Carotid Intima-Media Thickness Among Human Immunodeficiency Virus–Infected Patients Without Coronary Calcium

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Subjects infected with human immunodeficiency virus (HIV) have increased risk for atherosclerosis. Carotid artery intima-media thickness (IMT) assessed using ultrasound and coronary artery calcium (CAC) detected using computed tomography predict cardiovascular risk in the general population; however, their usefulness and comparability in patients with HIV are less well defined. The purpose of this study was to compare IMT and CAC in the detection of atherosclerosis in subjects with HIV. CAC and IMT were measured in 253 HIV-infected and 58 uninfected adults. Associations among HIV-related factors, traditional risk factors, and CAC and IMT were evaluated. The distribution of IMT among subjects with and without CAC was compared. Among the patients with HIV, 37% had detectable CAC compared to 28% of controls ($p = 0.19$); 16% of the patients with HIV had CAC >100 compared to 5% of controls ($p = 0.03$). With either detectable or undetectable CAC, HIV-infected subjects had higher IMT compared to controls (1.02 ± 0.34 vs 0.78 ± 0.12 mm, $p < 0.0001$), even after adjustment for traditional risk factors. Among those with undetectable CAC, 34% of patients with HIV had markedly increased IMT (≥1 mm) compared to no controls ($p < 0.0001$). HIV-related factors were associated with IMT but not with CAC. In conclusion, patients with HIV and controls had similar rates of detectable CAC, while absolute CAC scores were modestly higher in the HIV group. Conversely, carotid IMT detected advanced subclinical atherosclerosis in patients with HIV even in the absence of CAC. Thus, with HIV, IMT is associated with disease-related factors and may be a more sensitive indicator of subclinical atherosclerosis than CAC. **(Am J Cardiol 2012;109:742–747)**

Coronary artery calcium (CAC) is an accepted measure of atherosclerosis burden and a robust predictor of clinical outcomes in the general population.[1–3] The prevalence of CAC is also higher in patients with human immunodeficiency virus (HIV) than would be expected for their age.[4–6] Limited data are available comparing carotid artery intima-media thickness (IMT) and CAC in patients with HIV.[6] Therefore, we compared these 2 measurements prospectively in a large cohort of patients with HIV and in a control group of uninfected subjects. As previous studies have revealed that no CAC is detected in ≥1/2 of patients with HIV,[4–6] we were particularly interested in analyzing IMT measurements according to whether or not CAC was detected. In addition, we wished to determine the associations of HIV-related factors with CAC and with IMT.

Methods

Patients for the study were recruited from a clinic-based HIV cohort at San Francisco General Hospital (Study of the Consequences of the Protease Inhibitor Era [SCOPE]). Study participants were confirmed to be HIV infected using HIV antibody testing, letters of diagnosis, or medical records. Patients with histories of coronary heart disease (CHD) or atrial fibrillation were excluded. We recruited our control group through advertisements placed around the hospital and community. All control participants were HIV antibody negative. Study participants were selected independently of their cardiovascular risk factor profile. This study was approved by the University of California, San Francisco, Committee on Human Research, and all participants provided written informed consent.

We performed detailed interviews of all study participants, focusing on cardiovascular risk factors, past and present medication use, and illicit drug use. We calculated 10-year risk for CHD using the Framingham risk calculator.[7] For the patients with HIV, we recorded antiretroviral

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Table 1
Characteristics of patients infected with human immunodeficiency virus and controls

Characteristic	Patients With HIV (n = 253)	Controls (n = 58)
Age (years)	49 (43–53)	47.5 (42–55)
Men	225 (89%)	48 (83%)
Race		
European American	158 (63%)	36 (62%)
African American	55 (22%)	12 (21%)
Latino	25 (10%)	2 (3%)
Other	15 (6%)	8 (14%)
Hypertension	70 (28%)	12 (21%)
Diabetes mellitus	16 (6%)	1 (2%)
Low-density lipoprotein cholesterol (mg/dl)	108 (83–128)	113 (92–147)
High-density lipoprotein cholesterol (mg/dl)	43 (36–50)	48 (42–55)
Triglycerides (mg/dl)	144 (86–215)	107 (70–145)
Injection drug use		
Ever but not current	59 (23%)	5 (9%)
Current	11 (4.3%)	0 (0%)
Cigarette smoking (ever)	164 (65%)	32 (55%)
Hepatitis C	56 (22%)	1 (2%)
Duration of HIV infection (years)	15 (10–19)	—
Use of antiretroviral medication		—
Ever	193 (76%)	
Current	172 (68%)	
Duration of nucleoside reverse transcriptase inhibitor use (years)	6.1 (0–19)	—
Duration of non-nucleoside reverse transcriptase inhibitor use (years)	0 (0–15)	—
Duration of protease inhibitor use (years)	3.3 (0–13)	—
$CD4^+$ T cells/mm^3	471 (3–1960)	—
Nadir $CD4^+$ T cells/mm^3	185 (0–1200)	—
Plasma HIV ribonucleic acid copies/ml, % <75	146 (58%)	—

Data are expressed as median (interquartile range) or as number (percentage).

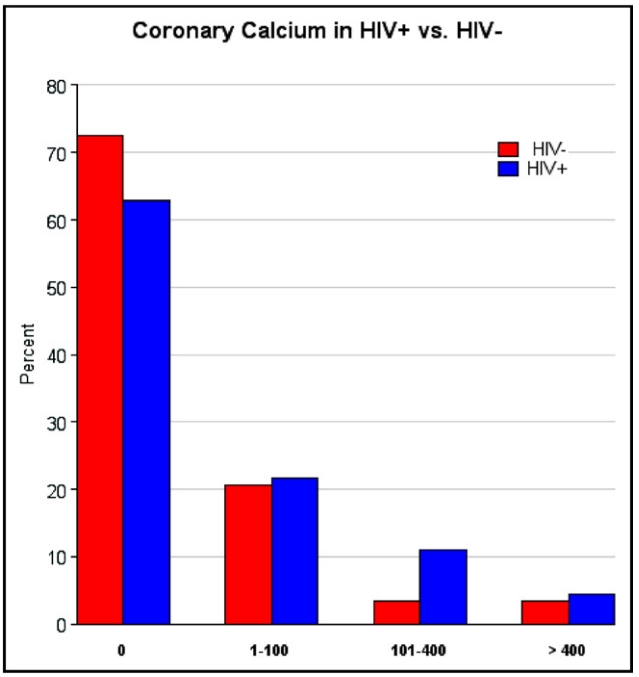

Figure 1. Frequency of CAC in patients with HIV and controls. CAC scores >0 were found in 37% of patients with HIV and in 28% of controls (p = 0.19). However, 16% of patients with HIV had CAC scores >100 compared to 5% of controls (p = 0.03). Finally, 13 patients with HIV (5%) had CAC scores >400.

therapy use by chart review, as well as history of opportunistic infections, CD4 counts, and HIV viral loads.

Blood was drawn in the fasting state and used to measure total cholesterol, high-density lipoprotein cholesterol, and triglycerides. Low-density lipoprotein cholesterol was calculated using Friedewald's formula[8] except when triglycerides were ≥400 mg/dl, in which case it was measured directly. $CD4^+$ T-cell counts were measured at the respective clinical laboratories associated with each of the SCOPE cohort clinic sites. The nadir $CD4^+$ T-cell count was the lowest laboratory-confirmed value before the date of computed tomography.

Multislice computed tomography to assess CAC score was performed using a 16-detector Philips MX8000 scanner (Philips Medical Systems, Andover, Massachusetts). Imaging used a slice thickness of 3 mm and electrocardiographic gating was used to trigger axial multislice scan acquisitions. Using this technique, a gantry rotation time of 420 ms results in a temporal resolution of 210 ms. CAC was measured using the Philips scoring software program and calculated as described by Agatston et al.[9] The sum of the scores for all arterial lesions was used to provide an overall score for each subject. The radiologists interpreting the scans were blinded with respect to the participants' HIV status.

We assessed carotid IMT using the GE Vivid 7 system (GE Healthcare, Milwaukee, Wisconsin) and a 10-MHz linear-array probe as described previously.[10] IMT was measured in a total of 12 segments in the near and far walls of the common carotid, bifurcation region, and internal carotid region according to the standardized protocol of the Atherosclerosis Risk in Communities (ARIC) study.[11–13] A single experienced technician who was blinded to the subjects' HIV status performed all the IMT studies and caliper measurements of the digital images. The CAC and carotid IMT studies were performed during the same time period.

Given 80% power and a 2-tailed α value of 0.05, the sample size required for this study was 159 patients with HIV and 40 controls, assuming a standard deviation of 20 and a difference in CAC score of 10. Because a major goal of our study was to compare predictors of CAC and IMT specifically in patients with HIV, we enrolled more of them compared to controls. Descriptive statistics including medians, interquartile ranges, and percentages were used to summarize all variables. We first compared characteristics of HIV-infected and controls using Mann-Whitney U tests for continuous variables and Pearson's chi-square tests for categorical variables. Mean IMT was compared using Student's *t* tests, and median values were compared using Mann-Whitney U statistics. Although only mean values are shown for IMT in the "Results" section, results obtained using Mann-Whitney U statistics were similar. All p values were 2 sided.

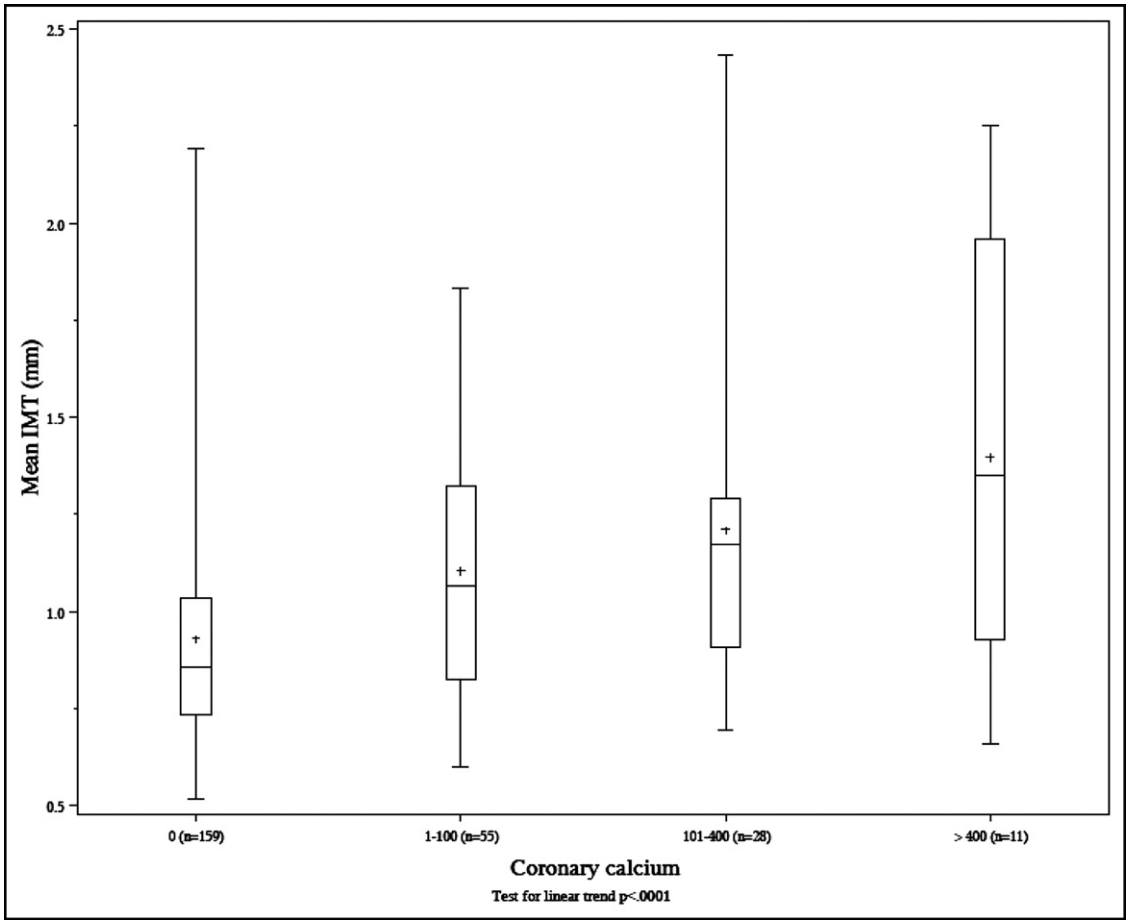

Figure 2. Comparison of carotid IMT among patients with HIV by CAC level. Box plot showing the distribution of carotid IMT per category of CAC among patients with HIV. The *asterisk* indicates the mean IMT value. The *middle bar* is the median value, the *upper and lower bars* are the first and third quintiles, and the *upper and lower whiskers* represent the minimum and maximum IMTs. There was a graded relation between CAC and IMT ($p < 0.0001$ test for trend); however, the IMT values for patients in each CAC category overlapped appreciably.

To assess the association of HIV status with any detectable CAC and with CAC >100, multiple logistic regression analyses adjusting for appropriate risk factors was used to estimate odds ratios and confidence intervals. Candidate covariates for a final model were determined by logistic regression with CAC (detectable or >100) as the response variable, HIV status as the explanatory variable, and each of the following risk factors 1 at a time: age, gender, race, antihypertensive medication, blood pressure, diabetes, ever smoked, years of cigarette smoking, family history of cardiovascular disease, use of lipid-lowering medication, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, C-reactive protein, Framingham risk score, body mass index, intravenous drug use, cocaine use, and methamphetamine use. Only risk factors significant in the smaller models ($p < 0.05$) were included in the final multiple logistic regression model. A separate multiple logistic regression of patients with HIV was carried out to assess the independent associations of antiretroviral use and other HIV-specific factors with detectable CAC. This analysis considered the aforementioned risk factors and HIV-specific risk factors. To evaluate the independent associations of HIV-specific risk factors and the increased prevalence of high levels of detectable CAC in HIV-infected subjects compared to controls (chi-square $p = 0.0042$), a separate but parallel multiple logistic regression analysis of high levels of detectable calcium (>100 vs ≤100) was carried out in HIV patients only.

Results

The clinical features of the 253 patients with HIV and 58 uninfected controls are listed in Table 1. The calculated 10-year Framingham CHD risk was low and similar in the 2 groups (HIV group: median 4%, interquartile range 2% to 6%; controls: median 3%, interquartile range 0.5% to 6%, $p = 0.09$). The median duration of HIV infection was 15 years, and most subjects were being actively treated with antiretroviral therapy.

CAC was detected in 63% of patients with HIV and 72% of controls ($p = 0.19$). Among those with detectable CAC, patients with HIV had higher CA scores compared to controls, as shown in Figure 1; for example, 40 of the patients with HIV (16%) compared to only 3 of the controls (5%) had CAC >100 ($p = 0.03$).

The mean IMT was higher in all patients with HIV (1.02 ± 0.34 mm) compared to all uninfected controls (0.78 ± 0.12

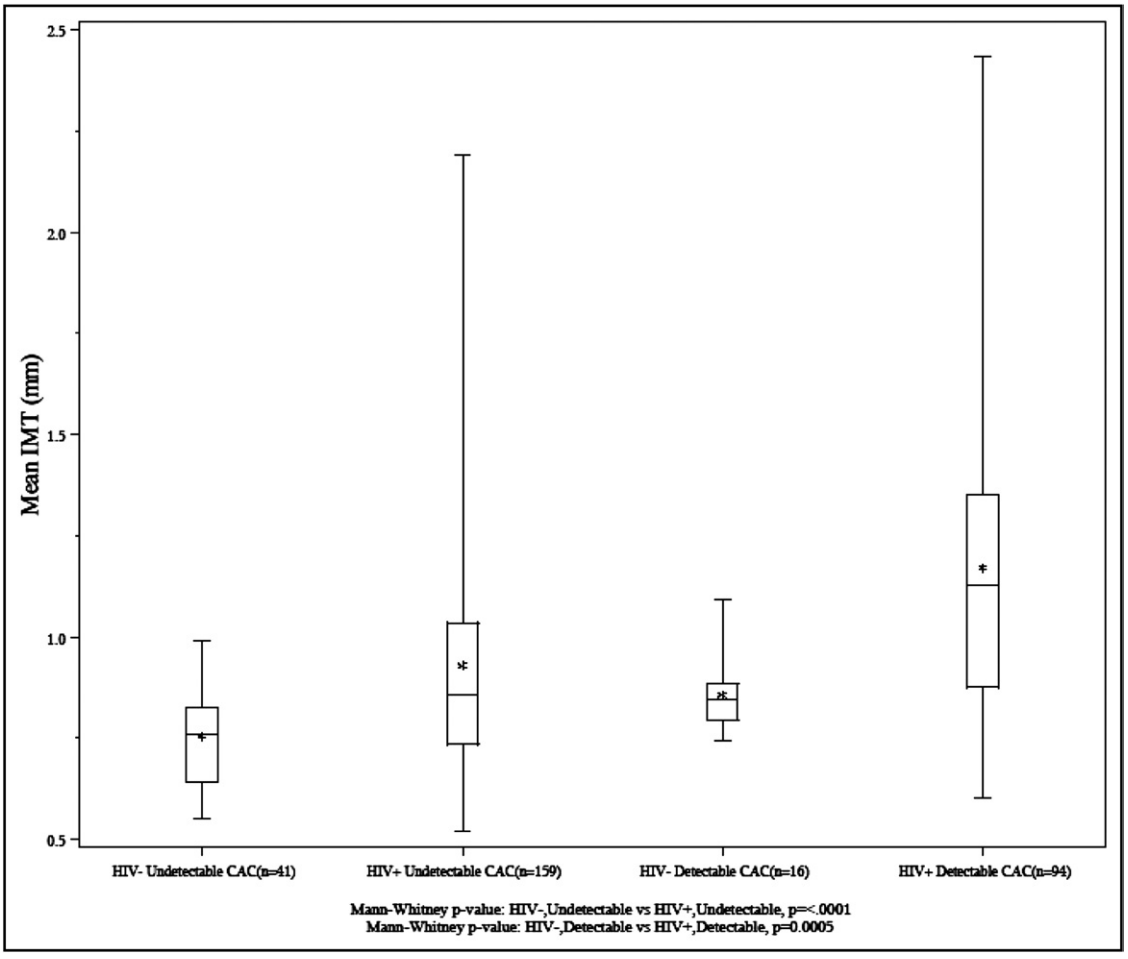

Figure 3. Carotid IMT levels in patients HIV and controls by CAC level. Mean IMT is indicated by the *asterisk*, median IMT is the *solid horizontal line*, the *upper and lower bars* are the first and third quintiles, and the *upper and lower whiskers* represent maximum and minimum IMT values. Among subjects with undetectable CAC, the mean IMT of the patients with HIV was higher than among controls ($p < 0.0001$). Among subjects with detectable CAC, the mean IMT was also higher among patients with HIV compared to controls ($p = 0.0005$).

mm, $p < 0.0001$), a difference that persisted even after adjusting for traditional risk factors and Framingham risk score ($p < 0.0001$). Among all subjects after adjustment for risk factors, HIV infection was independently associated with higher IMT, as was older age, male gender, and hypertension ($p < 0.05$ for all). Among the patients with HIV, older age, hypertension, and the HIV-related factors of lower nadir CD4 cell count and duration of antiretroviral therapy were associated with higher IMT ($p < 0.05$).

Among patients with HIV, those with detectable CAC had higher IMT compared to those with no detectable CAC (1.17 ± 0.38 vs 0.93 ± 0.28 mm, respectively, $p < 0.0001$). As shown in Figure 2, CAC and IMT were correlated ($p < 0.0001$ for trend).

Among subjects with no detectable CAC, carotid IMT was higher in patients with HIV compared to uninfected controls (0.93 ± 0.28 vs 0.75 ± 0.12 mm, $p < 0.0001$), as depicted in Figure 3. This difference persisted after adjusting for traditional risk factors ($p = 0.0004$). In the ARIC study, whose carotid imaging protocol we used, IMT ≥ 1 mm was predictive of a high incidence of CHD.[13] Among patients without detectable calcium, 54 patients with HIV (34%) and none of the controls met this threshold ($p < 0.0001$).

Among subjects with detectable calcium, IMT was higher in the 94 patients with HIV compared to the 16 controls (1.17 ± 0.38 vs 0.86 ± 0.09 mm, $p = 0.0005$), as shown in Figure 3. This difference also persisted after adjusting for traditional risk factors ($p < 0.0001$). For patients with detectable CAC, 28 (63%) of the patients with HIV had a mean IMT ≥ 1 mm, compared to 1 (6%) of controls ($p < 0.0001$).

Among all subjects in this study, older age and male gender were independently associated with detectable coronary calcium, while HIV infection was not ($p = 0.19$). Older age was the only variable associated with a CAC score >100, after adjusting for traditional risk factors. When the analysis was restricted to the patients with HIV, only older age (odds ratio 1.13, 95% confidence interval 1.09 to 1.12, $p < 0.0001$) was associated with detectable coronary calcium. Intravenous drug use, cocaine use, and methamphetamine use were not associated with detectable CAC in univariate analysis ($p > 0.05$ for all analyses).

Discussion

In this study, both CAC and IMT revealed more subclinical atherosclerosis in patients with HIV than in uninfected

controls. Although CAC was not present significantly more often in patients with HIV compared to controls, the CAC scores of patients with HIV were modestly but significantly higher. Among patients with HIV with detectable calcium, IMT and CAC scores were correlated (p for trend <0.0001). IMT was markedly higher in patients with HIV than in controls (1.02 ± 0.34 vs 0.78 ± 0.12 mm, $p < 0.0001$). To place this nearly 0.2-mm difference in context, it is similar to the difference observed between subjects with heterozygous familiar hypercholesterolemia and the general population in the era before statins.[14] This difference persisted after adjustment for traditional risk factors and was present for subjects with and those without detectable calcium. IMT, but not CAC, was higher in patients with HIV with a lower nadir CD4 cell count and a longer duration of antiretroviral therapy.

Arterial calcium deposition is a highly regulated process involving molecular determinants familiar from bony mineral formation, including matrix G1a protein, osteopontin, inorganic pyrophosphate, and osteoprotegerin.[15] Many diseases, including uremia, diabetes, osteoporosis, and hyperparathyroidism, stimulate arterial calcification, independently of atherosclerosis.[16] The extent of vascular calcification generally correlates with the extent of atherosclerosis but is also influenced by the specific underlying disease process. As a chronic inflammatory state with increased oxidative stress, HIV infection might be expected to promote calcification, leading to high CAC scores. In contrast, age is a very strong predictor of CAC score across many studies,[17] so the relatively younger patients with HIV might be expected to have no CAC or lower scores.

CAC scores in subjects with HIV have been reported in several cross-sectional studies.[5,6,18] A third to a half of patients were found to have detectable CAC, a high prevalence compared to the general population and notable given the relatively youthful age of these HIV cohorts. A study of 327 patients with HIV showed that high-sensitivity C-reactive protein was associated with CAC score in men, while in women, age and glucose were associated with CAC.[6] Although this study reported CAC and IMT, no uninfected controls were studied, and a detailed comparison and analysis of the 2 techniques was not the purpose of the study. Use of antiretroviral therapy was not associated with increased CAC in this study.

In the Multicenter AIDS Cohort Study, which included 947 HIV-infected male participants, increasing age was most strongly associated with the extent and prevalence of CAC, similar to our results.[5] After adjustment for traditional risk factors, highly active antiretroviral therapy exposure of >8 years was associated with a lower extent of CAC, the effect of which was more evident in nonusers of lipid-lowering medications. A study using computed tomographic angiography rather than CAC showed that patients with HIV had a higher prevalence of coronary atherosclerosis compared to uninfected controls.[19] Our study confirms the lack of an association detected between antiretroviral therapy and CAC as well as the importance of age.

The most important aspect of our study may be that a large subset of patients with HIV have no detectable CAC but have advanced subclinical atherosclerosis as assessed by IMT. A markedly increased IMT has been reported in patients with HIV in some studies.[10,20,21] We have previously reported thicker IMT and more rapid rates of IMT progression in patients with HIV compared to controls.[10] In the general population, IMT is a powerful predictor of cardiovascular outcomes[22] that improves coronary risk prediction beyond traditional risk factors.[13] For example, in the ARIC study, whose imaging protocol we used,[10] the hazard ratios for incident CHD with IMT ≥ 1 mm compared to <1 mm were approximately 5 in women and nearly 2 in men.[12] One third of the patients with HIV without detectable calcium in our cohort has IMTs above this threshold, compared to none in the control group. Whether IMT has similar predictive power in patients with HIV as it does in the general population has not yet been determined.

Our study had limitations. The control group was relatively small; however, the CAC and IMT findings in the control group are similar to what have been reported in other studies. The associations that we found do not prove causality. The significance of increased IMT in the absence of CAC in a large proportion of the patients with HIV has an uncertain prognostic importance. Will these patients have a high cardiovascular event rate, as suggested by their higher IMTs, or a low rate, as suggested by the absence of CAC? Further studies are needed to determine the best markers of CHD risk in patients with HIV.

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