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Predicting Subclinical Cardiovascular Disease in HIV-infected Women using a Biomarker Score

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Predicting Subclinical Cardiovascular Disease in HIV-infected Women using a Biomarker Score

By

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Abstract

Predicting Subclinical Cardiovascular Disease in HIV-infected Women using a Biomarker Score

By Caitlin Moran

HIV infection is a well-established independent risk factor for cardiovascular disease (CVD). The risk of CVD is especially increased among younger individuals and women with HIV, compared with age- and sex-matched controls in the general population. However, CVD risk-stratification tools underperform in this setting. Biomarkers, including high-sensitivity C-reactive protein (hsCRP), d-dimer, heat shock protein 70 (HSP-70), and bilirubin, are associated with CVD risk in the general population, but their utility in enhancing CVD prediction in the HIV-infected population is unknown. In this retrospective cohort study of HIV-infected and at-risk HIV-uninfected women in the Women's Interagency HIV Study (WIHS) vascular substudy, hsCRP, d-dimer, HSP-70, and bilirubin, levels measured at the initial substudy visit above or below pre-specified cutpoints made up a composite biomarker score from 0-4. Multivariable regression modeling was used to determine the association between the biomarker score and the presence and progression of subclinical CVD [increased carotid intima-media thickness (CIMT), development of common carotid artery (CCA) lesions (IMT >1.5 mm), and/or increased carotid artery echolucency] as measured by high resolution B-mode ultrasonography. 783 women (572 HIV-infected, 211 HIV-uninfected) were followed for a median of 6.6 years. The composite biomarker score was not associated with baseline prevalence or progression of subclinical CVD. However, in HIV-uninfected women, hsCRP was associated with CCA plaque progression [RR 2.00, 95% confidence interval (CI) 1.21-3.29, p=0.007] and CIMT progression (mean difference 5.4, 95% CI 0.2-10.7, p=0.04]. No association was seen in HIV-infected women. These findings suggest that the pathogenesis of CVD may be different in the setting of HIV infection and further research is warranted.

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INTRODUCTION

Human immunodeficiency virus (HIV) infection is associated with an increased risk of cardiovascular disease (CVD). Persons living with HIV experience CVD events including myocardial infarction (MI) and ischemic stroke at younger ages and with greater severity than persons in the general population (1, 2). Even though the total number of CVD events are greatest in older men, the effect modification of HIV infection on CVD risk is greatest in younger women (3, 4). However, frequently used CVD risk-prediction tools are not validated in persons under the age of 40, and women traditionally have been underrepresented in many of the large studies that inform these risk calculators.

Biomarkers, therefore, are an attractive non-invasive option for improving CVD risk prediction. However, when used individually, many biomarkers associated with CVD risk fail to improve CVD risk prediction beyond risk calculators (5). However, in a study of 3,763 HIV-uninfected individuals undergoing left heart catheterization, a composite biomarker score consisting of high-sensitivity C-reactive protein (hsCRP), fibrin degradation products or d-dimer, and heat shock protein-70 (HSP-70), the risk of MI or all-cause mortality was increased for each biomarker that was present above a specific threshold (6). We therefore sought to determine if a similar composite biomarker score could predict subclinical CVD in HIV-infected women.

We conducted a retrospective cohort study utilizing data from the Women's Interagency HIV Study (WIHS) cohort vascular substudy. In this prospective cohort substudy, women underwent up to 4 carotid ultrasounds between 2004-2013 to assess carotid plaque, carotid intima-media thickness, and carotid echolucency (7). Plasma and

serum was obtained at the start of the study. We hypothesized that baseline biomarker score would be associated with both the prevalence of subclinical CVD at the start of the substudy, as well as the progression of subclinical CVD.

BACKGROUND

Thanks to effective treatment with combination antiretroviral therapy (cART), the life expectancy of persons living with HIV is approaching that of the general population (8, 9). In high-income countries, non-AIDS causes of mortality now exceed AIDS-related causes of mortality in HIV-infected individuals, and CVD currently accounts for approximately 20% of non-AIDS related causes of mortality in this population (10, 11). As the HIV-infected population continues to age, the prevalence of CVD and other non-communicable diseases is expected to increase (12). Furthermore, many population-based studies suggest that HIV infection increases the risk for CVD: individuals infected with CVD experience MI and stroke 10-15 years earlier than the general population (13, 14), and have more frequent complications (1, 2, 15). This increased risk of CVD in the setting of HIV infection is particularly pronounced in younger women, despite the overall incidence of CVD being greater in men and in older patients. In a nested case-control study conducted in France of 360 cases of MI among HIV-infected individuals between 2000-2006 matched by age and gender to controls in the general population, the overall standardized morbidity ratio (SMR) for MI was 1.5 [95% confidence interval (CI) 1.3-1.7), with an SMR for women of 2.7 (95% CI 1.8-3.9) (3). The effect measure modification of HIV on MI rates was even more pronounced in the youngest age strata among women with SMRs of 4.9 (95% CI 2.1-3.9) and 5.1 (95% CI 2.8-8.0) for women ages 35-39 and 40-44, respectively (3). Additionally, in a United States cohort of 4,308 HIV-infected individuals and matched HIV-uninfected controls, an overall hazard ratio (HR) for ischemic stroke of 1.40 (95% CI 1.17-1.69) was observed, although the incidence rates for stroke were only statistically significantly greater in HIV-infected

individuals up to age 50 (4). Furthermore, the increased incidence of stroke was observed in women, but not in men: women with HIV had an adjusted HR of 1.76 (95% CI 1.24-2.52) for stroke compared with HIV-uninfected controls while men with HIV had an adjusted HR of 1.05 (95% CI 0.84-1.32), with the greatest incidence rate ratios occurring in women up to age 40 (4). Taken together, these data indicate that the effect of HIV on CVD is more pronounced in women compared with men, and in younger individuals compared with older individuals, suggesting that HIV infection may mediate a different pathway for vascular dysfunction in this population.

Despite this increased risk for CVD observed in HIV-infected patients, no good risk-prediction tool exists to identify those HIV-infected patients, particularly younger and female patients, who are at greater risk for CVD. Traditional CVD risk-prediction calculators, including the 2013 American College of Cardiology/American Heart Association Atherosclerotic Cardiovascular Disease (ACC/AHA ASCVD) risk calculator, are not validated for individuals under the age of 40 (16). The ASCVD risk calculator does not include HIV as a CVD risk factor and offers little guidance for risk-stratifying individuals with HIV due to lack of evidence (17). Furthermore, CVD risk scores have been shown to underperform in HIV-infected individuals, either overestimating or underestimating CVD risk (18). For example, when the ASCVD risk guidelines were applied to a cohort of HIV-infected patients, 74% of patients who were later found to have high-risk coronary plaques were initially classified as low risk (19). This misclassification is not inconsequential: had those patients been classified as moderate- or high-risk, it would have been recommended that they be treated with a statin, which have been shown to reduce plaque burden and cardiac events in the general

population and in HIV-infected individuals (20-22). Therefore, there exists a need for improved CVD risk prediction in the HIV-infected population, particularly among HIV-infected women.

Biomarkers have been studied extensively for their association with CVD in the general population. These soluble molecules are involved in inflammatory, hemostatic, and cellular damage pathways that are associated with CVD risk (23). However, when these biomarkers are added individually to CVD risk equations, they only modestly improve CVD risk prediction (5). This lack of improvement in CVD risk assessment is likely because the development of atherosclerosis is a complex process involving multiple pathways including inflammation, coagulation, cellular stress and oxidation (6), and individual biomarkers reflect only one or two of these processes. HsCRP is produced by hepatocytes in response to pro-inflammatory cytokines and in the setting of decreased nitric oxide production and increased endothelin-1 production, both of which lead to vasoconstriction and vascular dysfunction (24). D-dimer and other fibrin degradation products (FDPs) are end products of the coagulation cascade and are associated with CVD severity and adverse cardiac events (6). Heat shock proteins, including HSP-70, are intracellular proteins that serve as molecular chaperones and are produced in response to cellular stress (6). Finally, bilirubin is a potent antioxidant that, at physiologic levels, is associated with decreased atherosclerosis and markers of inflammation (25). When hsCRP, FDP, and HSP-70 were assessed in aggregate in 3,763 HIV-uninfected persons (median age 63 years, 65% male) undergoing coronary angiography, each biomarker above a pre-specified threshold was associated with an additional increased risk of MI or cardiac death (6).

However, it is unknown how these biomarkers will perform in women infected with HIV. CVD pathogenesis is further complicated by HIV infection due to chronic immune activation and persistent inflammation (26), and there is some evidence that, in the setting of HIV infection, women have a greater degree of immune activation than men (27, 28). Therefore, our study was designed to determine the association between a composite biomarker score consisting of hsCRP, d-dimer, HSP-70, and bilirubin and the prevalence and progression of subclinical CVD in HIV-infected women and HIV-uninfected women at risk for HIV infection in the WIHS cohort.

METHODS

Research Goal

The study aims were (1): to develop a composite biomarker score consisting of hsCRP, d-dimer, HSP-70 values above, and bilirubin values below, pre-specified cutpoints determined from the literature; and (2): to determine the relationship between the composite biomarker score and the prevalence and progression of subclinical cardiovascular disease (CVD) in HIV-infected women and HIV-uninfected women who are at risk for HIV infection.

Study Design

To determine if a biomarker score consisting of hsCRP, d-dimer, HSP-70, and bilirubin predicts the prevalence and progression of subclinical CVD in HIV-infected women and at-risk HIV-uninfected women, a retrospective cohort study utilizing data from the WIHS vascular substudy was conducted.

Characteristics of the Study population

Study data was obtained from the Women's Interagency HIV Study (WIHS) cohort. WIHS is an ongoing longitudinal cohort of HIV-infected women and HIV-uninfected women who are at risk for HIV infection who are frequency matched on demographics and key risk factors including age, race, level of education, injection drug use, and number of sexual partners (29). Women were recruited from primary care, infectious disease, and obstetrics/ gynecology clinics, HIV community organizations, churches, and social service organizations. The initial enrollment occurred between 1994 and 1995 and included 2,059 HIV-infected and 569 HIV-uninfected women with a smaller second enrollment of 1,143 (737 HIV-infected and 406 HIV-uninfected) in 2001-

02 at six clinical consortia in the United States: Brooklyn/Manhattan, NY; Bronx, NY; Chicago, IL; San Francisco/Bay Area, CA; Los Angeles/Southern California/Hawaii; and Washington, DC (30). Because of the growing burden of the HIV epidemic among minority populations in the Southeast, four additional sites: Chapel Hill, NC; Atlanta, GA; Miami, FL; and Birmingham, AL/Jackson, MS, were added to WIHS in 2013. WIHS participants have follow-up visits at six-month intervals during which detailed medical histories are obtained and comprehensive physical examinations are conducted. Plasma, serum, peripheral blood mononuclear cells (PBMCs), genital secretions, and urine samples are collected and placed in a central repository (SeraCare, Inc.). Data collected during WIHS core visits include age, race, body mass index (BMI), blood pressure, use of lipid-lowering medication, current smoking status, diabetes, CD4 counts, HIV viral load, and combination antiretroviral therapy (cART) use.

Description of the WIHS Vascular Substudy

In 2004, a prospective vascular disease substudy was initiated among WIHS participants that included high resolution B-mode ultrasonography of the common carotid artery (CCA) to measure carotid artery plaques, carotid intima-media thickness (CIMT) and carotid artery echolucency (figure 1). All WIHS participants were eligible for participation in the substudy, and 1331 HIV-infected and 534 HIV-uninfected WIHS participants were enrolled (31). Baseline substudy visits occurred between 2004-2006 and study participants completed up to three follow-up visits with a visit every 2-3 years through 2013; 1011 women completed at least 2 substudy visits (7). In addition to ultrasound measurements, plasma and serum samples were collected from participants at

their baseline vascular substudy visit. Demographic and clinical data from WIHS core visits were merged with substudy data.

Inclusion criteria. All WIHS participants who were enrolled in the vascular substudy, who completed the baseline study visit and at least one follow-up study visit, and who had serum specimens available for biomarker analysis were included.

Exclusion criteria. Participants who became infected with HIV during the course of the analysis period were excluded from the analysis.

Measurements

Description of study variables

Outcome variables: Subclinical CVD. Subclinical CVD was defined in three ways: carotid intima-media thickness (CIMT), carotid artery echolucency as measured by gray-scale media (GSM), and the presence of focal carotid artery plaques as defined by focal intima-media thickness (IMT) > 1.5 mm in any of the imaged segments. Progression of subclinical CVD was defined as increase in CIMT, increase in echolucency (decrease in GSM), and formation of new focal carotid plaques. Change in CIMT and GSM were calculated as the final measurement minus the baseline measurement. New plaque formation was calculated as the difference in the number of focal plaques measured between the baseline visit and final study visit.

Predictors: Biomarker score. Baseline serum and plasma levels of hsCRP, d-dimer, and HSP-70 above the established cutpoints were given a score of one that was added to the aggregate score, while serum levels of total bilirubin below the established cutpoint were given a score of one, such that the total biomarker score was measured from 0-4. The following cutpoints for the biomarkers were used based on previously published studies

using data from the general population: hsCRP 3.0 mg/l, d-dimer 0.5 μg/ml, HSP-70 0.625 ng/ml, and bilirubin 1.2 mg/dl (6, 25, 32, 33).

Description of covariates. Traditional CVD risk factors and HIV-related variables were controlled for in this analysis. CVD risk factors included age, race/ethnicity, systolic blood pressure, use of antihypertensive medications, low-density lipoprotein (LDL) cholesterol, cholesterol-lowering medication use, diabetes, estimated glomerular filtration rate (eGFR), smoking status, crack/cocaine use, and hepatitis C virus (HCV) infection. HIV-associated covariates included CD4 count, CD4 nadir, HIV viral load, and combination antiretroviral therapy (cART) use.

Demographic, clinical, and laboratory variables were collected by standardized protocols at semiannual core study visits and merged with baseline vascular substudy data. HIV infection was determined by serologic testing using ELISA and confirmed with Western blot assays. Plasma HIV RNA levels were quantified using nucleic-acid-sequence-based amplification commercial assays with a lower limit of quantification of 80 copies/ml (bioMérieux, Boxtel, NC), total peripheral CD4 T cell counts were measured with standard flow cytometric methods. CD4 T cell nadir was defined as the lowest CD4 T cell count prior to cART initiation. cART use was defined as the use of three or more antiretroviral medications, one of which had to be a protease inhibitor, non-nucleoside reverse transcriptase inhibitor, one of the nucleoside reverse transcriptase inhibitors tenofovir or abacavir, an integrase inhibitor, or an entry inhibitor. Total cholesterol, HDL cholesterol, LDL cholesterol, and triglycleride levels were obtained following a fast of at least 8 hours at the WIHS core visit most proximate to the baseline vascular substudy visit. Demographic information including age, race/ethnicity, income,

education, history of injection drug use, history of crack/cocaine use, and alcohol use, was obtained by participant self-report. Cardiometabolic data including current cigarette smoking and smoking history, current use of antihypertensives and lipid-lowering medications, and history of diabetes were also obtained by self-report.

Serum samples were obtained at the baseline vascular substudy visit, and ultrasound data were obtained by trained ultrasound technicians at the baseline substudy visit in 2004-2006 and at follow-up visits at 2-3 year intervals through 2013 (7). CIMT and carotid echolucency measurements were obtained at all follow-up visits; carotid plaque measurements were obtained only at the baseline and final visits. All sites followed a standardized protocol and all carotid artery outcome measures were obtained at a centralized reading center at the University of Southern California (7, 31).

Ultrasound data. High resolution B-mode ultrasonography was used to measure six locations of the right common carotid artery (CCA) according to previously published procedures (34): the near and far walls of the right CCA, the internal carotid artery (ICA), and the carotid bulb (31). In the same CCA segment, the echo-strength [measured as gray-scale median (GSM) based on the echo gray-white scale from 0 to 255, unitless] of each individual pixel within the intima-media complex was measured by methods described previously (35). New focal plaque formation was defined as an increase in the number of focal plaques measured over the six carotid artery locations between the baseline and final vascular study visit (7).

Measurement of biomarkers. Serum samples obtained at the baseline vascular substudy visit were stored in the WIHS central repository at -80 ^{o}C. Samples were obtained from the repository and kept frozen until assay time. As part of the vascular substudy, citrate

plasma hsCRP measurements were obtained at the baseline substudy visit by a nephelometric immunoassay (Dade-Behring BN II) (36). Bilirubin levels obtained at the WIHS core visit most proximate to the participant's vascular substudy visit were used. HSP-70 levels were measured in serum by quantitative ELISA (Affymetrix eBioscience, Santa Clara, CA); D-dimer levels were measured in serum by quantitative ELISA (Abcam, Cambridge, MA). Minimum detectable concentrations of the assays were 71 pg/ml and 0.052 ng/ml, respectively.

Missing data. Nine participants were missing data for CIMT, 47 were missing data for CCA plaques, and 111 were missing data for carotid echolucency. Among the biomarkers, 31 were missing hsCRP values, 15 were missing d-dimer values, 134 were missing HSP-70 values, and 3 were missing bilirubin values. WIHS is a well-curated dataset and less than 1% of all covariate data were missing, except for lipid data, of which 5% of the data were missing at random. Therefore, multiple imputation with five datasets were used for missing covariate data.

Sample size and power considerations

Data on the association between the composite biomarker score and CIMT, focal carotid plaque formation, and carotid artery echolucency are not available. However, a recent study of 3,415 patients undergoing elective or emergent coronary angiograms examined the association between a similar biomarker score (including only CRP, d-dimer, and HSP-70) and the severity of coronary artery disease (6). On this 0-3 scale, the distribution of aggregate biomarker scores was 37%, 43%, 17%, and 4% for 0, 1, 2, and 3 biomarkers, respectively. The annual rate of cardiac death or MI for all participants was approximately 2%, 4%, 11%, and 16% for 0, 1, 2, and 3 biomarkers. Although

subclinical CVD is not directly measured in this study, it is a precursor to MI and cardiac death; therefore, these rates are likely underestimates of the prevalence of subclinical CVD in the population. Additionally, since the early aggregate biomarker score does not contain bilirubin, it is likely an underestimate of the currently proposed biomarker score.

In the WIHS vascular study, the average right CIMT increased from 725 μm to 752 μm over a median of 7 years of follow-up. At baseline, 8% of study participants (43 HIV-infected, 16 HIV-uninfected) had focal carotid artery plaques. At the final substudy visit, 93 (16%) of HIV-infected participants and 21 (10%) of HIV-uninfected participants had any focal plaques; 70 (12%) of HIV-infected and 16 (8%) of HIV-uninfected participants had plaques that were new since the previous visit (7).

Assuming a prevalence of the biomarker score similar to what was observed in the previous biomarker study (6) and considering the event rates as estimates of the proportion of the population with subclinical CVD [which was seen in the WIHS vascular substudy (7)], and conservatively estimating 505 eligible WIHS participants, using a Cochran-Armitage test for trend in proportions with sample sizes of 185, 215, 85, and 20 for aggregate risk scores of 0, 1, 2, and 3, a total sample of 505 subjects achieves 91% power to detect a linear trend using a two-sided Z test with continuity correction and a significance level of 0.05, thus providing adequate power for both aims among HIV-infected participants.

Analytic plan

Descriptive analyses. Summary statistics for baseline demographic, clinical, biomarkers, and outcome variables were calculated. Frequencies were calculated for categorical

variables, and histogram plots were created for continuous variables to assess for normal distribution.

Biomarkers. 12 participants had 0 biomarkers above or below the pre-specified cutpoints, 153 had 1 biomarker, 298 had 2 biomarkers, 224 had 3 biomarkers, and 41 had 4 biomarkers. Therefore, those with 0 and 1 biomarkers were combined for further analysis, as were those with 3 and 4 biomarkers.

Statistical inference

Point estimates, confidence intervals, statistical tests. For continuous variables, mean (± standard deviation) or median (interquartile range q1-q3) were calculated; for categorical variables, proportions (%) were calculated. Analyses were performed for the entire cohort, and by biomarker score stratified by HIV status. Within-group and between-group differences were assessed by Student's t-test or Wilcoxon rank-sum test (continuous variables) or Chi-squared or Fisher's exact tests (categorical variables).

Bivariable and multivariable analyses. To assess for the relationship between the various biomarkers and the subclinical CVD outcomes, bivariable and multivariable regressions were performed using the individual biomarkers as predictors both categorically above/below the pre-specified cutpoints, and as continuous variables. HsCRP, d-dimer, and HSP-70 were right-skewed; therefore, these variables were natural log-transformed for normality prior to using as continuous variables. The overall biomarker score was then assessed with 0-1 biomarkers as the reference (2 vs. 0-1 and 3-4 vs. 0-1). Each subclinical CVD endpoint was assessed individually. For the multivariable regressions, covariates were chosen *a priori* based on clinical importance. Linear regression was used for continuous variables [CIMT or carotid echolucency

(GSM)]. Logistic regression was used for baseline carotid plaques; Poisson regression was used for the number of carotid plaques formed. The final multivariable models were:

Baseline prevalence of disease:

Logit (CCA plaques) = $\beta_0 + \beta_1 biomarker + \beta_2 age + \beta_3 race + \beta_4 CVDhx + \beta_5 BP + \beta_6 LDL + \beta_7 BPmed + \beta_8 statin + \beta_9 DM + \beta_{10} eGFR + \beta_{11} smoke + \beta_{12} crack + \beta_{13} HCV + (\beta_{14} CD4 + \beta_{15} cART + \beta_{16} VLoad)$

Baseline CIMT (or GSM) = $\beta_0 + \beta_1 biomarker + \beta_2 age + \beta_3 race + \beta_4 CVDhx + \beta_5 BP + \beta_6 LDL + \beta_7 BPmed + \beta_8 statin + \beta_9 DM + \beta_{10} eGFR + \beta_{11} smoke + \beta_{12} crack + \beta_{13} HCV + (\beta_{14} CD4 + \beta_{15} cART + \beta_{16} VLoad)$

Progression of disease:

Log (change in CCA plaques) = $\log(followuptime) + \beta_0 + \beta_1 biomarker + \beta_2 age + \beta_3 race + \beta_4 CVDhx + \beta_5 BP + \beta_6 LDL + \beta_7 BPmed + \beta_8 statin + \beta_9 DM + \beta_{10} eGFR + \beta_{11} smoke + \beta_{12} crack + \beta_{13} HCV + (\beta_{14} CD4 + \beta_{15} cART + \beta_{16} VLoad)$

Change in CIMT (or GSM) = $\beta_0 + \beta_1 biomarker + \beta_2 age + \beta_3 race + \beta_4 CVDhx + \beta_5 BP + \beta_6 LDL + \beta_7 BPmed + \beta_8 statin + \beta_9 DM + \beta_{10} eGFR + \beta_{11} smoke + \beta_{12} crack + \beta_{13} HCV + (\beta_{14} CD4 + \beta_{15} cART + \beta_{16} VLoad)$

Analyses were conducted using SAS v9.4 (SAS Institute, Cary, NC).

RESULTS

Baseline characteristics

783 participants (572 HIV-infected and 211 HIV-uninfected) were followed for a median of 6.6 years (IQR 6.4-7.0). Median (IQR) age at baseline was 40.6 (35.2-46.7) years. 62% of participants were Black; 29% were Hispanic. Overall, participants had low systolic blood pressure [median (IQR) 117 (109-127) mm Hg] and low low-density lipoprotein (LDL) cholesterol [median (IQR) 102 (79-124) mg/dL] at baseline. 42 (5%) of participants were on cholesterol-lowering medication, 14% had a diagnosis of diabetes mellitus, and 25 (3%) had a history of clinical CVD defined as a history of prior myocardial infarction, hospitalization for angina, ischemic stroke, or revascularization. 48% of participants were current smokers, and 28% were infected with hepatitis C virus. The overall 10-year CVD Framingham risk was 1%. Among HIV-infected participants, median (IQR) CD4 count was 452 (288-658) cells/mm^3, HIV viral load was 180 (80-6900) copies/mL. 62% of participants were on cART. CD4 count and cART use decreased as the biomarker score increased ($p=0.008$ and <0.0001, respectively) (table 1).

Subclinical CVD

At baseline, 54 (7%) of participants had carotid plaques; 39 (7%) HIV-infected and 65 (12%) HIV-uninfected ($p=0.79$). Baseline median (IQR) CIMT was 707 (649-776) μm among HIV-infected women and 714 (657-794) μm among HIV-uninfected women ($p=0.20$). Baseline median (IQR) GSM for HIV-infected women was 60 (48-71), unitless, and for HIV-uninfected women was 60 (47-72), unitless ($p=0.23$). There were also no differences in the progression of subclinical CVD between HIV-infected and HIV-uninfected women (table 2).

Biomarkers

Baseline median (IQR) hsCRP was 2.2 (0.8-5.3) mg/dL in HIV-infected women and 3.2 (0.9-7.7) mg/dL in HIV-uninfected women (p=0.005). Baseline median (IQR) d-dimer was 0.50 (0.28-0.92) μL/mL in HIV-infected women and 0.47 (0.30-0.69) μL/mL in HIV-uninfected women (p=0.15). Baseline median (IQR) HSP-70 was 0.40 (0.17-0.92) in HIV-infected women and 0.47 (0.30-0.69) in HIV-uninfected women (p=0.22). Baseline median (IQR) bilirubin was 0.4 (0.3-0.6) mg/dl in HIV-infected women and 0.4 (0.3-0.6) in HIV-uninfected women (p=0.90) (table 3).

Univariable analysis

Baseline subclinical CVD. In unadjusted regression analyses, no association was observed between any biomarker or the composite biomarker score and baseline CCA plaques or CIMT. However, baseline hsCRP was associated with lower carotid echolucency with a mean difference (MD) [95% confidence interval (CI)] of -2.5 (-3.5, -1.5) unitless, p<0.0001 for every unit increase in the natural log of hsCRP for the entire cohort, -1.9 (-3.1, -0.7) unitless, p=0.002 for HIV-infected women and -4.0 (-5.8, -2.2) unitless, p<0.0001 for HIV-uninfected women (table 4).

Subclinical CVD progression. In unadjusted regression analyses, hsCRP was associated with progression of CCA plaques among HIV-uninfected women with a rate ratio (95% CI) of 1.74 (1.25, 1.74), p=0.002 for every unit increase in the natural log of hsCRP. No association was observed between hsCRP and CCA plaque progression among HIV-infected women [RR (95% CI) 0.97 (0.81, 1.18), p=0.79]. D-dimer was associated with CCA plaque regression among HIV-uninfected women, RR (95% CI) 0.52 (0.35, 0.82), p=0.009, but not among HIV-infected women, RR (95% CI) 1.14 (0.90, 1.47), p=0.27 for

every unit increase in the natural log of d-dimer. No other biomarkers or the composite biomarker score were associated with CCA plaque progression (table 5). HsCRP was associated with CIMT progression for the entire cohort with a mean difference (MD) (95% CI) of 3.2 (0.0, 6.3) μm, p=0.047 for every unit increase in the natural log of hsCRP, although not when stratified by HIV status. HSP-70 was associated with CIMT regression among HIV-infected women, MD (95% CI) -4.5 (-8.2, -0.8), p=0.02 for every unit increase in the natural log of HSP-70. There was also a trend toward an association with CIMT progression the composite biomarker score of 2 vs. 0-1 among HIV-uninfected women, MD (95% CI) 17.1 (-1.0, 35.3) μm, p=0.06, and there was a stronger association with a biomarker score of 3-4 vs. 0-1 among HIV-uninfected women, MD (95% CI) 24.4 (6.1, 42.8), p=0.009, but not among HIV-infected women (table 5). There was no association between any biomarker or the composite biomarker score and the carotid echolucency progression (table 5).

Multivariable analysis

Baseline subclinical CVD. Adjusting for traditional CVD risk factors and HIV-associated risk factors described above, there was no association between any of the biomarkers or the composite biomarker score and baseline CCA plaques or baseline CIMT. However, hsCRP was associated with a decrease in carotid echolucency among HIV-infected women, MD (95% CI) -2.1 (-3.4, -0.9) unitless, p=0.001, and among HIV-uninfected women, MD (95% CI) -4.2 (-6.2, -2.2) unitless, p<0.0001 for every unit increase in the natural log of hsCRP. In addition, there was an association with decreased carotid echolucency and a composite biomarker score of 3-4 vs. 0-1 for HIV-infected

women, MD (95% CI) -5.3 (-9.7, -0.9) unitless, p=0.02 with a trend in HIV-uninfected women, MD (95% CI) -5.9 (-14.3, 2.4) unitless, p=0.16 (table 6).

Subclinical CVD progression. In multivariable regression, hsCRP was associated with progression of CCA plaques in HIV-uninfected women, RR (95% CI) 2.00 (1.21, 3.29), p=0.007, but not in HIV-infected women, RR (95% CI) 1.00 (0.81, 1.25), p=0.97 for every unit increase in the natural log of hsCRP. There was no association with any of the other biomarkers or the composite biomarker score and CCA plaque progression. Similarly, there was an association between hsCRP and CIMT progression among HIV-uninfected women, MD (95% CI) 5.4 (0.2, 10.7) μm, p=0.04, but not among HIV-infected women, MD (95% CI) 3.0 (-1.5, 7.5) μm, p=0.20 for every unit increase in the natural log of hsCRP. In addition, among HIV-uninfected women, there was a trend towards an association of CIMT progression and the composite biomarker score 2 vs. 0-1, MD (95% CI) 19.4 (-0.9, 39.7) μm, p=0.06 and a stronger association between CIMT progression and a composite biomarker score of 3-4 vs. 0-1, MD (95% CI) 29.5 (8.8, 50.3) μm, p=0.005. Similar associations were not seen among HIV-infected women (table 7).

DISCUSSION/CONCLUSIONS

In this retrospective cohort study of HIV-infected and at-risk HIV-uninfected women, a composite biomarker score consisting of hsCRP, d-dimer, HSP-70, and bilirubin was not associated with the prevalence or progression of subclinical CVD. Furthermore, with the exception of hsCRP and baseline carotid echolucency, there was no apparent association between the individual biomarkers and baseline prevalence of subclinical CVD: higher levels of hsCRP were associated with lower baseline carotid echolucency in all participants. Similarly, d-dimer, HSP-70, and bilirubin were not associated with subclinical CVD progression. However, higher levels of hsCRP were associated with the progression of CCA plaques and CIMT in HIV-uninfected, but not HIV-infected, participants. Since both HIV-infected and –uninfected women had similar CVD risk factors at baseline and similar rates of baseline subclinical CVD and subclinical CVD progression, these findings suggest that the pathogenesis of CVD in HIV-infected women is different than in HIV-uninfected women.

This association of hsCRP with subclinical CVD progression in HIV-uninfected women is consistent with a robust body of evidence that hsCRP is associated with CVD progression and severity in the general population (20, 23, 24). The evidence for an association between hsCRP and CVD in the HIV-infected population is much less clear. In one study of 70,357 patients (487 HIV-infected) in the United States, elevated CRP and HIV infection were both independently associated with acute MI when included in the same model; however, importantly, this model was not adjusted for smoking status (37). A retrospective analysis of the SMART study, in which HIV-infected patients were randomized to continuous cART or intermittent cART, similarly found that higher

plasma levels hsCRP and d-dimer were associated with CVD events in HIV-infected individuals (38). In contrast, a case-control study evaluating HIV-infected patients who did or did not experience a MI, hsCRP was not associated with MI (39). In a systematic review of inflammatory biomarkers and CVD in HIV-infected patients, Vos et al. reported an association of both CRP and d-dimer with an increased risk of CVD; interestingly, such an association did not exist between the inflammatory biomarkers and CIMT (40). Our study, which similarly included subclinical endpoints, rather than hard clinical endpoints, also did not find any association between these biomarkers and progression of subclinical CVD in HIV-infected women.

There are several possible explanations for this observed discrepancy between the association of hsCRP and subclinical CVD progression in HIV-uninfected, but not HIV-infected women. One likely possibility is that the pathogenesis of atherosclerosis is different in the setting of HIV infection. HIV infection is associated with chronic innate, as well as adaptive, immune activation even in the setting of viral suppression of cART, which has been shown to be associated with CVD in HIV-infected individuals (41-43). Furthermore, HIV-infected individuals experience a disruption in their intestinal microbiome that leads to increased GI permeability and endotoxin release, further contributing to the inflammatory milieu (44, 45). Finally, in women, HIV infection is associated with premature ovarian insufficiency and menopause, and estrogen depletion is considered to be a risk factor for many inflammatory conditions (46). In a study of pre-menopausal HIV-infected women, estrogen and testosterone levels were found to be lower than in age-matched HIV-uninfected controls, and that this relative decrease in sex hormones was associated with an increase in subclinical CVD (46). These causes of

inflammation could all have contributed to subclinical CVD progression in the HIV-infected group in our study, and would not be accounted for by the biomarkers studied in this analysis.

In addition, our study utilized subclinical endpoints rather than hard clinical endpoints of MI or death. The pathophysiologic processes that lead to atherosclerosis and plaque development are probably different from those that lead to stable plaque and plaque rupture (6). Therefore, it is not surprising that the aggregate biomarker score that was strongly associated with MI or cardiac death in previous studies (6), was not associated with subclinical CVD in our study. This difference between atherogenesis and plaque rupture may account for the lack of association observed between d-dimer, HSP-70, and bilirubin and subclinical CVD; however, it does not account for the hsCRP findings that were discussed above.

Our study had several strengths. The relatively large study population from six clinical sites represents different geographic locations in the US, and the demographics of the study population are generally representative of HIV-infected women in the US (30). Also, the long follow-up period of almost seven years allowed for observation of subclinical CVD progression over an extended period of time. We also focused on women, who traditionally have been underrepresented in both cardiovascular and infectious disease studies in the US, but are nevertheless important to study since disease processes often differ by sex. Finally, we chose our predictors *a priori* based on biologic plausibility and prior clinical data, rather than searching for one or two biomarkers out of several. In doing so, we were able to conclude that biomarkers that were effective at

predicting CVD in the general population did not perform well in HIV-infected individuals, and that research efforts may be better directed elsewhere.

This study also had several limitations. Its retrospective design did not allow for adjustment for all relevant covariates, such as sex hormones, that may be important in CVD pathogenesis in women. In limiting the study population to women, we are unable to generalize our findings to include men. Furthermore, although measures of subclinical CVD are associated with MI and cardiac death (47, 48), the use of subclinical endpoints limits the conclusions we can draw from the data. Since not all atherosclerosis leads to MI or stroke, we are unable to assess whether the biomarkers studied are associated with these important clinical outcomes in HIV-infected women. Our study population was relatively young and healthy and at low overall risk for MI, so the follow-up time may not have been long enough to capture subclinical CVD progression.

Despite these limitations, this study contributes to a growing body of data that inflammation-related comorbidities associated with HIV infection may be different that those seen in the general population, and that predictive biomarkers used in the general population may not be applicable to HIV-infected individuals. In addition, this study included over 700 HIV-infected and -uninfected women, approximately 90% of whom are racial or ethnic minorities, and contributed to the understanding of CVD in minority women, a population that is typically underrepresented in clinical research.

No causal relationships can be determined from the results of this study, and further research is needed to understand the pathogenesis of CVD in HIV-infected women, so that risk-prediction tools can be developed. Next steps include studying markers of immune activation as predictors of CVD, as well as studying the role HIV

infection plays in the pathogenesis of inflammatory comorbidities in women, possibly mediated through relative estrogen insufficiency or gut dysbiosis with the aim of better predicting disease progression.

REFERENCES

1. Boccara F, Mary-Krause M, Teiger E, et al. Acute coronary syndrome in human immunodeficiency virus-infected patients: characteristics and 1 year prognosis. *European heart journal* 2011;32(1):41-50.
2. Carballo D, Delhumeau C, Carballo S, et al. Increased mortality after a first myocardial infarction in human immunodeficiency virus-infected patients; a nested cohort study. *AIDS research and therapy* 2015;12:4.
3. Lang S, Mary-Krause M, Cotte L, et al. Increased risk of myocardial infarction in HIV-infected patients in France, relative to the general population. *AIDS (London, England)* 2010;24(8):1228-30.
4. Chow FC, Regan S, Feske S, et al. Comparison of ischemic stroke incidence in HIV-infected and non-HIV-infected patients in a US health care system. *Journal of acquired immune deficiency syndromes (1999)* 2012;60(4):351-8.
5. Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *The New England journal of medicine* 2006;355(25):2631-9.
6. Eapen DJ, Manocha P, Patel RS, et al. Aggregate risk score based on markers of inflammation, cell stress, and coagulation is an independent predictor of adverse cardiovascular outcomes. *Journal of the American College of Cardiology* 2013;62(4):329-37.
7. Hanna DB, Post WS, Deal JA, et al. HIV Infection Is Associated With Progression of Subclinical Carotid Atherosclerosis. *Clinical infectious diseases :*

an official publication of the Infectious Diseases Society of America 2015;61(4):640-50.

8. Samji H, Cescon A, Hogg RS, et al. Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. *PloS one* 2013;8(12):e81355.
9. Siddiqi AE, Hall HI, Hu X, et al. Population-Based Estimates of Life Expectancy After HIV Diagnosis: United States 2008-2011. *Journal of acquired immune deficiency syndromes (1999)* 2016;72(2):230-6.
10. Smith CJ, Ryom L, Weber R, et al. Trends in underlying causes of death in people with HIV from 1999 to 2011 (D:A:D): a multicohort collaboration. *Lancet (London, England)* 2014;384(9939):241-8.
11. Farahani M, Mulinder H, Farahani A, et al. Prevalence and distribution of non-AIDS causes of death among HIV-infected individuals receiving antiretroviral therapy: a systematic review and meta-analysis. *International journal of STD & AIDS* 2016.
12. Smit M, Brinkman K, Geerlings S, et al. Future challenges for clinical care of an ageing population infected with HIV: a modelling study. *The Lancet Infectious diseases* 2015;15(7):810-8.
13. Boccara F, Lang S, Meuleman C, et al. HIV and coronary heart disease: time for a better understanding. *Journal of the American College of Cardiology* 2013;61(5):511-23.

14. Finegold JA, Asaria P, Francis DP. Mortality from ischaemic heart disease by country, region, and age: statistics from World Health Organisation and United Nations. *International journal of cardiology* 2013;168(2):934-45.

15. Lorgis L, Cottenet J, Molins G, et al. Outcomes after acute myocardial infarction in HIV-infected patients: analysis of data from a French nationwide hospital medical information database. *Circulation* 2013;127(17):1767-74.

16. Goff DC, Jr., Lloyd-Jones DM, Bennett G, et al. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;129(25 Suppl 2):S49-73.

17. Stone NJ, Robinson JG, Lichtenstein AH, et al. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation* 2014;129(25 Suppl 2):S1-45.

18. Crane H, Nance R, Delaney JA, et al. Comparing Cardiovascular Disease Risk Scores for Use in HIV-Infected Individuals. *Conference on Retroviruses and Opportunistic Infections (CROI)*. Boston, MA, 2016.

19. Zanni MV, Fitch KV, Feldpausch M, et al. 2013 American College of Cardiology/American Heart Association and 2004 Adult Treatment Panel III cholesterol guidelines applied to HIV-infected patients with/without subclinical high-risk coronary plaque. *AIDS (London, England)* 2014;28(14):2061-70.

20. Ridker PM, Danielson E, Fonseca FA, et al. Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *The New England journal of medicine* 2008;359(21):2195-207.

21. Lo J, Lu MT, Ihenachor EJ, et al. Effects of statin therapy on coronary artery plaque volume and high-risk plaque morphology in HIV-infected patients with subclinical atherosclerosis: a randomised, double-blind, placebo-controlled trial. *The lancet HIV* 2015;2(2):e52-63.

22. Lang S, Lacombe JM, Mary-Krause M, et al. Is Impact of Statin Therapy on All-Cause Mortality Different in HIV-Infected Individuals Compared to General Population? Results from the FHDH-ANRS CO4 Cohort. *PloS one* 2015;10(7):e0133358.

23. Baldassarre D, De Jong A, Amato M, et al. Carotid intima-media thickness and markers of inflammation, endothelial damage and hemostasis. *Annals of medicine* 2008;40(1):21-44.

24. Mincu RI, Janosi RA, Vinereanu D, et al. Preprocedural C-Reactive Protein Predicts Outcomes after Primary Percutaneous Coronary Intervention in Patients with ST-elevation Myocardial Infarction a systematic meta-analysis. *Scientific reports* 2017;7:41530.

25. Kang SJ, Kim D, Park HE, et al. Elevated serum bilirubin levels are inversely associated with coronary artery atherosclerosis. *Atherosclerosis* 2013;230(2):242-8.

26. Longenecker CT, Sullivan C, Baker JV. Immune activation and cardiovascular disease in chronic HIV infection. *Current opinion in HIV and AIDS* 2016;11(2):216-25.

27. Meier A, Chang JJ, Chan ES, et al. Sex differences in the Toll-like receptor-mediated response of plasmacytoid dendritic cells to HIV-1. *Nature medicine* 2009;15(8):955-9.

28. Ticona E, Bull ME, Soria J, et al. Biomarkers of inflammation in HIV-infected Peruvian men and women before and during suppressive antiretroviral therapy. *AIDS (London, England)* 2015;29(13):1617-22.

29. Barkan SE, Melnick SL, Preston-Martin S, et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. *Epidemiology (Cambridge, Mass)* 1998;9(2):117-25.

30. Bacon MC, von Wyl V, Alden C, et al. The Women's Interagency HIV Study: an observational cohort brings clinical sciences to the bench. *Clin Diagn Lab Immunol* 2005;12(9):1013-9.

31. Kaplan RC, Kingsley LA, Gange SJ, et al. Low CD4+ T-cell count as a major atherosclerosis risk factor in HIV-infected women and men. *AIDS (London, England)* 2008;22(13):1615-24.

32. Kuller LH, Tracy R, Belloso W, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. *PLoS medicine* 2008;5(10):e203.

33. Ford ES, Greenwald JH, Richterman AG, et al. Traditional risk factors and D-dimer predict incident cardiovascular disease events in chronic HIV infection. *AIDS (London, England)* 2010;24(10):1509-17.

34. Hodis HN, Mack WJ, Lobo RA, et al. Estrogen in the prevention of atherosclerosis. A randomized, double-blind, placebo-controlled trial. *Annals of internal medicine* 2001;135(11):939-53.

35. Jung M, Parrinello CM, Xue X, et al. Echolucency of the Carotid Artery Intima-Media Complex and Intima-Media Thickness Have Different Cardiovascular Risk Factor Relationships: The Women's Interagency HIV Study. *Journal of the American Heart Association* 2015;4(2).

36. Palella FJ, Jr., Gange SJ, Benning L, et al. Inflammatory biomarkers and abacavir use in the Women's Interagency HIV Study and the Multicenter AIDS Cohort Study. *AIDS (London, England)* 2010;24(11):1657-65.

37. Triant VA, Meigs JB, Grinspoon SK. Association of C-reactive protein and HIV infection with acute myocardial infarction. *Journal of acquired immune deficiency syndromes (1999)* 2009;51(3):268-73.

38. Duprez DA, Neuhaus J, Kuller LH, et al. Inflammation, coagulation and cardiovascular disease in HIV-infected individuals. *PloS one* 2012;7(9):e44454.

39. Westhorpe CL, Schneider HG, Dunne M, et al. C-reactive protein as a predictor of cardiovascular risk in HIV-infected individuals. *Sexual health* 2014;11(6):580-2.

40. Vos AG, Idris NS, Barth RE, et al. Pro-Inflammatory Markers in Relation to Cardiovascular Disease in HIV Infection. A Systematic Review. *PloS one* 2016;11(1):e0147484.

41. Kaplan RC, Sinclair E, Landay AL, et al. T Cell Activation and Senescence Predict Subclinical Carotid Artery Disease in HIV-Infected Women. *Journal of Infectious Diseases* 2011;203(4):452-63.

42. Bernal E, Serrano J, Perez A, et al. The CD4:CD8 ratio is associated with IMT progression in HIV-infected patients on antiretroviral treatment. *Journal of the International AIDS Society* 2014;17(4 Suppl 3):19723.

43. Hearps AC, Maisa A, Cheng WJ, et al. HIV infection induces age-related changes to monocytes and innate immune activation in young men that persist despite combination antiretroviral therapy. *AIDS (London, England)* 2012;26(7):843-53.

44. Dillon SM, Kibbie J, Lee EJ, et al. Low abundance of colonic butyrate-producing bacteria in HIV infection is associated with microbial translocation and immune activation. *AIDS (London, England)* 2017;31(4):511-21.

45. Dillon SM, Lee EJ, Kotter CV, et al. An altered intestinal mucosal microbiome in HIV-1 infection is associated with mucosal and systemic immune activation and endotoxemia. *Mucosal immunology* 2014;7(4):983-94.

46. Karim R, Mack WJ, Kono N, et al. Gonadotropin and sex steroid levels in HIV-infected premenopausal women and their association with subclinical atherosclerosis in HIV-infected and -uninfected women in the women's interagency HIV study (WIHS). *The Journal of clinical endocrinology and metabolism* 2013;98(4):E610-8.

47. Lorenz MW, Markus HS, Bots ML, et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation* 2007;115(4):459-67.

48. Inaba Y, Chen JA, Bergmann SR. Carotid plaque, compared with carotid intima-media thickness, more accurately predicts coronary artery disease events: a meta-analysis. *Atherosclerosis* 2012;220(1):128-33.

TABLES/FIGURES

Table 1: Baseline demographic and clinical data for all women and by HIV serostatus and biomarker score

		HIV-infected					HIV-uninfected					p-value
			Biomarker score			p-value		Biomarker score			p-value	
Characteristic, median (IQR) or n (%)	All (n= 783)	HIV + (n= 572)	0-1 (n= 133)	2 (n= 211)	3-4 (n= 193)		HIV- (n= 211)	0-1 (n= 35)	2 (n= 87)	3-4 (n= 80)		
Age, years	40.6 (35.2 - 46.7)	40.8 (35.3 - 46.8)	41.8 (36.3 - 47.9)	40.2 (34.3 - 46.9)	40.4 (35.8 - 45.5)	0.33	40.6 (34.3 - 46.5)	39.3 (31.3 - 47.1)	37.1 (33.6 - 45.7)	42.9 (38.8 - 47.7)	0.006	0.51
Race						0.052					0.007	0.36
White, non-Hispanic	57 (7)	45 (8)	14 (11)	16 (8)	11 (6)		12 (6)	5 (14)	6 (7)	0 (0)		
Black, non-Hispanic	484 (62)	344 (60)	70 (53)	127 (60)	130 (67)		140 (66)	22 (63)	53 (61)	57 (71)		
Hispanic	225 (29)	169 (30)	42 (32)	64 (30)	50 (26)		56 (27)	6 (17)	27 (31)	23 (29)		
Other	17 (2)	14 (2)	7 (5)	4 (2)	2 (1)		3 (1)	2 (6)	1 (1)	0 (0)		
Systolic blood pressure, mm Hg	117 (109-127)	116 (108-127)	115 (108-124)	116 (107-125)	117 (109-128)	0.36	120 (111-132)	113 (105-124)	119 (112-135)	124 (113-138)	0.008	0.007
Diastolic blood pressure, mm Hg	71 (64-79)	71 (64-78)	70 (64-78)	70 (63-78)	73 (66-78)	0.45	71 (65-80)	68 (64-77)	71 (66-80)	72 (63-81)	0.39	0.55
Antihypertensive medication use	142 (18)	109 (19)	25 (19)	32 (15)	45 (23)	0.11	33 (16)	4 (11)	10 (12)	19 (24)	0.07	0.27
Total cholesterol, mg/dL	175 (150-201)	174 (149, 201)	181 (157-213)	171 (145-198)	174 (150-197)	0.14	177 (154-204)	169 (151-185)	186 (160-210)	176 (150-196)	0.16	0.28
HDL cholesterol, mg/dL	47 (39-58)	46 (37-56)	48 (41-58)	46 (37-58)	43 (33-53)	0.04	52 (43-63)	57 (46-66)	53 (42-65)	48 (41-57)	0.003	<0.001
LDL cholesterol, mg/dL	102 (79-124)	102 (78-123)	104 (83-126)	95 (76-122)	105 (80-122)	0.31	103 (79-125)	91 (74-106)	109 (87-132)	102 (80-124)	0.04	0.40
Triglycerides, mg/dL	104 (73-152)	112 (77-158)	116 (76-156)	108 (75-157)	113 (78-164)	0.77	90 (66-127)	78 (59-99)	80 (60-120)	105 (77-134)	0.09	<0.001
Cholesterol-lowering medication	42 (5)	36 (6)	9 (7)	15 (7)	9 (5)	0.56	6 (3)	0 (0)	2 (2)	4 (5)	0.41	0.06

use												
Diabetes	106 (14)	69 (12)	16 (12)	26 (12)	26 (13)	0.91	37 (18)	2 (6)	15 (17)	17 (21)	0.12	0.047
Body mass index, kg/m^2	28.3 (24.3 - 33.5)	27.6 (24.1 - 32.1)	26.9 (23.2 - 30.8)	27.3 (24.3 - 31.4)	29.3 (24.4 - 35.0)	0.0003	30.2 (25.3 - 37.8)	27.4 (22.8 - 33.3)	28.5 (25.0 - 37.9)	34.1 (28.5 - 39.0)	0.004	<0.0001
eGFR, ml/min/1.73 m^2 (CKD-epi)	101 (87-114)	102 (86-115)	100 (81-112)	101 (87-116)	104 (91-117)	0.24	100 (88-111)	99 (83-112)	102 (89-113)	96 (87-107)	0.12	0.81
History of clinical CVD*	25 (3)	18 (3)	4 (3)	5 (2)	8 (4)	0.59	7 (3)	1 (3)	5 (6)	0 (0)	0.07	0.90
Framingham risk score	8 (3-12)	8 (3-12)	7 (2-12)	8 (3-12)	9 (4-12)	0.27	9 (3-13)	7 (3-11)	7 (2-12)	10 (4-15)	0.08	0.41
10-year CVD risk by FRS, %	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1)	1 (1-1)	0.86	1 (1-2)	1 (1-1)	1 (1-1)	1 (1-3)	0.11	0.13
Current smoker	374 (48)	257 (45)	43 (33)	96 (46)	101 (52)	0.002	117 (55)	23 (66)	42 (48)	46 (58)	0.18	0.01
Crack/ cocaine use						0.19					0.14	<0.0001
Never	456 (58)	355 (62)	92 (70)	127 (60)	110 (57)		101 (48)	12 (34)	48 (55)	39 (49)		
Former	259 (33)	180 (32)	32 (24)	70 (33)	70 (36)		79 (37)	14 (40)	30 (34)	31 (39)		
Current	65 (8)	34 (6)	7 (5)	13 (6)	13 (7)		31 (15)	9 (26)	9 (10)	10 (13)		
Alcohol use						0.89					0.009	0.0002
None	399 (51)	314 (55)	70 (53)	109 (52)	110 (57)		85 (40)	10 (29)	32 (37)	39 (49)		
1-7 drinks/week	311 (40)	214 (38)	51 (39)	84 (40)	70 (36)		97 (46)	14 (40)	45 (52)	34 (43)		
> 7 drinks/week	70 (9)	41 (7)	10 (7)	17 (8)	13 (7)		29 (14)	11 (31)	10 (11)	7 (9)		
HCV antibody positive	218 (28)	172 (30)	45 (34)	63 (30)	53 (27)	0.47	46 (22)	10 (29)	18 (21)	17 (21)	0.62	0.02
CD4 count, cells/mm^3	--	452 (288-658)	505 (318-724)	452 (294-631)	403 (262-602)	0.008	--	--	--	--	--	--
CD4 nadir, cells/mm^3	--	281 (161-409)	274 (154-416)	275 (160-417)	283 (155-396)	0.71	--	--	--	--	--	--
HIV viral load, cop/mL	--	180 (80-6900)	80 (80-1800)	250 (80-6700)	580 (80-10000)	0.46	--	--	--	--	--	--

cART use	--	356 (62)	103 (77)	126 (60)	101 (52)	<0.001	--	--	--	--	--	--

Abbreviations: HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease; FRS, Framingham risk score; HCV, hepatitis C virus; cART, combination antiretroviral therapy

* Clinical CVD defined as a history of MI, stroke/TIA, angina, peripheral arterial disease, or revascularization procedure

Table 2. Subclinical CVD outcomes for all participants and by HIV serostatus and biomarker score

		HIV-infected					HIV-uninfected					p-value
			Biomarker score			p-value					p-value	
Characteristic, median (IQR) or n (%)	All (n= 783)	HIV + (n= 572)	0-1 (n= 133)	2 (n= 211)	3-4 (n= 193)		HIV- (n= 211)	0-1 (n= 35)	2 (n= 87)	3-4 (n= 80)		
Baseline CCA plaques present	54 (7)	39 (7)	12 (9)	14 (7)	11 (6)	0.52	65 (12)	0 (0)	5 (6)	9 (12)	0.08	0.79
CCA plaque progression*	80 (11)	65 (12)	13 (10)	30 (15)	18 (10)	0.26	15 (8)	1 (3)	4 (5)	7 (9)	0.45	0.11
Baseline CIMT, µm	709 (652-779)	707 (649-776)	707 (658-784)	704 (646-775)	703 (651-773)	0.46	714 (657-794)	719 (646-775)	694 (638-760)	747 (680-823)	0.09	0.20
Final CIMT, µm	727 (674-804)	724 (670-803)	737 (676-800)	724 (663-803)	720 (674-803)	0.87	740 (684-814)	731 (676-787)	717 (662-787)	758 (709-841)	0.02	0.25
CIMT change**, µm	25.4 (-1.0-49.0)	19 (-2.0-49.0)	22.0 (0.0-55.0)	19.0 (-3.0-46.0)	20.0 (0.0-51.0)	0.99	23.0 (1.0-48.0)	20.0 (-13.0-39.5)	23.5 (3.5-47.0)	24.0 (2.0-62.0)	0.03	0.83
Baseline GSM, unitless	59.0 (47.7 - 70.7)	59.9 (48.4 - 71.2)	59.9 (47.9 - 73.2)	59.4 (48.3 - 70.7)	57.2 (48.4 - 68.9)	0.39	56.5 (44.6 - 69.6)	59.7 (47.0 - 72.3)	60.2 (46.6 - 72.9)	55.2 (42.8 - 63.9)	0.07	0.24
Final GSM, unitless	73.4 (60.5 - 86.8)	73.0 (60.1 - 86.0)	70.6 (58.9 - 85.7)	70.8 (59.5 - 85.9)	73.5 (60.8 - 84.7)	0.99	77.4 (61.9 - 88.0)	82.0 (71.8 - 97.6)	77.1 (59.2 - 86.5)	74.8 (59.0 - 86.8)	0.07	0.38
GSM change**, unitless	12.5 (-1.6-27.2)	11.3 (-2.5-25.9)	8.2 (-2.7-23.9)	10.2 (-2.3-24.4)	13.0 (-2.2-28.9)	0.51	15.1 (1.6-31.9)	21.3 (-5.8-37.8)	9.7 (0.2-25.1)	16.4 (3.6-34.0)	0.14	0.08

Abbreviations: CCA, common carotid artery; CIMT, carotid artery intima-media thickness; GSM, gray-scale median

* Plaque progression defined as increase in number of total plaques (either from 0 to >0, or 1+ to >baseline number) from baseline visit to final visit

** CIMT change and GSM change defined as difference in CIMT or GSM, respectively, from baseline visit to final visit

Table 3: Median baseline biomarker values for all participants by HIV serostatus

	Entire cohort (n=780)	HIV-infected (n=569)	HIV-uninfected (n=211)	p-value
hsCRP, mg/L median (IQR)	2.4 (0.9-5.7)	2.2 (0.8-5.3)	3.2 (0.9-7.7)	0.005
d-dimer, μg/ml median (IQR)	0.49 (0.28-0.84)	0.50 (0.28-0.92)	0.47 (0.30-0.69)	0.15
HSP-70, ng/ml median (IQR)	0.40 (0.17-0.95)	0.40 (0.17-0.92)	0.43 (0.19-1.07)	0.22
Bilirubin, mg/dl median (IQR)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	0.90

p-value for median HIV-invected vs. HIV-uninfected by Wilcoxon-Mann-Whitney test

Abbreviations: hsCRP, high-sensitivity C-reactive protein; HSP-70, heat shock protein 70; IQR, interquartile range

Table 4: Unadjusted odds ratios or mean differences for baseline CCA plaque formation, CIMT, or carotid echolucency according to biomarker levels for all participants, and by HIV serostatus

Outcome	All participants	HIV-infected	HIV-uninfected
Baseline CCA plaque	OR (95% CI), p-value	OR (95% CI), p-value	OR (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=705)	1.11 (0.63, 1.97), 0.70	0.91 (0.46, 1.79), 0.77	1.87 (0.61, 5.69), 0.27
d-dimer ≥ 0.5 µg/ml (n=721)	0.97 (0.55, 1.70), 0.92	0.80 (0.41, 1.54), 0.50	1.68 (0.56, 5.03), 0.36
HSP-70 ≥ 0.625 ng/ml (n=724)	1.27 (0.71, 2.27), 0.43	0.79 (0.38, 1.67), 0.54	**3.66 (1.24, 10.82), 0.02**
Bilirubin < 1.2 mg/dl (n=733)	0.78 (0.30, 2.04), 0.61	0.69 (0.26, 1.84), 0.46	**
Continuous biomarkers*			
hsCRP (n=705)	1.01 (0.82, 1.25), 0.93	0.96 (0.75, 1.24), 0.76	1.12 (0.76, 1.67), 0.56
d-dimer (n=721)	0.92 (0.69, 1.22), 0.55	0.87 (0.63, 1.18), 0.36	1.27 (0.59, 2.63), 0.56
HSP-70 (n=618)	1.11 (0.92, 1.35), 0.28	1.01 (0.80, 1.28), 0.93	1.38 (0.98, 1.96), 0.07
Bilirubin (n=733)	0.79 (0.43, 1.46), 0.45	0.84 (0.46, 1.54), 0.58	0.35 (0.03, 4.47), 0.42
Categorical biomarker score (n=693)			
2 vs. ≤ 1	0.91 (0.43, 1.92), 0.79	0.72 (0.32, 1.61), 0.42	**
≥ 3 vs. ≤ 1	1.05 (0.50, 2.21), 0.90	0.62 (0.27, 1.45), 0.27	**
Baseline CIMT	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=743)	7.5 (-9.7, 24.7), 0.39	7.5 (-12.7, 27.7), 0.47	3.8 (-29.6, 37.1), 0.82
d-dimer ≥ 0.5 µg/ml (n=765)	-2.2 (-19.1, 14.6), 0.79	-3.7 (-23.2, 15.8), 0.71	2.7 (-30.7, 36.2), 0.87
HSP-70 ≥ 0.625 ng/ml (n=760)	**19.2 (0.8, 37.6), 0.04**	7.5 (-13.9, 29.0), 0.49	**50.1 (14.5, 85.7), 0.006**
Bilirubin < 1.2 mg/dl	-7.5 (-39.8, 24.8),	-11.3 (-45.2, 22.6),	3.4 (-104.3, 111.0),

(n=771)	0.65	0.51	0.95
Continuous biomarkers			
hsCRP (n=743)	5.5 (-0.8, 11.9), 0.09	4.3 (-3.2, 11.9), 0.26	7.1 (-5.0, 19.2), 0.25
d-dimer (n=759)	-2.0 (-10.9, 6.9), 0.66	-1.8 (-11.4, 7.9), 0.72	-2.1 (-24.7, 20.5), 0.86
HSP-70 (n=641)	**9.8 (3.5, 16.0), 0.002**	6.3 (-0.9, 13.5), 0.09	**19.4 (6.7, 32.2), 0.003**
Bilirubin (n=771)	3.9 (-10.8, 18.7), 0.60	4.2 (-11.0, 19.4), 0.59	12.3 (-45.4, 70.1), 0.68
Categorical biomarker score (n=730)			
2 vs. ≤ 1	-0.8 (-23.3, 21.7), 0.95	3.0 (-22.6, 28.6), 0.82	-12.2 (-59.7, 35.2), 0.61
≥ 3 vs. ≤ 1	14.4 (-8.5, 37.3), 0.22	7.2 (-18.9, 33.4), 0.59	29.4 (-18.5, 77.3), 0.23
Baseline GSM	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=643)	**-5.8 (-8.5, -3.1), <0.0001**	**-3.7 (-6.9, -0.5), 0.02**	**-11.0 (-16.1, -5.9), <0.0001**
d-dimer ≥ 0.5 µg/ml (n=663)	0.5 (-2.2, 3.2), 0.69	0.4 (-2.7, 3.5), 0.80	0.9 (-4.6, 6.4), 0.74
HSP-70 ≥ 0.625 ng/ml (n=661)	-1.1 (-4.1, 1.8), 0.44	-1.3 (-4.6, 2.1), 0.46	-0.8 (-6.7, 5.1), 0.80
Bilirubin < 1.2 mg/dl (n=669)	0.5 (-4.5, 5.5), 0.85	1.8 (-3.3, 7.0), 0.49	-10.2 (-28.2, 7.8), 0.27
Continuous biomarkers			
hsCRP (n=643)	**-2.5 (-3.5, -1.5), <0.0001**	**-1.9 (-3.1, -0.7), 0.002**	**-4.0 (-5.8, -2.2), <0.0001**
d-dimer (n=657)	0.3 (-1.1, 1.8), 0.66	0.3 (-1.2, 1.8), 0.71	0.4 (-3.5, 4.3), 0.85
HSP-70 (n=584)	-0.7 (-1.6, 0.3), 0.18	-0.3 (-1.4, 0.8), 0.56	-1.6 (-3.5, 0.4), 0.11
Bilirubin (n=669)	-0.5 (-2.7, 1.7), 0.65	-0.7 (-3.0, 1.6), 0.55	0.1 (-9.1, 9.3), 0.98
Biomarker score (n=632)			
2 vs. ≤ 1	-1.2 (-4.9, 2.4), 0.51	-1.3 (-5.4, 2.8), 0.54	-1.1 (-8.7, 6.6), 0.78
≥ 3 vs. ≤ 1	**-4.2 (-7.9, -0.5), 0.03**	-2.9 (-7.1, 1.3), 0.18	-7.2 (-14.9, 0.3), 0.06

Abbreviations: CCA, common carotid artery; CIMT, carotid intima-media thickness; GSM, gray-scale median; hsCRP, high sensitivity C-reactive protein; HSP-70, heat shock protein 70

* hsCRP, d-dimer and HSP-70 are natural log-transformed

** not enough events to run the model

Table 5: Unadjusted odds ratios or mean differences for change in CCA plaque formation, CIMT, or carotid echolucency according to biomarker levels for all participants, and by HIV serostatus

Outcome	All participants	HIV-infected	HIV-uninfected
CCA plaque progression	RR (95% CI), p-value	RR (95% CI), p-value	RR (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=705)	1.17 (0.75, 1.84), 0.49	0.93 (0.56, 1.55), 0.77	**7.08 (1.62, 30.88), 0.009**
d-dimer ≥ 0.5 µg/ml (n=727)	1.24 (0.79, 1.95), 0.35	1.45 (0.88, 2.40), 0.15	0.51 (0.15, 1.67), 0.26
HSP-70 ≥ 0.625 ng/ml (n=724)	0.74 (0.45, 1.20), 0.22	0.65 (0.38, 1.12), 0.12	1.31 (0.42, 4.07), 0.65
Bilirubin < 1.2 mg/dl (n=733)	0.67 (0.34, 1.32), 0.24	0.69 (0.34, 1.37), 0.29	**
Continuous biomarkers*			
hsCRP (n=705)	1.05 (0.89, 1.25), 0.54	0.97 (0.81, 1.18), 0.79	**1.74 (1.25, 2.44), 0.001**
d-dimer (n=721)	1.05 (0.81, 1.36), 0.70	1.15 (0.90, 1.47), 0.27	**0.52 (0.32, 0.85), 0.009**
HSP-70 (n=618)	1.01 (0.85, 1.19), 0.95	0.98 (0.81, 1.17), 0.80	1.22 (0.85, 1.75), 0.28
Bilirubin (n=733)	1.14 (0.85, 1.53), 0.38	1.09 (0.80, 1.48), 0.59	1.60 (0.66, 3.89), 0.30
Categorical biomarker score (n=693)			
2 vs. ≤ 1	1.33 (0.71, 2.51), 0.37	1.37 (0.71, 2.65), 0.34	2.12 (0.24, 18.76), 0.50
≥ 3 vs. ≤ 1	1.09 (0.56, 2.14), 0.79	0.94 (0.45, 1.96), 0.87	4.00 (0.50, 31.81), 0.19
CIMT change	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=743)	8.0 (-0.5, 16.4), 0.06	8.1 (-2.5, 18.8), 0.14	8.5 (-4.2, 21.3), 0.19
d-dimer ≥ 0.5 µg/ml (n=765)	**9.3 (1.0, 17.5), 0.03**	8.0 (-2.3, 18.2), 0.13	12.8 (-0.1, 25.7), 0.053
HSP-70 ≥ 0.625 ng/ml (n=760)	**-10.2 (-19.2, -1.2), 0.03**	**-16.0 (-27.2, -4.9), 0.005**	5.6 (-8.4, 19.5), 0.43
Bilirubin < 1.2 mg/dl	-3.1 (-19.0, 12.7),	-5.3 (-23.2, 12.5),	19.5 (-22.1, 61.2),

(n=771)	0.70	0.56	0.36
Continuous biomarkers			
hsCRP (n=743)	**3.2 (0.0, 6.30), 0.047**	2.8 (-1.2, 6.7), 0.17	4.5 (-0.1, 9.2), 0.053
d-dimer (n=759)	2.8 (-1.5, 7.2), 0.21	2.1 (-2.9, 7.2), 0.41	6.2 (-2.5, 14.9), 0.16
HSP-70 (n=641)	**-3.3 (-6.3, -0.4), 0.03**	**-4.5 (-8.2, -0.8), 0.02**	-0.4 (-4.9, 4.1), 0.88
Bilirubin (n=771)	-0.8 (-8.0, 6.4), 0.83	0.5 (-7.5, 8.5), 0.90	-19.8 (-42.0, 2.5), 0.08
Categorical biomarker score (n=730)			
2 vs. ≤ 1	3.9 (-7.3, 15.1), 0.49	0.6 (-13.1, 14.4), 0.93	17.1 (-1.0, 35.3), 0.06
≥ 3 vs. ≤ 1	5.7 (-5.7, 17.1), 0.33	0.1 (-13.9, 14.1), 0.99	**24.4 (6.1, 42.8), 0.009**
GSM change	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=643)	2.3 (-1.1, 5.7), 0.18	0.4 (-3.5, 4.4), 0.83	6.4 (-0.3, 13.2), 0.06
d-dimer ≥ 0.5 µg/ml (n=663)	2.1 (-1.3, 5.5), 0.22	2.8 (-1.1, 6.6), 0.16	0.3 (-6.7, 7.3), 0.94
HSP-70 ≥ 0.625 ng/ml (n=661)	-2.6 (-6.2, 1.0), 0.16	-1.2 (-5.3, 2.9), 0.57	-6.5 (-14.0, 0.91), 0.09
Bilirubin < 1.2 mg/dl (n=669)	-1.2 (-7.4, 5.0), 0.71	-2.1 (-8.5, 4.3), 0.52	0.5 (-22.5, 23.5), 0.97
Continuous biomarkers			
hsCRP (n=643)	1.0 (-0.30, 2.2), 0.13	0.6 (-0.9, 2.0), 0.45	1.5 (-0.9, 3.9), 0.23
d-dimer (n=657)	1.2 (-0.6, 3.0), 0.18	1.6 (-0.3, 3.5), 0.10	-0.6 (-5.5, 4.4), 0.83
HSP-70 (n=584)	-1.1 (-2.2, 0.1), 0.07	-1.2 (-2.5, 0.1), 0.07	-0.8 (-3.2, 1.5), 0.49
Bilirubin (n=669)	-0.008 (-2.8, 2.8), 1.00	0.40 (-2.4, 3.2), 0.78	-2.7 (-14.4, 9.1), 0.65
Categorical biomarker score (n=632)			
2 vs. ≤ 1	-0.9 (-5.4, 3.5), 0.68	1.1 (-4.0, 6.1), 0.68	-8.3 (-18.0, 1.5), 0.10
≥ 3 vs. ≤ 1	2.3 (-2.2, 6.8), 0.32	2.9 (-2.2, 8.0), 0.26	-1.7 (-11.4, 8.0), 0.73
Abbreviations: CCA, common carotid artery; CIMT, carotid intima-media thickness; GSM, gray-scale median; hsCRP, high sensitivity C-reactive protein; HSP-70, heat shock protein 70			

* hsCRP, d-dimer and HSP-70 are natural log-transformed ** not enough events to run the model

Table 6: Adjusted* odds ratios or mean differences for baseline CCA plaques, CIMT, or carotid echolucency according to biomarker levels for all participants, and by HIV serostatus			
Outcome	All participants	HIV-infected	HIV-uninfected
Baseline CCA plaque	OR (95% CI), p-value	OR (95% CI), p-value	OR (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=667)	1.36 (0.70, 2.63), 0.37	1.49 (0.67, 3.34), 0.33	0.63 (0.12, 3.35), 0.59
d-dimer ≥ 0.5 µg/ml (n=689)	1.44 (0.76, 2.74), 0.27	1.24 (0.55, 2.78), 0.60	3.68 (0.73, 18.49), 0.11
HSP-70 ≥ 0.625 ng/ml (n=686)	0.92 (0.47, 1.77), 0.79	0.52 (0.22, 1.24), 0.14	2.16 (0.52, 9.05), 0.29
Bilirubin < 1.2 mg/dl (n=698)	0.72 (0.25, 2.08), 0.54	0.77 (0.25, 2.40), 0.65	***
Continuous biomarkers**			
hsCRP (n=667)	1.11 (0.87, 1.42), 0.40	1.19 (0.88, 1.61), 0.25	0.79 (0.44, 1.43), 0.44
d-dimer (n=683)	1.10 (0.81, 1.49), 0.54	1.07 (0.75, 1.53), 0.71	2.41 (0.82, 7.07), 0.11
HSP-70 (n=589)	1.00 (0.80, 1.25), 0.97	0.86 (0.65, 1.15), 0.31	1.44 (0.84, 2.49), 0.19
Bilirubin (n=698)	0.62 (0.28, 1.37), 0.24	0.69 (0.32, 1.51), 0.36	0.03 (<0.001, 2.34), 0.11
Categorical biomarker score (n=656)			
2 vs. ≤ 1	1.01 (0.43, 2.40), 0.98	0.79 (0.30, 2.06), 0.62	***
≥ 3 vs. ≤ 1	1.34 (0.56, 3.21), 0.51	0.91 (0.33, 2.51), 0.85	***
Baseline CIMT	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=700)	-3.7 (-19.1, 11.8), 0.64	2.2 (-16.2, 20.7), 0.81	-21.6 (-52.0, 8.7), 0.16
d-dimer ≥ 0.5 µg/ml (n=722)	-4.2 (-19.1, 10.8), 0.58	1.6 (-17.1, 20.4), 0.86	-8.2 (-37.3, 20.9), 0.58
HSP-70 ≥ 0.625 ng/ml (n=717)	7.4 (-9.0, 23.8), 0.38	-1.2 (-20.6, 18.2), 0.90	21.9 (-10.3, 54.1), 0.18
Bilirubin < 1.2 mg/dl	-11.0 (-39.2, 17.3),	-5.9 (-36.6, 24.8),	-7.4 (-98.9, 84.2),

(n=731)	0.45	0.71	0.87
Continuous biomarkers**			
hsCRP (n=700)	2.2 (-3.6, 8.0), 0.45	4.4 (-2.7, 11.4), 0.22	-1.5 (-12.7, 9.7), 0.79
d-dimer (n=716)	0.6 (-7.5, 8.6), 0.89	2.3 (-7.3, 11.9), 0.64	-1.3 (-21.2, 18.6), 0.90
HSP-70 (n=610)	5.1 (-0.5, 10.6), 0.08	2.7 (-3.9, 9.3), 0.42	10.5 (-0.8, 21.7), 0.07
Bilirubin (n=731)	3.7 (-9.1, 16.5), 0.57	0.6 (-12.9, 14.1), 0.93	13.0 (-36.9, 62.9), 0.61
Categorical biomarker score (n=688)			
2 vs. $\leq$ 1	-1.7 (-21.6, 18.2), 0.87	6.2 (-17.1, 29.5), 0.60	-17.2 (-60.7, 26.4), 0.44
$\geq$ 3 vs. $\leq$ 1	-1.6 (-22.1, 18.9), 0.88	4.7 (-19.6, 29.0), 0.70	-13.8 (-58.3, 30.8), 0.54
Baseline GSM	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value
Categorical biomarkers			
hsCRP $\geq$ 3.0 mg/L (n=617)	**-5.8 (-8.6, -3.0), <0.0001**	**-4.4 (-7.7, -1.1), 0.009**	**-10.2 (-15.8, -4.6), 0.0004**
d-dimer $\geq$ 0.5 µg/ml (n=637)	-0.6 (-3.4, 2.2), 0.68	-2.2 (-5.6, 1.2), 0.20	0.5 (-5.1, 6.2), 0.85
HSP-70 $\geq$ 0.625 ng/ml (n=635)	-0.1 (-3.1, 2.9), 0.94	0.05 (-3.4, 3.5), 0.98	0.04 (-6.3, 6.3), 0.99
Bilirubin < 1.2 mg/dl (n=646)	-2.0 (-7.0, 3.1), 0.45	-1.2 (-6.6, 4.2), 0.67	-12.1 (-30.7, 6.6), 0.21
Continuous biomarkers**			
hsCRP (n=617)	**-2.6 (-3.6, -1.5), <0.0001**	**-2.1 (-3.4, -0.9), 0.001**	**-4.2 (-6.2, -2.2), <0.0001**
d-dimer (n=631)	-0.7 (-2.2, 0.8), 0.34	-1.3 (-3.0, 0.4), 0.13	-0.2 (-4.3, 3.9), 0.94
HSP-70 (n=561)	-0.5 (-1.5, 0.5), 0.34	-0.1 (-1.3, 1.0), 0.86	-1.2 (-3.3, 0.9), 0.26
Bilirubin (n=646)	0.4 (-1.8, 2.7), 0.72	0.5 (-1.8, 2.8), 0.67	2.5 (-7.2, 12.2), 0.61
Categorical biomarker score (n=607)			
2 vs. $\leq$ 1	-1.7 (-5.3, 2.0), 0.38	-3.0 (-7.3, 1.2), 0.16	1.9 (-6.3, 10.0), 0.65
$\geq$ 3 vs. $\leq$ 1	**-4.6 (-8.4, -0.8), 0.02**	**-5.3 (-9.7, -0.9), 0.02**	-5.9 (-14.3, 2.4), 0.16
Abbreviations: CCA, common carotid artery; CIMT, carotid intima-media thickness; GSM,			

gray-scale median; hsCRP, high sensitivity C-reactive protein; HSP-70, heat shock protein 70

* adjusted for age, race, CVD history, SBP, LDL, antihypertensive use, cholesterol-lowering med use, diabetes, eGFR, smoking status, crack/cocaine use, HCV ab for all participants and for CD4 count, HIV viral load, and cART use for HIV-infected participants

** hsCRP, d-dimer and HSP-70 are natural log-transformed

*** not enough events to run the model

Table 7: Adjusted* rate ratios or mean differences for change in CCA plaques, CIMT, or carotid echolucency according to biomarker levels for all participants, and by HIV serostatus			
Outcome	All participants	HIV-infected	HIV-uninfected
Carotid plaque progression	RR (95% CI), p-value	RR (95% CI), p-value	RR (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=667)	1.10 (0.71, 1.71), 0.67	0.87 (0.52, 1.45), 0.60	**5.26 (1.34, 20.6), 0.02**
d-dimer ≥ 0.5 µg/ml (n=689)	1.33 (0.84, 2.10), 0.22	1.41 (0.82, 2.44), 0.22	0.52 (0.18, 1.53), 0.24
HSP-70 ≥ 0.625 ng/ml (n=686)	0.74 (0.46, 1.19), 0.22	0.68 (0.39, 1.17), 0.16	0.83 (0.18, 3.73), 0.81
Bilirubin < 1.2 mg/dl (n=698)	0.64 (0.33, 1.22), 0.17	0.78 (0.38, 1.61), 0.50	***
Continuous biomarkers**			
hsCRP (n=667)	1.09 (0.92, 1.31), 0.32	1.00 (0.81, 1.25), 0.97	**2.00 (1.21, 3.29), 0.007**
d-dimer (n=683)	1.11 (0.87, 1.42), 0.40	1.13 (0.88, 1.44), 0.35	0.70 (0.43, 1.12), 0.14
HSP-70 (n=589)	1.03 (0.88, 1.21), 0.69	1.01 (0.85, 1.20), 0.95	1.12 (0.76, 1.67), 0.56
Bilirubin (n=698)	1.16 (0.89, 1.53), 0.28	1.05 (0.75, 1.48), 0.79	2.15 (0.60, 7.72), 0.24
Categorical biomarker score (n=656)			
2 vs. ≤ 1	1.57 (0.86, 2.86), 0.14	1.30 (0.69, 2.44), 0.41	1.26 (0.10, 15.49), 0.86
≥ 3 vs. ≤ 1	1.11 (0.58, 2.14), 0.75	0.77 (0.37, 1.59), 0.48	1.91 (0.20, 17.98), 0.57
CIMT change	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value	Mean difference, µm (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=700)	7.4 (-1.8, 16.6), 0.12	6.9 (-4.9, 18.7), 0.25	11.0 (-3.5, 25.4), 0.14
d-dimer ≥ 0.5 µg/ml (n=722)	**9.5 (0.6, 18.4), 0.04**	11.3 (-0.6, 23.1), 0.06	13.1 (-0.9, 27.2), 0.07
HSP-70 ≥ 0.625 ng/ml (n=717)	**-10.1 (-19.9, -0.5), 0.04**	**-17.3 (-29.5, -5.2), 0.005**	9.1 (-6.4, 24.7), 0.25
Bilirubin < 1.2 mg/dl	-9.5 (-26.2, 7.3), 0.27	-10.8 (-30.2, 8.5),	20.7 (-23.5, 65.0),

(n=731)		0.27	0.36
Continuous biomarkers			
hsCRP (n=700)	**3.5 (0.00, 6.9), 0.05**	3.0 (-1.5, 7.5), 0.20	**5.4 (0.2, 10.7), 0.04**
d-dimer (n=716)	2.8 (-2.0, 7.5), 0.26	3.5 (-2.5, 9.5), 0.25	6.1 (-3.5, 15.6), 0.21
HSP-70 (n=610)	-2.4 (-5.6, 0.9), 0.15	-3.6 (-7.7, 0.5), 0.08	1.5 (-3.5, 6.4), 0.56
Bilirubin (n=731)	1.4 (-6.2, 9.0), 0.71	2.8 (-5.7, 11.3), 0.51	**-24.4 (-48.3, -0.4), 0.046**
Categorical biomarker score (n=688)			
2 vs. ≤ 1	0.8 (-11.2, 12.8), 0.90	-2.7 (-17.7, 12.3), 0.72	19.4 (-0.9, 39.7), 0.06
≥ 3 vs. ≤ 1	3.4 (-8.9, 15.7), 0.59	-1.4 (-17.0, 14.3), 0.87	**29.5 (8.8, 50.3), 0.005**
GSM change	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value	Mean difference, unitless (95% CI), p-value
Categorical biomarkers			
hsCRP ≥ 3.0 mg/L (n=617)	2.2 (-1.4, 5.8), 0.23	0.7 (-3.4, 4.8), 0.73	6.2 (-1.4, 13.8), 0.11
d-dimer ≥ 0.5 µg/ml (n=637)	2.1 (-1.5, 5.6), 0.26	2.2 (-2.0, 6.5), 0.30	1.8 (-5.7, 9.3), 0.64
HSP-70 ≥ 0.625 ng/ml (n=635)	-2.5 (-6.3, 1.4), 0.21	-0.8 (-5.1, 3.5), 0.72	-7.8 (-16.0, 0.3), 0.06
Bilirubin < 1.2 mg/dl (n=646)	-0.9 (-7.4, 5.6), 0.79	-0.8 (-7.6, 5.9), 0.81	0.6 (-24.1, 25.2), 0.96
Continuous biomarkers			
hsCRP (n=617)	0.9 (-0.4, 2.3), 0.19	0.7 (-0.9, 2.2), 0.41	1.3 (-1.5, 4.1), 0.35
d-dimer (n=631)	1.2 (-0.7, 3.1), 0.21	1.3 (-0.9, 3.4), 0.25	0.4 (-5.0, 5.8), 0.89
HSP-70 (n=561)	-0.7 (-2.0, 0.5), 0.25	-0.6 (-2.1, 0.8), 0.38	-1.4 (-4.1, 1.2), 0.28
Bilirubin (n=646)	-0.02 (-2.9, 2.9), 0.99	-0.008 (-2.9, 2.9), 1.00	-3.2 (-15.9, 9.5), 0.62
Categorical biomarker score (n=607)			
2 vs. ≤ 1	-1.2 (-5.8, 3.5), 0.62	0.8 (-4.4, 6.0), 0.75	-10.4 (-21.1, 0.3), 0.06
≥ 3 vs. ≤ 1	2.1 (-2.7, 6.9), 0.38	3.2 (-2.2, 8.7), 0.25	-2.2 (-13.2, 8.7), 0.69
Abbreviations: CCA, common carotid artery; CIMT, carotid intima-media thickness; GSM, gray-scale median; hsCRP, high sensitivity C-reactive protein; HSP-70, heat shock protein 70 * adjusted for age, race, CVD history, SBP, LDL, antihypertensive use, cholesterol-lowering med use, diabetes, eGFR, smoking status, crack/cocaine use, HCV ab for all participants and for CD4			

count, HIV viral load, and cART use for HIV-infected participants

** hsCRP, d-dimer and HSP-70 are natural log-transformed

*** not enough events to run the model

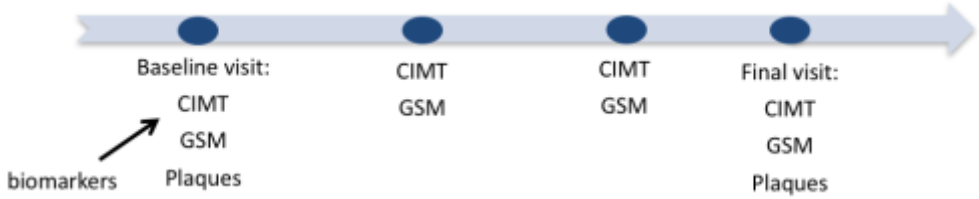

Figure 1. Study design for the WIHS vascular substudy. CCA ultrasound measurements of CIMT and GSM were obtained at study baseline and at 2-3 year intervals for a maxiumum of 4 study visits. CCA plaques were measured at baseline and final visits only. Biomarkers were analyzed from serum and plasma obtained at the baseline study visit.