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Persistent Immune Activation and Carotid Atherosclerosis in HIV-Infected Ugandans Receiving Antiretroviral Therapy

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Background. Human immunodeficiency virus (HIV) infection and associated immune activation predict the risk of cardiovascular disease in resource-rich areas. Less is known about these relationships in sub-Saharan Africa.

Methods. Beginning in 2005, we enrolled subjects in southwestern Uganda into a cohort at the time of antiretroviral therapy (ART) initiation. Multiple immune activation measures were assessed before and 6 months after ART initiation. Beginning in 2013, participants aged >40 years underwent metabolic profiling, including measurement of hemoglobin A1c and lipid levels and carotid ultrasonography. We fit regression models to identify traditional and HIV-specific correlates of common carotid intima media thickness (CCIMT).

Results. A total of 105 participants completed carotid ultrasonography, with a median completion time of 7 years following ART initiation. Age, low-density lipoprotein cholesterol level, and pre-ART HIV load were correlated with CCIMT. No association was found between CCIMT and any pre-ART biomarkers of immune activation. However, in multivariable models adjusted for cardiovascular disease risk factors, lower absolute levels of soluble CD14 and interleukin 6 and greater declines in the CD14 level and kynurenine-tryptophan ratio after 6 months of ART predicted a lower CCIMT years later ($P < .01$).

Conclusions. Persistent immune activation despite ART-mediated viral suppression predicts the future atherosclerotic burden among HIV-infected Ugandans. Future work should focus on clinical correlates of these relationships, to elucidate the long-term health priorities for HIV-infected people in the region.

Keywords. HIV/AIDS; Uganda; aging; inflammation; atherosclerosis; carotid intima media thickness; antiretroviral therapy.

The era of antiretroviral therapy (ART) has brought remarkable improvement in life expectancy for human immunodeficiency virus (HIV)–infected individuals [1, 2], but it has also introduced new challenges. Soon after it became evident that ART prolonged survival, data from the United States and Europe began demonstrating increasing incidence of cardiovascular disease [3]. Large cohort studies have corroborated that the risk of both myocardial infarction and cerebrovascular disease (CVD) is 40%–70% greater among HIV-infected individuals than among age- and sex-matched HIV-uninfected controls [4–7].

Whereas some ART medications have been implicated in CVD risk [3], the elevated risk in this population appears to be mediated to a greater extent by HIV infection itself. Studies have demonstrated an increased risk of CVD events among those discontinuing ART [8] and in those with detectable viral loads [7]. It has been hypothesized that the increased attributable risk among HIV-infected individuals is due to increased immune activation and chronic inflammation, which remain abnormally high among those infected with HIV even after viral suppression [9, 10], and are associated with preclinical and clinical atherosclerosis [11–16].

To date, there are few data describing these same correlations between HIV infection, immune activation, and CVD risk in sub-Saharan Africa, where >70% of the world's population of HIV-infected individuals reside [17]. We measured a well-validated surrogate marker of CVD risk [18–21], common carotid intima media thickness (CCIMT), a median of 7 years after ART initiation among participants enrolled in a prospective, longitudinal cohort of HIV-infected individuals. We sought to assess relationships between ART, immune activation, and risk of atherosclerotic disease among HIV-infected individuals in Uganda.

METHODS

Study Participants and Procedures

Beginning in 2005, participants were enrolled in the Ugandan AIDS Rural Treatment Outcomes study. Full cohort details

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have been described previously [22, 23]. Briefly, participants provided blood samples just prior to ART initiation (baseline) and then every 3–4 months thereafter for determination of the $CD4^+$ T-cell count and viral load. At baseline, for all participants, and 6 months after ART initiation, for those who achieved viral suppression by that time (n = 90), we assessed cryopreserved plasma samples for levels of soluble CD14 (sCD14; R&D Systems), soluble CD163 (sCD163; Trillium Diagnostics), interleukin 6 (IL-6; Human IL-6 Ultra-Sensitive Kit, Meso Scale Diagnostics), D-dimer (Diagnostico Stago), lipopolysaccharide (LPS; Limulus Amebocyte Assay, Cambrex) and the ratio of kynurenine to tryptophan (KT) [24]. The percentage of $CD38^+DR^+$ T cells among $CD8^+$ T-cells was assessed in fresh whole-blood specimens processed the same day, as described previously [22, 24]. Approximately 95% of samples were stored in acid citrate dextrose (ACD), with the remainder stored in ethylenediaminetetraacetic acid. To account for differences in diluents, an adjustment factor of 1.276 was multiplied to the results of plasma markers tested from ACD tubes. Depending on the year of enrollment, HIV type 1 (HIV-1) RNA load testing was performed with the Roche Amplicor HIV Monitor assay or the Roche Cobas Taqman HIV-1 test, version 1.0.

In December 2013, we began a substudy of cardiovascular disease risk assessment, titled the Ugandan Noncommunicable Diseases and Aging Cohort (clinical trials registration NCT02445079). Eligibility criteria for the substudy are age >40 years and a minimum of 3 years of ART use. Additional substudy procedures included completion of a questionnaire to assess smoking history, measurement of anthropomorphic characteristics, testing of blood specimens for the serum lipid profile and hemoglobin A1c level, measurement of blood pressure, and performance of carotid ultrasonography for CCIMT estimation. Serum chemistry tests were performed with the Abbott Architect Clinical Chemistry Analyzer (Abbott Diagnostics, Abbot Park, Illinois). Hemoglobin A1c levels were measured with the Bayer A1c Now+ point of care assay (Bayer, Pittsburgh, Pennsylvania).

CCIMT Measurement

CCIMT measurements were performed using a standardized protocol [25] by a single operator who completed the CCIMT training course at the University of Wisconsin [26]. All ultrasonography was performed with a Sonosite M-Turbo (Sonosite, Bothell, Washington). We collected images from the anterior, lateral, and posterior position on both the left and right carotid artery for a total of 6 images per participant. All study images were scored by a board-certified cardiologist. Images with a score of 3 were discarded from the analysis. Far-wall CCIMT was measured in 1-cm segments directly proximal to the carotid bulb, using semiautomated border-detection software (SonoCalc, version 5.0; Sonosite), as supported by published recommendations of CCIMT interpretation [25]. The mean value of all adequate images was summarized as the mean CCIMT estimate for each participant. Study staff were blinded to participant information during image quality assurance and CCIMT measurement.

Ultrasonography Quality Assurance

The ultrasonography technologist completed paired, prestudy ultrasonography on 2 separate days for 10 volunteers. The mean CCIMTs on day 1 and day 2 were both 0.538 mm ($t = 0.0012$ and $P = .999$, by the paired t test), with an absolute value mean difference (±standard deviation [SD]) between days of 0.017 ± 0.018 mm. The coefficient of regression (β) between the 2 days was 1.001 ($P < .001$ and $R^2 = 0.87$), and the coefficient of variation was 4.9%. The mean image quality score (±SD) for images collected during the study, as graded by the study cardiologist (L. H.), was 1.40 ± 0.71, with 1 indicating a high-quality image and 3 indicating a poor-quality image. This mean score falls well within recommended thresholds for adequate image quality [26]. Approximately three quarters of images (453 of 616 [74%]) were scored as high quality, 13% (78 of 616) were scored as average quality, and 14% (85 of 616) were scored as unsuitable for interpretation and discarded from the analysis. After discarding poor-quality images, most participants (60 of 105 [57%]) contributed all 6 anatomical images to the calculation of CCIMT, and nearly all participants (100 of 105 [95%]) contributed at least 4 images, a proportion higher than reported in other similar published studies [27].

Statistical Analyses

We used standardized statistical summarization techniques to describe cohort characteristics. To confirm assumptions about linear relationships, we graphically depicted scatterplots between mean CCIMT and relevant predictors of interest, including standard CVD risk factors and markers of immune activation. We then fit linear univariable regression models for each predictor of interest, including age, sex, smoking status (ever vs never), hemoglobin A1c level on the date of CCIMT, high blood pressure (defined as a systolic pressure of >140 mm Hg or a diastolic pressure of >90 mm Hg on the date of CCIMT), low-density lipoprotein (LDL) cholesterol level on the date of CCIMT, high-density lipoprotein (HDL) cholesterol level on the date of CCIMT, $CD4^+$ T-cell count on the date of CCIMT, $CD4^+$ T-cell count nadir, pre-ART viral load, current viral load (dichotomized as less than or greater than 400 copies/mL), and total ART duration through the date of CCIMT. To determine covariates for inclusion in final models, we performed backward stepwise multivariable logistic regression, retaining all variables with a level of significance of ≤0.25.

We added each marker of immune activation to the multivariable model individually, using the pre-ART absolute level, the 6-month post-ART absolute level, and the difference between 6 months and the baseline value (meaning that a positive value represents an increase in the level of the inflammatory marker from baseline to 6 months). All markers of immune activation

were log transformed and divided by the cohort interquartile range (IQR), such that each unit of increase in the coefficient represented a change in the IQR of the marker. Because any association between markers of immune activation and atherosclerosis risk might be mediated by HIV viremia, we also fit additional models excluding pre-ART viral load. Last, we assessed for interaction in associations between immune activation and atherosclerosis, by sex. All statistical analyses were conducted with Stata, version 13 (Statacorp, College Station, Texas).

Ethical Considerations

Study procedures were reviewed and approved by the institutional review committees of the Mbarara University of Science and Technology, the Ugandan National Council of Science and Technology, and Partners Healthcare. All participants gave written informed consent.

RESULTS

One hundred and five participants completed the CVD substudy. A total of 51% of participants were female, with a median age of 49 years (IQR, 45–51 years; Table 1). Twenty-eight participants (27%) were overweight or obese (body mass index, >25), and 39 (37%) were former or current smokers. The median LDL cholesterol level was 74 mg/dL (IQR, 56–93 mg/dL), and the median HDL cholesterol level was 44 mg/dL (IQR, 37–53 mg/dL). At the time of CCIMT, participants had a median of 7.0 years of ART exposure (IQR, 6.4–7.5 years) and a median $CD4^+$ T-cell count of 430 cells/mm^3 (IQR, 334–546 cells/mm^3), and 85 (81%) had a viral load of <400 copies/mL. The median nadir $CD4^+$ T-cell count was 122 cells/mm^3 (IQR, 80–175 cells/mm^3). Distributions of each inflammatory marker at baseline and for those with an undetectable viral load at 6 months are displayed in Figure 1. Levels of most biomarkers decreased substantially ($P < .001$) between the pre-ART and 6-month measurements, with the exception of sCD14 (3.32 vs 3.29; $P = .04$) and LPS (1.33 vs 1.34; $P = .90$).

The mean CCIMT (±SD) in the cohort was 0.656 ± 0.10 mm (range, 0.513–1.153 mm). In univariable models, greater mean CCIMT was associated with older age ($\beta = 0.088$ for each 10-year increase; $P < .001$), higher hemoglobin A1c level (0.018; $P = .02$), higher LDL (0.048 for each 50-mg/dL increase; $P = .01$), lower baseline viral load (−0.041 for each $\log_{10}$ copies/mL increase; $P = .02$), and higher nadir $CD4^+$ T-cell count (0.014 for each 50-cell/mm^3 increase; $P = .02$; Table 2). In multivariable models, only age ($\beta = 0.081$; $P < .001$), LDL cholesterol level (0.038; $P = .01$), and baseline viral load (−0.038; $P = .01$) were independently associated with mean CCIMT (Table 2 and Figure 2).

None of the pre-ART markers of immune activation (sCD14, sCD163, IL-6, d-dimer, and LPS levels; KT ratio; and proportion $CD38^+DR^+$ T cells among $CD8^+$ T-cells) were associated with future mean CCIMT either in univariable models or after adjustment for age, LDL cholesterol level, and baseline viral load (Table 3). In contrast, in models restricted to those with an undetectable viral load at 6 months, higher absolute values of sCD14 (0.022 mm per IQR increase; $P = .04$) and KT ratio (0.024 mm per IQR increase; $P = .04$) were associated with a higher mean CCIMT (Figure 2). Although not statistically significant, a similar relationship was seen with IL-6 levels at 6 months after ART initiation (0.018 mm per IQR increase; $P = .120$). These relationships remained (or became) significant after adjustment of models for age, LDL cholesterol level, and baseline viral load for sCD14 level (0.026 mm per IQR increase; $P = .006$) and IL-6 level (0.020 mm per IQR increase; $P = .04$) but not for KT ratio (0.013 mm per IQR increase; $P = .25$; Table 3). Similarly, after adjustment for age, LDL cholesterol level, and baseline viral load, greater decreases in sCD14 level

Table 1. Cohort Characteristics

Characteristic	Summary Statistic (n = 105)
At time of CCIMT measurement	
Female sex	54 (51)
Age, y	49 (45–51)
Body mass index	
<18	6 (6)
18–25	71 (68)
25–30	22 (21)
>30	6 (6)
Smoking history	
Never	66 (63)
Former	35 (33)
Current	4 (4)
Hb A1c level, % of total Hb	5.3 (5.0–5.7)
High blood pressure	12 (11)
LDL cholesterol level, mg/dL	74 (56–93)
HDL cholesterol level, mg/dL	44 (37–53)
Creatinine level, mg/dL	0.77 (0.72–0.84)
ART duration, y	7.0 (6.4–7.5)
ART regimen	
AZT/3TC/NVP	68 (65)
AZT/3TC/EFV	21 (20)
TDF/3TC/LPV/r	9 (9)
Other	7 (7)
Historical HIV-specific indicators	
$CD4^+$ T-cell count, cells/mm^3	
Nadir	122 (80–175)
6 mo after ART initiation	218 (152–301)
At time of CCIMT measurement	430 (334–546)
Viral load, $\log_{10}$ copies/mL	
Baseline	5.1 (4.8–5.6)
<400 6 mo after ART initiation	96 (91)

Data are no. (%) of subjects or median value (interquartile range). Blood test results were collected on the day of carotid ultrasonography, unless otherwise indicated.

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; CCIMT, common carotid intima media thickness; Hb, hemoglobin; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir.

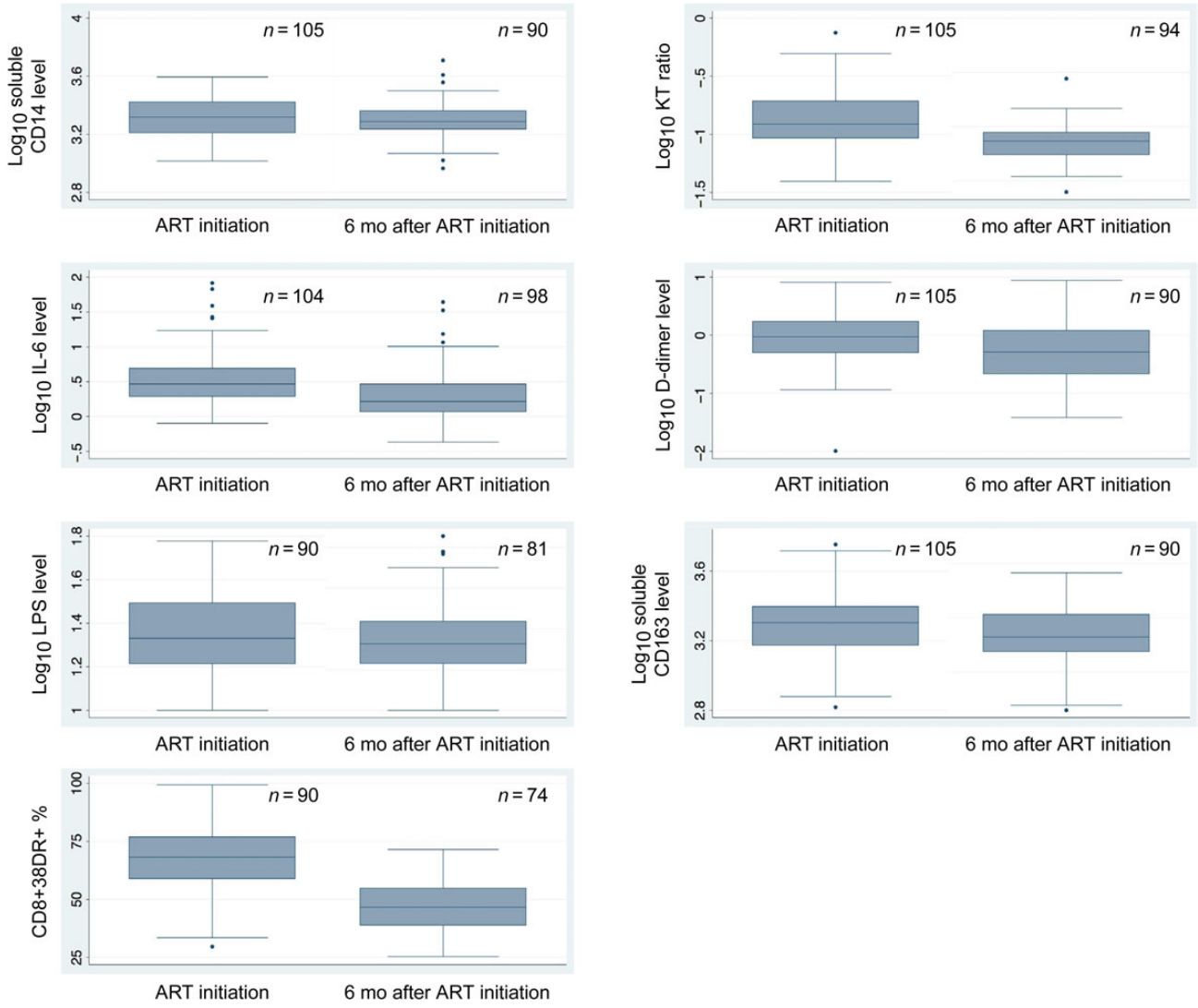

Figure 1. Distribution of markers of immune activation before and 6 months after initiation of antiretroviral therapy (ART) among a cohort of people in southwestern Uganda infected with human immunodeficiency virus. Abbreviations: IL-6, interleukin 6; KT, kynurenine-tryptophan; LPS, lipopolysaccharide; sCD14, soluble CD14; sCD163, soluble CD163.

(0.041 mm per IQR decrease; $P = .003$) and KT ratio (0.032 mm per IQR decrease; $P = .005$) between baseline and 6 months after ART-mediated viral suppression were associated with lower CCIMT years later (Table 3 and Figure 3). Decreases in IL-6 and d-dimer levels during the first 6 months of ART had similar but nonsignificant associations with future mean CCIMT (Table 3). We found no association between changes in sCD163 level, plasma LPS level, or percentage of $CD38^+HLA\text{-}DR^+$ T cells among $CD8^+$ T cells during the first 6 months of ART and future CCIMT (Table 3). Removal of the pre-ART viral load from models did not alter any of the above associations. Last, we found that the association of sCD14 appeared to be independent of other inflammatory markers. For example, in a multivariable model including changes between the pre-ART value and the 6-month value for both the sCD14 level and KT ratio, we found only minimal confounding effects in either marker, with regression coefficients decreasing by approximately 30% in each and unchanged P values ($P = .03$ vs .04 for the sCD14 level, and $P = .04$ vs .06 for the KT ratio). In a model with 6-month levels of both sCD14 and IL-6, the sCD14 coefficient was largely unchanged (β changed from 0.026 to 0.022 mm per IQR), and the association remained significant ($P = .03$), whereas the IL-6 coefficient decreased substantially and became nonsignificant ($P = .38$). We found no evidence of interaction between markers of immune activation and atherosclerosis, by sex.

DISCUSSION

To our knowledge, we present the first study describing prospective associations between markers of immune activation and future risk of atherosclerotic burden in HIV-infected individuals in sub-Saharan Africa. Our data demonstrate that

Table 2. Correlates of Common Carotid Intima Media Thickness

	Univariable Model		Multivariable Model[a]	
Correlate	β coefficient (95% CI)	*P* Value	β coefficient (95% CI)	*P* Value
Female sex	0.028 (−.012 to .068)	.17		
Age (each 10-y increase)	0.088 (.060–.116)	<.001	0.081 (.054–.108)	<.001
Body mass index				
<18	0.017 (−.072 to .106)	.70		
18–25	Reference			
25–30	0.010 (−.041 to .061)	.71		
>30	0.010 (−.079 to .099)	.82		
Ever smoker	−0.022 (−.064 to .019)	.29		
Hemoglobin A1c level	0.018 (.003–.341)	.02		
High blood pressure	0.010 (−.053 to .074)	.75		
LDL cholesterol level (each 50-mg/dL increase)	0.048 (.012–.083)	.01	0.038 (.008–.068)	.01
HDL cholesterol level (each 50-mg/dL increase)	0.006 (−.077 to .090)	.88		
Creatinine level	−0.037 (−.179 to .104)	.60		
Nadir $CD4^+$ T-cell count (each 50-cell increase)	0.014 (.002–.026)	.02		
Baseline viral load (each $\log_{10}$ copies/mL increase)	−0.041 (−.074 to −.008)	.02	−0.038 (−.066 to .010)	.01
Current viral load undetectable	0.010 (−.042 to .061)	.71		
Duration of ART (each additional year)	−0.006 (−.026 to .014)	.54		
ART regimen (no. [%])				
AZT/3TC/NVP	Reference			
AZT/3TC/EFV	0.035 (−.017 to .086)	.19		
TDF/3TC/LPV/r	0.023 (−.050 to .097)	.53		
Other	0.038 (−.044 to .120)	.36		

Abbreviations: 3TC, lamivudine; ART, antiretroviral therapy; AZT, zidovudine; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPV/r, lopinavir/ritonavir; NVP, nevirapine; TDF, tenofovir.

[a] Backward stepwise regression with a *P* value threshold of .25 was used to select covariates for the multivariable model.

persistent immune activation, despite 6 months of ART-mediated viral suppression, is associated with an increased burden of carotid atherosclerosis a median of 7 years later in a population of older-aged people receiving stable ART in southwestern Uganda. Further data are needed to corroborate these findings and to assess the population health relevance of these associations. If our results are confirmed, they reinforce the important need to consider expanding the breadth of HIV care programs in the region to include CVD screening, prevention, and treatment [28–30].

The association we found between sCD14, a macrophage-associated marker of immune activation, and subclinical atherosclerosis among HIV-infected individuals in Uganda builds upon prior work from the United States and Europe [31, 32]. This specific marker is of particular relevance to this population because it appears to remain elevated in HIV-infected individuals,

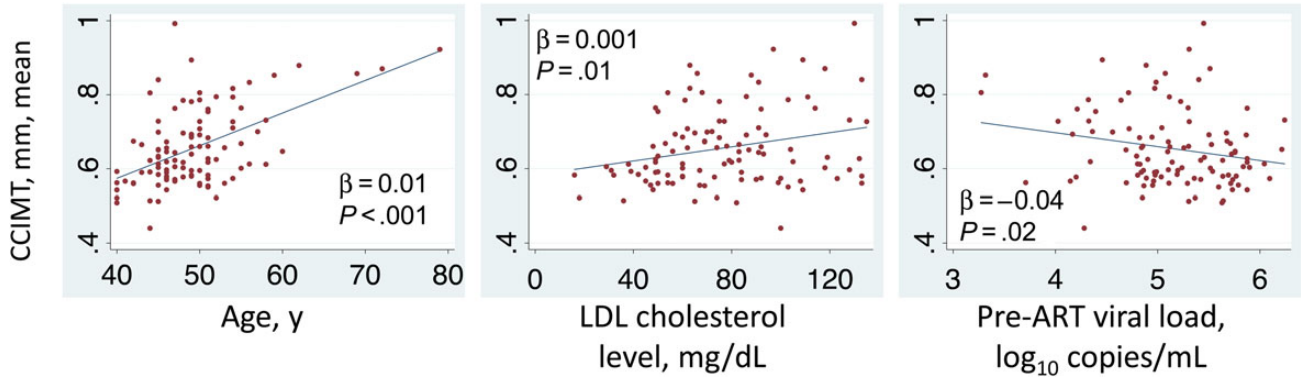

Figure 2. Scatterplots demonstrating relationships between common carotid intima media thickness (CCIMT) and age, low-density lipoprotein (LDL) cholesterol level, and viral load before antiretroviral therapy (ART) initiation among a cohort of people in southwestern Uganda receiving therapy for a median of 7 years. Fit lines, β statistics, and *P* values were generated by bivariate linear regression models.

Table 3. Relationships Between Carotid Intima Media Thickness a Median of 7 Years After Antiretroviral Therapy (ART) Initiation and Levels of Soluble Biomarkers of Immune Activation at the Time of ART Initiation, Levels 6 Months After ART Initiation, and Changes in Levels Over the First 6 Months of Therapy

	Level Before ART Initiation, Per IQR Increase[a]		Level 6 Mo After ART Initiation, Per IQR Increase[a]		Difference in Levels Between 6 Mo After ART and Before ART[a]	
Marker	β coefficient (95% CI)	*P* Value	β coefficient (95% CI)	*P* Value	β coefficient (95% CI)	*P* Value
Soluble CD14	−0.005 (−.032 to .020)	.68	0.026 (.008–.044)	.006	0.041 (−.067 to .014)	.003
KT ratio	−0.017 (−.040 to .006)	.15	0.013 (−.009 to .035)	.25	0.032 (−.055 to −.010)	.005
IL-6	−0.002 (−.019 to .016)	.85	0.020 (.001–.039)	.04	0.015 (−.032 to .002)	.08
D-dimer	−0.000 (−.022 to .021)	.98	0.016 (−.010 to .041)	.24	0.019 (−.042 to .005)	.11
Lipopolysaccharide	0.006 (−.015 to .028)	.58	−0.002 (.024–.020)	.87	−0.012 (−.016 to .040)	.40
Soluble CD163	0.016 (−.035 to .003)	.09	−0.016 (−.040 to .008)	.18	−0.004 (−.021 to .029)	.78
Percentage of $CD38^{+}DR^{+}$ T-cell lymphocytes	−0.006 (−.031 to .018)	.61	−0.014 (−.042 to .015)	.35	0.002 (−.028 to .024)	.86

All models were adjusted for age, low-density lipoprotein cholesterol level, and baseline viral load.

Abbreviations: CI, confidence interval; IL-6, interleukin 6; IQR, interquartile range; KT, kynurenine-tryptophan.

[a] Inflammatory markers are log-transformed and divided by the IQR of the cohort, such that the β coefficient signifies the estimated change in mean common carotid intima media thickness for each IQR in the marker of inflammation. In the models with a change in inflammatory markers, the variable is calculated as 6 months – baseline, such that a larger number represents less of a decrease over that period.

compared with HIV-uninfected controls, despite receipt of long-term suppressive ART [33] and because of its association with both cardiovascular disease risk and all-cause mortality in the general population [34]. A subanalysis of HIV-infected persons within the SATURN randomized controlled trial, which evaluated the effect of rosuvastatin on atherosclerotic disease among people with elevated C-reactive protein (CRP) levels, noted independent cross-sectional associations between sCD14 and coronary artery calcification [35]. More recently, a large analysis among participants in the Multicenter AIDS Cohort Study also noted significant cross-sectional correlations between sCD14 levels and noncalcified coronary artery plaque, coronary stenosis >50%, and total plaque score [13]. Other studies that have assessed inflammatory markers and progression of carotid atherosclerosis have found no association between sCD14 or high-sensitivity C-reactive protein levels and CCIMT at baseline, but strong associations between sCD14 levels and future CCIMT progression were observed [36, 37]. An important and unique feature of our study is that we were able to assess relationships among a population before and after ART-mediated viral suppression and subsequent atherosclerosis years later. We found that those with the highest levels of sCD14 after 6 months of ART had the greatest future CCIMT. One potential explanation for this association is that persistent monocyte activation despite suppression of HIV viremia could be a key driver of cardiovascular disease risk in this setting. Alternatively, other potential drivers of immune activation that are not directly affected by ART (eg, helminthic infection and subclinical malaria) could also be responsible. Future studies involving HIV-uninfected persons will help to elucidate these relationships.

Our study is the first report, to our knowledge, to correlate the KT ratio with the atherosclerotic disease risk among an HIV-infected population. The KT ratio largely reflects the activity of host indoleamine 2,3-dixoygenase-1 (IDO), an inducible enzyme in macrophages and dendritic cells that catabolizes tryptophan to kynurenine. IDO is activated in response to type I and II interferons and microbial products [38, 39]. We have previously shown that the KT ratio is elevated in HIV-infected Ugandans initiating ART, that levels decrease after ART, and that levels before initiation of and during ART independently predict all-cause mortality in this setting [24]. Whereas IDO activity has been associated with both cardiovascular disease risk factors [40] and coronary artery disease [41] in uninfected populations, as well as with CCIMT among patients undergoing dialysis [42], less is known about the KT ratio and cardiovascular disease risk among HIV-infected populations. Our finding that the failure to decrease KT ratios despite 6 months of suppressive ART is associated with future CCIMT, independent both of traditional cardiovascular risk factors and sCD14, suggests that this pathway might also play a similarly unique role in atherogenesis in HIV-infected persons and should prompt further investigation of these relationships.

We also detected associations between persistent elevations in IL-6 levels despite 6 months of ART-mediated viral suppression and carotid atherosclerosis. Data from the SMART study demonstrated that IL-6 is an independent correlate of both CVD outcomes and all-cause mortality in HIV-infected persons [12, 43]. Notably, as in our study, that study detected associations between IL-6 levels and clinical outcomes even among those receiving suppressive ART. Similarly, a cross-sectional study of people with HIV infection who were receiving suppressive ART found nonsignificant associations between plasma IL-6 levels and abnormal carotid atherosclerosis ($P = .08$), defined as an internal carotid intima media thickness of >1.0 mm [44].

We did not find relationships between carotid atherosclerosis and baseline or early post-ART changes in sCD163 levels, another marker of monocyte activation and, like sCD14 levels, a

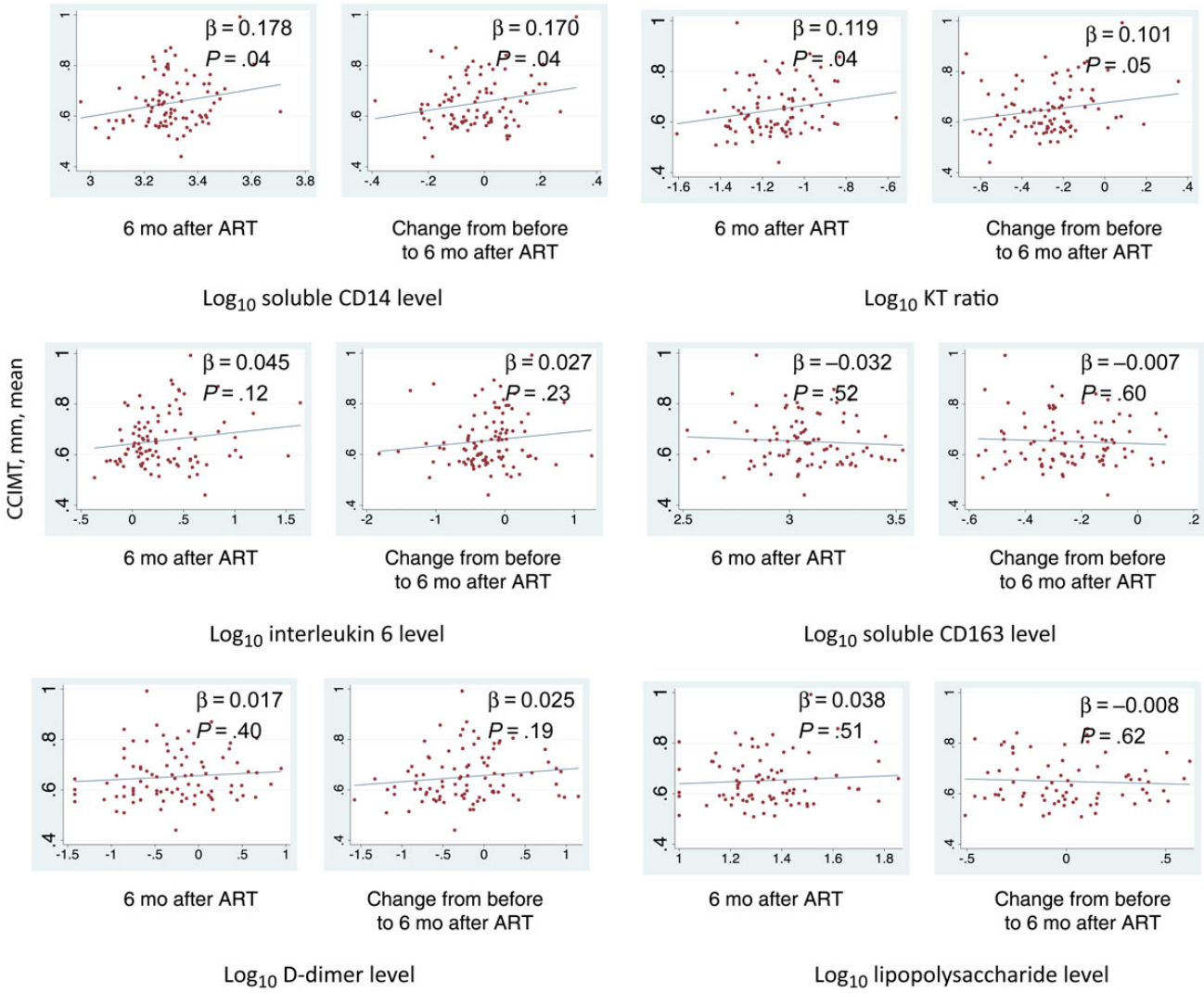

Figure 3. Unadjusted scatter plots demonstrating relationships between common carotid intima media thickness (CCIMT) and markers of immune activation at 6 months after antiretroviral therapy (ART) initiation (left) and changes from pre-ART to 6 months after initiation of ART (right) among a cohort of people in southwestern Uganda receiving therapy for a median of 7 years. Inflammatory markers are log transformed. Fit lines, β-statistics, and *P* values were generated by bivariate linear regression models. Abbreviation: KT, kynurenine-tryptophan.

marker of HIV-related immune activation [45]. This finding is in contrast to prior work noting relationships between sCD163 and noncalcified coronary artery plaque, coronary artery stenosis, and arterial wall inflammation [11, 13, 46]. Ours is the first study, to our knowledge, to assess this interaction in sub-Saharan Africa. Interestingly, while associations between sCD163 levels and CCIMT have been noted in the general population [47], the only other study, to our knowledge, to assess correlations between sCD163 levels and carotid atherosclerosis among an HIV-infected population also failed to find a relationship between the two [48].

An unexpected finding of our study was that higher pre-ART viral load and lower nadir CD4$^+$ T-cell count predicted decreased future CCIMT. This finding is similar to that from a prior study in the United States, which found that higher nadir CD4$^+$ T-cell counts were associated with an increased risk of CCIMT progression during ART [49], but it differs from those from larger cohort studies in the United States that have noted a decreasing risk of stroke and myocardial infarction with increasing nadir CD4$^+$ T-cell counts [6, 50]. Because our study evaluated participants who remained alive and in care a median of 7 years after ART initiation, we could be observing a survivorship bias. Alternatively, there could be a lagged relationship between nadir CD4$^+$ T-cell count and atherosclerosis, such that the risk begins once a relative return to health is achieved. Future work will be necessary to help elucidate the precise relationships between HIV viremia, immune activation, and atherosclerotic disease burden in this setting.

Our study should be considered while keeping several limitations in mind. Most importantly, only study participants who remained in the parent study from enrollment through initiation of the cardiovascular disease procedures in late 2013 were eligible to be included in the substudy. Notably only 80 of 762 participants (10%) in the parent cohort died, withdrew, or were lost to follow-up from the parent study in the interim. Of these, only 49 (6%) would have been >40 years of age in 2014 and eligible for this study, including 35 (5%) who died in the interim. Furthermore, our 6-month measures of immune activation were restricted to those with viral suppression at that time. As such, our study should be taken to represent associations between baseline inflammatory markers and atherosclerotic progression among those who suppress HIV during the first 6 months of ART and survive in the 5–10-year period after ART initiation. As with all nonrandomized observational studies, our study could also by limited by unmeasured or residual confounding from the associations between immune activation and atherosclerosis. Last, we currently lack similar data from an HIV-uninfected control group sampled from the same environment, so we are not able to address whether the associations between inflammatory markers and atherosclerosis observed in this study are unique to HIV infection. We plan to address this issue in the future as part of an ongoing extension of this study.

In conclusion, we found that persistent immune activation following ART initiation is strongly and independently associated with the subsequent extent of carotid atherosclerosis among ART-treated, HIV-infected individuals in Uganda. These data are among the first to demonstrate this association in the sub-Saharan African region and signal an important need to further explore the long-term health priorities for the large and aging population of HIV-infected individuals in the region. Future work should focus on relationships between HIV infection, immune activation, and clinical outcomes and, if significant relationships are confirmed, should evaluate preventive and interventional strategies to reduce the CVD risk in this population.

Notes

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