Increased prevalence of subclinical coronary atherosclerosis detected by coronary computed tomography angiography in HIV-infected men

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Objective: The degree of subclinical coronary atherosclerosis in HIV-infected patients is unknown. We investigated the degree of subclinical atherosclerosis and the relationship of traditional and nontraditional risk factors to early atherosclerotic disease using coronary computed tomography angiography.

Design and methods: Seventy-eight HIV-infected men (age 46.5 ± 6.5 years and duration of HIV 13.5 ± 6.1 years, CD4 T lymphocytes 523 ± 282; 81% undetectable viral load), and 32 HIV-negative men (age 45.4 ± 7.2 years) with similar demographic and coronary artery disease (CAD) risk factors, without history or symptoms of CAD, were prospectively recruited. 64-slice multidetector row computed tomography coronary angiography was performed to determine prevalence of coronary atherosclerosis, coronary stenosis, and quantitative plaque burden.

Results: HIV-infected men demonstrated higher prevalence of coronary atherosclerosis than non-HIV-infected men (59 vs. 34%; $P = 0.02$), higher coronary plaque volume [55.9 (0–207.7); median (IQR) vs. 0 (0–80.5) μl; $P = 0.02$], greater number of coronary segments with plaque [1 (0–3) vs. 0 (0–1) segments; $P = 0.03$], and higher prevalence of Agatston calcium score more than 0 (46 vs. 25%, $P = 0.04$), despite similar Framingham 10-year risk for myocardial infarction, family history of CAD, and smoking status. Among HIV-infected patients, Framingham score, total cholesterol, low-density lipoprotein, CD4/CD8 ratio, and monocyte chemoattractant protein 1 were significantly associated with plaque burden. Duration of HIV infection was significantly associated with plaque volume ($P = 0.002$) and segments with plaque ($P = 0.0009$) and these relationships remained significant after adjustment for age, traditional risk factors, or duration of antiretroviral therapy. A total of 6.5% (95% confidence interval 2–15%) of our study population demonstrated angiographic evidence of obstructive CAD (>70% luminal narrowing) as compared with 0% in controls.

Conclusion: Young, asymptomatic, HIV-infected men with long-standing HIV disease demonstrate an increased prevalence and degree of coronary atherosclerosis compared with non-HIV-infected patients. Both traditional and nontraditional risk factors contribute to atherosclerotic disease in HIV-infected patients.

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Introduction

Survival of HIV-infected patients worldwide has remarkably improved with the use of HAART and increasing access to treatment; however, even among treated HIV patients, survival is still decreased compared with the general population due, in large part, to excess mortality from noninfectious illnesses, including cardiovascular disease [1,2]. Traditional cardiovascular risk factors, including dyslipidemia, diabetes mellitus, and smoking are highly prevalent in the HIV population [3]. Furthermore, immune activation, chronic inflammation, and viral factors may predispose this patient population to increased cardiovascular risk above and beyond that conferred by traditional cardiovascular risk factors [4].

Prior retrospective studies utilizing healthcare system-based databases have shown the rate of cardiovascular events to be higher in young men and women with HIV infection than non-HIV-infected individuals [5,6]; however, prospective studies examining early subclinical coronary artery disease (CAD), assessing detailed measures of plaque burden beyond calcium scoring, using coronary CT angiography (CTA) to quantitatively measure the extent of coronary plaque, including noncalcified, mixed calcified and noncalcified, and calcified plaque and to assess for coronary artery luminal narrowing in HIV-infected patients compared with concurrently enrolled uninfected controls are still lacking. Therefore, we investigated the prevalence and degree of early subclinical coronary atherosclerosis in young asymptomatic HIV-infected individuals without previously known cardiovascular disease using 64-slice cardiac multidetector row computed tomography (MDCT) and coronary CTA in a prospectively recruited cohort of HIV-infected patients compared with concurrently recruited HIV-seronegative controls with similar demographic and traditional cardiovascular risk factors.

Methods

Study design

In a prospectively recruited cohort, the prevalence of subclinical CAD in asymptomatic HIV-infected men was compared with that in HIV-seronegative control men. The prevalence of coronary plaque was the primary endpoint. Secondary endpoints included plaque volume, number of coronary segments with plaque, coronary stenosis, and Agatston calcium score.

Participant selection

One hundred and ten men participated in this study. Seventy-eight men with HIV infection were recruited from HIV clinics and community health centers in the Boston area as well as by newspaper advertisements. HIV-negative control men were identically recruited from the same communities using the same advertisements, seeking asymptomatic young men without known cardiac disease. Family members, partners, and friends of the HIV-infected patients were also encouraged to enroll in attempt to ensure the two groups would be similar with respect to demographic characteristics and cardiovascular risk factors. Other than HIV disease, inclusion and exclusion criteria were identical for both groups. Participants aged 18–55 years, without known cardiac disease or symptoms suggestive of cardiac disease (any current or prior heart disease, including angina, arrhythmias, valvular heart disease, pericarditis, congestive heart failure, or any prior treatment for CAD or any heart disease) were recruited. HIV and control participants were not recruited with regard to any changes in body composition, weight, or metabolic criteria. HIV and control participants with known renal disease or creatinine more than 1.5 mg/dl or estimated creatinine clearance less than 70 ml/min were excluded to minimize risk of contrast nephropathy. Participants with contraindications to administration of contrast agent, β-blockade, or nitroglycerin were also excluded. HIV-infected patients receiving HAART at the time of the study were required to be on stable therapy for more than 3 months. HIV-infected patients not receiving antiretroviral therapy (ART) were also recruited. All participants gave informed consent to participate. This study was approved by the institutional review boards of Massachusetts General Hospital and Massachusetts Institute of Technology.

Study procedures and assessment of cardiovascular risk factors

All participants underwent a detailed interview and physical examination by a single investigator. Detailed information on sociodemographic factors, medical history, family history, behavior, including smoking, recreational drug use, and medications was obtained. Duration of known HIV diagnosis and detailed history of prior antiretroviral use were determined. All participants fasted at least 12 h before blood draws and the 75-g oral glucose tolerance test (OGTT). Assessment of traditional cardiovascular risk factors was determined through comparison of individual risk factors and an aggregate risk score using the Framingham risk equation [4].

Cardiac multidetector row computed tomography and computed tomography angiography

CT imaging was performed using a 64-slice CT scanner (Sensation 64; Siemens Medical Solutions, Forchheim, Germany). In preparation for the scan, participants with heart rate more than 60 beats/min received intravenous β-blocker (metoprolol 5–20 mg) unless contraindications to β-blockers were present or if systolic blood pressure was less than 100 mmHg. Participants also received 0.6 mg sublingual nitroglycerin. Image acquisitions were

performed during breathhold in inspiration. As per standard protocol, a test bolus of 20 ml contrast agent was administered with a flow rate of 5 ml/s to determine the optimal timing of contrast injection. Coronary CTA datasets were acquired with 64×0.6 mm slice collimation, a gantry rotation time of 330 ms, tube voltage of 120 kVp, and an effective tube current of 850 mAs using ECG-correlated tube current modulation when appropriate. Contrast agent (80–100 ml, iopamidol, Isovue; Bracco Diagnostics, Inc., Princeton, New Jersey, USA) was injected intravenously at a rate of 5 ml/s to ensure homogeneous enhancement of the entire coronary artery tree. Axial images were reconstructed with a slice thickness of 0.75 mm and increment of 0.4 mm using a half-scan algorithm with a temporal resolution of 165 ms. Images were initially reconstructed at 60, 65, 70, and 35% of the cardiac cycle. Additional reconstructions were performed to minimize motion artifacts, if necessary. All reconstructions were transferred to an offline workstation for analysis (Leonardo; Siemens Medical Solutions). Assessment of coronary atherosclerotic plaque, including number of segments with plaque, and degree of stenosis, was determined by a consensus reading between two investigators, including a cardiologist and a radiologist with significant experience in the interpretation of cardiac CT. Physicians analyzing the scans were blinded to the participants' clinical history or HIV status. Agatston calcium score was calculated using the noncontrast CT images by standardized techniques [7].

Plaque volume measurements were performed by a single experienced reader blinded to participants' HIV status using an offline Vitrea 2 workstation (Vital Images, Minnetonka, Minnesota, USA) and software for plaque analysis (SUREPlaque) [8]. Plaques were identified in the curved multiplanar reconstruction after readers selected the phase of the cardiac cycle with no motion artifact. The plaque length was established visually with a marker in the proximal and distal plaque limits. The inner and outer borders of the vessel/plaque were established using a semiautomatic tracer and plaque volume was calculated automatically. When the contour was visually out of the vessel/plaque border, manual correction was performed. Lesions with motion artifact in all phases of the cardiac cycle were excluded. Interobserver and intraobserver intraclass correlation coefficients determined in 25 patients were more than 0.99 ($P < 0.001$).

The presence of any coronary atherosclerotic plaque, whether calcified or noncalcified, was assessed. Quantification of atherosclerotic plaque was measured by counting the number of coronary segments with evidence of plaque present (using a modified 17-segment model of the coronary artery tree) [9]; and by plaque volume. Presence of severe coronary artery stenosis was defined as luminal obstruction more than 70% diameter in any coronary segment.

Body composition and dietary assessment

Weight and anthropometric measurements were determined in the morning, prior to breakfast. To assess abdominal visceral and abdominal subcutaneous adipose tissue area (VAT and SAT, respectively), a cross-sectional abdominal CT scan at the level of the L4 pedicle was performed [10]. Four-day food records were completed by the participants and analyzed using Minnesota Nutrition Data System software.

Metabolic, biochemical, and immunologic parameters

Total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides, glucose, and creatinine were determined using standard techniques. Monocyte chemoattractant protein-1 (MCP-1) and C-reactive protein (CRP) were measured using enzyme-linked immunosorbent assay (ELISA). Insulin was measured using either a radioimmunoassay (RIA) or a chemiluminescence immunoassay. Cross validation analysis indicated a strong linear correlation ($r = 0.99$, $P < 0.0001$). $CD4^+$ and $CD8^+$ T-cell counts were assessed by flow cytometry. $CD4^+$ nadir was obtained by patient report. HIV viral load was determined by ultrasensitive real-time PCR (lower limit of detection = 50 copies/ml). HIV testing was performed by chemiluminometric immunoassay and confirmed by western blot. Cytomegalovirus IgG (CMV IgG) titers were measured using an enzyme-linked fluorescent immunoassay.

Statistical analysis

Presence of plaque (yes or no) as a dichotomized variable was compared between the HIV group and the control group using the χ^2 test. Plaque burden as a continuous variable was quantified using total number of coronary segments with plaque and by plaque volume. Comparisons between two groups were performed using Student's *t*-test for normally distributed continuous variables and Wilcoxon rank-sum test if the distribution was nonnormal. Spearman correlation coefficient was used to assess correlations with plaque burden as number of segments with plaque, plaque volume, and Agatston calcium score had nonnormal distributions. Logistic regression analysis was used to perform adjusted analyses for binary outcome of presence of plaque. Linear regression analysis was used for adjusted analyses for continuous outcome variables. For the assessment of differences between HIV and non-HIV-infected participants, known cardiovascular disease risk markers were included as covariates in regression modeling. In multivariate modeling, among HIV-infected patients, assessing the relationship of duration of HIV to measures of plaque burden, modeling was performed including HIV-related parameters and traditional risk factors as covariates. Additional analyses were performed comparing cardiovascular risk and HIV-related parameters in HIV-infected patients with ($n = 46$, 59%) and without ($n = 32$, 41%)

coronary plaque. Sensitivity analyses were performed comparing the presence of plaque in the subset excluding any patient with triglyceride more than 200 mg/dl or fasting insulin more than 75th centile for men of this age group (>11.9 μU/ml), using normative data from the Framingham Offspring study (personal communication with Dr James Meigs). Two-tailed probability values are reported and statistical significance was assumed when *P* value was less than 0.05. All statistical analyses were performed using JMP (SAS Institute Inc., Cary, North Carolina, USA) and SPSS (SPSS Inc., Chicago, Illinois, USA).

Results

Characteristics of participants

One hundred and ten men participated in this study, including 78 HIV-infected men and 32 HIV-seronegative men. Demographic and clinical characteristics of the study participants are described in Table 1. HIV-infected patients had a long duration of known HIV infection of approximately 13.5 years. The great majority (95%) were receiving ART, with an average duration of treatment of 7 years. The percentages receiving each major class of ART use and the duration of this use are also shown in Table 1. Overall, immunological control was good, with CD4 cell count 523 ± 282 cells/μl and 81% with undetectable viral load. A total of 89% of patients had a viral load under 1000 copies/ml. The four patients who were not on ART had never been on ART and had CD4 cell count 815 ± 190 cells/μl and viral load 1039 (156–7038) copies/ml.

Age, race, sex, BMI, and Framingham risk score were similar between both groups (Table 1). Family history of premature coronary heart disease, prevalence of hypertension, prevalence of diabetes mellitus, % of current smokers, blood pressure, fasting glucose, 2-h glucose, hemoglobin A1c, total cholesterol, HDL, LDL, CRP, and IL-6 were also similar between the HIV and control groups. The HIV and non-HIV-infected group had similar Framingham scores (7.7 ± 5.1 vs. 7.0 ± 4.6, $P = 0.50$) and predicted median 10-year myocardial infarction (MI) risk scores 4 vs. 4.5% risk. The HIV group had a higher triglyceride concentration and borderline higher fasting insulin and these variables were investigated in sensitivity analyses and regression modeling. Dietary intake of total calories, fat, protein, carbohydrates, saturated fat, fiber, and cholesterol were similar between the HIV and non-HIV-infected groups (data not shown).

Prevalence of coronary artery disease and coronary artery stenosis

Significant difference in the prevalence of coronary atherosclerosis was seen between the two groups with 59% of the HIV group having evidence of plaque present in the coronary arteries visualized by CTA vs. 34% of the control group ($P = 0.02$; Fig. 1; Table 2). Controlling for known cardiovascular risk factors, including age, race, BMI, smoking pack-years, Framingham risk score, hypertension, diabetes mellitus, cocaine use, total cholesterol, LDL, HDL, and triglycerides, prevalence of CAD remained higher in the HIV group ($P = 0.02$).

Plaque burden

Plaque burden measured by number of segments with plaque [1 (0–3) vs. 0 (0–1) segments, $P = 0.03$] and plaque volume [55.9 (0–207.7) vs. 0 (0–80.5) μl, $P = 0.02$] indicate higher plaque burden in the HIV group when compared with the control group (Table 2).

Agatston calcium score

Overall Agatston calcium score tended to be higher in HIV patients ($P = 0.08$) and percentage with score more than 0 was higher in the HIV group (46.2 vs. 25.0%, $P = 0.04$; Table 2). Among participants with Agatston calcium score of 0, 10 out of 42 HIV patients [23.8% (95% confidence interval 13.5–38.5%)] had evidence of noncalcified plaque seen on CTA and three out of 24 controls [12.5% (4.3–31.0%)] had evidence of noncalcified plaque.

Critical coronary stenosis

A total of 6.5% (95% confidence interval 2–15%) of HIV patients had greater than 70% stenosis in one or more coronary segments compared with 0% in controls (Table 2). The median Framingham 10-year risk among HIV patients with stenosis more than 70% was 12% (IQR 6–19%) vs. 4% (2–6%) ($P = 0.006$) among HIV-infected patients without stenosis more than 70%. Further clinical follow-up information was obtained in the five asymptomatic HIV-infected patients we identified with severe CAD (>70% coronary luminal narrowing) by CTA. Due to the data obtained by cardiac CTA from our study, these patients were referred to cardiologists by their primary care physicians. One patient underwent cardiac stress testing, which yielded normal results, and continues to do well. Another patient was recommended by his provider to undergo cardiac stress testing, which has not yet been obtained. After formal evaluation by cardiologists, three patients underwent subsequent cardiac catheterization and in all three patients, coronary angiography confirmed their cardiac CTA findings. Of these three patients, one patient underwent coronary artery bypass graft surgery for four-vessel revascularization and is doing well; the second patient was found to have 85% stenosis of the proximal left anterior descending artery (LAD) on cardiac catheterization for which he underwent single vessel stent placement and is doing well; and the third patient was found to have severe stenosis of the proximal left circumflex artery and diffuse mild-moderate coronary disease in other vessels. After failed stent placement, he received medical therapy but was unable to tolerate statins. Two and a half years

Table 1. Demographic and clinical characteristics of study population.

	Controls ($n=32$)	HIV ($n=78$)	P value
Demographics			
Age (years)	45.4 ± 7.2	46.5 ± 6.5	0.44
Race (%)			0.17
White	59	68	
Black	19	18	
Asian	9	1	
Hispanic	3	9	
Native American	6	4	
Family history of premature CHD by NCEP (%)	14	19	0.53
Framingham risk score	7.0 ± 4.6	7.7 ± 5.1	0.50
Hypertension (%)	16	29	0.14
Diabetes milletus (%)	3	9	0.23
Current smoker (%)	31	35	0.70
Past cocaine use, no. of uses	0 (0–1)	0 (0–6)	0.12
HIV disease-related parameters			
Duration since HIV diagnosis (years)	N/A	13.5 ± 6.1	N/A
Ever on antiretroviral therapy (%)	N/A	95	N/A
Currently on antiretroviral therapy (%)	N/A	95	N/A
Duration of antiretroviral therapy (years)	N/A	7.1 ± 4.6	N/A
Current PI treatment (%)	N/A	53	N/A
Duration of PI treatment (years)	N/A	3.8 ± 4.2	N/A
Current NRTI treatment (%)	N/A	91	N/A
Duration of NRTI treatment (years)	N/A	6.8 ± 4.5	N/A
Current NNRTI treatment (%)	N/A	49	N/A
Duration of NNRTI treatment (years)	N/A	2.6 ± 3.5	N/A
$CD4^+$ T lymphocytes (cells/μl) (current)	N/A	523 ± 282	N/A
$CD4^+$ T-lymphocytes nadir (cells/μl)	N/A	169 (54–263)	N/A
HIV RNA viral load (copies/ml)	N/A	<50 (<50, <50)	N/A
Undetectable HIV RNA < 50 copies/ml (%)	N/A	81	N/A
Anthropometric parameters			
BMI (kg/m^2)	26.9 ± 5.2	26.1 ± 4.3	0.43
VAT area (cm^2)	149 ± 111	172 ± 121	0.35
SAT area (cm^2)	212 ± 138	167 ± 98	0.06
Hemodynamic parameters			
Systolic blood pressure (mmHg)	118 ± 11	120 ± 12	0.30
Diastolic blood pressure (mmHg)	76 ± 9	77 ± 8	0.55
Metabolic parameters			
Serum creatinine (mg/dl)	1.08 ± 0.16	1.06 ± 0.19	0.58
Fasting glucose, mmol/l (mg/dl)	5.1 ± 0.5 (92 ± 9)	5.2 ± 0.6 (94 ± 11)	0.40
2-h glucose, mmol/l (mg/dl)	6.2 ± 2.3 (112 ± 41)	6.8 ± 2.6 (123 ± 47)	0.27
Fasting insulin (μU/ml)	5.1 ± 3.6	8.2 ± 8.4	0.05
Fasting insulin >11.9 μU/ml[a] (%)	7	14	0.31
Lipid panel			
Total cholesterol, mmol/l (mg/dl)	4.7 ± 1.1 (180 ± 44)	4.7 ± 1.1 (182 ± 44)	0.75
HDL cholesterol, mmol/l (mg/dl)	1.3 ± 0.3 (49 ± 13)	1.2 ± 0.4 (48 ± 14)	0.76
LDL cholesterol, mmol/l (mg/dl)	2.8 ± 1.0 (110 ± 37)	2.6 ± 0.8 (101 ± 32)	0.22
Triglycerides, mmol/l (mg/dl)	1.2 ± 0.7 (103 ± 62)	1.9 ± 1.6 (167 ± 142)	0.02
Triglycerides >2.3 mmol/l (>200 mg/dl), %	9	24	0.06
Hemoglobin A1c (%)	5.5 ± 0.4	5.3 ± 0.6	0.17
Inflammatory and CMV-related parameters			
C-reactive protein, median (IQR; mg/l)	1.8 (0.6–3.5)	1.6 (0.7–4.0)	0.82
Interleukin-6, median (IQR; pg/ml)	2.4 (1.8–3.5)	2.6 (1.7–3.6)	0.93
Cytomegalovirus IgG seropositivity (%)	65	91	0.001
Cytomegalovirus IgG titers, median (IQR)	36.0 (3.0–68.3)	94.5 (65.8–189.5)	<0.0001

Data reported as mean ± standard deviation (SD) or percentage, except for variables with nonnormal distributions, which are reported as median (IQR = interquartile range). CHD, coronary heart disease; CMV, cytomegalovirus; HDL, high-density lipoprotein; IgG, immunoglobulin G; IQR, interquartile range; LDL, low-density lipoprotein; 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.
[a]Fasting insulin more than 11.9 μU/ml represents measurements above the 75th percentile among men 26–60-year-old in the Framingham Offspring study.

later, he developed new symptom of angina manifested as left arm pain with exertion. A subsequent repeat cardiac catheterization showed rapid progression of his coronary disease to near-complete stenosis of five coronary branches requiring coronary artery bypass graft surgery and patient is currently doing well.

Characteristics of HIV patients with evidence of coronary atherosclerosis

HIV patients with evidence of plaque seen on CTA were more likely to be older ($P=0.004$) and have longer duration of known HIV infection ($P=0.02$), higher Framingham risk score ($P=0.002$), higher total cholesterol

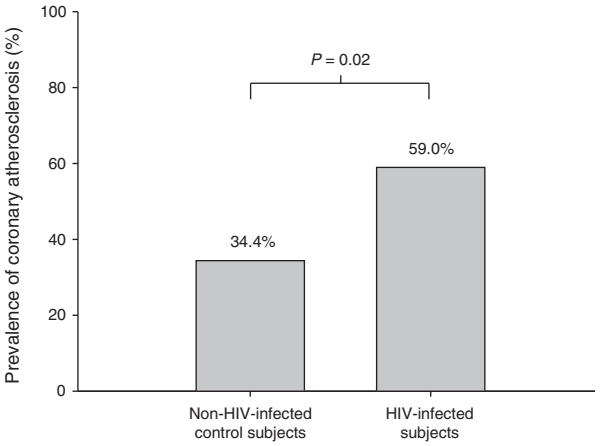

Fig. 1. Prevalence of coronary atherosclerosis in non-HIV-infected control participants vs. HIV-infected patients. $P = 0.02$ for comparison by χ^2 test.

($P = 0.01$), higher triglycerides ($P = 0.04$), higher LDL ($P = 0.046$), and lower CD4/CD8 ratio ($P = 0.001$). However, there were no differences in BMI, VAT, SAT, duration of protease inhibitor use, duration of ART, HDL, fasting glucose, 2-h glucose, fasting insulin, 120-min insulin, viral load, CRP, or IL-6 among HIV patients with coronary atherosclerosis vs. HIV patients without coronary atherosclerosis. In addition, HIV patients with coronary atherosclerosis were not more likely to have been on ARTor have CMV IgG seropositivity, but tended to have higher CMV IgG titers ($P = 0.05$; Table 3).

Relationship of cardiovascular risk factors to plaque burden and calcium score in HIV patients

Age, Framingham risk score, duration of known HIV infection, duration of protease inhibitor use, CD4/CD8 ratio, total cholesterol, LDL, CMV IgG antibody titers, and MCP-1 were associated with total number of coronary segments with plaque (Table 4). Plaque volume was associated with age, Framingham risk score, duration of known HIV infection, CD4/CD8 ratio, total cholesterol, and LDL. Agatston coronary calcium score was related to age, Framingham risk score, duration of known HIV infection, total cholesterol, LDL, triglycerides, glucose area under the curve (AUC), VAT, and MCP-1.

In multivariate regression analysis, controlling for age and Framingham risk score, the duration of known HIV infection remained significantly associated with plaque burden measured by number of segments with plaque ($P = 0.04$) and plaque volume ($P = 0.05$). The relationship between plaque volume and duration of HIV infection also remained significant in a model controlling for age, duration of protease inhibitor use, triglycerides, LDL, and HDL ($P = 0.047$). Controlling for parameters associated with HIV disease, including current CD4 cell count, nadir CD4 count, HIV viral load, and duration of ART, duration of HIV disease remained significantly associated with number of segments with plaque ($P = 0.007$).

Sensitivity analysis (in those with triglyceride <200 mg/dl, with insulin <75th centile of Framingham offspring study)

In participants without hypertriglyceridemia (triglyceride <200 mg/dl, $n = 88$), prevalence of coronary atherosclerosis was increased in HIV patients (54%) compared with controls (31%; $P = 0.04$); the number of segments with plaque was higher in HIV patients [1 (0–3)] compared with controls [0 (0–1)] ($P = 0.03$); and plaque volume was higher in HIV patients [36 (0–166) μl] compared with controls [0 (0–50) μl] ($P = 0.02$). In participants without hyperinsulinemia (fasting insulin ≤11.9 μU/ml, $n = 98$), prevalence of coronary atherosclerosis was increased in HIV patients (59%) compared with controls (33%) ($P = 0.02$); the number of segments with plaque was higher in HIV patients [1 (0–3)] compared with controls [0 (0–1)] ($P = 0.03$); and plaque volume was higher in HIV patients [42 (0–197) μl] compared with controls [0 (0–64) μl] ($P = 0.02$).

Discussion

The current study shows an increased prevalence and greater degree of subclinical CAD in asymptomatic young HIV-infected men without prior history of cardiovascular disease. The prevalence in the HIV-infected patients was significantly greater than that seen in concurrently recruited HIV-seronegative men with similar demographics, Framingham risk scores, and similar traditional risk factors (age, sex, family history, BMI, smoking, total cholesterol, and LDL).

Table 2. Cardiac computed tomography and coronary computed tomography angiography parameters.

	Controls ($n = 32$)	HIV ($n = 78$)	P value
Presence of coronary plaque (%)	34.4	59.0	0.02
Agatston calcium score, median (IQR); mean ± SD	0 (0–4.5); 21.6 ± 64.1	0 (0–20.7); 37.4 ± 93.3	0.08
Agatston calcium score >0 (%)	25.0	46.2	0.04
Segments with plaque, n, median (IQR); mean ± SD	0 (0–1); 1.2 ± 2.2	1 (0–3); 2.2 ± 2.7	0.03
Plaque volume, μl, median (IQR); mean ± SD	0 (0–81); 85 ± 193	56 (0–208); 173 ± 250	0.02
Participants found to have coronary stenosis >70% (%)	0	6.5%	0.06

P value by Wilcoxon rank-sum test. IQR, interquartile range.

Table 3. Characteristics of HIV patients with evidence of coronary atherosclerosis.

	No plaque ($n=32$)	Plaque present ($n=46$)	P value
Demographics			
Age (years)	43.5 ± 6.8	48.6 ± 5.4	0.0004
Race (%)			0.03
White	59	73	
Black	13	22	
Asian	3	0	
Hispanic	16	4	
Native American	9	0	
Family history of premature CHD by NCEP (%)	10	25	0.09
Framingham risk score	5.6 ± 5.4	9.2 ± 4.2	0.002
Hypertension (%)	19	35	0.14
Diabetes mellitus (%)	6	11	0.47
Current smoker (%)	44	29	0.18
Any past cocaine use (%)	75	70	0.60
HIV disease-related parameters			
Duration since HIV diagnosis (years)	11.6 ± 6.4	14.9 ± 5.5	0.02
Ever on antiretroviral therapy (%)	90.6	97.8	0.16
Duration of antiretroviral therapy (years)	6.2 ± 4.5	7.9 ± 4.7	0.19
Current PI treatment (%)	53	52	0.93
Duration of PI treatment (years)	2.9 ± 4.3	4.4 ± 4.1	0.17
Current NRTI treatment (%)	88	93	0.37
Duration of NRTI use (years)	6.1 ± 4.3	7.4 ± 4.6	0.28
Current NNRTI treatment (%)	44	52	0.46
Duration of NNRTI treatment (years)	2.4 ± 3.1	2.7 ± 3.8	0.78
$CD4^+$ T lymphocytes (cells/μl) (current)	577 ± 257	486 ± 294	0.16
$CD4^+$ nadir (cells/μl)	235 ± 208	171 ± 144	0.17
$CD4^+/CD8^+$ T-lymphocyte ratio	0.82 ± 0.46	0.54 ± 0.27	0.001
HIV RNA viral load (copies/ml)	<50 (<50, <50)	<50 (<50, <50)	0.99
Undetectable HIV RNA <50 copies/ml (%)	81	81	0.98
Anthropometric and fat depot parameters			
BMI (kg/m^2)	25.9 ± 0.8	26.3 ± 0.6	0.70
VAT area (cm^2)	148.7 ± 117.2	188.7 ± 123.0	0.16
SAT area (cm^2)	170.9 ± 110.5	164.2 ± 89.9	0.77
Metabolic parameters			
Serum creatinine (mg/dl)	1.02 ± 0.14	1.08 ± 0.21	0.16
Fasting glucose, mmol/l (mg/dl)	5.2 ± 0.7 (93.7 ± 11.9)	5.2 ± 0.6 (93.9 ± 10.3)	0.93
2-h glucose, mmol/l (mg/dl)	6.5 ± 2.6 (116.9 ± 46.2)	7.0 ± 2.6 (126.8 ± 47.1)	0.36
Fasting insulin (μU/ml)	8.9 ± 10.6	7.7 ± 6.6	0.55
2-h insulin (μU/ml)	56.5 ± 63.8	48.3 ± 53.6	0.54
Lipid panel			
Total cholesterol, mmol/l (mg/dl)	4.3 ± 1.0 (167.3 ± 38.9)	5.0 ± 1.1 (193.0 ± 44.3)	0.01
HDL cholesterol, mmol/l (mg/dl)	1.2 ± 0.4 (47.2 ± 14.7)	1.2 ± 0.4 (48.2 ± 13.9)	0.74
LDL cholesterol, mmol/l (mg/dl)	2.4 ± 0.8 (92.6 ± 31.7)	2.8 ± 0.8 (107.9 ± 31.5)	0.046
Triglycerides, mmol/l (mg/dl)	1.4 ± 0.8 (127.6 ± 75.2)	2.2 ± 1.9 (194.8 ± 169.7)	0.04
Inflammatory and CMV-related parameters			
C-reactive protein, median (IQR; mg/l)	1.8 (0.6–5.0)	1.6 (0.7–3.4)	0.56
Interleukin-6, median (IQR; pg/ml)	2.1 (1.7–3.3)	2.7 (1.6–4.0)	0.57
Cytomegalovirus IgG seropositivity (%)	88	94	0.37
Cytomegalovirus IgG titers, median (IQR)	86.5 (41.3–132.0)	113.0 (76.0–243.3)	0.05

CHD, coronary heart disease; CMV, Cytomegalovirus; HDL, high-density lipoprotein; IgG, immunoglobulin G; IQR, interquartile range; LDL, low-density lipoprotein; NCEP, National Cholesterol Education Program; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside/nucleotide reverse transcriptase inhibitors; PI, protease inhibitor; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue.

Surprisingly and of important clinical relevance, even among asymptomatic young HIV-infected men, 6.5% had evidence of severe CAD defined as coronary artery stenosis more than 70% as compared with 0% in the control group. The information obtained from the coronary angiography led to further work-up confirming coronary disease and cardiac procedures that may have prevented major adverse cardiac events. The fact that 6.5% of the HIV patients, all without any known cardiac disease or symptoms, were found to have severe obstructive CAD is of potential clinical significance. In contrast, none of the controls had severe obstructive CAD.

We found increased prevalence of atherosclerosis and increased plaque burden by indices of plaque volume and number of coronary segments affected in the HIV vs. non-HIV participants. Prior studies to assess atherosclerotic vascular disease in the HIV population have used brachial artery flow-mediated vasodilation, carotid intima–media thickness (IMT), and electron beam computed tomography (EBCT) calcium score without CT coronary angiography [11–21]. Despite evidence from data registry studies showing a greater prevalence of MIs in HIV vs. non-HIV-infected patients [22], studies using surrogate markers show mixed results, though

Table 4. Univariate relationships with coronary computed tomography parameters in HIV patients.

	Segments of plaque		Plaque volume		Agatston calcium score	
	Rho	*P* value	Rho	*P* value	Rho	*P* value
Demographics						
Age	0.40	0.0004	0.30	0.008	0.34	0.002
Framingham risk score	0.43	0.0001	0.37	0.001	0.39	0.0005
HIV disease parameters						
Duration since HIV diagnosis	0.37	0.0009	0.35	0.002	0.34	0.003
Total duration of antiretroviral therapy	0.26	0.07	0.19	0.18	0.20	0.16
Duration of PI use	0.27	0.04	0.24	0.06	0.16	0.22
Duration of NRTI use	0.21	0.12	0.18	0.20	0.18	0.19
Duration of NNRTI use	−0.08	0.54	−0.08	0.54	−0.07	0.59
$CD4^+$ T-cell count	−0.14	0.22	−0.16	0.18	−0.11	0.34
Nadir $CD4^+$ T-cell count	−0.09	0.49	−0.06	0.66	−0.03	0.83
$CD8^+$ T-cell count	0.13	0.27	0.10	0.39	0.09	0.44
$CD4^+/CD8^+$ ratio	−0.25	0.03	−0.24	0.04	−0.19	0.10
HIV viral load	0.13	0.30	0.14	0.29	−0.05	0.70
Anthropometric parameters						
BMI	0.02	0.85	−0.04	0.75	−0.01	0.94
Visceral adipose tissue	0.19	0.10	0.13	0.28	0.23	0.04
Waist circumference	−0.01	0.94	−0.13	0.37	−0.05	0.73
Waist-to-hip ratio	0.03	0.81	−0.13	0.38	0.001	0.99
Lipid panel						
Total cholesterol	0.35	0.002	0.32	0.005	0.34	0.002
HDL cholesterol	−0.05	0.64	0.01	0.92	−0.02	0.89
LDL cholesterol	0.32	0.005	0.27	0.03	0.25	0.03
Triglycerides	0.21	0.07	0.20	0.08	0.28	0.01
Metabolic parameters						
Serum creatinine	0.13	0.26	0.22	0.06	0.16	0.16
Fasting glucose	0.03	0.82	−0.01	0.93	0.09	0.43
2-h glucose	0.16	0.17	0.11	0.33	0.22	0.05
Glucose AUC	0.20	0.07	0.18	0.12	0.26	0.02
Fasting insulin	0.10	0.39	0.09	0.43	0.13	0.27
2-h Insulin	0.06	0.61	−0.01	0.94	0.18	0.13
Insulin AUC	0.03	0.80	0.02	0.84	0.17	0.16
Hemoglobin A1C	0.04	0.74	0.03	0.77	0.06	0.62
Inflammatory and CMV-related parameters						
MCP-1	0.23	0.047	0.16	0.16	0.27	0.02
C-reactive protein	−0.02	0.88	−0.07	0.56	−0.02	0.87
Interleukin-6	0.17	0.14	0.16	0.18	0.11	0.34
Cytomegalovirus IgG antibody titer	0.25	0.03	0.22	0.06	0.22	0.05

AUC, area under the curve; CMV, Cytomegalovirus; IgG, immunoglobulin G; NNRTI, nonnucleoside reverse transcriptase inhibitors; NRTI, nucleoside/nucleotide reverse transcriptase inhibitors; PI, protease inhibitor.

studies of Hsue *et al*. [16] found carotid IMT to be higher in HIV patients than in age-matched controls and to progress at a more rapid rate than in noninfected controls. In a recent large study, carotid IMT was found to be higher in HIV patients from the study of Fat Redistribution and Metabolic Change in HIV (FRAM), compared with uninfected controls even after adjustment for traditional risk factors [23].

Previous studies utilizing CT to assess CAD in HIV patients assessed only coronary calcifications by coronary calcium scoring, but did not investigate noncalcified plaques nor visualized lumen caliber [18,20]. In a study by Kingsley *et al*. [21], HIV infection and long-term HAART use increased the likelihood of coronary calcium being present; however, after adjustment for traditional cardiovascular risk factors, HAART had no significant association with presence or extent of coronary calcium.

Although coronary calcification is specific for atherosclerotic lesions and the Agatston calcium score has been associated with cardiac event risk in non-HIV populations [24], atherosclerotic plaques can be present without detectable coronary calcium at the site. Coronary calcium score alone may not provide a true measure of early atherosclerosis in young HIV patients as plaque calcification usually occurs at a later stage and may not detect more vulnerable plaque lesions, which tend to be noncalcified or mixed calcified and noncalcified. In addition, the atherosclerotic disease process in HIV may be different from conventional atherosclerosis [25]. No published studies to date have used coronary CTA to assess noncalcified plaques in HIV patients. Our data using CTA show that a significant proportion of patients with coronary atherosclerosis would have been missed if calcium score was used as the sole criterion for coronary atherosclerosis as 23.8% (95% confidence interval 13.5–38.5%) of HIV patients had evidence of noncalcified

plaque seen on CTA among those with calcium score of zero. Moreover, the differences between the HIV and control groups were more consistent comparing indices of plaque that included noncalcified plaques beyond calcium score alone (e.g. presence of any plaque, segments with plaque, and plaque volume, all $P < 0.05$). Thus, our data suggest that CTA gives different and potentially more sensitive information than the coronary calcium score alone.

Participants in our study were screened to exclude patients with known ischemic heart disease or any symptoms of CAD, and were, on average, young, with an estimated 10-year Framingham risk of only 4%, similar to that of the controls. Clinicians might not consider such patients at risk for CAD, yet 6.5% of these asymptomatic young patients had critical stenosis in at least one vessel. These patients with critical stenosis did, however, have the highest Framingham score and thus this score still appears to be useful to identify those with the greatest degree of subclinical coronary atherosclerosis, even among a population of young asymptomatic patients. Moreover, those HIV patients with plaque clearly had elevated traditional risk factors, including age, Framingham risk score, total cholesterol, and LDL.

Our data suggest relationships between traditional and nontraditional risk factors to indices of plaque burden. For example, traditional risk factors such as age, total cholesterol, and LDL were strongly related to all indices of plaque burden as is also evidenced in the strong relationship between the Framingham risk score and plaque burden. 2-h glucose and triglycerides were more strongly related to Agatston score than to plaque volume or segments with plaque.

Our data also support the hypothesis that there is a relationship between HIV infection and coronary artery atherosclerosis independent of traditional cardiovascular risk factors, as traditional risk factors were generally similar between the groups, yet significant differences in plaque prevalence and plaque burden were seen between the HIV and non-HIV groups. Age, smoking, family history and overall Framingham score, as well as LDL, HDL, and glucose parameters were similar between the groups. Triglyceride and insulin levels were higher among the HIV-infected group, but we performed sensitivity analyses excluding any patient in either group with hypertriglyceridemia and hyperinsulinemia, and found similar results. We also confirmed our results in regression analyses, controlling for traditional cardiovascular risk factors, as well as triglyceride and insulin levels.

Data from the Strategies for Management of Antiretroviral Therapy (SMART) study [26,27] suggest that inflammatory, immune, or viral factors might contribute to increased CAD in the HIV population. We found that CD4/CD8 cell ratio was negatively associated with plaque volume and the relationship between CD4/CD8 ratio and plaque volume was stronger than that seen with CD4 cell count or viral load with plaque volume. Atherosclerosis is an inflammatory disease in which T lymphocytes can play a role in atheroma development [28,29]. Activated T cells, including CD8 cells, are present in atherosclerotic plaques [30] and may contribute to atherosclerosis [31,32]. Hsue *et al.* [33] have demonstrated increased carotid IMT in HIV-infected individuals who are able to maintain undetectable HIV RNA without ART, a group with heightened T-lymphocyte activation.

MCP-1 was significantly associated with the number of segments with plaque and with calcium score in our study. MCP-1 is a chemokine important for monocyte migration into the vascular intima during the development of atherogenesis [34]. HIV-1 *Tat* protein promotes MCP-1 secretion, leading to increased transmigration of monocytes across endothelium [35]. Furthermore, HIV patients with the MCP-1 2518G allele were found to have an associated five-fold increased risk for atherosclerosis [36] and this same polymorphism has also been associated with severity of CAD in non-HIV patients [37].

In this study, we have also shown that CMV IgG antibody titers are positively associated with degree of coronary atherosclerosis, as measured by number of coronary segments with plaque and Agatston calcium score, suggesting that immune activation as well as CMV-specific immune response may also be contributory to atherosclerosis in HIV patients. CMV infection has been associated with cardiac transplant vasculopathy and also implicated in the pathogenesis of atherosclerosis in HIV [38–40].

We found a significant and robust relationship between measures of coronary atherosclerosis and duration of known HIV infection. We controlled for duration of antiretroviral treatment and traditional cardiovascular risk factors in regression modeling. The relationship between duration of HIV and indices of coronary atherosclerosis (number of coronary segments with plaque, plaque volume, and calcium score) remained significant controlling for age, traditional cardiovascular risk factors, duration of ART, and specific HIV-related markers of disease and immune function. Duration of HIV disease may, therefore, reflect a relevant integrated measure of chronic subacute inflammation and altered immune function, processes that may contribute to increased CAD beyond the risk due to traditional risk factors. CRP and IL-6 were not increased in our participants; however, the participants in this study were specifically selected to have no history of CAD and were not acutely ill, in contrast to other studies investigating CRP in relationship to rates of MIs between HIV and non-HIV-infected patients [41].

Taken together, our data suggest that both traditional and nontraditional HIV-specific risk factors contribute to increased CAD in young asymptomatic HIV-infected men.

The current study has limitations, including the cross-sectional design, from which causality cannot be inferred. Second, the study was performed in men only and thus results may not be generalizable to HIV-infected women. Our results also cannot be generalized to a more high-risk population of HIV-infected patients or to symptomatic patients, in whom traditional risk may dominate more and in whom an even greater prevalence of plaque and potentially calcified plaque might be seen. Based on the data in our study, further studies are now needed to investigate the clinical implications and clinical course of CAD in HIV-infected patients with subclinical coronary atherosclerosis.

Our findings suggest a high prevalence and significant degree of coronary atherosclerosis among young HIV-infected men with a long duration of HIV disease, without any symptoms of cardiac disease or a prior diagnosis of cardiac disease and with a generally low Framingham risk score. Participants were recruited with a goal to have a similar traditional cardiac risk profile in HIV and control participants, yet various measures of atherosclerosis, including unique indices of plaque volume and, the number of coronary segments of plaque were different between the groups. These data suggest that both traditional and nontraditional risk factors contribute to increase subclinical atherosclerosis in HIV-infected patients. The findings from the current study support the need for increased efforts and attention to reducing modifiable traditional and nontraditional cardiovascular risk factors in this patient population. Our data highlight the need to address cardiac risk reduction early on in the course of HIV disease, before significant subclinical disease accrues and before cardiac events occur.

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