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ELEVATED MICROPARTICLE TISSUE FACTOR ACTIVITY IS ASSOCIATED WITH CAROTID ARTERY PLAQUE IN HIV INFECTED WOMEN

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Abstract

Background: Expression of tissue factor (TF) on the surface of activated monocytes may trigger thrombosis, leading to clotting risk, inflammation, and atherosclerosis. TF-positive microparticles (MP-TF) represent a functionally active form of TF that may be promulgated by long-term HIV infection. We hypothesized that greater MP-TF activity is associated with carotid artery plaque in HIV+ women.

Setting: In a case-control study nested within the Women's Interagency HIV Study (WIHS), eligible HIV+ participants underwent B-mode carotid artery ultrasound at 2 study visits occurring 7 years apart. Cases were defined by presence of at least 1 carotid artery plaque assessed at either visit. Cases were matched 1:2 to controls who were found not to have carotid artery plaques.

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Methods: Conditional logistic regression estimated the association of MP-TF activity with presence of carotid artery plaque, adjusting for demographic and behavioral characteristics, HIV-related factors, cardiometabolic risk factors, and serum inflammation biomarkers (hsCRP, IL-6, sCD14, sCD163, Gal-3, Gal-3BP).

Results: Elevated MP-TF activity (>0.537 pg/mL) was found to be significantly associated with greater odds of plaque (adjusted odds ratio 3.86, 95% CI 1.06–14.07, p=0.04). The association was attenuated after further adjustment for IL-6, but was unaffected by adjustment for other biomarkers including those denoting monocyte activation.

Conclusions: Our findings suggest a link among HIV infection, innate immune system perturbation, coagulation, and atherosclerosis.

Keywords

Tissue factor; atherosclerosis; coagulation; carotid plaque; HIV infection; women

Introduction

Compared to the general population, people living with HIV have a higher risk of cardiovascular disease (CVD) and other chronic inflammatory diseases [1, 2]. While the increased risk of CVD in people living with HIV has been consistently demonstrated [3], relevant underlying mechanisms have not yet been clearly delineated beyond a high prevalence of traditional CVD risk factors. Widespread use of antiretroviral therapy (ART) and improved CVD preventive care have not eliminated the differential risk of CVD-related morbidity and mortality by HIV serostatus [4, 5].

Tissue factor (TF) is a transmembrane protein that mediates signaling in the initiation of the coagulation cascade [6]. TF may be expressed in response to cell damage [7], inflammatory stimuli [8] and multiple cytokines [9], promoting the formation of thrombin and fibrin which trigger thrombosis. This leads to clotting events and further perpetuates inflammation and atherosclerosis [10]. TF is also correlated with the fibrinolysis biomarker D-dimer [11], which is consistent with its likely role upstream in the coagulation cascade. TF exists in a soluble cell-free form, on cell surfaces, and in association with cell-derived microparticles (MPs). TF-positive MPs represent a functionally active form of TF which may be clinically relevant, even though microparticles account for only a small portion of circulating TF [12].

In people living with HIV, elevated inflammation may lead to an increase in TF activity and initiation of the coagulation cascade [11]. In clinical samples, certain TF-expressing monocytes have been recently found to persist after virologic suppression and trigger coagulation via formation of factor Xa *in vitro* [13]. Increased TF expression has been associated with higher HIV RNA levels in blood serum [14], with hepatitis C virus coinfection [15], and with chronic immune activation [11] which may all contribute to increased risk of cardiovascular morbidity [16,17]. The impact of tissue factor-positive microparticles (MP-TFs) on subclinical atherosclerosis among people living with HIV has not been extensively explored.

Therefore, we conducted a nested case-control study to assess the association between MP-TF activity and carotid artery plaque formation in the Women's Interagency HIV Study (WIHS). Our aim was to identify a link between this marker of potential clotting risk and subclinical atherosclerosis in HIV-positive women, who have high rates of cardiovascular and cerebrovascular disease [5, 18].

Methods

Source Population.

The source population for our study was the WIHS, a prospective multicenter cohort study of women with or at risk for HIV infection. The WIHS was initiated in 1994 and at the time of this study enrolled over 3,000 women at six U.S. sites [19,20] Recruitment in the WIHS occurred in two waves (1994–1995, 2001–2002) from HIV primary care clinics, hospital-based programs, community outreach, support groups, and other locations. The WIHS protocol involves semi-annual follow-up visits with detailed examinations, specimen collection, and structured interviews. All individuals provided informed consent, and the studies were approved by each site's Institutional Review Board.

Study Design and Case Definition.

A vascular substudy was initiated in the WIHS in 2004. All WIHS participants were invited to undergo high-resolution B-mode carotid artery ultrasound to image six locations in the right carotid artery according to published procedures [21, 22]: the near and far walls of the common carotid artery, carotid bifurcation, and internal carotid artery. A standardized protocol was used at all sites, and measurements were obtained at a centralized reading center (University of Southern California).

We conducted a case-control study of HIV-positive women nested within the vascular substudy. All eligible participants underwent B-mode carotid artery ultrasound during at least one of two vascular study visits occurring 7 years apart. Carotid artery plaque was defined as an area (i.e., lesion) with localized IMT >1.5 mm measured in at least one of the six aforementioned artery locations. We use the term subclinical CVD (sCVD) to denote the case definition in this report.

We defined cases as women having at least 1 carotid artery plaque assessed at either vascular substudy visit. Cases were individually matched at a 1:2 ratio with available controls, who were vascular substudy participants with no plaque detected at either visit. Matching variables included age, smoking status, CD4+ count, and ART use at the first ultrasound scan (baseline). If 2 controls were not found, a single control was used, which occurred for 19 out of 98 cases.

Main Exposure of Interest.

From plasma specimens that had been frozen at the core study visit corresponding to the vascular substudy baseline visit, MP-TF activity was determined by the method of Key et al. [23] at the University of Vermont. MP-TF was initially categorized using quintiles and deciles defined in the subset of values above the minimum detectable limit (0.05 pg/mL)

among both cases and controls, to examine if its distribution varied linearly with case status. Because the data were highly skewed, we winsorized the data by eliminating the 1% and 99% percentiles (N=2 controls eliminated) and examined MP-TF continuously as well as categorically using quartiles and deciles. In addition, because other studies have suggested a threshold effect [24], we examined the existence and magnitude of a threshold for MP-TF for its association with sCVD in our study.

Covariates and Potential Confounders.

Demographic and behavioral variables included race/ethnicity, education, alcohol use, current crack/cocaine and alcohol use, and HCV infection (based on antibody or viral RNA testing). HIV-related risk factors included detectable plasma HIV RNA levels (≥80 copies/mL), prior AIDS-defining illness, and both baseline and historically lowest measured CD4 count prior to ART use (nadir CD4). Cardiometabolic risk factors included body mass index (BMI), systolic blood pressure, total and high-density lipoprotein cholesterol, current use of antihypertensive and lipid-lowering medications, history of diabetes mellitus, and estimated glomerular filtration rate (eGFR) [25]. All variables were measured based on the core study visit closest to the baseline scan. We also incorporated into the analysis levels of six serum inflammation and innate immunity markers that were measured concurrently with MP-TF: IL-6, hsCRP, sCD14, sCD163, Gal-3 and Gal-3BP. Similarly, these markers were measured from thawed frozen blood specimens originally obtained during the core study visit closest to the baseline scan. Enzyme-linked immunosorbent assay (ELISA) methods were used to measure sCD14, sCD163, Gal-3 (all R&D Systems, Minneapolis, MN), and Gal-3BP (s90K/Mac-2BP; eBioscience, San Diego, CA). The marker hsCRP was measured using a nephelometric immunoassay (Quest, Madison, NJ). IL-6 was measured with a multiplex assay by electrochemiluminescence (Meso Scale Discovery, Rockville, MD).

Statistical Methods.

The distribution of MP-TF activity was examined for all participants and for the case (sCVD+) and control (sCVD-) groups separately. To examine correlations between MP-TF activity and potential risk factors, including available markers related to inflammation or innate immunity (hsCRP, IL-6, sCD14, sCD163, Gal-3, Gal-3BP), age, total cholesterol, LDL cholesterol, non-HDL cholesterol, baseline and nadir CD4 count, and baseline HIV RNA level, we determined Spearman rank correlations among all participants. We also did this on an exploratory basis for three markers of coagulation (D-dimer, IP-10 [CXCL10], and fibrinogen) that have previously been associated with HIV and its complications [26, 27, 28] in a small subset of participants. To account for individual matching between cases and controls, conditional logistic regression was used to examine the association between MP-TF activity and case status. To control for potential confounding, demographic and behavioral characteristics, HIV-related factors and cardiometabolic risk factors were sequentially adjusted for in regression models. We additionally adjusted for the aforementioned serum inflammation biomarkers (hsCRP, IL-6, sCD14, sCD163, Gal-3, Gal-3BP) that may either confound or mediate the hypothesized association [28]. In exploratory analyses, we additionally adjusted for aspirin use and a history of cancer diagnosis, which have been shown to alter tissue factor levels [29, 30].

In secondary analyses, we examined the associations of MP-TF with sCVD case status in subgroups defined *a priori* by HIV-related characteristics: detectable HIV RNA levels (≥80 copies/mL) (Yes vs No), nadir CD4 count ≤200 cells/μL (Yes vs No), history of AIDS (Yes vs No), HCV co-infection (Yes vs No), and current ART users (Yes vs No). In exploratory analyses, we further separated ART users into protease inhibitor (PI) users, nucleoside reverse transcriptase inhibitor (NRTI) users, and non-nucleoside reverse transcriptase inhibitor (NNRTI users). We also assessed two-way multiplicative interaction terms based on the product of these variables and MP-TF. For the secondary analyses, we used unconditional logistic regression because the participants were no longer matched, while controlling for the matching factors: continuous age, smoking status, continuous baseline CD4+ count, and ART use. Statistical significance thresholds for main effects and interactions were determined based on a 2-sided *P* value <.05.

All analyses were performed using SAS 9.4 (SAS Institute Inc., Cary, NC) and R 3.3.2 (R Project for Statistical Computing, Geneva) [31]. To account for the <1% missing covariate data, we used IVEware software to conduct multiple imputation using multivariate sequential regression based on 5 imputed datasets [32]. All regression analyses were performed using these imputed datasets.

Results

Study Population Characteristics.

A total of 275 women living with HIV were included in our analysis. Among them, 98 participants (36%) had one or more carotid artery focal plaques identified (sCVD cases), while 177 had no carotid artery focal plaques identified (controls). As shown in Table 1, participants were well-matched by age, smoking status, baseline CD4+ count, and recent ART use. Median age was 46 years (IQR 39–50) at baseline. There were 59% of black race and 30% of Hispanic ethnicity. Current smokers made up 51% of the study population, and 8% were on lipid-lowering therapy at baseline, almost all (95%) of whom were on a statin. While 75% were on ART, only 44% had undetectable HIV RNA levels (<80 copies/mL) at baseline. Case and control groups were generally similar, although control participants had higher BMI at baseline and were more likely to use anti-hypertensive medications. There were no significant differences in levels of biomarkers by case status except for sCD14, which was significantly higher among sCVD cases than controls (Table 1, p=0.04).

Distribution of MP-TF Activity, and Correlation with Other Markers of Inflammation.

Median MP-TF activity was 0.10 pg/mL (interquartile range 0.05–0.22 pg/mL). Levels of MP-TF activity were not significantly correlated with age, HIV RNA levels or CD4+ T cell counts (Table 2), but were moderately correlated with the coagulation markers D-dimer and IP-10 (r=0.48, and r=0.47, respectively). Lipid markers were slightly negatively correlated with MP-TF activity (range: r=−0.09 to −0.12). Among other serum inflammation and innate immunity markers, we found MP-TF activity to be correlated significantly, albeit weakly, with the systemic inflammation markers IL-6 and hsCRP (r=0.15 and 0.13, respectively). Correlations with other markers were negligible and not statistically significant (range: r= −0.02 to 0.08).

Association of Elevated MP-TF Activity with Carotid Artery Plaque Presence.

When analyzing MP-TF as a continuous variable, median MP-TF activity did not differ significantly between cases and controls (p=0.59) (Table 3). Categorizing MP-TF activity into deciles or quintiles did not detect a linear association with case status (data not shown). Graphical examinations suggested that categorizing MP-TF at the highest decile (>0.537 pg/mL) provided the greatest separation between sCVD cases and controls. Cases had higher levels of MP-TF activity when using this threshold (13% vs. 3%, p=0.01) (Table 3). We therefore used this value as the threshold for all subsequent analyses.

Table 4 shows the association of elevated MP-TF activity (>0.537 pg/mL) with carotid artery plaque presence (i.e., sCVD case status) in serially adjusted multivariable models. There was an increased odds of plaque presence with elevated MP-TF activity. In unadjusted analyses, elevated MP-TF activity was associated with a four-fold increased odds of plaque presence (odds ratio [OR] 4.07, 95% confidence interval [CI] 1.54–10.74, p=0.005). This association was slightly attenuated after taking into account demographic and behavioral characteristics and HIV-related and cardiometabolic risk factors, but still remained statistically significant (aOR 3.86, 95% CI 1.06–14.07, p=0.04). The association was further attenuated after additional adjustment for aspirin use (aOR 3.23, 95% CI 0.95–10.93, p=0.06) or a history of cancer diagnosis (aOR 3.47, 95% CI 1.05–11.47, p=0.04).

As a sensitivity analysis, a published threshold of 0.500 pg/mL[33] was applied to the data, which revealed a greater proportion of high MP-TF activity in cases compared with controls (14% vs. 5%, p=0.02) (Table 3). Results from models that used this threshold were not meaningfully different (data not shown). This analysis further demonstrated a threshold effect rather than a linear association between MP-TF activity and sCVD.

In a subsequent model additionally adjusting for all of the serum inflammation markers together, the association of elevated MP-TF activity and plaque formation was further attenuated and became marginally statistically significant (aOR 3.32, 95% CI 0.86–12.75, p=0.08). In order to assess whether one or more of these inflammation markers may have had a greater influence in attenuating this association, we also adjusted for inflammation one serum marker at a time. We determined that the association was attenuated after adjustment for IL-6, but not for other biomarkers including those denoting monocyte activation (e.g., sCD14), suggesting that IL-6 may contribute to the relationship between MP-TF activity and plaque formation (Table 5).

We also explored potential effect modification of the association between MP-TF activity and carotid artery plaque formation by a set of characteristics determined *a priori*. No significant (p<0.05) effect modification of MP-TF effects was observed by baseline viral load (suppressed vs unsuppressed), history of AIDS, nadir CD4 count (≤200 cells/μL and >200 cells/μL), HCV co-infection or recent ART use (all interaction p-values >0.15). Despite this, we observed more pronounced associations between MP-TF activity and presence of carotid artery plaque in certain subgroups, including participants with undetectable HIV RNA (<80 copies/mL) (N=121, aOR 10.34, 95% CI 1.35–79.24, p=0.02), with nadir CD4+ count >200 cells/uL(N=151, aOR 17.75, 95% CI 2.5–126.21, p=0.004), with a history of AIDS (N=116, aOR 8.83, 95% CI 1.18–65.88, p=0.03), with HCV co-

infection (N=155, aOR 6.42, 95% CI 1.07–38.43, p=0.04) and with current use of ART (N=206, aOR 4.34, 95% CI 1.24–15.24, p=0.02), although all of these results had wide confidence intervals. Similarly, exploratory analyses by ART class suggested stronger associations among NNRTI and NRTI users as compared with PI users, albeit ART class-specific odds ratios had overlapping confidence intervals (data not shown).

Discussion

A growing body of literature has shown that, in the general population, aberrant TF activity plays a role in thrombosis, which may lead to CVD events and death [6, 7, 9, 11]. Our study lends credence to the hypothesis that microparticle-related TF activity may influence the development of subclinical atherosclerotic plaque in people living with HIV. TF expression has previously been found to be associated with higher carotid artery intima-media thickness in an HIV-positive population, although only the inactive state of plasma TF was tested in this small (N=121) study [34]. This finding identifies a possible mediator of the complex relationship of increased inflammation, altered coagulation, atherosclerosis and CVD in people living with HIV. Our study focused on the functionally active MP-TF activity and found an association of elevated TF activity with the presence of carotid artery plaque. The study was conducted in a well-characterized longitudinal cohort of HIV-positive women, allowing us to control for demographic characteristics and traditional and non-traditional CVD risk factors.

The association between MP-TF activity and carotid artery plaque generally remained after adjustment for other markers of inflammation. However, we observed some evidence that adjustment for IL-6, a marker of systemic inflammation, attenuated the association, which was no longer statistically significant. In HIV populations, high levels of IL-6 have been independently linked to a higher risk of non–AIDS-related death and mortality [35, 36], even in persons treated with ART [37]. Our findings may reflect the possibility that upregulated MP-TF activity explains, in part, IL-6 activity and that a state of inflammation characterized by elevated circulating IL-6 may contribute to CVD-related coagulation abnormalities by increasing TF activity. Furthermore, the interplay between inflammation and coagulation might be bi-directional [38, 39].

We also observed that inclusion of sCD14 in regression models did not attenuate the observed association between MP-TF and disease, and in fact we observed little relationship between MP-TF and monocyte activation markers, including sCD14. This was unexpected. One potential explanation for this finding is that these markers in our highly comorbid study population may be affected by other pathways beyond monocyte activation or MP-TF expression. For example, others have reported that hepatocytes produce sCD14 both in the presence of hepatitis B and C virus infection [40], as well as in absence of infection but with non-alcoholic fatty liver disease (NAFLD) [41]. We have noted previously that while high sCD14 levels may reflect receptor shedding from activated monocytes, they also may be related to liver function and/or other aspects of HIV-related and non-HIV-related disease processes [42]. Therefore, the relatively small correlations we observed may reflect this heterogeneity. Further examination of these correlations in populations with fewer comorbid conditions that may potentially confound associations with MP-TF is warranted.

In contrast, we did identify a significant correlation between MP-TF activity and circulating D-dimer, even in a small subset of the study population, providing face validity that MP-TF activity represents part of the coagulation pathway. Circulating D-dimer is a strongly predictive marker of CVD and mortality in HIV-positive populations [43, 44]. Because of the limited sample size and one-time measurement of MP-TF, this preliminary report will need to be followed by larger studies to understand the relationship between MP-TF activity, D-dimer, inflammation and CVD in HIV-positive individuals.

Our observations suggest that baseline CD4 count and HIV RNA are not correlated with MP-TF activity. This may be because the use of ART in 75% of the sample may have led to higher CD4 counts and lower HIV RNA, thereby weakening correlations that would be more apparent in treatment-naïve individuals. Exploratory analyses found some suggestion that the association between MP-TF and carotid plaque is more pronounced in NRTI and NNRTI users, but the confidence limits were very wide in these analyses.

No statistically significant interactions between MP-TF activity and other virus-related factors were identified, possibly because of low statistical power due to the relatively few individuals with elevated MP-TF activity. However, in exploratory analyses, stronger associations between MP-TF activity and carotid artery plaque formation were found in certain subgroups including participants with undetectable HIV RNA, with nadir CD4+ count >200 cells/uL, with a history of AIDS, with HCV co-infection and with current use of ART. We speculate that this might be reflective of long-term immune activation and low levels of sustained inflammation. Chronic inflammation has been increasingly recognized as a common denominator underlying a host of progressive and age-related diseases [1, 45]. For example, the sCVD risk association with MP-TF activity was stronger in participants co-infected with HCV. While HCV infection does not directly target CD4 cells as HIV does, it does trigger immune activation and contributes to systemic immune dysregulation [26].

Our study has some limitations. First, while we focused on women living with HIV, who are an understudied population, our results may not be generalizable to men. Due to the cross-sectional nature of this study we are not able to determine if elevated MP-TF activity on its own increases cardiovascular risk or whether it is simply a consequence of inflammation without a direct causal role. Longitudinal data on MP-TF activity would allow for better assessment of temporality. While extensive longitudinal data were available in the cohort, there may still be uncontrolled confounding related to exposure to HIV RNA in different biological compartments, and other unmeasured variables. Lastly, assays of functionally active TF have not yet been standardized, which makes comparisons across independent laboratories difficult. Accordingly, we used both published and data-driven approaches to define thresholds of high MP-TF activity.

In summary, we demonstrated that MP-TF activity is significantly associated with carotid artery plaque formation in HIV-infected women. Our results identified a potential link between innate immune system perturbation and subclinical atherosclerosis in this at-risk population, and support the need for more research to better understand the mechanisms driving coagulation abnormalities among people living with HIV.

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J.L. drafted the paper. A.L.L., R.P.T. and R.C.K. conceived the work. X.X., H.N.H., A.L.L., R.P.T. and D.B.H. contributed to design of the work. K.A., M.H.C., S.J.G., J.M.L., C.L., W.J.M., P.C.T., R.P.T., and D.B.H. contributed to acquisition of data. C.T. performed the tissue factor assays. J.L. and X.X. performed statistical analyses. J. L., X.X., K.A., M.H.C., S.J.G, J.M.L., C.L., W.J.M., P.C.T., C.T., H.N.H., A.L.L., R.P.T., R.C.K. and D.B.H. contributed to the interpretation of data for the work, revised the work critically for important intellectual content, approved the version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy of integrity of any part of the work are appropriately investigated and resolved.

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Table 1.

Study population characteristics, by subclinical cardiovascular disease case status (N=275)

Characteristic	Control (sCVD-), N=177 % or median (IQR)	Case (sCVD+), N=98 % or median (IQR)	p-value
Demographic characteristics			
Age at baseline vascular study visit, years (median, IQR) *	46 (39–50)	46 (40–51)	-
Race/ethnicity			0.22
Black (non-Hispanic)	107 (60.5)	56 (57.1)	
Hispanic	56 (31.6)	27 (27.6)	
White (non-Hispanic)	14 (7.9)	15 (15.3)	
Other	-	-	
Education (at study entry)			0.10
Did not complete high school	63 (35.6)	45 (45.9)	
Completed high school	56 (31.6)	24 (24.5)	
At least some college	58 (32.8)	29 (29.6)	
Behavior-related characteristics			
Current crack/cocaine use	15 (8.5)	12 (12.2)	0.42
Current alcohol use			0.95
Abstainer	102 (57.6)	53 (54.1)	
Light (<3 drinks/week)	56 (31.6)	34 (34.7)	
Moderate (3–13 drinks/week)	15 (8.5)	8 (8.2)	
Heavier (14+ drinks/week)	4 (2.3)	3 (3.1)	
History of hepatitis C infection	72 (40.7)	48 (49.0)	0.21
Metabolic risk factors			
Current smoker *	90 (50.9)	51 (52.0)	-
Body mass index, kg/m^2 (median, IQR)	27.6 (24.4–31.8)	26.4 (23–31.2)	0.05
Systolic blood pressure, mmHg (median, IQR)	116 (107–124)	119 (110–132)	0.28
Total cholesterol, mg/dL (median, IQR)	172 (149–203)	178 (145–205)	0.47
HDL cholesterol, mg/dL (median, IQR)	47 (39–59)	46 (37–53)	0.19
Current use of anti-hypertensive medications	35 (19.8)	31 (31.6)	0.04
Current use of lipid-lowering medications **	12 (6.8)	10 (10.2)	0.26
Current use of aspirin	15 (8.6)	15 (15.5)	0.08
History of cancer diagnosis	5 (3.6)	1 (1.3)	0.93
History of diabetes	19 (10.7)	17 (17.4)	0.14
Estimated glomerular filtration rate (median, IQR)	94.0 (74.5–112.7)	100.0 (82.0–110.9)	0.09
HIV-specific characteristics			
Baseline CD4+ T-cell count, cells/mm^3 (median, IQR) *	397 (262–602)	370.5 (239–579)	0.93
Baseline HIV-1 viral load, copies/mL (median, IQR)	140 (80–6000)	365 (80–9800)	0.67
Undetectable baseline HIV-1 viral load	83 (46.9)	38 (38.8)	0.25
History of clinical AIDS	78 (44.1)	38 (38.2)	0.39
Potent ART use in past 6 months *	133 (75.1)	73 (74.5)	-

Characteristic	Control (sCVD-), N=177 % or median (IQR)	Case (sCVD+), N=98 % or median (IQR)	p-value
Cumulative exposure of potent ART[a], years (median, IQR)	3.5 (1.5–6)	4.5 (2.5–6.5)	0.14
of PIs, years (median, IQR)	1 (0–4.5)	2.5 (0–5)	0.23
of NNRTIs, years (median, IQR)	1 (0–3)	1.5 (0–3)	0.20
of NRTIs, years (median, IQR)	6 (2.5–8.5)	6 (3–9)	0.31
PI use in past 6 months	84 (48.0)	45 (46.4)	0.80
NNRTI use in past 6 months	58 (33.1)	26 (26.8)	0.28
NRTI use in past 6 months	131 (74.9)	74 (76.3)	0.79
Nadir CD4+ T-cell count before ART use[a], cells/mm^3 (median, IQR)	233 (122–362)	242 (152–363)	0.50
hsCRP (ug/mL)	2.2 (0.6–5.3)	1.7 (0.7–4.8)	0.29
IL-6 (pg/mL)	1.0 (0.6–1.5)	1.09 (0.7–2.1)	0.21
Galectin-3 (ng/mL)	9.4 (7.6–11.9)	9.66 (7.91–12.0)	0.44
Galectin-3 binding protein (ug/mL)	13.75 (8.3–23.8)	15.13 (9.36–23.6)	0.62
Soluble CD163 (ng/mL)	958.55 (738.5–1236.0)	996.83 (728.8–1357.5)	0.37
Soluble CD14 (ng/mL)	2134.5 (1730.3–2419.6)	2233.8 (1908.1–2633.5)	0.04

All characteristics assessed at baseline unless otherwise noted.

[a]Among those using ART at baseline.

[*]Cases of subclinical CVD (sCVD) and controls matched 1:2 based on baseline CD4+ count, ART use, age ±5 yr, current smoking at baseline. 19 cases had only 1 available control.

[**]21/22 participants reported statin use (N=11, atorvastatin; N=7, pravastatin; N=3, simvastatin), with 3/22 also reporting fibrate use.

AIDS = acquired immunodeficiency syndrome, ART = antiretroviral therapy, sCVD = subclinical cardiovascular disease,

HIV = human immunodeficiency virus, IQR = interquartile range, NNRTI = non-nucleoside reverse transcriptase inhibitor, NRTI = nucleoside reverse transcriptase inhibitor, PI = protease inhibitor.

Table 2.

Spearman correlation coefficients (r) for MP-TF with other serum biomarker levels and risk factors (N=275 unless otherwise noted).

	r	p-value		r	p-value
hsCRP	0.13	0.04	**IP-10 (N=18)**	0.47	0.05
IL-6	0.15	0.02	**Age**	0.01	0.93
Gal-3	0.03	0.65	**Nadir CD4 count**	0.12	0.05
Gal-3BP	0.05	0.45	**Baseline CD4 count**	0.01	0.88
sCD163	0.08	0.22	**Baseline HIV RNA**	0.03	0.58
sCD14	−0.02	0.81	**Total Cholesterol**	−0.12	0.07
Fibrinogen (N=8)	0.02	0.95	**LDL Cholesterol**	−0.11	0.07
D-dimer (N=18)	0.48	0.05	**Non-HDL Cholesterol**	−0.09	0.17

Table 3.

Distribution of MP-TF, by subclinical cardiovascular disease[1] case status (N=275)

Characteristic	Control (sCVD-), N=177 N (%) or median (IQR)	Case (sCVD+), N=98 N (%) or median (IQR)	p-value[2]
MP-TF activity (pg/mL)	0.10 (0.05–0.19)	0.10 (0.05–0.27)	0.59
MP-TF activity, >0.500 pg/mL	9 (5)	14 (14)	0.02
MP-TF activity, >0.537 pg/mL	6 (3)	13 (13)	0.01

[1] Subclinical CVD was defined based on focal plaque assessed using B mode ultrasound of the carotid artery.

[2] p-value for Wilcoxon Rank-sum test or Chi-Square test

Table 4.

Association between elevated MP-TF activity (>0.537 pg/mL) and subclinical cardiovascular disease case status[1] (N=275)

	Odds ratio	95% CI	P-value[2]
Model 1: Unadjusted model	4.07	(1.54, 10.74)	0.0046
Model 2: Model 1 plus adjustment for demographic and behavioral characteristics	3.63	(1.33, 9.90)	0.0119
Model 3: Model 2 plus adjustment for HIV-related factors	3.75	(1.28, 11.02)	0.0162
Model 4: Model 3 plus adjustment for cardiometabolic risk factors	3.86	(1.06, 14.07)	0.0407
Model 5: Model 4 plus adjustment for serum inflammation markers	3.32	(0.86, 12.75)	0.0805

[1] Subclinical CVD was defined based on focal plaque assessed using B mode ultrasound of the carotid artery.

[2] P-values based on conditional logistic regression.

Table 5.

Association between elevated MP-TF activity (>0.537 pg/mL) and subclinical cardiovascular disease case status (N=275)

	Odds ratio	95% CI	P-value[3]
Model 1: Fully adjusted model[1] without serum markers of inflammation	3.86	(1.06, 14.07)	0.0407
Model 2: Model 1 plus all serum markers of inflammation[2]	3.32	(0.86, 12.75)	0.0805
Model 3: Model 1 plus sCD14 only	3.84	(1.06, 13.93)	0.0408
Model 4: Model 1 plus sCD163 only	3.89	(1.06, 14.25)	0.0404
Model 5: Model 1 plus Gal-3 only	4.09	(1.11, 15.06)	0.0341
Model 6: Model 1 plus Gal-3BP only	3.72	(1.03, 13.47)	0.0453
Model 7: Model 1 plus IL-6 only	3.09	(0.83, 11.44)	0.0913
Model 8: Model 1 plus hsCRP only	4.07	(1.10, 15.09)	0.0358

[1]Adjusted for demographic and behavioral characteristics and HIV-related and cardiometabolic risk factors

[2]Including sCD14, sCD163, Gal-3, Gal-3BP, IL-6 and hsCRP

[3]P-values based on conditional logistic regression.