01–1.26	0.02
Obesity	C3	2.02	1.14–3.75	0.01
Dyslipidemia	C3	1.87	0.98–4.03	0.07
	Ethnicity—white	10.7	2.39–68	0.004
	PI use	8.01	1.24–81	0.04
	NNRTI use	8.01	1.28–83	0.04
Hypertension ^c	C3	1.66	0.94–3.12	0.09

^aAll models included age, sex, ethnicity, cART drug classes, and smoking as covariates in addition to those that passed the covariate screen. Reported here are terms that reached significance ($p<0.05$).

^bOdds ratios reported per 50 mg/dl increase in C3 level and per year increase for age (see Materials and Methods).

^cNo terms reached significance in the model for hypertension.

FIG. 2. Comparison of cystatin C among MetS and comorbidity groups. (A) Comparison of $\log_{10}$ cystatin C values among HIV⁺ participants using three clinical definitions of MetS. $\log_{10}$ cystatin C values are higher in MetS cases with each definition (IDF 2006: $p=0.02$; Standard ATP III: $p=0.02$; Modified ATP III: $p=0.01$; one-tailed t -test). (B) Comparison of $\log_{10}$ cystatin C values across HIV⁺ participants with 0, 1–2, or 3–4 MetS component comorbidities. Individuals with 3–4 comorbidities showed higher $\log_{10}$ cystatin C than those with 0 ($p=0.002$) but not 1–2 comorbidities ($p>0.05$; one-way ANOVA followed by Tukey's post hoc test).

–0.02; 3–4: –0.12). Differences in cystatin C among comorbidity burden groups were significant overall ($p=0.007$) and pairwise comparison was significant for three to four comorbidities vs. 0 ($p=0.005$).

Discussion

In this study we found an association between higher plasma complement C3 levels and MetS, obesity, type II diabetes, and dyslipidemia in an older cohort of cART-treated HIV⁺ individuals. This association was present in univariable analysis and after adjustment for potential confounders including age, sex, ethnicity, and cART drug class, among others. The association was specific to the HIV⁺ group versus HIV[–] controls. We found limited evidence of similar associations in the HIV⁺ group for IL-6, which was associated with the composite MetS variable but not with individual MetS components. We found that the association between C3 and MetS in HIV⁺ individuals was robust to several definitions of MetS. In ROC curve analysis, a threshold C3 value of 163.5 mg/dl resulted in 90% specificity for the diagnosis of MetS among HIV⁺ participants. Finally, we performed similar analyses for the HIV[–] control group and found associations between cystatin C and multiple cardiometabolic outcomes.

C3 is the central component of the complement system, a primary mechanism of innate immunity. C3 is primarily produced by hepatocytes but is also produced by adipocytes as an adipokine; in the setting of obesity, higher C3 levels are associated with a low-level systemic inflammatory state and are hypothesized to contribute to the development of insulin resistance, though the exact causal mechanisms remain unclear.^{10–15} Higher C3 is also associated with nonalcoholic fatty liver disease, the primary liver manifestation of MetS, and may contribute to the impaired liver function and dyslipidemia observed in cardiometabolic disease.^{16,17} The complement system has been hypothesized to play a role in the development of MetS and may be involved in deranged postprandial lipid metabolism in this setting.^{18–20}

C3 may play a particularly strong role in HIV-associated cardiometabolic disease. In this study, C3 was higher in HIV⁺ patients with MetS but was not correlated with other inflammatory biomarkers, potentially indicating a unique role for C3 in this population. HIV both directly and indirectly activates complement pathways during active infection; however, the sequelae of virus-induced inflammation in the context of long-term treated HIV disease remain unclear.⁷ Chronic treated HIV disease is associated with immune dysregulation and ongoing low-level systemic inflammation, which may further increase the levels of proinflammatory cytokines, increase C3 production, and heighten subsequent risk for MetS. The etiology of this chronic inflammation is poorly understood but may include residual viral replication, coinfections, or leakage of bacteria from the gut.²⁶ The lipodystrophy syndrome associated with cART (fat redistribution from the periphery to primarily the abdomen and dorsocervical regions) has been proposed to cause an increase in proinflammatory adipokine production, including C3, that may underlie HIV-associated MetS, and increased visceral adiposity is highly predictive of cardiovascular complications across populations.^{4,21–23}

In this study, we found little evidence for a relationship between C3 and MetS or its components in our HIV[–] control group; instead, cystatin C was most strongly associated with the outcomes in this group. As our participants were older (median age 59 years), this may reflect the increasing cardiometabolic importance of cystatin C with advancing age in the HIV[–] population. Others have reported a link between higher cystatin C and the nexus of advanced age, cardiometabolic disease, and declining renal function in the general population.^{24,25}

We found that dyslipidemia was more prevalent in the HIV⁺ group while the prevalence of MetS and the other components (hypertension, obesity, hyperglycemia) did not differ among groups. This difference appears to be driven by higher average triglycerides and lower HDL cholesterol among HIV⁺ participants. In correlating C3 to these measures, we found associations with total cholesterol and LDL that were present in HIV⁺ but not HIV[–] individuals. In the HIV⁺ participants, C3 was not correlated with continuous measures underlying other components of MetS (blood pressure, BMI, waist circumference, plasma glucose). Finally, in the multivariable model for dyslipidemia in HIV⁺ individuals, PI use, NNRTI use, and white ethnicity were strongly associated with the outcome. Taken together, these data point to an interaction between HIV infection and/or cART, complement, and lipid regulation that could be partly responsible for driving MetS in this population.

Our study is subject to important limitations. Although we found a link between our inflammatory biomarkers and MetS using three MetS definitions, we did not use quantitative criteria (e.g., for low HDL) to categorize participants and relied instead on current drug treatment and self-reported diagnosis. Though the IDF criteria allow for replacing quantitative criteria in this way, the published ATP III definition does not (see Materials and Methods).^{8,9} A truly quantitative method of defining MetS and its component morbidities could reveal different associations with inflammatory biomarkers. Finally, our findings apply to a subset of patients whose HIV disease was well-controlled with an undetectable plasma viral load.

Findings from the present study point toward several lines of future investigation. C3 warrants additional prospective study as a marker of metabolic and cardiovascular disease risk in the aging, cART-treated HIV⁺ population. Moreover, data are needed to determine the precise role of C3 and potential interactions with latent virus, cART regimens or specific drugs, and lipid balance. Future studies will determine whether C3 plays a unique role in HIV-associated MetS compared to MetS in the general population and also compared to the other inflammatory molecules produced in HIV disease.

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D.J.M. designed the study. A.K.B. wrote the manuscript. S.L.L. and T.H.B. chose the biomarkers for assessment. P.L.F. conducted patient assessments and performed initial data screening. A.K.B. and S.L.L. performed the analysis. M.P. and T.H.B. performed biomarker assays. All authors contributed to the overall study conceptualization and provided critical revision of the manuscript.

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Author Disclosure Statement

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