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Carotid Intima-Media Thickness Among HIV-Infected Patients Without Coronary Calcium

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Abstract

HIV-infected subjects have increased risk for atherosclerosis. Both carotid artery intima-media thickness (IMT) assessed by ultrasound and coronary artery calcium (CAC) detected by computerized tomography predict cardiovascular risk in the general population; however, their usefulness and comparability in HIV patients is less well defined. The purpose of this study was to compare IMT and CAC in the detection of atherosclerosis in HIV subjects. We measured CAC and IMT in 253 HIV-infected and 58 uninfected adults. We evaluated associations between HIV-related factors, traditional risk factors and CAC and IMT. We compared the distribution of IMT among individuals with and without CAC. Among the HIV patients, 37% had detectable CAC versus 28% of controls ($p=0.19$); 16% of the HIV patients had CAC > 100 vs. 5% of controls ($p=0.03$). With either detectable or undetectable CAC, HIV-infected individuals had a 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 HIV patients 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, HIV patients 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 among HIV patients 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.

Keywords

atherosclerosis; carotid arteries; calcium; coronary risk; HIV

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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 HIV patients than would be expected for their age.⁴⁻⁶ Limited data is available comparing IMT and CAC in HIV patients.⁶ Therefore we compared these 2 measurements prospectively in a large cohort of HIV patients and in a control group of uninfected subjects. As prior studies have revealed that no CAC is detected in half or more of HIV patients,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 (SCOPE). Study participants were confirmed to be HIV-infected using HIV-antibody testing, letter of diagnosis, or medical records. Patients with history of 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 UCSF 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 usage and illicit drug use. We calculated 10-year risk of CHD using the Framingham risk calculator.⁷ For the HIV patients, we recorded antiretroviral therapy usage via 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, HDL-C, and triglycerides. LDL-C was calculated by Friedewald's formula⁸ except for triglycerides were 400mg/dL where 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 prior to the CT scan date.

Multislice CT to assess CAC score was performed using a 16-detector Philips MX8000 scanner. Imaging utilized a slice thickness of 3mm, and EKG gating was used to trigger axial multi-slice scan acquisitions. Using this technique, a gantry rotation time of 420 msec results in a temporal resolution of 210 msec. CAC was measured using the Philips scoring software program and calculated as described by Agatston.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 participant's HIV status.

We assessed the carotid IMT using the GE Vivid 7 system 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 two-tailed alpha of 0.05, the sample size required for this study was 159 HIV patients and 40 controls assuming a standard deviation of 20 and a difference in CAC score of 10. Since a major goal of our study was to compare predictors of CAC and IMT specifically among HIV patients, we enrolled more of them compared to controls.

Descriptive statistics including medians, inter-quartile ranges, and percentages, were used to summarize all variables. We first compared characteristics of HIV-infected and controls using the Mann Whitney U test for continuous variables and Pearson Chi-squared test for categorical variables. Mean IMT was compared using T-tests and median values were compared using Mann-Whitney statistics. While only mean values are shown for IMT in the results section, results obtained using Mann-Whitney statistics were similar. All p-values were 2 sided.

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 explanatory variable and each of the following risk factors one at a time: age, gender, race, antihypertensive medication, blood pressure, diabetes, ever smoked, years of cigarette smoking, family history of CVD, use of lipid lowering medication, LDL-C, HDL-C, C-reactive protein, Framingham risk score, body mass index (BMI), 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 HIV patients 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 among HIV patients only.

Results

The clinical features of the 253 HIV patients 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%, IQR 2-6%, controls: median 3%, IQR 0.5-6%, $p = 0.09$). 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 the HIV patients and 72% of controls ($p = 0.19$). Among those with detectable CAC, HIV patients had higher CA scores compared to controls, as shown in Figure 1; for example, 40 (16%) of the HIV patients compared to only 3 (5%) of the controls had CAC >100 ($p = 0.03$).

The mean IMT was higher among all HIV patients (1.02 ± 0.34 mm) compared to all uninfected controls (0.78 ± 0.12 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 HIV patients, 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 HIV patients, those with detectable CAC had a higher IMT thickness compared to those with no detectable CAC (1.17 ± 0.38 mm 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 HIV patients compared to uninfected controls, (0.93 ± 0.28 mm 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, an IMT ≥ 1 mm was predictive of a high incidence of CHD.¹³ Among patients without detectable calcium, 54 HIV patients (34%) and none of the controls met this threshold ($p<0.0001$). Among individuals with detectable CAC, 28 HIV patients (63%) had a mean IMT ≥ 1 mm compared to one (6%) control ($p<0.0001$).

Among subjects with detectable calcium, IMT was higher in the 94 HIV patients compared to the 16 controls (1.17 ± 0.38 mm 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, 63% of the HIV patients had a mean IMT ≥ 1 mm compared to 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 HIV patients, only older age (OR 1.13, 95% CI 1.09 - 1.12, $p<0.0001$) was associated with detectable coronary calcium. Intravenous drug use, cocaine use, or 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 HIV patients than in uninfected controls. Although CAC was not present significantly more often in HIV patients compared to controls, the CAC scores of HIV patients were modestly but significantly higher. Among HIV patients with detectable calcium, IMT and CAC scores were correlated (p for trend <0.0001). IMT was markedly higher in HIV patients than in controls (1.02 ± 0.34 versus 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 familial hypercholesterolemia and the general population in the era before statins.² This difference persisted after adjustment for traditional risk factors and was present both for subjects with and without detectable calcium. IMT, but not CAC, was higher in HIV patients 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 Gla protein, osteopontin, inorganic pyrophosphate and osteoprotegerin.¹⁴ Many diseases, including uremia, diabetes, osteoporosis, and hyperparathyroidism stimulate arterial calcification, independently of atherosclerosis.¹⁵ 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. On the other hand, age is a very strong predictor of CAC score across many studies,¹⁶ so that the relatively younger HIV patients might be expected to have no CAC or lower scores.

CAC scores in HIV subjects have been reported in several cross-sectional studies.^{5,6,17} 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 HIV patients showed that hs-CRP was associated with CAC score in men while in women age and glucose were associated with CAC.⁶ While this study reported both CAC and IMT, no uninfected controls were studied and a detailed comparison and analysis of the

two 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, HAART exposure of more than 8 years was associated with a lower extent of CAC, the effect of which was more evident among non-users of lipid-lowering medication. A study using CT angiography rather than CAC showed that HIV patients had a higher prevalence of coronary atherosclerosis compared to uninfected controls.³ 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 HIV patients have no detectable CAC but have advanced subclinical atherosclerosis as assessed by IMT. A markedly increased IMT has been reported in HIV patients in some studies.^{10,18,19} We have previously reported both thicker IMT and more rapid rates of IMT progression in HIV patients compared to controls.¹⁰ In the general population, IMT is a powerful predictor of cardiovascular outcomes²⁰ 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 an IMT ≥ 1 mm compared to <1 mm were approximately 5 in women and nearly 2 in men.¹² One third of the HIV patients without detectable calcium in our cohort has an IMT above this threshold, compared to none in the control group. Whether IMT has similar predictive power in HIV patients as it does in the general population has not yet been determined.

Our study has limitations. The control group is 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 a thickened IMT in the absence of CAC in a large proportion of the HIV patients has an uncertain prognostic import. Will these patients have a high cardiovascular event rate as suggested by their thickened IMT, or a low rate, as suggested by the absence of CAC? Further studies are needed to determine the best markers of CHD risk in HIV patients.

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Figure 1. Frequency of CAC in HIV Patients and Controls

A CAC score >0 was found in 37% of HIV patients and in 28% of controls ($p=0.19$). However, 16% of HIV patients had a CAC score >100 compared to 5% of controls ($p=0.03$). Finally, 13 HIV patients (5%) had a CAC score >400.

Figure 2. Comparison of Carotid IMT Levels Among HIV-Infected Individuals by CAC Levels
 Box plot showing the distribution of carotid IMT per category of CAC among HIV patients. The asterisk is the mean IMT value. The middle bar is the median value, the upper and lower bars are the 1st and 3rd quintiles, and the upper and lower whiskers represent the minimum and maximum IMT. There is a graded relationship between CAC and IMT ($p < 0.0001$ test for trend); however, the IMT values for patients in each CAC category overlap appreciably.

Figure 3. Carotid IMT levels in HIV-Infected Individuals and Uninfected Controls by CAC Levels

Mean IMT depicted using the asterisk, median IMT is the solid horizontal line, the upper and lower bars are the 1st and 3rd quintiles, and the upper and lower whickers represent maximum and minimum IMT values. Among individuals with undetectable CAC, the mean IMT of the HIV patients is higher than controls ($p < 0.0001$). Among individuals with detectable CAC, the mean IMT is also higher among HIV patients compared to controls ($p = 0.0005$).

Table 1

Characteristics of Human Immunodeficiency Virus-Infected Patients and Controls

Characteristic	HIV-Infected (N=253)	Controls (N=58)
Age (years)	49 (43-53)	47.5 (42-55)
Male	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 (0.0%)
Cigarette smoking (ever)	164 (65%)	32 (55%)
Hepatitis C	56 (22%)	1 (2%)
Duration of HIV infection (years)	15 (10 to 19)	-
Use of antiretroviral medication		-
Ever	193 (76%)	
Current	172 (68%)	
Duration of NRTI [*] use (years)	6.1 (0-19)	-
Duration of NNRTI [*] use (years)	0.0 (0-15)	-
Duration of PI [*] 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 RNA copies/ml, % < 75	146 (58%)	-

Values are expressed as number (%) or median (interquartile range)

* Denotes nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase, protease inhibitor, respectively