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MAJOR ARTICLE

Association of Soluble Markers of Inflammation With Peri-coronary Artery Inflammation in People With and Without HIV Infection and Without Cardiovascular Disease

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Background. Inflammation is linked to elevated cardiovascular disease (CVD) risk in people with HIV (PWH) on antiretroviral therapy (ART). Fat attenuation index (FAI) is a measure of peri-coronary inflammation that independently predicts CVD risk in HIV-uninfected persons. Whether FAI is associated with soluble inflammatory markers is unknown.

Methods. Plasma levels of inflammatory markers were measured in 58 PWH and 16 controls without current symptoms or prior known CVD who underwent coronary computed tomography angiography and had FAI measurements. A cross-sectional analysis was performed, and associations of markers with FAI values of the right coronary artery (RCA) and left anterior descending artery (LAD) were assessed using multivariable regression models adjusted for the potential confounders age, sex, race, low-density lipoprotein cholesterol, body mass index, and use of lipid-lowering medication.

Results. Several inflammatory markers had significant associations with RCA or LAD FAI in adjusted models, including sCD14, sCD163, TNFR-I, and TNFR-II, CCL5, CX3CL1, IP-10.

Conclusions. The associations between indices of systemic and peri-coronary inflammation are novel and suggest that these systemic markers and FAI together are promising noninvasive biomarkers that can be applied to assess asymptomatic CVD in people with and without HIV; they also may be useful tools to evaluate effects of anti-inflammatory interventions.

Keywords. atherosclerosis; coronary computed tomography angiography (CCTA); fat attenuation index (FAI); HIV infection; inflammation.

Despite virological suppression on antiretroviral therapy (ART) and independent of traditional cardiovascular risk factors, people with HIV (PWH) have an approximately 2-fold increased risk of developing cardiovascular disease (CVD) compared with people without HIV (PWoH) [1–3]. This increased risk has been associated with persistently elevated plasma levels of inflammatory mediators potentially due to factors including low-level HIV replication, persistent functional immunosuppression, microbial translocation, and coinfections [4, 5]. Inflammation is associated with the development of CVD, including atherosclerosis, but whether circulating soluble inflammatory cytokines are drivers of CVD remains unresolved. Identification of pathogenic mechanisms contributing to CVD risk has been impeded by the inability to accurately measure vascular inflammation, particularly in those without history or symptoms of CVD.

Recent advances in noninvasive imaging of coronary inflammation have resulted in the development of the peri-coronary fat attenuation index (FAI) using coronary computed tomography angiography (CCTA). Lower (less negative) FAI values in both the proximal right coronary artery (RCA) and left anterior descending artery (LAD), but not the left circumflex coronary artery, were found to independently predict CVD risk in PWoH, illustrating the spatially heterogeneous process of atherosclerosis [6]. FAI can be used to assess inflammation in the coronary artery by measuring perivascular fat changes and may be a better marker for coronary inflammation than coronary

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calcium score, which is determined by late-stage structural changes and not early-stage events that may lead to atherosclerosis [7]. One study quantified peri-coronary FAI in PWH but did not compare findings with those among PWoH, nor were measurements of soluble markers of inflammation reported [8].

Our group conducted a longitudinal, observational study in those without prior atherosclerotic CVD (ASCVD) and without symptoms of CVD using CCTA to characterize FAI in PWH on ART and in PWoH [9]. Only participants asymptomatic for CVD were enrolled to determine if associations between systemic and peri-coronary inflammation could be detected early in the disease process, where inflammation could contribute to disease, rather than late, where it may be a consequence of ongoing events. Our initial observations suggested that while asymptomatic PWH did not have worse peri-coronary inflammation than did asymptomatic PWoH at enrollment, FAI progressed significantly in PWH over 2 years [9].

In the present cross-sectional study, our goal was to investigate associations between baseline FAI measurements and plasma levels of select soluble inflammatory markers and chemokines in PWH and PWoH. We hypothesized that these markers could promote T-cell and myeloid-cell activation and infiltration into inflamed vasculature and would be associated with FAI values.

METHODS

Study Participants and Procedures

PWH virologically suppressed on ART for at least 6 months (n = 58) and adult volunteers without HIV or hepatitis C (HCV; n = 21), all without history of ASCVD and asymptomatic for CVD, were prospectively enrolled from clinics at University of Maryland (UMB) and elsewhere in and around Baltimore, Maryland. Summary of study design and methods are illustrated in Figure 1.

Seronegative volunteers (controls) were age- and sex-matched to seropositive study participants with otherwise identical inclusion/exclusion criteria and enrolled from the same clinics and campus newsletter for faculty, staff, and students. In our initial cohort, we found that PWoH had significantly greater body mass index (BMI) than PWH (33.24 ± 9.73 vs 27.16 ± 6.10; $P < .001$). In our previous study, BMI was strongly associated with FAI in both PWH and PWoH [9], and given the strong links with BMI and inflammation and CVD, we excluded the 5 PWoH with the highest BMI levels from further analysis; this adjustment resulted in the mean BMI of the PWoH controls (29.13 ± 6.09) being more comparable to the mean BMI of the PWH ($P = .256$). Exclusion criteria included presence of unstable coronary syndromes or symptoms suggestive of acute coronary syndromes, systolic blood pressure >160 mmHg, low-density lipoprotein cholesterol (LDL-c) >160 mg/dL, poorly controlled diabetes mellitus (defined as a need for antiglycemic therapies and/or HbA1c >7%), hemoglobin ≤7 g/dL, creatinine clearance <45 mL/min, self-reported and/or chart-identified active illicit drug use within the past 3 months, history of cardiac conduction abnormalities without pacemaker, left ventricular ejection fraction <40%, history of severe asthma or currently uncontrolled asthma, known contraindications to β-blocker, sublingual nitroglycerin, or known contraindication to obtaining CCTA.

Patient Consent

All data were derived from an observational study that was approved by the UMB Institutional Review Board (IRB# HP-00074189). All study participants signed informed consent for study enrollment.

Imaging Technique and FAI Measurement

CCTA was acquired and analyzed as described previously [9]. Briefly, CCTA images were acquired using a third-generation dual-source computed tomography scanner (SOMATOM Force; Siemens Medical Solutions, Forchheim, Germany) scanned from September 2017 to June 2021. CCTA acquisition was performed at 120 kVp with a collimation of $2 \times 192 \times 0.6$ mm. Quantitative plaque analysis was performed on a dedicated workstation using validated software (Aquarius iNtuition; TeraRecon Inc., Durham, NC, USA).

Peri-coronary fat attenuation was measured around the proximal right coronary artery (RCA) and left anterior descending artery (LAD) and defined as the mean attenuation of all adipose-containing voxels between −190 and −30 Hounsfield units (HU) obtained from the outer wall of the vessel within a radius equal to the vessel diameter. A total length of 40 mm from the proximal portion of the RCA and LAD was measured, and the proximal 10 mm of the RCA was excluded to limit artifacts from the aortic wall. Several studies have confirmed that FAI of inflamed coronary arteries is shifted from more negative (near to −190 HU) to less negative (closer to −30 HU) values [7, 10, 11].

Multiplex Analysis of Soluble Analytes

Peripheral blood was collected from all participants in EDTA at enrollment, and stored plasma samples were thawed to measure levels of monocyte chemoattractant protein-1 (MCP-1 or CCL2), interferon-inducible protein–10 (IP-10 or CXCL10), fractalkine (CX3CL1), regulated upon activation normal T cell expressed and secreted (RANTES or CCL5), CD14, CD163, interleukin-6 (IL-6), tumor necrosis factor receptor–I (TNFR-I), and TNFR-II using the ELLA assay (ProteinSimple) per the manufacturer's instructions. Separately, whole blood was sent to Labcorp reference laboratory for white blood cell (WBC) count and high-sensitivity C-reactive protein (hs-CRP) testing.

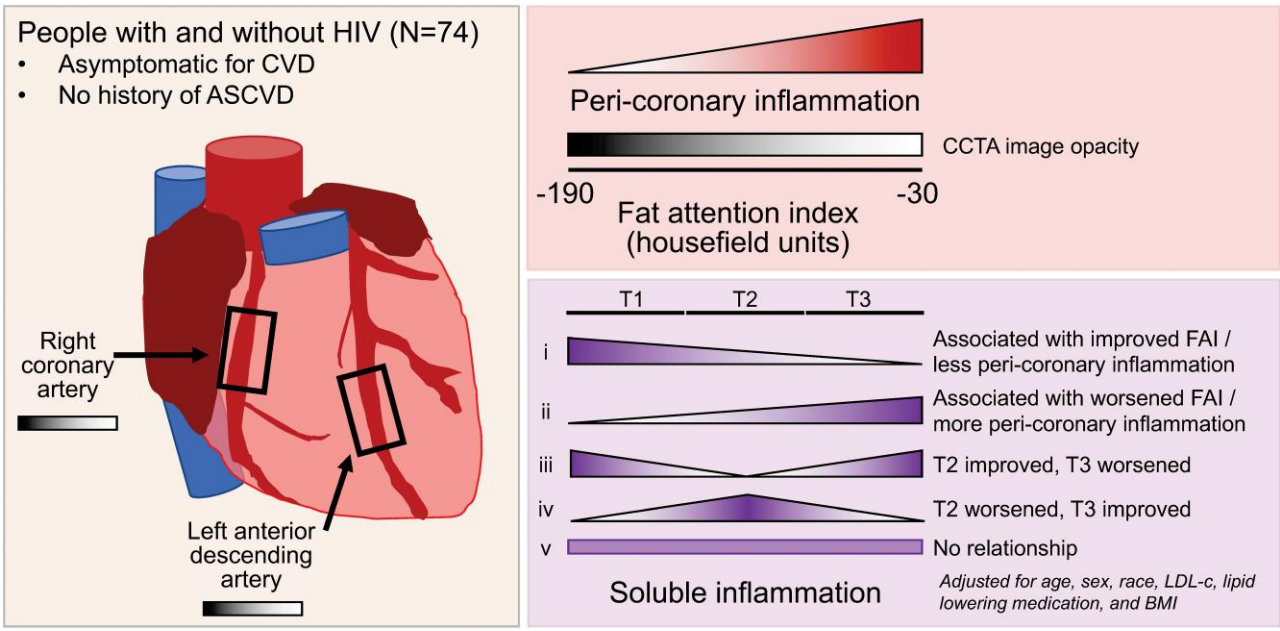

Figure 1. Summary of research design. Fat attenuation index (FAI) was measured in the right coronary artery (RCA) and left anterior descending artery (LAD) in a cohort of people with and without HIV (n = 74 total) who were asymptomatic for cardiovascular disease (CVD) and had no history of atherosclerotic CVD (ASCVD). FAI is an assessment of peri-coronary inflammation measured by coronary computed tomography angiography (CCTA) and is defined as the mean attenuation of all adipose-containing voxels between −190 and −30 Hounsfield units (HU) obtained from the outer wall of the vessel within a radius equal to the vessel diameter. Soluble inflammatory mediators and chemokines were measured in plasma and assessed as tertiles (*T*) for their relationship with RCA and LAD mean FAI, after adjustment for age, sex, race, low-density lipoprotein cholesterol (LDL-c), use of lipid lowering medication, and body mass index (BMI), with T1 as the reference level. Each soluble marker can be associated with mean FAI (and peri-coronary inflammation) in 1 of 5 ways: (i) an association with improved FAI, less peri-coronary inflammation; (ii) an association with worsened FAI, more peri-coronary inflammation; (iii) a nonlinear relationship where T2 is associated with improved FAI and T3 is associated with worsened FAI; (iv) a nonlinear relationship where T2 is associated with worsened FAI and T3 is associated with improved FAI; and (v) no relationship with FAI.

Statistical Analyses

We performed a cross-sectional analysis to determine whether plasma levels of inflammatory markers were associated with FAI measurements in PWH and PWoH.

Demographic and radiological features, behavioral and clinical indicators, and plasma concentrations of inflammatory markers among PWH and PWoH were summarized and compared. Assumption of normality was checked for each continuous variable using Q-Q plots and the Shapiro-Wilk test. Summary statistics were reported using mean ± SD or median with first and third quartiles as indicated based upon data distribution. As appropriate, an independent-samples *t* test or a nonparametric Mann-Whitney *U* (Wilcoxon rank-sum) test was used to examine the differences between groups for continuous variables, and a chi-square test or Fisher exact test was used to examine significant differences for categorical variables.

Tertiles of plasma concentrations of individual markers were analyzed to examine the association with RCA and LAD mean FAI. Clinically relevant covariates and covariates with significant associations ($P < .20$) with serostatus or dependent-variable mean FAI were included in the adjusted analysis as potential confounders: age, sex, race, LDL-c, lipid-lowering medication use, and BMI. Presence of prediabetes, hypertension, and current smoking were considered covariates but excluded from the analysis because they were not statistically significant in the bivariate analyses with serostatus or dependent-variable FAI. Diabetes mellitus, antiglycemic medication use, and aspirin use were also considered covariates but excluded from the model as no participants had diabetes and only 1 was using aspirin. Presence of coronary plaque did not improve the multivariable model and had no bivariate associations with serostatus or dependent-variable mean FAI.

A multivariable regression analysis was performed to evaluate the association between tertiles of plasma concentrations of individual markers and mean FAI adjusting for potential confounders. The lowest tertile (T1) was the reference category, and the regression coefficients for T2 (medium tertile) and T3 (highest tertile) were reported with 95% CIs. The residual plots were examined and checked for normality.

In addition, for each model evaluating the associations of tertiles of individual markers and FAI of the RCA and LAD, the interaction between serostatus and individual marker plasma concentration was assessed. The analysis was performed after adjustment for the same potential confounders: age, sex, race, LDL-c, lipid-lowering medication use, and BMI. A multivariable regression analysis was performed to evaluate the association of

individual markers (tertiles) and mean FAI. The lowest tertile (T1) was the reference category. The interaction effect of serostatus and plasma was reported with 95% CI. The residual plots were examined and checked for normality. Statistical significance was defined as $P < .05$, and the analysis was performed using STATA 17.0 (StataCorp, College Station, TX, USA).

RESULTS

Participant Characteristics

The final analysis after removing the 5 PWoH with the highest BMIs included 74 participants (35 women, 58 PWH, and 16 PWoH), and their clinical and sociodemographic characteristics are shown in Table 1. PWH and PWoH were similar for age and sex. A greater proportion of PWH had lower incomes than PWoH, but there were no significant differences between groups for past or current cigarette use, ASCVD risk score, or lipid panel measurements. Current alcohol use was higher among PWoH ($P = .045$), and prior alcohol use was higher among PWH ($P = .028$). Peri-coronary inflammation in the RCA and LAD at baseline was modestly but significantly lower in PWH than in PWoH. Of the plasma levels of inflammatory markers examined, levels of CCL2 ($P = .046$) and soluble CD163 ($P = .008$) were significantly higher in PWH than in PWoH, with a nonsignificant trend toward more IP-10 among PWH (Supplementary Table 1). Correlations among soluble analytes are shown in Supplementary Figure 1. Most analytes were positively correlated with each other, and the effects were largely driven by the relationships among PWH. However, among all participants, CCL5 consistently trended toward negative associations with all other analytes tested. Furthermore, the relationship of CD14 and CX3CL1 was significantly negative in both PWH and PWoH.

Associations of Soluble Inflammatory Markers and FAI in all Participants

We investigated the associations between mean FAI values of RCA and LAD with inflammatory markers in all participants, regardless of HIV serostatus, assessed as tertiles in unadjusted analyses (Supplementary Table 2). Each β-coefficient represents an association between the RCA or LAD FAI values and markers for participants in the indicated tertile in reference to T1. A positive β-coefficient (rather than sign of individual FAI value) indicates worsened mean FAI (ie, greater peri-coronary inflammation) for the participants in the corresponding tertile, compared with those in T1. For LAD mean FAI, T2 CD14 ($\beta = 1.26$; $P < .001$), T3 CD14 ($\beta = 0.81$; $P = .009$), and T3 TNFR-I ($\beta = 0.70$; $P = .037$) were associated with worsened peri-coronary inflammation, as was T2 CD14 with RCA mean FAI ($\beta = 0.93$; $P = .009$). Interestingly, 2 chemokines were negatively associated with both RCA and LAD mean FAI (meaning a trend toward reduced peri-coronary inflammation): T3 CCL2 (RCA $\beta = -0.79$; $P = .036$; LAD $\beta = -0.87$; $P = .010$) and T3 CX3CL1 (RCA $\beta = -0.77$; $P = .036$; LAD $\beta = -0.67$; $P = .049$).

We next investigated the associations between the mean FAI values of RCA and LAD with inflammatory markers assessed as tertiles and adjusted for potential confounders in all 74 participants (Table 2). For RCA mean FAI, T2 CD163 levels were associated with reduced peri-coronary inflammation ($\beta = -0.74$; $P = .031$). For LAD mean FAI, T2 CD14 levels were associated with greater peri-coronary inflammation ($\beta = 0.77$; $P = .028$). There were no other significant associations noted between mean FAI (RCA or LAD) and soluble inflammatory markers in adjusted analyses (Supplementary Table 3).

Comparisons of Soluble Inflammatory Markers and FAI in People With and Without HIV

We assessed the effects of HIV serostatus on the associations of inflammatory markers with RCA and LAD mean FAI by examining the interactions between marker and HIV serostatus in unadjusted (Supplementary Table 4) and adjusted analyses (Table 3; Supplementary Table 5). A positive interaction term indicates that the difference in the mean FAI in each tertile group among PWH is larger (ie, increased peri-coronary inflammation) than a similar difference in the corresponding tertile group of PWoH; a negative interaction indicates the reverse. Alternatively stated, a positive β-coefficient represents directionality of the interaction term for FAI rather than individual FAI value, and therefore indicates greater peri-coronary inflammation compared with T1, whereas a negative β-coefficient indicates the opposite. In the adjusted analyses, we found that TNFR-I was the plasma cytokine most substantially related to FAI. T3 TNFR-I had significant positive interactions (worsened FAI, greater peri-coronary inflammation) with HIV serostatus for both RCA mean FAI (interaction coefficient = 2.10; $P = .041$) and LAD mean FAI (interaction coefficient = 2.64; $P = .005$). Furthermore, T2 TNFR-I was significantly positively associated with RCA mean FAI (interaction coefficient = 2.58; $P = .010$) and trended toward a positive association with LAD mean FAI (interaction coefficient = 1.60; $P = .081$). Additionally, T3 TNFR-II was significantly positively associated with LAD mean FAI (interaction coefficient = 2.05; $P = .003$), suggesting that the TNF signaling pathway is particularly important for inflammation in the coronary artery in PWH. The other significant and positive interaction with HIV serostatus was for T3 IP-10 with RCA mean FAI (interaction coefficient = 2.22; $P = .040$). Interestingly, 2 chemokines had a significantly negative interaction with serostatus associated with LAD mean FAI, indicating that the difference in the mean FAI among PWH is smaller (ie, decreased peri-coronary inflammation) than a similar difference in PWoH: T2 CX3CL1 (interaction coefficient = −1.71; $P = .035$) and T2 CCL5 (interaction coefficient = −1.56; $P = .049$).

DISCUSSION

In this study investigating the association between soluble inflammatory mediators and chemokines with peri-coronary

Table 1. Sociodemographic and Clinical Characteristics of Study Population (n = 74)

	People With HIV, n = 58 (78.4%)	People Without HIV, n = 16 (21.6%)[a]	*P* Value
Sociodemographic factors			
Sex at birth, No. (%)			
Female	27 (46.6)	8 (50.0)	.807*
Age at enrollment, mean ± SD, y	51.57 ± 9.37	51.60 ± 5.00	.990***
Race, No. (%)			
Black/African American	47 (82.5)	8 (50.0)	**.018****
White	10 (17.5)	8 (50.0)	
Education, No. (%)			
High school education or less	46 (79.3)	5 (33.3)	**.001***
Post high school education	12 (20.7)	10 (66.7)	
Income, No. (%)			
<$25 000	39 (67.2)	2 (13.3)	**<.001***
Behavioral indicators			
Cigarette smoker			
Current	22 (37.9)	4 (26.7)	.417*
Past	27 (51.9)	4 (30.8)	.172*
Alcohol use			
Current	31 (53.4)	13 (81.3)	**.045***
Past	43 (84.3)	5 (50.0)	**.028****
Illicit use			
Current	3 (5.2)	0 (0)	
Past	16 (27.6)	1 (6.3)	.097**
Clinical indicators			
Duration of HIV diagnosis, mean ± SD, y	17.83 ± 9.31		
ART duration, median (Q1, Q3), y	6.00 (2.00–8.00)		
CD4 cell count/mm^3, median (Q1, Q3)	682.00 (543.00–1015.00)	1076.00 (1016.00–1079.00)	
Hepatitis B infected, No. (%)	5 (8.6)	0 (0)	.576**
Hepatitis B and C infected, No. (%)	0 (0)	0 (0)	
Family history CVD, No. (%)	19 (32.8)	6 (40.0)	.598*
Baseline ASCVD risk score, median (Q1, Q3)	.05 (0.02–0.09)	0.04 (0.04–0.09)	.931****
BMI, mean ± SD, kg/m^2	27.16 ± 6.10	29.13 ± 6.09	.256***
Antihypertensive use, No. (%)	21 (36.2)	7 (43.8)	.303*
Other lipid-lowering medication use, No. (%)	8 (16.3)	4 (33.3)	.229**
Systolic blood pressure, mean ± SD, mm Hg	125.24 ± 13.94	125.19 ± 13.69	.989***
Total cholesterol, median (Q1, Q3), mg/dL	183.00 (151.00–203.00)	187.00 (164.00–213.00)	.780****
HDL, median (Q1, Q3), mg/dL	50.50 (46.00–66.00)	53.00 (38.00–78.00)	.795****
LDL, median (Q1, Q3), mg/dL	102.50 (76.00–123.00)	109.00 (94.00–126.00)	.517****
Triglycerides, median (Q1, Q3), mg/dL	94.50 (69.00–132.00)	84.00 (59.00–107.00)	.224****
RCA mean FAI,[b] mean ± SD	−85.80 ± 1.14	−85.00 ± 1.43	**.028*****
LAD mean FAI, mean ± SD	−86.11 ± 1.00	−84.93 ± 1.29	**<.001*****

Bolded values represent statistically significant results.

Abbreviations: ART, antiretroviral therapy; ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; CVD, cardiovascular disease; HDL, high-density lipoprotein; LAD, left anterior descending artery; LDL, low-density lipoprotein; RCA, right coronary artery.

**P* value using chi-square test; ***P* value using Fisher exact test; ****P* value using independent *t* test; *****P* value using Mann-Whitney *U* test.

[a]Five high-BMI cases from the HIV-uninfected group were removed.

[b]Six missing observations: 5 in HIV-infected and 1 in HIV-uninfected.

FAI, we found that inflammatory markers such CD14 (greater inflammation) and CD163 (lesser inflammation) demonstrated significant associations with FAI when all study participants were included (n = 74). These results suggest that key soluble inflammatory markers are associated with pericoronary inflammation, as assessed by FAI, in both PWH and PWoH and with very low ASCVD risk scores (<1%). Together, these inflammatory markers and FAI are promising noninvasive biomarkers that can be applied to assess pericoronary inflammation in people with asymptomatic CVD with and without HIV. Some prior studies have shown different findings between some of these markers of inflammation and CVD or other non-AIDS events than our study, but these differences may be due to numerous factors including different study end points and differences in study populations [12, 13].

Table 2. Significant Adjusted Associations Between Tertiles of Soluble Markers of Inflammation and Peri-coronary Fat Attenuation in All Study Participants (n = 74)

		RCA Mean FAI		LAD Mean FAI	
	Ref:T1	[a]β-Coefficient (95% Confidence Interval)	*P* Value	β-Coefficient (95% Confidence Interval)	*P* Value
CD14[b]	T2	0.46 (−0.28 to 1.20)	0.214	0.77 (0.09 to 1.44)	**.027**
	T3	0.27 (−0.54 to 1.08)	.505	.55 (−0.17 to 1.27)	.131
CD163[b]	T2	−0.72 (−1.39 to −0.05)	**.037**	−0.21 (−0.86 to 0.43)	.506
	T3	−0.24 (−0.94 to 0.46)	.499	−0.01 (−0.68 to 0.66)	.983

Bolded values represent statistically significant values. Each β-coefficient corresponding to T2 represents an association between RCA or LAD peri-coronary inflammation (assessed by FAI values) and soluble marker for study participants in the second tertile of marker values in reference to the first tertile of marker values. A similar interpretation applies to the β-coefficient corresponding to T3.

Abbreviations: BMI, body mass index; LAD, left anterior descending; RCA, right coronary artery; T1, first tertile; T2, second tertile; T3, third tertile.

[a]Regression β-coefficients adjusted for age, sex, race, LDL-c level, use of lipid-lowering medication, BMI, and current smoking status and T1 as reference category.

[b]n = 60 (14 missing observations).

In our study, when the interactions between soluble markers and HIV serostatus were accounted for in evaluating the association of marker and FAI, IP-10, TNFR-I, and TNFR-II were additionally associated with increased FAI (greater inflammation), raising the possibility of an HIV-specific significant TNF signaling in PWH. Intriguingly, the association of LAD mean FAI with the chemokines CX3CL1 and CCL5 had negative interactions with HIV serostatus in our models after adjustment, suggesting their possible role in dampening inflammation, the relative importance of other mediators in PWH, or their presence downstream of immune-regulatory processes among PWH. Thus, our data suggest that not all inflammatory events may be comparable across all populations, and some markers that drive cell traffic to sites of vascular inflammation may be linked to protection in certain compartments.

As the risk of developing CVD is elevated ∼2-fold in PWH on ART compared with PWoH [1–3], HIV-associated CVD is a major public heath burden, and increasingly so since the aging population represents a growing proportion of PWH [14]. Immune activation and inflammatory events may promote CVD beyond traditional risk factors [2, 4], but the links among systemic inflammation, inflammation in cardiovascular tissues, and infiltrating immune cells have yet to be fully elucidated. Our findings suggest that certain systemic inflammatory markers may be early indicators of peri-coronary inflammation and underlying asymptomatic CVD, and strategies to target these factors may prove beneficial in people at risk for CVD with or without HIV infection. We limited our assessment of inflammatory mediators to known factors associated with either mortality or CVD risk in PWH as well as certain chemokines we anticipated could promote trafficking of immune cells to the vasculature, such as CCL2, CCL5, and CX3CL1. We have recently shown that both CCL2 and CX3CL1 are produced by primary vascular endothelial cells following activation with TNF [15], suggesting that these chemokines may be involved in the recruitment of CCR2- and CX3CR1-expressing monocytes to sites of endothelial dysfunction. Additional evidence from our group suggests that the CX3CL1/CX3CR1 axis may be particularly important for the homing of cytolytic CX3CR1+ effector T cells to the vasculature in atherosclerotic CVD in PWH and PWoH [15, 16]. Moreover, CCL5 deficiency has been shown to reduce diet-induced atherosclerosis in mice [17], and increased CCR5+ CD8 and CCR5+ CD4 naïve and effector T-cell levels and monocyte markers were observed in circulation of PWH at least 1 year before first acute coronary syndrome (ACS) among those who experienced an ACS event compared with matched controls who did not [18]. That these chemokines were associated with reduced peri-coronary inflammation especially among PWH in unadjusted (CCL2 and CX3CL1) and adjusted analyses (CCL5) is intriguing and may indicate a protective role for immune cell trafficking in individuals without symptomatic CVD. IP-10 is mainly thought of as a proinflammatory cytokine, and when expressed at sites of vascular damage it could induce the infiltration of CXCR3-expressing T cells and activated monocytes. Evidence from mouse models and humans suggests a mechanistic role for IP-10 in CVD development [19, 20]. Finally, other chemokines, such as CCL3 interacting with CCR5 expressed on T cells, may also be important and are worth further consideration [16].

Our study has important strengths. We used a stepwise approach to examine the associations of soluble inflammatory and immune-activating markers with peri-coronary inflammation at 2 branches of the coronary artery that have predictive value for all-cause and cardiac mortality [6] while controlling for conventional CVD risk factors. We first considered the effects on FAI in all participants, including those with and without HIV, and found novel soluble inflammatory markers that are associated with either improved or worsened peri-coronary inflammation in the RCA and LAD even in those asymptomatic for CVD and with very low risk for CVD. To our knowledge, this is the first report of relationships among these soluble markers and FAI in these populations. Next, we included

Table 3. Significant Comparisons of Adjusted Associations of Tertiles of Soluble Markers of Inflammation and Peri-coronary Fat Attenuation in People With HIV and People Without HIV (n = 74)

		IP-10[a]	CX3CL1[a]	CCL5[b]	TNFR-I[b]	TNFR-II[b]
RCA	T2*Serostatus	1.40	−0.68	−1.13	**2.63**	−0.77
Mean		(−0.41 to 3.21)	(−2.44 to 1.07)	(−3.14 to 0.87)	**(0.69 to 4.56)**	(−3.08 to 1.54)
FAI		*0.125*	*0.435*	*0.260*	***0.009***	*0.505*
	T3*Serostatus	**2.26**	−0.72	0.80	1.95	0.63
		(0.15 to 4.37)	(−2.56 to 1.11)	(−1.17 to 2.77)	(−0.06 to 3.96)	(−0.99 to 2.26)
		0.037	*0.431*	*0.416*	*0.057*	*0.437*
LAD	T2*Serostatus	−1.39	**−1.63**	**−1.55**	1.61	−0.05
Mean		(−2.91 to 0.14)	**(−3.17 to −0.08)**	**(−3.07 to −0.02)**	(−0.18 to 3.41)	(−1.98 to 1.89)
FAI		*0.074*	***0.039***	***0.047***	*0.077*	*0.962*
	T3*Serostatus	−0.12	−0.58	0.29	**2.54**	**2.02**
		(−1.94 to 1.70)	(−2.22 to 1.05)	(−1.28 to 1.85)	**(0.75 to 4.32)**	**(0.70 to 3.33)**
		0.895	*0.475*	*0.715*	***0.006***	***0.003***

Bolded values represent statistically significant results. A positive interaction between T2 and serostatus (T2*serostatus) indicates that compared with the seronegative group, the HIV-seropositive group has a more peri-coronary inflammation (as assessed by worsened mean FAI change) when going from those in the T1 marker group to those in the T2 marker group. A negative interaction indicates the opposite. A similar interpretation applies to the interaction between T3 and serostatus.

Abbreviations: LAD, left anterior descending; RCA, right coronary artery; T1, first tertile; T2, second tertile; T3, third tertile; TNFR, tumor necrosis factor receptor.

*Data presented as β-coefficient (95% CI) *P* value. Regression coefficients adjusted for age, sex, race, LDL-c level, use of lipid-lowering medication, and BMI, with T1 as the reference category.

[a]n = 57 (17 missing observations).

[b]n = 60 (14 missing observations).

HIV serostatus in our model to determine the influence of serostatus on these relationships. Both of these models were considered among tertiles of plasma marker concentrations and in both unadjusted and adjusted analyses, and those significant and borderline significant associations between soluble inflammatory markers (CD14, CD163, hs-CRP, TNFR-I, TNFR-II, IP-10, CCL2, CCL5, and CX3CL1) and peri-coronary inflammation suggest targets for further investigation in mechanistic studies.

There are some notable limitations of this study. Our current analysis only considered soluble inflammation and FAI values cross-sectionally at study entry. In our previous report, we found that PWH had significant progression of peri-coronary inflammation as measured by FAI in ∼2 years [9]. Future analyses will need to examine both whether baseline inflammation levels predict that FAI progression and whether the associations of soluble mediators with FAI values are sustained at the later time point. In addition, local vascular inflammatory events may differ from what we can detect systemically. Additionally, our study has a modest sample size, so we may be underpowered to detect associations when the effect sizes are not robust. Furthermore, we did not correct for multiple comparisons of these associations, and therefore our findings are hypothesis-generating for future studies. Lastly, we did not consider the effects of other pathogens of interest in our models, particularly HCV and cytomegalovirus (CMV), both of which have known associations with both inflammation and CVD risk in PWH and PWoH [21–28]. Future work will need to consider the effects of HCV, CMV, and other pathogens with known CVD risk potential in our cohort.

In conclusion, we found that certain inflammatory factors had significant associations with RCA or LAD FAI values in models adjusted for potential confounders, including IP-10, CX3CL1, CCL5, sCD163, TNFR-I, and TNFR-II, some of which were associated with improved FAI (less peri-coronary inflammation) and some with worsened FAI (more peri-coronary inflammation) (Figure 1). Longitudinal FAI measurements have shown that peri-coronary inflammation advances in ∼2 years in PWH on ART [9], and our current work provides insights into possible mechanisms of vascular inflammation preceding structural or clinical CVD in this population that is highly susceptible to CVD that warrant further investigation. The associations of FAI values with systemic inflammation and markers of immune activation suggest that inflammation may be an early driver of CVD in asymptomatic individuals and that FAI may be a robust marker of peri-coronary inflammation in those without history of ASCVD and low CVD risk. Our findings support the use of FAI as a noninvasive biomarker to assess asymptomatic CVD in people with and without HIV. Future investigations are needed to assess the utility of soluble inflammatory markers in assessing progression of peri-coronary inflammation, to determine the relationship between cellular activation and expression of key chemokines and peri-coronary inflammation, and to understand the role of certain coinfections such as HCV and CMV contributing to peri-coronary inflammation. Such studies could elucidate the pathogenic mechanisms of peri-coronary inflammation preceding atherosclerosis and clinical CVD in both HIV-infected and uninfected populations. Finally, peri-coronary inflammation as assessed by FAI could be a useful tool for testing or monitoring anti-inflammatory

interventions for CVD in HIV-infected and uninfected populations.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Availability of data. Data are not publicly available but may be made available upon reasonable request to the corresponding author.

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