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Brain vascular intima vulnerability among Human Immunodeficiency Virus positive and negative individuals

Madeleine D HUNTER, BA¹, Adele SHENOY, BA², Andrew DWORK, MD³, Mitchell SV ELKIND, MD, MS^{4,5}, Randolph MARSHALL, MD, MS⁴, Jay P MOHR, MD⁴, Susan MORGELLO, MD⁶, and Jose GUTIERREZ, MD, MPH⁴

¹Vagelos College of Physicians and Surgeons, Columbia University, New York, NY

²New York Medical College, Valhalla, NY

³Departments of Pathology and Cell Biology and Psychiatry, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY

⁴Department of Neurology, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY

⁵Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY

⁶Departments of Neurology and Pathology, Icahn School of Medicine at Mount Sinai, New York, NY

Abstract

OBJECTIVE: To test whether HIV is associated with brain large artery vulnerable intima.

DESIGN: Cross-sectional study of autopsied HIV+ cases sex- and age-matched to HIV-controls.

METHODS: Brain large arteries from 302 autopsied cases (50% HIV+) were evaluated morphometrically for the presence of atherosclerosis, size of necrotic core and fibrous cap thickness. Intima vulnerability was measured as intima elastolytic score (0–5, based on intimal metalloproteinases [MMP]-2, -3, and -9 and tissue inhibitor for (MMP) -1 and -2 staining), intima inflammatory score (0–3, based on intimal presence of CD3+ and CD68+ cells and TNF α staining), neoangiogenesis (Factor VIII staining), and apoptosis (caspase 3 staining). Hierarchical generalized linear models were used to obtain the beta estimates and their 95% confidence intervals, adjusting for demographics and vascular risk factors.

RESULTS: The prevalence of atherosclerosis did not differ by HIV status. Necrotic cores filled larger proportions of the intima in HIV+ individuals with CD4 count >200 at death compared to HIV-controls (adjusted B=11.6%, P=0.04). HIV+ individuals had greater elastolytic scores (adjusted B= 0.34; P=0.02), especially those with < 200 CD4+ cells/ μ l at death (adjusted B= 0.41;

CONTACT AUTHOR: Jose Gutierrez, MD, MPH, Address: 710 W 168th Street, 6th floor, Suite 639, New York, NY, 10032, jg3233@cumc.columbia.edu, Phone: (212) 305-1710, Fax: (212) 305-3741.

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P=0.01). Intima inflammation, neoangiogenesis, and apoptosis were not different among HIV+ cases versus HIV-controls.

CONCLUSION: Individuals with HIV and CD4 ≥ 200 at death had relatively larger necrotic cores while those with HIV and CD4 < 200 at death had evidence of increased connective tissue remodeling in the intima. These findings suggest an increased potential for endothelial erosion, thrombosis, and plaque rupture that may relate to higher risk for vascular events.

Keywords

Atherosclerosis; HIV; immunosuppression; immunohistochemistry; cerebral arteries

INTRODUCTION

HIV+ individuals have a higher incidence of stroke than those without HIV. This association has been consistent across various cohorts representing different regions within the United States [1, 2, 3] and other countries.[4] While traditional risk factors among those with HIV increase the risk of stroke, the use of antiretroviral therapy, HIV-associated vasculopathy, and opportunistic infections may play a role in stroke causation in this population.[5] Immunosuppression among those with HIV has also been associated with an incidence rate of stroke 2.7 times higher than in HIV infected individuals who are not immunosuppressed.[6]

Most studies that evaluated HIV as a vascular risk factor controlled for possible confounders. It is known that vascular risk factors such as diabetes and hypertension may be overrepresented among those with HIV.[7] Also, HIV populations in the United States are predominantly underserved and this may add social determinants of vascular risk.[8] Whether the mechanism of stroke in those with HIV differs from those without HIV is less well known, given that the majority of studies have used administrative datasets to ascertain stroke.[4] Such datasets have as a limitation the lack of adjudication of stroke mechanisms. The variability in the samples used to study stroke has led to the inclusion of a broad range of stroke etiologies, which tend to vary by the underlying distribution of CD4 counts in the population. For example, the prevalence of intracranial large artery atherosclerosis (ILAA) is higher in samples with higher CD4 counts[6] compared to those with lower CD4 counts.[9] In a pathology-based study, we reported that the prevalence of stenosis was not significantly higher compared with sex- and age-matched HIV-controls.[10]

ILAA may lead to cerebrovascular events through either plaque rupture or endothelial vulnerability. Though both these processes can cause strokes, the underlying plaques that predispose to either mechanism differ. Plaques that are thought to cause thrombosis by endothelial vulnerability or erosion typically have more smooth muscle cells and proteoglycans,[11] smaller lipid cores,[12] larger luminal areas,[13] are less likely to be calcified.[13] Increased plaque vulnerability in HIV+ patients has been previously found in the coronary arteries. However, some populations including women, younger patients, and smokers are more likely to generate thrombi after eroding the endothelium of their vessels,[11] which would also be possible in the HIV+ population.

In that context, we hypothesized that even if the prevalence of ILAA is not higher among those with HIV, the endothelium may be more vulnerable to erosion, making plaques more vulnerable to rupture and providing a substrate for coagulation. This could potentially explain the higher incidence of stroke events despite a relatively similar prevalence of arterial disease.

METHODS

Sample:

Autopsied cases from the Brain Arterial Remodeling Study (BARS) were included in this study. HIV cases were obtained mostly from the Manhattan HIV Brain Bank (MHBB; U24MH100931), while HIV negative controls were collected from MHBB, the New York Presbyterian Brain Bank/Macedonia Tissue Collection, and the University of Miami Brain Endowment Bank. The characteristics of each brain bank, as well as the characteristics of the cases by brain bank, have been reported elsewhere.^[14] Demographic and risk factor information, including HIV and immune status, were gathered by self-report, performance of laboratory testing during MHBB study visits, and/or by review of the electronic medical record.

Processing of brain arteries:

Brain large arteries forming the circle of Willis and the vertebral arteries were dissected from available cases after formalin fixation for at least 7 days. All the visualized arteries were extracted. The first segments of the supraclinoid internal carotid, middle, anterior, posterior, basilar and vertebral arteries were identified, and a 5-mm cut was performed at their most proximal ends (after the bifurcation) as well as the more distal ends (preceding a subsequent bifurcation). Each segment was labelled accordingly, embedded in paraffin, and sectioned in 6-micron cuts for staining with hematoxylin & eosin, Van Gieson (for elastin), Congo Red (for amyloid deposition), and trichrome and Sirius Red (for collagen visualization). Each artery was digitized with Olympus Soft Imaging Solutions software running on a high-speed, high-resolution Olympus VS110 virtual slide scanning system (Olympus, Center Valley, Pennsylvania, USA), and a microscope with a 10× objective lens under constant illumination with a 0.643 micron per pixel resolution. Each artery was assigned a phenotype as recommended by the revised AHA classification system.^[15] For the purpose of this study, we segregated “atherosclerotic” arteries as arteries with evident cholesterol deposition, either diffusely as in pathological intima thickening, or coalescent as in an atheroma, with or without a thin fibrous cap. Arteries with evidence of fibrosis and/or calcification without cholesterol deposition (i.e fibrocalcific plaques) were not considered atherosclerotic for the purpose of this analysis given lack of evident cholesterol deposition. The inter- and intra-reader reliabilities of the categorical variables described above have been reported as moderate to good.^[15, 16, 17, 18] In arteries with atheromas, we determined the atheroma area, the thickness of the fibrous cap separating the atheroma from the lumen, the percentage of the intima occupied by the atheroma, the number of atheromas in a given individual, whether the atheroma was located deep against the media, and whether the intima thickening extended around the total circumference of the lumen (Figure 1).

Additionally, we calculated the wall thickness, lumen diameters, and the lumen-to-wall ratio using color thresholding with good to excellent reliability.^[19]

Immunohistochemistry:

A variety of immunohistochemical stains have been performed on vessels in the BARS collection, as previously described.^[20] For each specimen, paraffin was removed from the sections by first applying xylene and graded alcohols and then rinsing with distilled water. Then, one of various antigen retrieval methods was used depending on the antigen of interest (described in the Supplemental Table 1). Primary and secondary antibodies were then applied and tailored to the antigen of interest (specific antibodies are described in Supplemental Table 1). For all specimens, we used an ABC kit at 1:50 dilution for thirty minutes at room temperature as a tertiary antibody (Vector Laboratories, #PK-6100). Slides were visualized after use of a diaminobenzidine chromogen (DAB visualization kit, DAKO Corp., #K3467) and hematoxylin counterstain. All staining was done at the Herbert Irving Cancer Center Molecular Pathology Laboratory at Columbia University.

Measures of Intima Vulnerability:

In addition to the structural features of atheromas described above, we used immunohistochemical staining to assess other features of vulnerability. We determined whether the strongest staining for MMP-2, -3, and -9 within the wall of an artery was found in the luminal aspect of the artery by visual rating while blinded to HIV status. We also determined whether an artery demonstrated staining for TIMP-1 and -2 that was in the lowest quartile of the arteries stained after adjusting for average pixel intensity, as previously described.^[21] We then created an intima elastolytic score that combined the pro-elastolytic measures indicated by stronger MMP staining in luminal aspect of the intima and the lower anti-elastolytic measures indicated by whether an artery fell within the lowest staining of a TIMP quartile. The score ranged from 0 to 5, with a 5 indicating the greatest elastolytic potential given the combined effects of MMPs, unopposed by TIMP-1 or -2 (Figure 2). The inclusion of high MMP staining and low TIMP staining in the elastolytic score was based on previous evidence which had found that MMPs -3 and -9 were found predominantly in vulnerable plaque and that MMP -2 was found more ubiquitously, but was accompanied by less of its inhibitors in vulnerable plaque.^[22] We also created an intima inflammation score that assessed whether the intima had the strongest staining of CD3+ cells, CD68+ cells, and TNF α . The score ranged from 0 to 3, with 3 implying greater lymphocytic and macrophage infiltration plus increased intimal production of TNF α (Figure 2). We evaluated for intima apoptosis using activated caspase-3 staining since endothelial apoptosis is thought to be part of the erosion pathway^[11] (Figure 3, in Supplemental Material). We rated intima neoangiogenesis by the presence of vascular channels in the intima, and confirmed these with Factor-VIII staining (Figure 3, in Supplemental Material). Neoangiogenesis was investigated because plaque vessels have been associated with plaque progression.^[23] The reliability of the topographical staining distribution is discussed below.

Statistical Analysis:

We assessed inter-reader reliability in the sample by having each artery rated twice by two different readers who were blinded to HIV status and to each other's reads. First, we used

Cochran's alpha and gamma correlation to assess the reliability of a series of scales. We proposed to visually rate the area of the artery with strongest brown staining for a given antibody, but detailed characterization of staining intensity within the intima or media was in general suboptimal. The best performing scale rated the arterial layer with the strongest staining. We then restricted further analyses to the location with the strongest staining and did not use the other variables. The same topographic description was used for TNF- α and Factor-VIII staining. The reliability of the CD68 and CD3 assessments have been previously described. The intra-class correlation coefficient for CD68 was 0.93.^[20] The kappa value for inter-rater reliability for CD3+ T cell presence vs absence in the intima was $\kappa=0.59$ (further detail provided in a paper currently under review). We provided the descriptive statistics in percentages or mean $\pm$ standard deviation, as indicated. We used hierarchical multilevel models to assess the association between HIV and measures of ILAA vulnerability (generalized linear models for binary and ordinal variables, with a binomial or Poisson binomial distribution as fitted and mixed linear models for continuous variables). We then stratified by CD4 count to assess an association with ILAA vulnerability because studies have found that the prevalence of ischemic stroke^[2] and ILAA-related stroke^[10] vary with CD4 count.

Three models were generated for the investigations in the association with HIV status and with CD4 count. The first model adjusted for age, sex, ethnicity, inter-adventitial diameter, and artifact variables. The second model adjusted for vascular risk factors, which would allow us to interpret the degree of confounding effects from modifiable risk factors. The last model added atherosclerosis to isolate endothelial vulnerability from the effects associated with atheromas.

We reported beta estimates and their 95% confidence intervals for the associations found here. A P value of 0.05 was considered significant. The analysis was carried out with SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Demographics/Baseline

Sample characteristics comparing HIV+ and HIV-subjects are shown in Table 1.

Prevalence of Atherosclerosis and Structural Variables

HIV status was associated with a lower prevalence of ILAA in an unadjusted model (B= -0.67; CI -1.19, -0.16), but not in one adjusting for demographics and risk factors (B= -0.15; -0.87, 0.57). Those with HIV and CD4 < 200 cells/ μ l or CD4 $\geq$ 200 cells/ μ l were no more likely to have ILAA than HIV-controls (Supplemental Table 2). Atheromas in HIV+ individuals with CD4 counts $\geq$ 200 cells/ μ l had a higher percentage of plaque occupied by the necrotic core (B=11.6%, P=0.04) compared to HIV-controls. Atheromas in HIV+ individuals had greater cross-sectional areas compared to HIV-controls, though the difference was not statistically significant. HIV+ individuals and controls had similar fibrous cap thickness (Supplemental Table 3).

ILAA and intima vulnerability

In separate models adjusted for demographics, vascular risk factors, and arterial size, ILAA was associated with intima elastolytic score ($B=0.48$, CI 0.15–0.80, $P=0.004$), intima inflammation score ($B=1.72$, 1.27–2.17, $P<0.001$), and intima neoangiogenesis ($B=4.01$, 2.40; 5.62, $P<0.001$), but not with intima apoptosis ($B=0.55$; -0.13 , 1.23; $P=0.11$). Adjusting for the four scores in the same model showed a persistent association between ILAA and intima inflammation score ($B=1.87$; 1.13, 2.59; $P<0.001$) and intima neoangiogenesis ($B=3.27$; 0.67, 5.87; $P=0.01$) but not with intima elastolytic score ($B=0.67$; -0.38 , 1.69; $P=0.$) or intima apoptosis ($B=0.21$; -1.14 , 1.56; $P=0.75$). There was no statistical interaction with HIV status for any of these associations.

Intima Vulnerability and HIV-related immune status

Higher intima elastolytic score was associated with HIV ($B=0.39$; 0.12, 0.67, $P=0.005$) and the association remained significant after adjusting for vascular risk factors in addition to demographics and prevalent ILAA ($B=0.34$; 0.05, 0.64, $P=0.02$). Stratifying those with HIV by CD4 counts demonstrated that the association was stronger among those with $CD4 < 200$ cells/ μ l compared with HIV-controls in the fully adjusted model ($B=0.41$; 0.10, 0.71, $P=0.01$). In contrast, the association for HIV+ individuals with $CD4 > 200$ cell/ μ l compared to HIV-negative individuals had a smaller effect size and was borderline significant in the fully adjusted model ($B=0.35$; 0.00, 0.71, $P=0.05$).

HIV was associated with less intima neoangiogenesis in a model adjusting for demographics alone ($B=-1.55$; -3.09 , -0.01 , $P=0.05$), but the association became non-significant after adjusting for demographics, vascular risk factors and atherosclerosis. There was no association between HIV, either categorically or stratified by CD4 counts, with the intima inflammation score or with intima apoptosis (Table 2).

Co-expression of intima vulnerability scores

In models adjusting for demographics, risk factors, and ILAA, intima elastolytic score was associated with intima apoptosis ($B=0.23$; 0.05, 0.41; $P=0.01$) but not with intima neoangiogenesis ($B=0.09$; -0.37 , 0.56; $P=0.69$) nor intima inflammation score ($B=0.03$; -0.07 , 0.12; $P=0.55$). Intima inflammation score, intima apoptosis, and intima neoangiogenesis were not mutually associated, independently of ILAA. There was no statistical interaction by HIV in any of these associations.

DISCUSSION

People living with HIV/AIDS are at an increased risk of stroke compared to uninfected controls. Among this population, intracranial atherosclerosis is an important cause of stroke. [10,24] Because the prevalence of pathologically-defined ILAA is not different between those with HIV compared to HIV-controls (after adjusting for appropriate risk factors), we hypothesized that intima vulnerability may differ among those with HIV and thus partially explain a higher rate of ILAA-related events. We presented here data partially validating our hypothesis. Supporting our hypothesis, we found that individuals with HIV, and especially those whose last CD4 prior to death was < 200 cells/ μ l, exhibit more intima connective

tissue remodeling as suggested by the imbalance of matrix metalloproteases (MMPs) and tissue inhibitors of MMPs (TIMPs). Specifically, there was relatively higher staining of matrix metalloproteases 2, 3, and 9 in the intima and the relatively less staining of tissue inhibitors of matrix metalloproteases 1 and 2. Such an imbalance between MMP and their inhibitors suggests an enhanced elastolytic activity. This could indicate that individuals with HIV and CD4 < 200 cells/μl are more likely to have plaques vulnerable to rupture, or, in the absence of an atheroma, may be more likely to erode intima compared either to those with HIV and better immune status or to HIV-controls. In the setting of equivalent prevalence of atheromas between the two populations as we found in this study, the population with more vulnerable plaques, found here to be HIV+ individuals, would be expected to have more stroke events. In addition, in the hypercoagulable state found in HIV, [25,26] the simple erosion of intima may be sufficient substrate for triggering *in-situ* thrombosis and potentially occluding the artery or embolizing distally, causing stroke. In fact, stroke due to hypercoagulable state is overrepresented among those with HIV.[9] We did not find a significant difference by HIV status in other traditional measures of plaque vulnerability such as the thickness between the atheroma and the lumen or raw atheroma size.

The interpretation of our results becomes more complicated in the context of seemingly contradictory literature. One report indicates that ischemic strokes are more prevalent among immunosuppressed HIV patients, with no correlating additional risk in HIV patients with a CD4 count above 500 cells/μl.[2] In the context of this study, the greater elastolytic activity in immunocompromised HIV patients might be thought to contribute to the vulnerability of the plaque directly, perhaps thinning fibrous caps. For arteries without atheromas, eroded intima could provide a nidus for clots.

However, another study has found that the prevalence of ILAA-related strokes was higher among HIV patients with higher CD4 counts,[10] which suggests a different possible role for the increased elastolytic activity in immunocompromised HIV+ individuals. Taking into account these studies, the greater elastolytic activity in immunocompromised HIV+ individuals may be thought to lead to remodeling which manifests in changes that could contribute to the plaque's vulnerability when immunocompetence is regained. The atheromas with more prominent necrotic cores which we found in our study could be an indication of such changes.

Regarding the possible mechanism by which immunocompromised HIV+ individuals were found to have had greater elastolytic activity, it may be that the immune activation which is suspected to deplete CD4+ cells[27] may also activate elastolytic enzymes. Supporting this idea, TNFα has been shown to act through MMP-9 to allow vascular smooth muscle cell migration across the internal elastic lamina.[28] Prolonged release of TNFα present in chronic immune activation could therefore cause elevated MMP-9 levels in the vessels of the immunosuppressed individuals included in our study.

These results should be interpreted in the context of the limitations of this study, which include the use of qualitative measurements for certain variables such as determining if the intima had the strongest staining. This method was used to determine if the strongest staining was found in the intima on stains for caspase 3, MMPs, and TNF α. Also, though

this sample includes 302 study subjects, 140 of the subjects had atheromas, which restricted numbers for analyses investigating atheroma characteristics. Similarly, the prevalence of brain infarcts due atherosclerosis was too small to allow for multivariate analyses. Also, HIV + individuals and controls differed in a few characteristics which were found to have effects as covariates. First, there was a marked difference in ethnicity. HIV-negative controls were predominantly non-Hispanic White while HIV+ individuals were more often non-Hispanic Black or Hispanic.

Ethnicity was associated with elastolytic score and apoptosis in HIV status and both HIV status and CD4 models respectively. However, these associations lost statistical significance in models that adjusted for vascular risk factors, which suggest to us that the initial association was mediated by a higher prevalence of vascular risk factor among ethnic minorities with HIV. Furthermore, cocaine use, hepatitis C and hepatitis B were higher in those with HIV, and the lack of detailed information regarding this confounder in the HIV-negative sample prevents from excluding a role of these factors in modifying the vulnerability noted among those with HIV.

Despite this study's limitations, the Brain Arterial Remodeling Study is a unique and relatively large sample of age and sex matched controls for the HIV cases. There is no precedent for the premise and scope of this study. Because of the novelty of the results and limitations discussed above, these result should be used as preliminary evidence for studies of intracranial plaque vulnerability in living individuals with HIV.

CONCLUSION

HIV and persistent immunosuppression at the time of were associated with a greater degree of elastolysis in the intima of intracranial vessels, potentially leading to more vulnerable plaques or to endothelial erosion and thrombosis. This may help explain the higher stroke incidence in HIV despite a prevalence of atherosclerosis that was no higher in HIV+ individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1 legend:

Panel a: Measurement of the fibrous cap of an atheroma. **Panel b:** The green arrow points to the necrotic core found in the atheroma. To determine the percentage of plaque occupied by the necrotic core, the quotient of the area of the necrotic core and that of the atheroma was taken. **Panel c:** The atheroma in panel c was determined to be media based and the intima thickening encircled > 75% of the arterial circumference. Following the vessel to either side shows that the atheroma is located deep against the media. **Panel d:** Multiple atheromas are found in this artery cross-section.

Figure 2 legend:

Panel a: Top row: The three panels show a cellular infiltrate (in H&E) co-existing with increased collagen deposition (in Trichrome) and internal elastic lamina degradation (in Verhoeff-van Gieson). In the same area of tissue remodeling, we found relatively higher intensity staining for MMP2, MMP3 and MMP9 with a relatively weaker staining for TIMP-1 and TIMP2. **Panel b:** Using CD68+ antibody, we identified two regions of macrophage infiltrates in an area of an atheroma. The same area also exhibited CD3+ cells as well as increased staining for TNF α in the periphery of CD68+ cells. The same region exhibited scattered foam cells and cholesterol deposition (Sirius red) but not coalescing into a necrotic core.

Table 1:

Characteristics of sex and age matched HIV negative and positive subjects

Characteristic *	HIV negative subjects (n=151)	HIV positive subjects (n=151)	p Value
Male sex	109 (72.2%)	108 (71.5%)	0.90
Age	49.4 + 10.0 years	49.4 + 8.9 years	0.98
Ethnicity			< 0.01
Non-Hispanic White	112 (74.2%)	25 (16.6%)	
Non-Hispanic Black	16 (10.6%)	72 (47.7%)	
Hispanic	23 (15.2%)	54 (35.8%)	
Hypertension	53 (35.1%)	90 (59.6%)	< 0.01
Diabetes Mellitus	21 (13.9%)	25 (16.6%)	0.52
Dyslipidemia	19 (12.6%)	31 (20.5%)	0.06
Smoking	85 (56.3%)	81 (53.6%)	0.64
Cocaine Use	10 (6.6%)	59 (39.1%)	< 0.01
Ischemic Infarct	18 (11.9%)	32 (21.2%)	0.03
Coronary artery disease	45 (29.8%)	54 (35.8%)	0.27
Myocardial infarction	28 (18.5%)	15 (9.9%)	0.032
Valvulopathy	22 (14.9%)	8 (5.1%)	0.029
Hepatitis B	17 (11.3%)	76 (50.3%)	< 0.01
Hepatitis C	21 (13.9%)	69 (45.7%)	< 0.01
Antiretroviral use at death	N/A	90 (59.6%)	N/A
Nucleoside Reverse Transcriptase Inhibitor Use	N/A	83 (55.0%)	N/A
Non-Nucleoside Reverse Transcriptase Inhibitor Use	N/A	30 (19.9%)	N/A
Protease Inhibitor Use	N/A	59 (39.1%)	N/A
CD4+ count <200 cells/μl	N/A	96 (66.2%)	N/A
CD4+ count (cells/ μl)	N/A	88 (13 – 249)	N/A
CD4+ nadir (cells/ μl)	N/A	48 (9 – 153)	N/A
Time with HIV	N/A	11.8 + 5.9 years	N/A
Viral load (copies per ml)	N/A	11531 (0 – 237722)	N/A

* Data are presented as n (%) or median (IQR) unless otherwise noted

Abbreviations: not available or not applicable

Table 2:

Associations between Intima Vulnerability and HIV positive or HIV immunosuppressed status compared to HIV negative status

	Model 1* B (95% CI)	Model 2[†] B (95% CI)	Model 3[‡] B (95% CI)
Intima elastolytic score			
HIV (all cases)	0.39 (0.12, 0.67)	0.32 (0.02, 0.61)	0.34 (0.05, 0.64)
HIV (by CD4 counts)			
CD4 ≥ 200 cell/ul		0.33 (−0.03, 0.68)	0.35 (0.00, 0.71)
CD4 < 200 cell/ul		0.39 (0.09, 0.70)	0.41 (0.10, 0.71)
Intima inflammation score			
HIV (all cases)	0.01 (−0.12, 0.13)	−0.01 (−0.14, 0.13)	0.04 (−0.09, 0.18)
HIV (by CD4 counts)			
CD4 ≥ 200 cell/ul		0.01 (−0.14, 0.17)	0.05 (−0.09, 0.19)
CD4 < 200 cell/ul		0.03 (−0.13, 0.19)	0.05 (−0.11, 0.21)
Intima neoangiogenesis			
HIV (all cases)	−1.55 (−3.09, −0.01)	−1.22 (−3.62, 1.17)	−0.05 (−2.03, 1.92)
HIV (by CD4 counts)			
CD4 ≥ 200 cell/ul		−0.70 (−3.21, 1.82)	0.07 (−1.91, 2.06)
CD4 < 200 cell/ul		−0.90 (−2.83, 1.03)	−0.52 (−3.02, 1.97)
Intima apoptosis			
HIV (all cases)	0.65 (−0.08, 1.38)	0.52 (−0.46, 1.49)	0.54 (−0.46, 1.54)
HIV (by CD4 counts)			
CD4 ≥ 200 cell/ul		0.72 (−0.33, 1.78)	0.74 (−0.32, 1.81)
CD4 < 200 cell/ul		0.14 (−0.95, 1.23)	0.10 (−1.03, 1.23)

* **Model 1:** Adjusted for age, sex, ethnicity, interadventitial diameter, and artifact variables

[†] **Model 2:** Model 1 plus hypertension, diabetes mellitus, dyslipidemia, smoking and cocaine.

[‡] **Model 3:** Model 2 plus prevalence of atherosclerosis

Abbreviations: B, beta coefficient; CI, confidence interval