Human Immunodeficiency Virus 1 Infection, Cocaine, and Coronary Calcification

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Background: Although cocaine use and human immunodeficiency virus (HIV) infection have been linked with clinical cardiovascular disease, the effects of cocaine use and HIV infection, especially the combination of the 2, on subclinical disease have rarely been reported. The objective of this study was to evaluate whether cocaine use alone, HIV infection alone, or a combination of the 2 is associated with coronary calcification, a marker of subclinical atherosclerosis.

Methods: Between May 20, 2000, and March 31, 2003, 224 black study participants from Baltimore were enrolled in an observational study of subclinical atherosclerosis as related to HIV and cocaine use. Interviews about sociodemographic characteristics and drug use behaviors, clinical examinations, echocardiographic examinations, lipid profiles, high-sensitivity C-reactive protein tests, and computed tomographic scans for coronary calcification were performed. Although the overall investigation is a cohort study, the data presented herein are cross sectional only.

Results: The highest proportion (37.6%) of presence of coronary calcification was in the HIV-positive and cocaine-positive group, followed by 29.8% in the HIV-negative and cocaine-positive group, 28.6% in the HIV-positive and cocaine-negative group, and 18.8% in the HIV-negative and cocaine-negative group. Univariate analysis showed that HIV, cocaine use, and both were associated with a higher number of lesions, calcified area, volume, and calcium score. In multiple regression analysis with adjustment for age, body mass index, low-density lipoprotein cholesterol level, triglyceride level, mean corpuscular volume, and systolic blood pressure, HIV, cocaine use, and both were independently associated with coronary calcification.

Conclusion: These results suggest that HIV infection alone, cocaine use alone, or the 2 combined may contribute to early subclinical atherosclerotic cardiovascular disease.

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OCAINE ABUSE IS AN IMPORTANT public health problem in the United States. As it has become widespread, the number of cocaine-related cardiovascular events, including angina pectoris, myocardial infarction, cardiomyopathy, and sudden death from cardiac causes, has increased dramatically.1,2 In vitro studies have shown that cocaine causes structural defects in the endothelial-cell barrier, thereby increasing its permeability to low-density lipoprotein (LDL) and escalating the expression of endothelial adhesion molecules and leukocyte migration—effects associated with the progression of atherosclerosis.3,4 Most studies of the impact of cocaine on cardiovascular complications have focused on clinical diseases; to our knowledge, the impact of cocaine use on subclinical coronary artery disease has not been thoroughly investigated.

Despite recognition that human immunodeficiency virus (HIV) infection is an important causal factor for developing cardiovascular complications, such as dilated cardiomyopathy and pericardial effusion,5 most investigations have focused on clinical disease. Virtually no reported studies have addressed the combined effects of HIV infection and cocaine use on subclinical atherosclerosis.

The objective of this study was to examine the association of HIV infection alone, cocaine use alone, and both with coronary artery calcification, a marker of subclinical atherosclerosis,6,7 using only baseline cross-sectional data from a cohort of participants aged 25 to 45 years.

STUDY SUBJECTS

Between May 20, 2000, and March 31, 2003, 224 black study participants from Baltimore were enrolled in an observational study investigating the effects of HIV infection and cocaine use on subclinical atherosclerosis. The goals of the overall study were to (1) describe prospectively the cause
and natural history of atherosclerosis and the ability of noninvasive tools to measure atherosclerotic burden in black men and women with HIV infection who abuse cocaine and (2) investigate whether HIV infection, cocaine abuse, and protease inhibitor (PI) treatment accelerate atherosclerosis. The study enrolled black men and women with HIV infection and without cardiovascular diseases, including PI users and non-PI users, and black men and women without HIV infection or cardiovascular diseases. Interviews about sociodemographic characteristics and drug use behaviors, clinical examinations, electrocardiograms, echocardiographic examinations, lipid profiles, high-sensitivity C-reactive protein tests, and spiral computed tomographic (CT) scans for coronary calcium (CAC) were performed. The HIV-positive (HIV+) cocaine users were recruited from the AIDS Links to the Intravenous Experience study, which is an ongoing prospective study of the natural history of HIV infection among injection drug users in Baltimore. Those who were HIV+ and cocaine negative (cocaine−) (ie, did not use cocaine) were recruited from the Sexually Transmitted Disease Clinic at The Johns Hopkins Hospital and clinics. Those who were HIV− and cocaine positive (cocaine+) (ie, did use cocaine) and those who were HIV− and cocaine− were recruited from the eastern part of Baltimore, where the AIDS Links to the Intravenous Experience study participants reside.

Inclusion criteria were age between 25 and 45 years and black race. Exclusion criteria were as follows: (1) any evidence of hypertension or ischemic heart disease, based on clinical history, previous hospitalization for myocardial infarction, angina pectoris, or electrocardiographic and/or echocardiographic evidence of previous myocardial damage by ischemic heart disease; (2) any symptoms believed to be related to cardiovascular disease; and (3) pregnancy. Information about sociodemographic characteristics, medical history, medication use, and cocaine use behaviors was obtained by interviewer-administered questionnaires. The Committee on Human Research at The Johns Hopkins University Bloomberg School of Public Health approved the study protocol, and all study participants provided written informed consent. All procedures used in this study were in accordance with institutional guidelines. Although the overall investigation is a cohort study, the data presented herein are cross sectional only.

MEASUREMENT OF LIPIDS
Venous blood samples were obtained after an overnight fast from a large antecubital vein. Serum was separated by centrifugation (2000g for 15 minutes at 4°C) and stored at −75°C until assayed. Serum lipid variables, including total cholesterol, triglycerides, high-density lipoprotein (HDLC) cholesterol, and LDL cholesterol levels, were directly determined with an analyzer (Hitachi 747 analyzer; Roche, Englewood, NJ).

BLOOD PRESSURE MEASUREMENT
Systolic blood pressure (SBP) and diastolic sitting BP were measured twice with a standard mercury sphygmomanometer. A nurse at the clinic measured the study participant’s arm circumference and applied a correctly sized cuff. The participant sat quietly for 5 minutes, and then the nurse obtained the SBP and diastolic BP measurements; a second measurement was made 3 minutes later. The average of the 2 readings was reported.

PROSPECTIVE ELECTROCARDIOGRAPHIC-GATED CARDIAC CT SCAN PROTOCOL
Scanning was performed with a zoom scanner (Siemens Somatom Plus 4 Volume Zoom Multislice; Siemens AG, Forchheim, Germany). Participants underwent scanning in the nonspiral 240° partial sequence scan mode, slice thickness was 2.5 mm, tube current was 50 mA, and tube voltage was 140 kV with electrocardiographic triggering. Images were acquired by a single breathholding technique on full inspiration, with an average breathholding of 20 to 30 seconds. Each scan took 0.36 second, and the cycle time was 1.3 seconds. Four 2.5-mm-thick slices were taken by each scan. On average, a total of 12 scans—equal to 48 slices—were obtained for each patient. The scans started just below the carina, and the entire coronary tree was imaged. Scoring was by the method of Agatston and colleagues, with a threshold of 130 Hounsfield units. The lesion score was calculated by multiplying the lesion area by a density factor derived from the maximal Hounsfield unit within this area, as originally described by Agatston and colleagues for electron beam CT scanning.3,10 Numbers of lesions, mean calcium scores, peak calcium scores, the areas affected, and volumes were calculated for the left main artery, left anterior descending coronary artery, left circumflex artery, and right coronary artery. The total calcium score, total number of lesions, mean score, peak value, total affected area, and total volume score were determined by summing individual scores from each of 4 anatomical sites in all 48 slices. A radiologist from the Department of Radiology at The Johns Hopkins Hospital (E.K.F), who has extensive experience in cardiac anatomical features and CAC scanning, scored the spiral CT scans. He was blinded with regard to the HIV/risk factor status of the study participants examined.

STATISTICAL ANALYSIS
Statistical analysis was performed with SAS statistical software.31 All continuous variables were described by means and standard deviations, and all categorical variables were summarized as proportions. Because CAC scores were not normally distributed, log, transformations were taken for all CT variables as follows: for values of 1 or less, the loge-transformed values were zeroes; and for values greater than 1, the loge-transformed values were log, (actual values). To compare between-group differences in CT characteristics and other factors, we used the analysis of variance and t test for continuous variables and the χ2 test (or Fisher exact test if necessary) for categorical variables. Multiple regression analysis was performed to investigate the effects of HIV infection, cocaine use, and both on CT variables. Three indicator variables were created for the regression analysis: HIV+ and cocaine−, HIV− and cocaine+, and HIV+ and cocaine+ (HIV− and cocaine− was the reference group). Because age, body mass index (BMI), SBP, LDL cholesterol level, triglyceride level, and mean corpuscular volume were significantly different among the 4 study groups (HIV− and cocaine−, HIV− and cocaine+, HIV− and cocaine+, and HIV+ and cocaine), and because these factors were also associated with CT variables according to univariate analyses, these factors were treated as potential confounding factors to be controlled for in a multiple regression model. Other variables, including sex, HDL cholesterol level, and cigarette smoking, did not enter the multiple regression model because they were either not associated with CT variables or not different among the 4 study groups. All P values reported are 2-sided.

RESULTS
GENERAL CHARACTERISTICS
Of the 224 participants in this study, 153 (68.3%) were cocaine users and 124 (55.4%) were infected with HIV. Their mean ± SD age was 38.1 ± 5.2 years. General char-
Among the 192 participants who completed CT examinations, 60 (31.2%) had detectable calcium (total calcium score, >0 [presence of coronary calcification]). Six (18.8%) of 32 participants in the HIV− and cocaine− group, 8 (28.6%) of 28 in the HIV+ and cocaine− group, 14 (29.8%) of 47 in the HIV− and cocaine+ group, and 32 (37.6%) of 85 in the HIV+ and cocaine+ group had detectable calcium (Figure 1). The proportions of presence of coronary calcification in the HIV+ and cocaine− group (P=0.37) and the HIV− and cocaine+ groups (P=0.27) were not significantly higher than that in the HIV− and cocaine− group, but the proportion of presence of coronary calcification in the HIV+ and cocaine+ group was marginally significantly higher than that in the HIV− and cocaine− group (P=0.05).

CRUDE ASSOCIATIONS OF HIV, COCAINE, AND BOTH WITH CORONARY CALCIFICATION

As shown in Table 2, among nonusers of cocaine, the differences between HIV+ and HIV− in several CT characterisitics of the study participants by HIV and cocaine use status are presented in Table 1.

There were significant differences in age (P<.001), cigarette smoking (P<.001), BMI (P<.001), HDL cholesterol level (P=0.008), LDL cholesterol level (P=.02), and total cholesterol level (P=.01) among the 4 HIV/cocaine groups. Those who were HIV− and cocaine− were younger, smoked less, used less alcohol, and had lower levels of triglycerides than did other groups. Nevertheless, those who were HIV− and cocaine+ had a higher BMI and higher levels of LDL and total cholesterol than did other groups.

Of the 224 participants in this study, 192 (85.7%) completed CT examinations and interviewer-administered questionnaires by the end of July 30, 2003, and were included for analysis. Among these 192 participants, 68.8% were cocaine users. There were no statistically significant differences between those who underwent the CT examinations and the 32 persons who did not in terms of age, sex, BMI, SBP, diastolic BP, cigarette smoking status, alcohol use, and HDL, LDL, and total cholesterol level (P>0.05 for all). Overall, 129 (67.2%) of these 192 participants were male, and among 113 (58.9%) who were HIV+, 64 had been taking PIs for at least 3 months, whereas 49 had never taken PIs.

PRESENCE OF CORONARY ARTERY CALCIFICATION IN RELATION TO HIV, COCAINE, AND BOTH
The results of multiple regression analyses are presented in Table 3. Compared with those who were HIV− and cocaine−, those who were HIV+ and cocaine− had significantly more lesions, a larger total calcified area, a higher total volume, and a higher total calcium score; those who were HIV− and cocaine+ had significantly more lesions, a larger total calcified area, a higher total volume, and a higher total calcium score, after controlling for age, BMI, LDL cholesterol level, triglyceride level, mean corpuscular volume, and SBP. After controlling for these same covariates, those who were HIV+ and cocaine+ had a significantly higher total number of lesions, total calcified area, total volume, and total calcium score than did those who were HIV− and cocaine−. Thus, compared with the total volume in those who were HIV− and cocaine−, the total volume in those who were HIV+ and cocaine+ was higher on average by a factor of 1.23-5.42; and in those who were HIV+ and cocaine+, higher by a factor of 3.21 (95% CI, 1.03-6.57); in those who were HIV− and cocaine+, higher by a factor of 3.49 (95% CI, 1.66-7.34). Compared with the total calcium score in those who were HIV− and cocaine−, the total calcium score in those who were HIV+ and cocaine+ was higher by a factor of 2.92 (95% confidence interval [CI], 1.15-7.41); in those who were HIV− and cocaine+, higher by a factor of 2.59 (95% CI, 1.23-5.42); and in those who were HIV+ and cocaine+, higher by a factor of 3.21 (95% CI, 1.53-6.71). Because approximately 57% of HIV+ participants were using PIs and PIs may be associated with accelerated atherosclerosis,12,13 the HIV− and cocaine− group was also compared with the HIV+ and cocaine− group in which those who used PIs were excluded. The difference between the HIV− and cocaine− group and the HIV+ co-

<table>
<thead>
<tr>
<th>Calcium Score Variable</th>
<th>Cocaine− and HIV− (n = 32)</th>
<th>Cocaine− and HIV+ (n = 28)</th>
<th>Cocaine+ and HIV− (n = 47)</th>
<th>Cocaine+ and HIV+ (n = 85)</th>
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<tbody>
<tr>
<td>Total Values</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No. of lesions</td>
<td>0.11 ± 0.36</td>
<td>0.48 ± 0.89†</td>
<td>0.23 ± 0.63</td>
<td>0.26 ± 0.54</td>
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<tr>
<td>Calcified area, mm²</td>
<td>0.08 ± 0.22</td>
<td>0.54 ± 1.20‡</td>
<td>0.55 ± 1.33†</td>
<td>0.50 ± 0.97§</td>
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<tr>
<td>Volume, mm³</td>
<td>0.22 ± 0.53</td>
<td>0.75 ± 1.54‡</td>
<td>0.78 ± 1.65扬尘</td>
<td>0.82 ± 1.37扬尘</td>
</tr>
<tr>
<td>Score</td>
<td>0.08 ± 0.21</td>
<td>0.55 ± 1.34扬尘</td>
<td>0.69 ± 1.66扬尘</td>
<td>0.69 ± 1.35扬尘</td>
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<tr>
<td>Values in the LM Artery</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. of lesions</td>
<td>0.00 ± 0.00</td>
<td>0.05 ± 0.19</td>
<td>0.22 ± 1.07扬尘</td>
<td>0.11 ± 0.42</td>
</tr>
<tr>
<td>Calcified area, mm²</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.22</td>
<td>0.32 ± 1.08扬尘</td>
<td>0.15 ± 0.64扬尘</td>
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<tr>
<td>Volume, mm³</td>
<td>0.00 ± 0.00</td>
<td>0.12 ± 0.43</td>
<td>0.42 ± 1.34扬尘</td>
<td>0.22 ± 0.85扬尘</td>
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<tr>
<td>Score</td>
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<td>0.04 ± 0.19</td>
<td>0.41 ± 1.39扬尘</td>
<td>0.19 ± 0.84扬尘</td>
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<tr>
<td>Values in the RCA</td>
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<td></td>
<td></td>
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<tr>
<td>No. of lesions</td>
<td>0.02 ± 0.12</td>
<td>0.03 ± 0.13</td>
<td>0.04 ± 0.28</td>
<td>0.02 ± 0.13</td>
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<tr>
<td>Calcified area, mm²</td>
<td>0.00 ± 0.00</td>
<td>0.08 ± 0.32</td>
<td>0.07 ± 0.46</td>
<td>0.05 ± 0.27</td>
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<tr>
<td>Volume, mm³</td>
<td>0.03 ± 0.14</td>
<td>0.15 ± 0.54</td>
<td>0.11 ± 0.61</td>
<td>0.09 ± 0.44</td>
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<tr>
<td>Score</td>
<td>0.00 ± 0.00</td>
<td>0.10 ± 0.49</td>
<td>0.07 ± 0.49</td>
<td>0.06 ± 0.40</td>
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<td>Values in the LAD Artery</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No. of lesions</td>
<td>0.02 ± 0.12</td>
<td>0.18 ± 0.51</td>
<td>0.07 ± 0.30</td>
<td>0.09 ± 0.28</td>
</tr>
<tr>
<td>Calcified area, mm²</td>
<td>0.04 ± 0.16</td>
<td>0.20 ± 0.66</td>
<td>0.33 ± 0.96扬尘</td>
<td>0.25 ± 0.69扬尘</td>
</tr>
<tr>
<td>Volume, mm³</td>
<td>0.11 ± 0.39</td>
<td>0.30 ± 0.93</td>
<td>0.46 ± 1.24扬尘</td>
<td>0.51 ± 1.23扬尘</td>
</tr>
<tr>
<td>Score</td>
<td>0.04 ± 0.16</td>
<td>0.23 ± 0.84扬尘</td>
<td>0.43 ± 1.25扬尘</td>
<td>0.39 ± 1.05扬尘</td>
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<tr>
<td>Values in the LCx Artery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of lesions</td>
<td>0.02 ± 0.12</td>
<td>0.30 ± 0.65扬尘</td>
<td>0.02 ± 0.16</td>
<td>0.05 ± 0.25</td>
</tr>
<tr>
<td>Calcified area, mm²</td>
<td>0.01 ± 0.07</td>
<td>0.42 ± 1.10扬尘</td>
<td>0.09 ± 0.48扬尘</td>
<td>0.08 ± 0.31扬尘</td>
</tr>
<tr>
<td>Volume, mm³</td>
<td>0.06 ± 0.25</td>
<td>0.59 ± 1.41扬尘</td>
<td>0.14 ± 0.66扬尘</td>
<td>0.15 ± 0.54扬尘</td>
</tr>
<tr>
<td>Score</td>
<td>0.01 ± 0.05</td>
<td>0.44 ± 1.26扬尘</td>
<td>0.11 ± 0.85扬尘</td>
<td>0.07 ± 0.30扬尘</td>
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</table>

Abbreviations: CT, computed tomographic; HIV, human immunodeficiency virus; LAD, left anterior descending; LCx, left circumflex; LM, left main; RCA, right coronary artery; −, negative; +, positive.

*Data are given as mean ± SD.
†P<.05, using the t test to compare with the cocaine− and HIV− group.
‡P<.10, using the t test to compare with the cocaine− and HIV− group.
§P<.01, using the t test to compare with the cocaine− and HIV− group.
caine−, and PI-negative group in detectable CAC was not statistically different (25.0% vs 18.8%; P = .21). However, when BMI, a potential confounding factor, was adjusted in a regression model, HIV infection was significantly associated with a higher CAC level (P = .02).

**COMMENT**

This study in young adults demonstrates a positive association of HIV infection, cocaine use, and both with coronary artery calcification. These findings suggest that HIV infection and cocaine use may be involved in the development of subclinical atherosclerosis.

Coronary artery calcification detected by CT occurs early in plaque development as part of the inflammatory pathophysiologic cascade of coronary heart disease. A solid base of knowledge has been established recently regarding the relation between unstable coronary plaque and cocaine-related cardiovascular events, including angina pectoris, myocardial infarction, cardiomyopathy, and sudden death from cardiac causes, has increased dramatically. However, to our knowledge, no data have been published on the association between cocaine use and coronary calcification, one exception being a recent report demonstrating that cocaine use was independently associated with the CAC score. Nevertheless, this previous report was based on a much smaller sample size (N = 139) and it was not possible to examine the effect of cocaine in its own right on CAC given that many of the cocaine users were infected with HIV and many of the HIV-infected persons were treated with PIs.

The analysis to explore the association between HIV infection and coronary calcification was first performed by comparing all HIV-infected cocaine nonusers with HIV− cocaine nonusers. The data suggest a possible association between HIV infection and coronary calcification. Nevertheless, because many HIV-infected persons were treated with PIs, and PIs may accelerate subclinical atherosclerosis, the association between HIV infection and subclinical atherosclerosis has to be explained as follows. First, it is well established that atherosclerosis is accompanied by adaptive immune responses and that the early phase of atherosclerosis is dominated by immune cells, particularly macrophages and T lymphocytes. Potential mechanisms through which HIV-1 may promote subclinical atherosclerosis are activation of cytokines and cell adhesion molecules and alteration of major histocompatibility complex class I molecules on the surface of smooth muscle cells. Human immunodeficiency virus–infected cells may also generate reactive oxygen species with the activation of factors that induce apoptosis. Growing evidence suggests that fractalkine may be involved in atherosclerosis and cardiovascular pathophysiologic features. Second, HIV-infected individuals take PIs, which themselves have an adverse effect on subclinical atherosclerosis.

To examine the effect of HIV infection in its own right on CAC, an analysis was performed to compare the HIV+...
noncocaine users who did not use PIs with HIV–noncocaine users. We have demonstrated that HIV infection itself may accelerate subclinical atherosclerosis.

This study has several limitations. First, the study used a factorial design to recruit participants from 4 populations: HIV– and cocaine–, HIV+ and cocaine–, HIV– and cocaine+, and HIV+ and cocaine+. Nevertheless, it was not possible to obtain a representative sample from each population because the target populations are hidden. Second, because most HIV-infected participants were infected through injection drug use and most HIV-infected study participants were treated with antiretroviral medications, these 3 factors (HIV infection, cocaine use, and PI use) were highly correlated with each other. The multicollinearity with these 3 factors makes it difficult to separate the effect of one factor from others. Third, because of unbalanced data (approximately 70% were cocaine users) and the limited number of participants, this study failed to identify the synergistic effects of HIV infection and cocaine use on coronary calcification. The question of whether cocaine use plays an equal role in atherosclerotic heart disease across HIV status deserves further study. Finally, causal relations cannot be assessed in this cross-sectional study, and the results from this study need to be explained with caution.

Despite these limitations, this study suggests that HIV infection, cocaine use, or both may contribute to early subclinical atherosclerotic cardiovascular disease. Studies with a larger sample size are needed to test the interaction between HIV infection and cocaine use, and clinical trials are needed to examine whether reduction in cocaine use is an effective means of preventing atherosclerosis and, thus, ameliorating the burden of coronary disease.

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