MAJOR ARTICLE

Noncalcified Coronary Atherosclerotic Plaque and Immune Activation in HIV-Infected Women

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(See the editorial commentary by Boccara and Cohen on pages 1729–31.)

Background. Little is known about coronary plaque in human immunodeficiency virus (HIV)–infected women.

Methods. Sixty HIV-infected and 30 non–HIV-infected women without symptoms or history of cardiovascular disease were recruited to assess coronary plaque with coronary computed tomographic angiography and immune activation. Data from 102 HIV-infected men and 41 non–HIV-infected male controls were compared.

Results. HIV-infected women demonstrated significantly higher percentages of segments with noncalcified plaque (mean ± SD, 74% ± 28% vs 23% ± 39% compared to female control subjects; median [interquartile range], 75% [63%–100%] vs 0% [0%–56%]; $P = .007$) and more segments with noncalcified plaque (mean ± SD, 0.92 ± 1.48 vs 0.40 ± 1.44; median [interquartile range], 0 [0–2] vs 0 [0–0]; $P = .04$). Immune activation parameters, including soluble CD163 (sCD163; $P = .006$), CXCL10 ($P = .002$), and percentages of $CD14^+CD16^+$ monocytes ($P = .008$), were higher in HIV-infected women than in female control subjects, but no differences were seen in general inflammatory markers. Among HIV-infected women with noncalcified coronary plaque, sCD163 levels were significantly higher than in HIV-infected women without noncalcified plaque ($P = .04$). In multivariate modeling for sCD163 levels among male and female subjects, significant effects of HIV ($P < .0001$), age ($P = .002$), and sex ($P = .0002$) were seen.

Conclusions. Young, asymptomatic, HIV-infected women, demonstrate increased noncalcified coronary plaque and increased immune activation, particularly monocyte activation. Independent effects of sex, HIV status, and aging on immune activation may contribute to cardiovascular disease in this population.

Clinical Trials Registration. NCT00455793.

Keywords. HIV; atherosclerosis; cardiovascular disease; non-calcified coronary plaque; immune activation; age.

Cardiovascular disease (CVD) is increased approximately 2-fold in human immunodeficiency virus (HIV) infection [1] and may have a unique relationship to sex in this population. Two large registry studies have recently shown that the relative increases in myocardial infarction rates are higher in HIV-infected vs non–HIV-infected women than in HIV-infected vs non–HIV-infected men [2, 3]. Worldwide, women account for a growing percentage of HIV-infected patients and more than half of all HIV infections [4]. Few studies have assessed CVD exclusively among HIV-infected women [5–7], and, to our knowledge, none have explored sex differences with respect to coronary atherosclerotic plaque.

In the context of HIV infection, CVD is probably related to the interplay between traditional CVD risk factors, effects of antiretroviral therapy (ART), and the

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proinflammatory and immune activation effects of HIV. Among HIV-infected men, Lo et al [8] previously demonstrated a higher prevalence of atherosclerotic plaque and in particular, noncalcified plaque. In more recent data from the same cohort, we also demonstrated that soluble CD163 (sCD163) levels are increased in HIV-infected men and are associated with noncalcified plaque [9]. CD163 is a monocyte-macrophage specific scavenger receptor cleaved from activated monocytes and macrophages during inflammation. Among non–HIV-infected patients, increased levels of sCD163 have been found to be associated with coronary artery disease [10]. These prior studies have not investigated indices of immune activation with respect to atherosclerotic plaque features among HIV-infected women. In the current study, we examined atherosclerotic plaque features and detailed indices of immune activation among HIV-infected women and investigated the relationships of age, sex, and HIV infection to these indices.

METHODS

Study Participants

Ninety women were recruited for the study. Sixty women with HIV infection were recruited from HIV clinics and community health centers in the Boston area and by newspaper advertisements. Thirty HIV-negative women were recruited as control subjects from the same communities to ensure that the groups would be similar with respect to demographic and cardiovascular risk factors.

Other than HIV disease, inclusion and exclusion factors were identical for both groups. Subjects recruited were aged 18–60 years and had no known cardiac disease or symptoms suggestive of any current or prior cardiac disease (including angina, arrhythmias, valvular disease, pericarditis, and congestive heart failure). Patients with renal disease, creatinine levels >1.0 mg/dL or creatinine clearance <60 mL/min were excluded to minimize the risk of contrast nephropathy. Subjects with contraindications to administration of contrast agent, β-blockade, or nitroglycerin were also excluded. HIV-infected patients receiving ART at the time of the study were required to be have been receiving stable therapy with no change in ART medications for > 3 months.

All participants provided informed consent to participate. This study was approved by the Institutional Review Board of Massachusetts General Hospital. Data from the HIV-infected and non–HIV-infected women were also compared with available data from 102 HIV-infected and 41 non–HIV-infected men recruited for a prior study, based on similar criteria and similar techniques to assess coronary angiography and immune activation. Cardiac computed tomography (CT) [8] and monocyte activation [9] data among the male subjects have been published elsewhere but have not been compared with data in HIV-infected women.

Study Procedures and Assessment of Cardiovascular Risk Factors

Detailed information was obtained on demographic factors, medical history, family history, smoking, recreational drug use, and medications. Cocaine or intravenous drug use was classified as active if subjects reported use in the past 6 months. Duration of known HIV diagnosis was determined by number of years since HIV diagnosis. For HIV-infected patients, a detailed history of prior ART use was obtained. Women were classified as menopausal if they reported a history of amenorrhea for ≥12 consecutive months [11]. All blood samples were obtained from fasting subjects. Assessment of traditional cardiovascular risk factors was determined by comparing individual risk factors and an aggregate risk score using the Framingham equation [12].

Cardiac CT Angiography

Cardiac CT imaging using a 64-slice dual-source CT scanner (Siemens Medical Solutions) was performed using a standardized protocol identical to that used previously in men and described elsewhere, including assessment of calcified and noncalcified plaque segments [8, 9].

Metabolic, Biochemical, Immunologic and Body Composition Parameters

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglyceride, and glucose levels were determined using standard techniques. $CD4^+$ and $CD8^+$ T-cell counts were assessed with flow cytometry and HIV loads were determined with ultrasensitive reverse-transcription polymerase chain reaction (Roche COBAS Amplicor; lower limit of detection, 50 copies/mL). HIV testing was performed with enzyme-linked immunosorbent assay, and results confirmed with Western blot analysis. Plasma sCD163 (Trillium); monocyte chemoattractant protein-1 (MCP-1), CXCL10, sCD14, high-sensitivity interleukin 6 (hsIL-6) (all R&D Systems); high-sensitivity C-reactive protein (hsCRP) (Labcorp) were quantified with enzyme-linked immunosorbent assay. Abdominal visceral and subcutaneous fat were assessed with cross-sectional CT [13].

Flow Cytometry

Peripheral blood samples were collected in ethylenediaminetetraacetic acid and prepared according to standard methods [14, 15]. Eight fluorochrome-conjugated antibodies were combined with 100 μL of whole blood, incubated for 15 minutes, and then washed and fixed with 2 mL of 1X BD FACS Lysing solution (Becton Dickinson), followed by 500 μL of 1% paraformaldehyde (Sigma). Samples were processed on a FACSCanto II cytometer (Becton Dickinson) and 50 000 events were collected and analyzed using FACSDiva software. Data analysis was performed using FACSDiva software with side light scatter versus CD14 gating used to identify monocytes and forward versus

side light scatter gating used to identify lymphocytes. CD16 expression on monocytes was assessed by using negative lymphocyte populations to define negative controls. T lymphocytes were identified using populations of brightly stained $CD4^+$ or $CD8^+$ cells. HLA-DR and CD38 expression on $CD4^+$ or $CD8^+$ T cells was assessed by using negative lymphocyte populations to define negative controls.

Statistical Analysis

The primary end point for comparison in this study was noncalcified plaque. Data are presented as means ± SDs or medians (interquartile ranges [IQRs]), depending on normality of the distribution; all categorical variables are reported as proportions. Comparisons were made first between HIV-infected and non–HIV-infected women using the Student's *t* test for normally distributed continuous variables, the Wilcoxon rank sum test for nonnormally distributed data, and the x^2 test for categorical variables. For percentage of plaque and immune activation parameters, the Bonferroni *P* values for significance, adjusted for the number of comparisons made, were .02 and .01, respectively.

Comparisons were subsequently made with previously collected data in HIV-infected and non–HIV-infected male control subjects. Variables were compared between the 4 groups (HIV-infected women and men and non–HIV-infected women and men) by using overall analysis of variance for continuous variables and by x^2 test for categorical variables. For outcomes that were not normally distributed, the Kruskal-Wallis test was used. For variables with overall significance at $P < .05$, between-group comparisons were made. Multivariate regression modeling was performed for immune activation indices and noncalcified plaque, including all the subjects in the 4 groups, with assessment for individual effects of HIV status, sex, and age and the interaction between these terms in a fully saturated model with all possible interaction terms. Statistical significance was defined as $P < .05$. All statistical analysis was performed using SAS JMP software (version 9.0; SAS Institute).

RESULTS

Demographic and Clinical Characteristics of HIV-Infected and Non–HIV-Infected Women

Demographic and clinical characteristics are described in Table 1. HIV-infected women had a long duration since diagnosis, approximately 15 years, and 98% had been receiving ART for a mean of 8 years. Table 1 details the major classes of ART use and the duration of treatment. Among HIV-infected patients, immunologic control was good, with a mean $CD4^+$ T-cell count of 597 ± 297 cells/μL; 84% of patients had undetectable viral load.

Table 1. Characteristics of Control and HIV-Infected Women[a]

	Control Women (n = 30)	HIV-Infected Women (n = 60)	*P* Value
Demographics			
Age, y	47 ± 5	47 ± 7	.91
Race, %			.41
White	33	25	
Nonwhite	67	75	
Family history of premature CHD by NCEP, %	34	22	.20
Framingham estimate of 10-y CHD risk, %	2 ± 4	2 ± 2	.87
Hypertension, %	23	17	.45
Current statin treatment, %	3	8	.37
Diabetes mellitus, %	13	15	.83
Current smoker, %	53	50	.77
Menopausal, %	43	47	.76
Active intravenous drug use, %	7	5	.74
Active cocaine use, %	0	10	.07
HIV disease–related parameters			
Duration since HIV diagnosis, y	NA	15 ± 6	NA
Ever on ART, %	NA	98	NA
Currently on ART, %	NA	98	NA
Duration of ART, y	NA	8 ± 5	NA
Current PI treatment, %	NA	58	NA
Duration of PI treatment, y	NA	5 ± 4	NA
Current NRTI treatment, %	NA	92	NA
Duration of NRTI treatment, y	NA	7 ± 5	NA
Current NNRTI treatment, %	NA	17	NA
Duration of NNRTI treatment, y	NA	2 ± 4	NA
$CD4^+$ T lymphocytes, cells/μL	NA	597 ± 297	NA
Nadir $CD4^+$ T lymphocytes, cells/μL	NA	191 ± 160	NA
$CD8^+$ T lymphocytes, cells/μL	NA	880 ± 407	NA
$CD4^+/CD8^+$ T-lymphocyte ratio	NA	0.84 ± 0.57	NA
Log HIV RNA viral load, copies/mL	NA	4.1 ± 0.9	NA
Undetectable HIV RNA <50 copies/mL, %	NA	84	NA
Anthropometric parameters			
BMI, kg/m^2	29 ± 5	28 ± 6	.35
VAT area, cm^2	95 ± 80	88 ± 64	.68
SAT area, cm^2	371 ± 148	301 ± 143	.05
VAT/SAT ratio	0.26 ± 0.18	0.30 ± 0.18	.35
Metabolic parameters			
Systolic blood pressure, mm Hg	115 ± 18	118 ± 15	.58
Diastolic blood pressure, mm Hg	76 ± 10	75 ± 10	.87
Fasting glucose, mg/dL	82 ± 13	90 ± 42	.22
2-h glucose, mg/dL	135 ± 76	134 ± 83	.98
Hemoglobin A1c, %	5.9 ± 0.7	5.8 ± 1.1	.64
Total cholesterol, mg/dL	184 ± 27	187 ± 47	.70
HDL cholesterol, mg/dL	61 ± 16	61 ± 20	.97
LDL cholesterol, mg/dL	102 ± 26	105 ± 39	.72

Table 1 continued.

	Control Women (n = 30)	HIV-Infected Women (n = 60)	*P* Value
Triglycerides, mg/dL	106 ± 67	108 ± 59	.87

[a] Data represent means ± SDs or percentages.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; CHD, coronary heart disease; HDL, high-density lipoprotein; HIV, human immunodeficiency virus; LDL, low-density lipoprotein; NA, not applicable; NCEP, National Cholesterol Education Program; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse-transcriptase inhibitor; PI, protease inhibitor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue;

Age, race, body mass index (BMI), and traditional risk factors, including family history of premature coronary heart disease, Framingham 10-year CVD risk, hypertension, diabetes mellitus, current smoking, and active intravenous drug or cocaine use did not differ between HIV-infected and noninfected women (Table 1). The percentages of postmenopausal women were similar (47% vs 43%; $P = .76$). Metabolic parameters, including blood pressure and levels of glucose, cholesterol, HDL, LDL, and triglycerides, were similar in HIV-infected and noninfected women. Visceral adipose tissue was comparable in the 2 groups (Table 1).

Coronary Plaque Characteristics by HIV Status Among Female Subjects

The prevalence of plaque was similar between female HIV-infected and control subjects (37% vs 38%; $P = .88$); however, HIV-infected women demonstrated significantly higher prevalence of noncalcified coronary plaque (35% vs 12% in female control subjects; $P = .04$), percentage of segments with noncalcified plaque (mean ± SD, 74% ± 28% vs 23% ± 39%; median [IQR], 75% [63%–100%] vs 0% [0%–56%]; $P = .007$), and segments with noncalcified plaque (mean ± SD, 0.92 ± 1.48 vs 0.40 ± 1.44; median [IQR], 0 [0–2] vs 0 [0–0]; $P = .04$) (Table 2). The prevalence of noncalcified plaque remained higher among HIV-infected women ($P = .004$) when known cardiovascular risk factors were controlled for, including age; race; Framingham score; smoking status; levels of triglycerides, HDL, and LDL; and BMI.

In contrast, HIV-infected women demonstrated a lower prevalence of calcified plaque (6% vs 26%; $P = .01$), percentage of segments with calcified plaque ($P = .001$), and number of segments with calcified plaque than female control subjects ($P = .01$; Table 2). Although the total Agatston calcium scores did not differ, a higher percentage of non–HIV-infected women had calcium scores >100 (15% vs 2%; $P = .02$, Table 2). Similar results were seen in a comparison between HIV-infected women with undetectable viral loads and non–HIV-infected women (Table 2).

Immune Activation and Inflammation Indices by HIV Status Among Female Subjects

HIV-infected women demonstrated increased immune activation compared with female non–HIV-infected control subjects. Levels of sCD163 ($P = .006$), sCD14 ($P = .05$), and CXCL10 ($P = .002$) and percentages of $CD8^+$ ($P \leq .0001$), $CD8^+$ $HLA{-}DR^+$ ($P < .0001$), $CD4^+HLA{-}DR^+$ ($P = .0004$), $HLA{-}DR^+CD38^+CD4^+$ ($P < .0001$), and $CD14^+CD16^+$ monocytes ($P = .008$) were all significantly higher in HIV-infected women (Table 2; Figure 1). In contrast, markers of inflammation, such as hsCRP ($P = .88$) and hsIL-6 ($P = .92$), did not differ between the 2 groups (Table 2). Similar results were seen in a comparison between HIV-infected women with undetectable viral loads and non–HIV-infected women (Table 2).

Characteristics of HIV-infected Women With Evidence of Noncalcified Coronary Plaque

HIV-infected women who had noncalcified coronary plaque detected by CT angiography were more likely to be older ($P = .005$) and have higher sCD163 levels ($P = .04$) than HIV-infected women without evidence of noncalcified coronary plaque (Supplementary Table 1). However, there were no differences between HIV-infected women with or without noncalcified plaque in smoking status, race, lipid levels, blood pressure, BMI or glucose levels, HIV disease–related parameters, inflammatory markers, or additional markers of monocyte activation.

Comparison of Female HIV-Infected and Control Subjects With Male HIV-Infected and Control Subjects

Demographic and clinical characteristics are shown in Supplementary Table 2. HIV-infected and non–HIV-infected women had higher hemoglobin A1c ($P < .05$) and HDL ($P < .05$) levels than HIV-infected and non–HIV-infected men, and HIV-infected and non–HIV-infected men had higher Framingham 10-year CVD risk scores ($P < .05$). HIV-infected men had significantly higher triglyceride levels ($P < .05$) than the other 3 groups. In contrast, age, BMI, and other traditional risk factors, such as family history of premature coronary heart disease, hypertension, diabetes mellitus, current smoking, and active intravenous drug or cocaine use, did not differ between the groups. Similar percentages of HIV-infected women and men had undetectable viral loads (84% vs 81%; $P = .70$) and $CD4^+$ T-cell counts were similar in the HIV-infected women and men (597 ± 297 vs 530 ± 287 cells/μL; $P = .18$).

All diabetic patients had type 2 diabetes. Among diabetic patients, there were no differences between HIV-infected and non–HIV-infected patients in antidiabetic medication use (87% vs 100%; $P = .39$), duration of diabetes (3.4 ± 2.3 vs 6.3 ± 9.5 years; $P = .53$), duration of antidiabetic medication use (2.7 ± 2.2 vs 2.7 ± 2.2 years; $P = 1.0$), or hemoglobin A1c (5.5% ± 0.8% vs 5.6% ± 0.5%, $P = .13$). There was no difference

Table 2. Coronary Plaque Characteristics and Immune Activation Among Women[a]

Characteristic	Control Women (n = 30)	HIV-Infected Women (n = 60)	*P* Value[b]	HIV-Infected Women With Undetectable Viral Loads (n = 51)	*P* Value[c]
Calcium score					
Agatston calcium score	40 ± 98 (0 [0–39])	8 ± 30 (0 [0–0])	.11	9 ± 32 (0 [0–0])	.11
Log calcium score	1.9 ± 3.1	0.9 ± 1.9	.11	0.9 ± 2.0	.11
Agatston calcium score >0, %	33	20	.19	19	.17
Agatston calcium score >10, %	30	13	.06	13	.07
Agatston calcium score >100, %	15	2	.02	2	.04
Plaque characteristics					
Presence of coronary plaque, %	38	37	.88	39	.96
Presence of noncalcified segments, %	12	35	.04	37	.03
Presence of mixed segments, %	28	20	.46	22	.58
Presence of calcified segments, %	26	6	.01	2	.003
No. of segments with plaque	1.38 ± 2.32 (0 [0–3])	1.41 ± 2.41 (0 [0–2])	.93	1.44 ± 2.44 (0 [0–2])	.98
No. of segments with noncalcified plaque	0.40 ± 1.44 (0 [0–0])	0.92 ± 1.48 (0 [0–2])	.04	0.95 ± 1.52 (0 [0–2])	.03
No. of segments with mixed plaque	0.56 ± 1.00 (0 [0–1])	0.43 ± 1.15 (0 [0–0])	.41	0.46 ± 1.23 (0 [0–0])	.51
No. of segments with calcified plaque	0.44 ± 0.89 (0 [0–1])	0.06 ± 0.24 (0 [0–0])	.01	0.02 ± 0.16 (0 [0–0])	.003
% of segments with noncalcified plaque	23 ± 39 (0 [0–56])	74 ± 28 (75 [63–100])	.007	75 ± 29 (78 [67–100])	.007
% of segments with mixed plaque	38 ± 32 (33 [11–58])	23 ± 28 (23 [0–33])	.13	23 ± 29 (23 [0–33])	.15
% of segments with calcified plaque	44 ± 38 (42 [0–75])	4 ± 9 (0 [0–0])	.001	2 ± 6 (0 [0–0])	.0004
Markers of monocytes or macrophage activation					
sCD163, ng/mL	1245 ± 759 (856 [649–1718])	1777 ± 901(1548 [1091–2397])	.006	1750 ± 906 (1537 [1091–2397])	.01
Log sCD163, ng/mL	3.0 ± 0.2	3.2 ± 0.2	.006	3.2 ± 0.2	.01
sCD14, ng/mL	1452 ± 1086 (1732 [242–2391])	2367 ± 1781 (2057 [1424–2684])	.05	2463 ± 1887 (2071 [1424–2777])	.04
CXCL10, pg/mL	155 ± 121 (118 [90–163])	306 ± 258 (202 [115–394])	.002	292 ± 265 (191 [105–385])	.01
MCP-1, pg/mL	208 ± 102 (192 [132–247])	250 ± 118 (223 [164–303])	.09	254 ± 126 (223 [153–305])	.11
Markers of immune activation					
$CD8^+$ (% of lymphocytes)	24 ± 8	43 ± 14	<.0001	43 ± 14	<.0001
$CD8^+CD38^+$ (% of $CD8^+$)	5 ± 6	5 ± 4	.94	5 ± 4	.94
$CD8^+HLA–DR^+$ (% of $CD8^+$)	37 ± 24	60 ± 18	<.0001	59 ± 18	<.0001
$HLA–DR^+CD38^+CD8^+$ (% of $CD8^+$)	6 ± 7	4 ± 3	.16	4 ± 3	.16
$CD4^+$ (% of lymphocytes)	47 ± 8	26 ± 12	<.0001	27 ± 12	<.0001
$CD4^+CD38^+$ (% of $CD4^+$)	7 ± 12	7 ± 4	.73	6 ± 4	.64
$CD4^+HLA–DR^+$ (% of $CD4^+$)	19 ± 9	28 ± 13	.0004	26 ± 13	.004
$HLA-DR^+CD38^+CD4^+$ (% of $CD4^+$)	0.7 ± 0.6	1.9 ± 1.6	<.0001	1.8 ± 1.5	<.0001
$CD14^+CD16^+$ (% of monocytes)	15 ± 12	24 ± 15	.008	23 ± 15	.02
Inflammatory and CMV-related parameters					
hsCRP, mg/L	2.6 ± 3.2 (1.5 [0.4–4.5])	4.2 ± 7.0 (1.2 [0.3–4.8])	.88	4.3 ± 7.2 (1.2 [0.3–4.8])	.93
hsIL-6, pg/mL	1.8 ± 1.0 (1.5 [1.1–2.2])	1.9 ± 1.2 (1.5 [1.0–2.3])	.92	1.9 ± 1.2 (1.5 [1.1–2.3])	.95
CMV IgG seropositivity, %	90	92	.77	90	.94

Abbreviations: CMV, cytomegalovirus; HIV, human immunodeficiency virus; hsCRP, high-sensitivity C-reactive protein; hsIL-6, high sensitivity interleukin-6; IgG, immunoglobulin G; MCP-1, monocyte chemoattractant protein-1; sCD14, soluble CD14; sCD163, soluble CD163.

[a] Normally distributed data reported as means ± SDs or percentages; variables with nonnormal distributions are also reported as (median [interquartile range]). Data from computed tomographic angiography were available in 27 control women and 55 HIV-infected women.

[b] Control vs HIV-infected women.

[c] Control vs HIV-infected women with undetectable viral loads.

in percentage with diabetes by hepatitis C virus (HCV) status either among HIV-infected patients (14% for those with vs 8% for those without HCV; $P = .28$) or non–HIV-infected patients (0% vs 8%, respectively; $P = .48$).

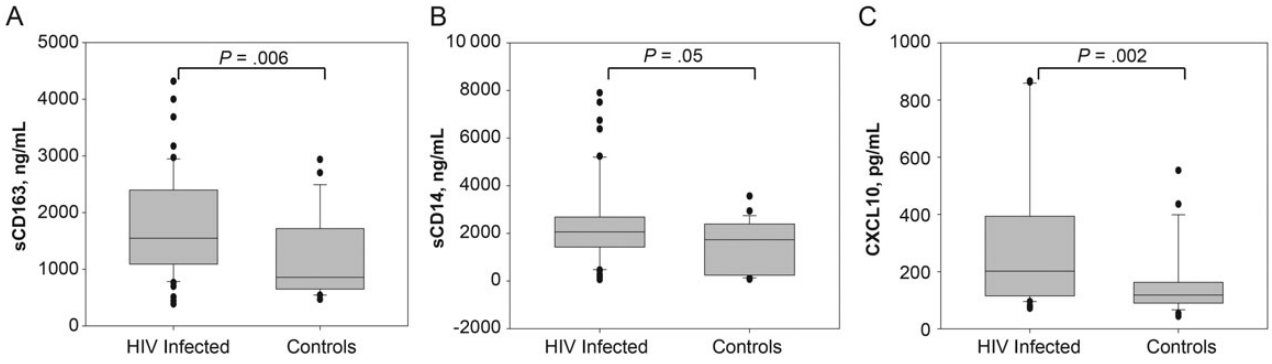

Figure 1. Markers of monocytes or macrophage activation in human immunodeficiency virus (HIV)–infected women compared with non–HIV-infected control women. *A,* Soluble CD163 (sCD163). *B,* Soluble CD14 (sCD14). *C,* CXCL10. Results are reported as medians (*horizontal lines in boxes*), interquartile ranges (*top and bottom edges of boxes*), and the 90th and 10th centiles (*small horizontal lines above and below boxes*).

Coronary Atherosclerosis Characteristics by HIV Status Among Male and Female Subjects

The percentage of coronary segments with noncalcified plaque was significantly higher in HIV-infected women (median, 75%; IQR, 63%–100%) than in HIV-infected men (median, 50%; IQR, 3%–100%; $P < .05$) or non–HIV-infected male controls (median, 33%; IQR, 4%–66%; $P < .05$) (Supplementary Table 2, Figure 2). The log Agatston calcium score was higher in HIV-infected men and significantly different from that in HIV-infected women ($P = .05$).

Immune Activation and Inflammation Indices by HIV Status Among Male and Female Subjects

HIV-infected women demonstrated significantly higher sCD163 and sCD14 levels than both HIV-infected and non–HIV-infected men ($P < .05$; Supplementary Table 2; Figure 2). In contrast, significant differences in MCP-1 levels and the percentage of $CD14^+CD16^+$ monocytes were not seen between HIV-infected men and women. CXCL10 levels could not be compared, because these data were not available for the HIV-infected men. The percentage of $CD4^+HLA{-}DR^+$ cells was higher in HIV-infected women than in HIV-infected or non–HIV-infected men. Levels of sCD163 and sCD14, as well as the percentage of $CD4^+HLA{-}DR^+$ cells were also higher in non–HIV-infected women than in non–HIV-infected men ($P < .05$). The hsCRP and hsIL-6 levels did not differ significantly between groups by HIV status or sex (Supplementary Table 2).

In sensitivity analyses, similar results were seen when noncalcified plaque and immune activation markers were compared between groups excluding any patients with diabetes (data not shown). We also performed multivariate modeling to look at the impact of diabetes on immune activation and found that HIV was independently associated with increased sCD163 levels ($P = .0003$), controlling simultaneously for sex and

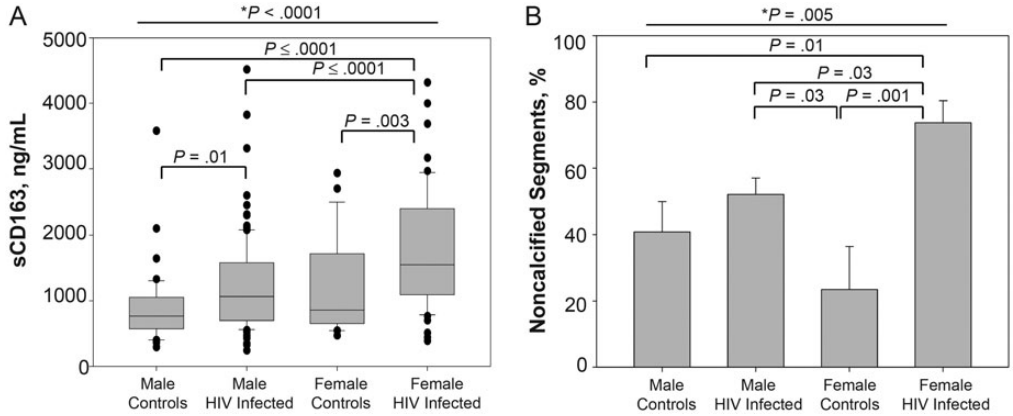

Figure 2. Immune activation as shown by soluble CD163 (sCD163) levels (*A*) and percentage of noncalcified plaque segments (*B*) in human immunodeficiency virus (HIV)–infected and non–HIV-infected men and women. For sCD163, results are shown as medians (*horizontal lines in boxes*), interquartile ranges (*top and bottom edges of boxes*), and the 90th and 10th centiles (*small horizontal lines above and below boxes*). For percentage of noncalcified plaque segments, results are shown as means ± SEMs. *Overall P value by ANOVA.

diabetes. No effects of cocaine or intravenous drug use on sCD163 levels or noncalcified plaque were seen.

Multivariate Modeling to Assess Effects of HIV Status, Sex, and Age on Monocyte Activation and Noncalcified Plaque

Because differences in monocyte activation markers were greatest between the HIV-infected women and the other groups, we performed multivariate regression modeling to explore the simultaneous effects of HIV status, sex and age with respect to these markers among all subjects. Significant effects of HIV infection ($P < .0001$; higher in HIV-infected than noninfected subjects), female sex ($P = .0002$; higher in women than in men), and age ($P = .002$; increased with age) were independently and significantly associated with sCD163 (Table 3; Figure 3). There was also a 2-way interaction between sex and age ($P = .046$), such that sCD163 levels increased more with age among women. Similarly, significant effects of HIV infection ($P = .001$), sex ($P < .0001$), and age ($P = .01$) were seen in multivariate modeling for sCD14 levels (Table 4).

We also performed multivariate regression modeling to examine the simultaneous effects of HIV status, sex, and age on noncalcified plaque among all subjects. In this model, we found that HIV infection ($P = .0002$; higher in HIV-infected than in noninfected subjects) was independently and significantly related to the percentage of noncalcified plaque. There was also a 2-way interaction between female sex and HIV infection ($P = .03$), such that the percentage of noncalcified plaque was more increased among HIV-infected women (Supplementary Table 3).

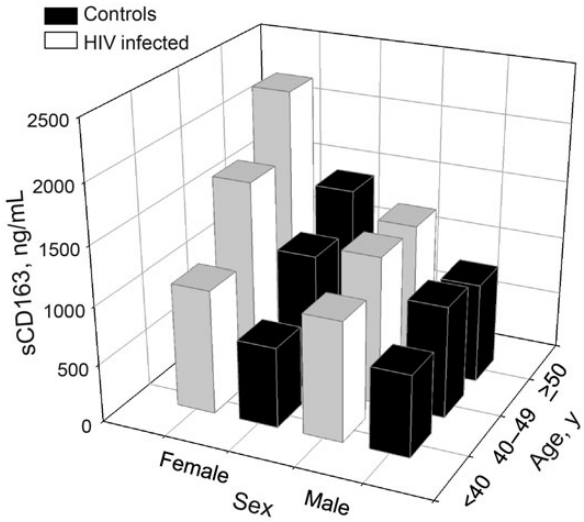

Figure 3. Impact of human immunodeficiency virus (HIV) status, sex, and age on soluble CD163 (sCD163). Results are shown as means. See Table 3 for statistical analysis.

DISCUSSION

To our knowledge, this study represents the first investigation of coronary plaque and the first to relate detailed parameters of immune activation to coronary plaque parameters in HIV-infected women. We demonstrate a higher prevalence of noncalcified coronary plaque and increased immune activation parameters among HIV-infected women compared with non–HIV-infected women. We were also able to show that these features (ie, noncalcified plaque and immune activation) were more increased in HIV-infected women than in HIV-infected men, and these results remained significant when we compared those with undetectable viral loads with the non–HIV-infected group. The HIV-infected women in this study were relatively young, had no symptoms or prior diagnosis of CVD, had low Framingham Risk scores, and had good viremic control.

Table 3. Model to Assess Effects of Interaction Between HIV Status, Female Sex, and Age on Soluble CD163 Levels Among Entire Cohort (n = 221)

	($R^2 = 0.22$; $P < .0001$)	
Variables[a]	β Estimate	*P* Value
HIV infection	219.3649	<.0001
Female sex	211.205	.0002
Age	26.609632	.002
HIV infection × female sex	48.36615	.38
HIV infection × age	8.769391	.30
Female sex × age	17.05738	.046
HIV infection × female sex × age	0.5383128	.95

Abbreviation: HIV, human immunodeficiency virus. R^2 represents the coefficient of determination and the proportion of variance explained by the model. P value represents significance by the whole model ANOVA test.

[a] Crossing of 2 or 3 variables was used in statistical modeling to determine the interaction between those variables.

Table 4. Model to Assess Effects of Interaction Between HIV Status, Female Sex, and Age on Soluble CD14 Levels Among Entire Cohort (n = 221)

	($R^2 = 0.46$, $P < .0001$)	
Variable[a]	β Estimate	*P* Value
HIV infection	243.8785	.001
Female sex	783.7037	<.0001
Age	−29.77958	.01
HIV infection × female sex	204.73177	.007
HIV infection × age	−4.2584888	.71
Female sex × age	32.641781	.005
HIV infection × female sex × age	−1.336222	.91

Abbreviation: HIV, human immunodeficiency virus. R^2 represents the coefficient of determination and the proportion of variance explained by the model. P value represents significance by the whole model ANOVA test.

[a] Crossing of 2 or 3 variables was used in statistical modeling to determine the interaction between those variables.

The primary observation in this study is that HIV-infected women have significantly more noncalcified coronary plaque than well-matched non–HIV-infected women, both absolutely and as a percentage. HIV-infected women do not have more coronary plaque in general but demonstrate an altered phenotype comprising more noncalcified plaque than seen in either non–HIV-infected women or HIV-infected men. The observation of more noncalcified plaque in this population is novel and of potential clinical relevance, because noncalcified plaque is known to have larger necrotic cores with increased focal inflammation. This type of plaque is considered "vulnerable" and may be associated with acute coronary syndromes because of its susceptibility to rupture [16]. Results of prior sex-stratified analyses suggest that the relative risk of acute myocardial infarction between HIV-infected and non–HIV-infected patients was higher among women than among men (relative risk, 2.98 vs 1.40), after adjustment for age, race, hypertension, diabetes, and dyslipidemia [2]. Thus, our current data offer a potential mechanism for the increase in acute myocardial infarction rates seen among HIV-infected women.

Despite excellent viremic control, HIV-infected women demonstrated significant increases in immune activation, which included markers of monocyte and T-cell activation. In contrast, markers of generalized inflammation were not increased among the HIV-infected women. These increases in immune activation were not only seen in comparison with non–HIV-infected women but were also seen in more detailed comparisons with HIV-infected men.

Sex is known to play a significant role in immune activation, and more specifically, the innate immune response. Recently, Martin et al [17] compared markers of innate immune activation in HIV-infected women and female control subjects, including sCD163, CXCL10, sCD14, and the percentage of $CD14^{+}CD16^{+}$ monocytes. We extend these findings in a larger study, including male HIV-infected and control subjects, and show that both HIV infection and sex simultaneously and independently contribute to increased immune activation, as indicated by sCD163 and sCD14 levels.

Monocyte and macrophage activation plays a critical role in the atherosclerotic process. Burdo et al [9] have reported elsewhere that sCD163 is associated with noncalcified plaque burden in HIV-infected men, independent of traditional risk factors. We now show increased sCD163 levels among women with noncalcified plaque. In contrast, traditional risk factors did not differ between the HIV-infected and noninfected women, and the participants in our study had a low overall estimate of 10-year CVD risk.

Findings of other studies have suggested that bacterial translocation from the gut is a mechanism for immune activation and the resultant accelerated risk of CVD. Kelesidis et al [18] reported elevated sCD14 levels in association with subclinical atherosclerosis, as measured by carotid IMT in HIV-infected patients. Elevated sCD14 levels can also predict mortality in HIV-infected patients [19]. In the current study, we extend the data of Kelesidis et al [18] to demonstrate increased sCD14 levels in HIV-infected women. The HIV-infected women in our study demonstrated elevated sCD14 levels compared with the non–HIV-infected female and HIV-infected male subjects. Further studies are needed to determine mechanisms, clinical consequences, and treatment strategies for monocyte activation in HIV-infected women.

Recent studies have explored the effect of HIV on the immune system and have shown that HIV may induce a premature immune aging phenotype with accelerated immune activation among HIV-infected patients as they age [17, 20]. We assessed the interaction between age and sex on sCD163 and sCD14 levels, as markers of monocyte activation. Among all participants, we performed multivariate modeling with independent terms of HIV status, age, and sex, as well as the interactions of all 3 in a fully saturated model with the relevant 2- and 3-way interaction terms. Prior studies have not considered all 3 relevant variables—age, sex, and HIV status—simultaneously in a single model. In novel data, we found strong independent relationships such that increased sCD163 was independently associated with HIV positivity, female sex and increased age. Moreover, there was a 2-way interaction between age and sex, such that sCD163 levels increased more with age among women than among men. Similarly, independent effects of HIV, age, and sex, as well as interactions between these covariates, were seen for sCD14 levels.

Among older women who are postmenopausal, estrogen deprivation may account, in part, for the increased monocyte and macrophage activation demonstrated by sCD163. Changes in the immune system during menopause have been attributed to estrogen deprivation with investigators reporting increased secretion of proinflammatory cytokines, such as interleukin 1, interleukin 6, and tumor necrosis factor α, during menopause [21]. Indeed, aging and menopause have been associated with both immune activation [22] and atherosclerosis [23] among non–HIV-infected women. Macaques in early menopause treated with estradiol had a significantly lower expression of CD163 in carotid artery tissue than macaques not treated with estradiol as well as those in late menopause [24]. In addition, T-cell–specific markers were reduced in carotid artery tissue after estradiol treatment in both early and late menopause groups.

Estrogen may thus have protective effects in premenopausal HIV-infected women relative to older HIV-infected women. Using sCD163 as a marker of monocyte activation, we showed in the current study that older women have more activation than younger women. Importantly, estrogen deprivation may have differential effects on immune activation indices with age, being more proinflammatory with aging [24–26]. Further

studies are needed to discern the role of estrogen with respect to immune activation in HIV-infected women.

The current study has a number of limitations as well as strengths. This is the first study to our knowledge to investigate coronary plaque specifically in HIV-infected women and to relate indices of immune activation to such morphologic characteristics. Because this study is cross-sectional, definitive conclusions on causality cannot be made, and further longitudinal studies relating changes in immune activation to coronary plaque characteristics in HIV-infected men and women are necessary. Finally, a number of comparisons were made in this study, but the main findings remained significant when multiple comparisons were accounted for.

In summary, this study adds new information on unique coronary atherosclerosis features in HIV-infected women. HIV-infected women demonstrate more noncalcified, potentially vulnerable plaque, which is not a function of increased traditional CVD risk factors or generalized inflammation. In contrast, immune activation is strikingly increased among HIV-infected women. Independent contributions of aging, HIV status, and female sex to increased immune activation were observed, suggesting that women, and older HIV-infected women in particular, are likely to demonstrate very high levels of monocyte activation, as indicated by sCD163 levels, which could contribute to noncalcified plaque and disproportionate CVD in this group. Further research into the mechanisms of immune activation and the relationship to CVD in HIV-infected women is critically needed.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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