Brain large artery inflammation associated with HIV and large artery remodeling

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Objective: To test the hypothesis that brain arteries from HIV+ cases have a greater degree of inflammation than brain arteries from HIV− cases, and that inflammation is associated with brain arterial remodeling.

Design: Case–control study, cross-sectional.

Methods: Brain arteries from 162 autopsy cases (84 with HIV) were systematically analyzed for thickness of the intima, media, and adventitia, and atherosclerosis and dolichoectasia. Inflammation was assessed with $CD68^+$ immunohistochemistry, and measured with a semiquantitative score reflecting the number and location (i.e., arterial layer) of activated macrophages infiltrating the arterial wall. Latent varicella zoster virus (VZV) was assessed with anti-VZV gene 63 product immunohistochemistry. Demographic and clinical variables were available in all cases, and longitudinal data about $CD4^+$ cell counts were available among cases with HIV. Multilevel generalized linear models were used to test the association between inflammation and HIV.

Results: Arteries from HIV+ cases had a higher inflammation score ($B = 0.36$, $P = 0.05$) compared with arteries from HIV− cases, although the association was attenuated after controlling for demographic variables, vascular risk factors, and latent VZV ($B = 0.20$, $P = 0.18$). Although intimal inflammation was similar in cases with and without HIV, adventitial inflammation was associated with HIV. Intimal inflammation was associated with intracranial atherosclerosis independent of HIV status, but adventitial inflammation was associated with HIV-associated dolichoectasia in arteries with a thin media.

Conclusions: Adventitial inflammation is associated with HIV and dolichoectasia independent of intracranial atherosclerosis. This suggests that differential inflammatory responses may play a role in intracranial atherosclerosis and dolichoectasia.

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Introduction

Infection with HIV has evolved from a nearly universal fatal disease to a chronic infection with the administration of combined antiretroviral therapy (cART). In spite of great advances in the management of HIV infection, vascular disease has emerged as a major cause of morbidity and mortality in the treated HIV population, with stroke among the most worrisome of cerebrovascular complications [1–3]. Early in the HIV pandemic, neurological

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complications and brain damage were noted in the majority of individuals who died with profound immunosuppression [4,5]. With the advent of cART, the progressive restoration of the immune system led to a sharp decrease in the rates of death because of opportunistic infections and neoplasia [3,4]. Stroke and HIV-associated neurocognitive disorders, however, have continued to afflict those with HIV despite the use of cART [2,6]. Some reports even suggest that the stroke incidence among those with HIV is higher than in uninfected control samples [7].

Although clinical guidelines for treating HIV emphasize the value of $CD4^+$ cell counts as markers of restored immunity, a growing body of evidence suggests that HIV infection has profound effects on the host immune system leading to chronic immune activation [8]. The central nervous system, although selectively impermeable to most blood elements, may be rendered vulnerable to systemic inflammation and may alter the course of cerebrovascular disease [9–11]. Aortic inflammation has been reported in individuals with HIV with normalized $CD4^+$ cell counts while on cART, and the inflammation correlated with the atherosclerotic burden [12]. Inflammatory cells are frequently noted in arterial specimens from patients with 'HIV vasculopathy' [13,14]. Few studies have systematically assessed for the presence of inflammatory cells in brain arteries. Because the brain arteries do not remodel outward in the same way as systemic arteries do in the setting of atherosclerosis [15], determining whether inflammation in brain arteries may play a role in brain arterial remodeling may lead to a better understanding of the pathophysiology of stroke in the HIV population.

We present here results from a sample of autopsied brains from HIV+ and HIV− individuals. In this sample, we tested the hypothesis that brain arteries from HIV+ cases have a greater degree of inflammation than brain arteries from HIV− cases, and that inflammation is associated with brain arterial remodeling.

Materials and methods

Arterial specimens were selected from the Brain Arterial Remodeling Study, a collection of large brain and penetrating arteries from 336 cases with and without HIV. Four different brain collections were the source of the specimens in the Brain Arterial Remodeling Study. We have previously reported in detail the characteristics of these four brain collections [16]. A subsample of Brain Arterial Remodeling Study cases was selected for various immunohistochemical stains. We prioritized cases with the greatest number of brain arteries available for analysis and cases with premortem follow-up (only available for those with HIV). For the HIV− cases, we attempted matching by sex and age ± 3 years to HIV+ cases. All the HIV+ cases and some HIV− cases included in this study came from the Manhattan HIV Brain Bank (U24MH100931) [4]. Individuals enrolled in this study were followed antemortem prospectively in routine research visits where cognitive testing, physical examination, and laboratory evaluation were performed. These individuals agreed to donate their brains for research purposes upon death in an institutional review board-approved protocol. The rest of the HIV− cases were selected from the Macedonian/New York Psychiatric Institute brain collection, with no longitudinal antemortem data available for any of these cases.

All brains were fixed in 10% buffered formalin after autopsy. A total of 5 mm segments were selected from the proximal and distal segments of the large arteries of circle of Willis and the posterior circulation. Arteries were embedded in paraffin and 6-μm-thick cuts were obtained for hematoxylin and eosin and elastic Van Gieson. The area of each of the arterial layers was quantified with ImageJ software (WS Rasband, ImageJ, US National Institutes of Health, Bethesda, Maryland, USA, imagej.nih.gov/ij/, 1997–2011) using color-based thresholding. With a series of standard geometric formulas, we derived arterial stenosis, lumen-to-wall ratio (LWR), wall thickness, and interadventitial diameters. Additionally, each artery was rated visually to determine the atherosclerosis phenotype using the revised American Heart Association atherosclerosis classification [17]. For this analysis, intracranial large artery atherosclerosis (ILAA) was considered present in arteries with at least an atheroma, independent of whether or not the atheroma had a thin fibrous cap. Dolichoectasia was defined by an arterial size-adjusted LWR > 2 SDs above the mean. The distribution of LWR was obtained by sex and by HIV status to allow a comparable number of arteries with dolichoectasia in each group. The intra and interreader reliabilities of our methods have been reported as good to excellent [18,19].

Immunohistochemistry: A total of 5-μm-thick sections were obtained. The slides were deparaffinized with a series of xylene and graded alcohols, and rinsed in distilled water. Antigen retrieval was carried out with Trilogy (Cell Marque Corp., Rocklin, California, USA, #920P), preceded by heat exposure in steamer for 25 min (for $CD68^+$ slides) and citrate buffer, and was further preceded by heat exposure in a pressure cooker for 20 min [for varicella zoster virus (VZV) slides]. We used anti-$CD68^+$ to identify activated macrophages (primary 1 : 100, DAKO Corp., Carpinteria, California, USA, #M0814, mouse monoclonal antibody; secondary biotinylated horse anti-mouse IgG antibody, Vector Laboratories, Burlingame, California, USA, #BA-2000) and anti-VZV open reading frame 63 gene product to identify latent VZV infection (primary 1 : 100, rabbit polyclonal antibody was provided by Dr Paul

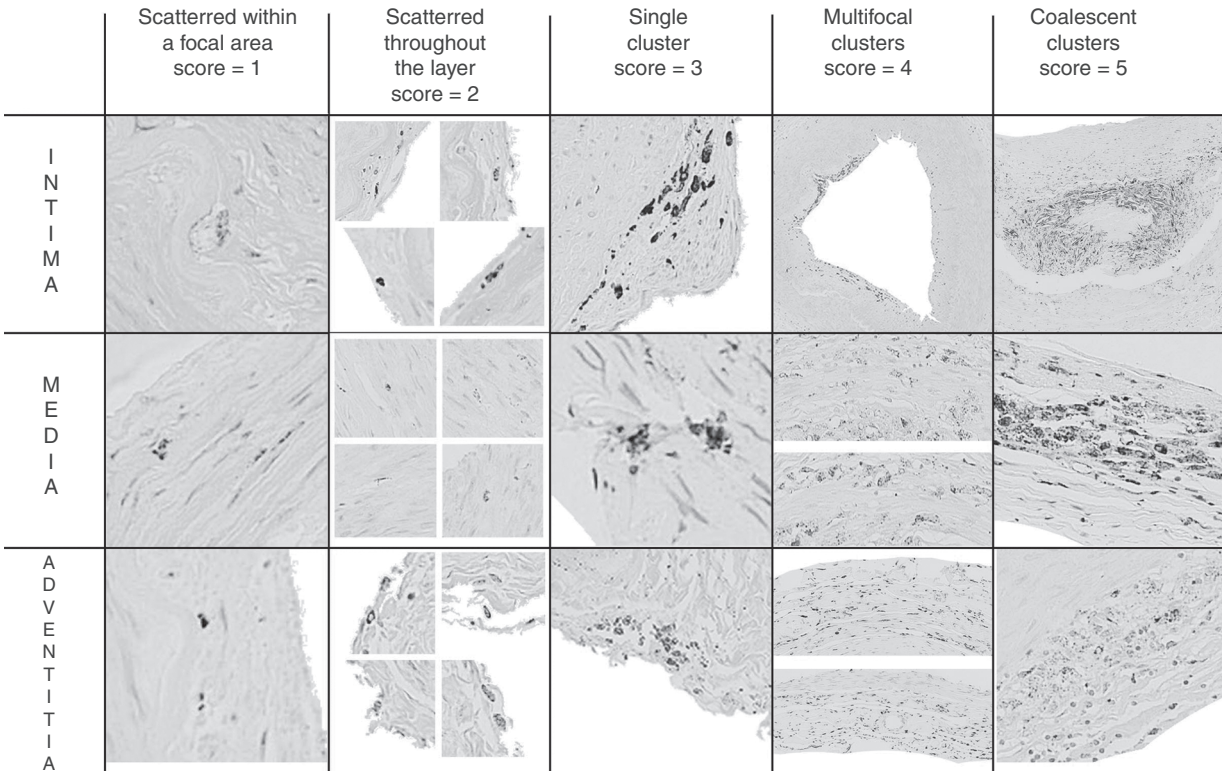

Fig. 1. Inflammation score reflects the relative distribution and extent of CD68$^+$ cells in the artery by arterial layer, with the global score reflecting the extent and intensity of the inflammatory activity.

Kinchington, The Campbell Laboratory for Infectious Eye Diseases, University of Pittsburgh; secondary biotinylated goat anti-mouse IgG antibody, Vector Laboratories, #BA-1000) [20,21]. Visualization of primary antibody binding to the arterial antigens was carried out with diaminobenzidine kit (DAKO Corp., #K3467) for CD68$^+$ and Red Alkaline Phosphatase Substrate Kit (Vector Laboratories), #SK5100 for VZV. We used hematoxylin to counterstain the CD68$^+$ and the VZV slides. The staining was carried out at the Herbert Irving Cancer Center molecular pathology laboratory at Columbia University. Each section was photographed with constant illumination using Olympus Soft Imaging Solutions software (Olympus, Center Valley, Pennsylvania, USA) and a microscope with constant illumination, with a 10× objective lens and scale = 0.643 μm/pixel in the Histology Shared Resource Facility of the Icahn School of Medicine at Mount Sinai.

Imaging postprocessing: Each microphotograph was systematically postprocessed by removing the background, debris, and folding artifacts to avoid an artificial increase in pixel intensity, as reported before [16]. In case of uneven staining, excessive folding, or tissue loss, the staining was repeated (17/200 CD68$^+$ slides and 9/200 latent VZV were repeated). Average pixel intensity in the case of VZV and arterial area with positive CD68$^+$ staining were obtained automatically using Visiopharm Integrator System version 4.6.3.857 (Hoersholm, Denmark) by color thresholding (see supplementary data, Figures e1–e4, http://links.lww.com/QAD/A806). Because of the inability to automatically detect the degree of inflammation by arterial layer in the CD68$^+$ slides, we created a semiquantitative scale based on visual assessment (Fig. 1) with a possible range from 0 to 15 for a summation of all three arterial layers. The intraclass correlation coefficient was 0.95 for the intima inflammation score, 0.90 for the media inflammation score, 0.71 for the adventitia inflammation score, and 0.93 for the overall inflammation score. To further validate this scale, we contrasted the overall inflammation score against the total arterial area occupied by CD68$^+$ cells, obtained automatically with Visiopharm Integrator System. As the inflammation score increased, so did the arterial area with CD68$^+$ cells (Figure e5, supplementary data, http://links.lww.com/QAD/A806). We also used visual observation and average pixel intensity measurement to ascertain latent VZV. Visually, we first assessed each artery for areas with a bright red staining and rated these as positive. Then, we obtained the average pixel intensity (for red) and the background staining in each artery using Visiopharm Integrator System version 4.6.3.857 by color thresholding and contrasted the visual rating with the average pixel intensity, after adjusting the average pixel intensity by the background staining to homogenize the variability between different batches of slides

(Figure e6, supplementary data, http://links.lww.com/QAD/A806). Using this system, the mean adjusted average pixel intensity for arteries rated as latent VZV positive was 2.37 ± 1.35, compared with 1.78 ± 1.12 in the arteries rated as negative ($P = <0.001$).

Statistical analysis

Differences in demographic and clinical variables among HIV+ and HIV− cases were assessed with chi-square or Student's *t*-test, as indicated. We used logistic regression to carry out a multivariable analysis and identify differences among HIV+ and HIV− cases as defined by odds ratio (OR) and their 95% confidence intervals (CIs) (OR ± 95% CI). The main outcome variable in this study was inflammation defined by the inflammation score globally and by arterial layer. The inflammation score had a mean ± SD of 1.2 ± 1.8. Based on this distribution, we used a Poisson regression to assess the association of inflammation with the independent variables used in our models. For LWR and media thickness, their distribution was adjusted by arterial size, normalized, and used continuously with mixed models to assess relationships with arterial inflammation. To account for the lack of independence among arteries extracted from the same individual, we used multivariable, multilevel generalized linear models with subject as the random effect to obtain the β estimates with a P value ≤ 0.05 considered statistically significant. The analysis was carried out with SAS software, version 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Sample studied

Brain arteries from 162 autopsied cases were included in this study, and 84 had HIV. The average number of arteries per case was 6.7 (range 3–11) in HIV+ cases and 5.8 (range 2–9) in HIV− ($P = 0.001$). The demographic and clinical variables of cases included in this sample are presented in Table 1. In multivariable logistic regression analysis, cases with HIV were more likely to be non-Hispanic blacks (OR 4.3, 1.4–13.7), to be smokers (OR 2.93, 1.2–7.2), and to have used cocaine (OR 5.2,

Table 1. Characteristics of the sample studied by HIV status.

	HIV+, $N = 84$	HIV−, $N = 78$	P value[a]
Age (years)			
Mean ± SD	49 ± 9	49 ± 9	0.91
Median (IQR)	48 (42–54)	48 (42–54)	
Range	30–84	30–82	
Male sex (%)	70	71	0.55
Ethnicity (%)			
NH white	23	67	<0.01
NH black	52	13	
Hispanic	25	21	
Residence in the United States (%)	100	58	<0.01
Hypertension (%)	60	44	0.03
Diabetes (%)	17	18	0.49
Dyslipidemia (%)	25	17	0.13
Smoking (%)	56	54	0.45
Cocaine use (%)	52	6	<0.01
Cardiovascular disease (%)	48	40	0.19
Number of years with HIV infection, median (IQR)	12 (7–16)	NA	–
Numbers of months in follow-up, median (IQR)	19 (3–54)	NA	–
$CD4^+$ cell count at death, median (IQR) (cells/μl)	107 (19–269)	NA	–
$CD4^+$ cell count nadir, median (IQR) (cells/μl)	64 (11–187)	NA	–
Unsuppressed viral load (>50 RNA copies/ml, %)	68	NA	–
History or evidence of opportunistic infections (%)	74	NA	–
Candida	18		
PCP	16		
Varicella zoster (%)	9		
Tuberculosis	3		
Cryptococcus	3		
HIV encephalitis (%)	2		
CNS lymphoma (%)	2		
cART use (%)[b]	69	NA	–
Nucleotide reverse transcriptase inhibitors	62		–
Nonnucleotide reverse transcriptase inhibitors	24		–
Protease inhibitors	49		–
Others	6		–

cART, combined antiretrovirals; CNS, central nervous system; IQR, interquartile range; NA, not applicable; NH, non-Hispanic; PCP, Pneumocystis carinii pneumonia.
[a]Univariate analysis, chi-squared used for categorical variables and Student's *t*-test used for continuous variables.
[b]cART use recorded at the time of death.

1.7–15.8). None of the other variables used in Table 1 remained significant.

HIV status and brain arterial inflammation

A total of 1018 segments of large brain arteries were studied. In arteries from HIV− cases, 174/452 (38%) had evidence of inflammation (i.e. inflammation score ≥1): 124 of 152 (27%) in the intima, 18 of 452 (4%) in the media, and 98 of 452 (22%) in the adventitia. In arteries from HIV+ cases, 308 of 566 (54%) had evidence of inflammation: 135 of 566 (24%) in the intima, 25 of 566 (4%) in the media, and 250 of 566 (44%) in the adventitia. In the whole sample, inflammation in the media was observed accompanying intima inflammation in 39 of 43 of the cases; only four of 43 arteries had media inflammation without intima inflammation. The prevalence of latent VZV infection was 55% in HIV+ cases versus 60% in HIV− cases ($P=0.11$). In univariate analysis, arteries from HIV+ cases had a higher inflammatory score ($B=0.36$, $P=0.05$) compared with arteries from HIV− cases. After adjusting for demographic variables, vascular risk factors, and latent VZV, the association between inflammation score and HIV was no longer statistically significant ($B=0.20$, $P=0.18$).

The localization of inflammation in the arterial wall, however, varied significantly by HIV status (Fig. 2). Compared with arteries from HIV− cases, arteries from HIV+ cases were more likely to have adventitial inflammation ($B=0.80$, $P<0.001$). Adjusting for demographic, vascular risk factors, and latent VZV ($B=0.70$, $P=0.01$), or categorizing inflammation as 'predominantly adventitial' ($B=1.05$, $P=0.003$) did not eliminate the association. There were no significant differences in the distribution of intima ($B=-0.16$, $P=0.46$) or media inflammation ($B=-0.14$, $P=0.78$) by HIV status.

Brain arterial remodeling and brain arterial inflammation

In univariate analysis, arterial inflammation was associated with smaller LWR ($B=-0.33$, $P=<0.001$). This association was enhanced when the inflammation was restricted to the intima ($B=-0.88$, $P=<0.001$). Inflammation in the adventitia was associated with larger LWR ($B=0.21$, $P=0.02$). We did not find evidence of a nonlinear association between inflammation and LWR (data not shown).

In adjusted models, arterial inflammation was associated with smaller LWR and with ILAA. These associations were stronger when the inflammation was predominantly localized to the intima. These associations varied little by HIV status (Table 2). The predictors for media thickness,

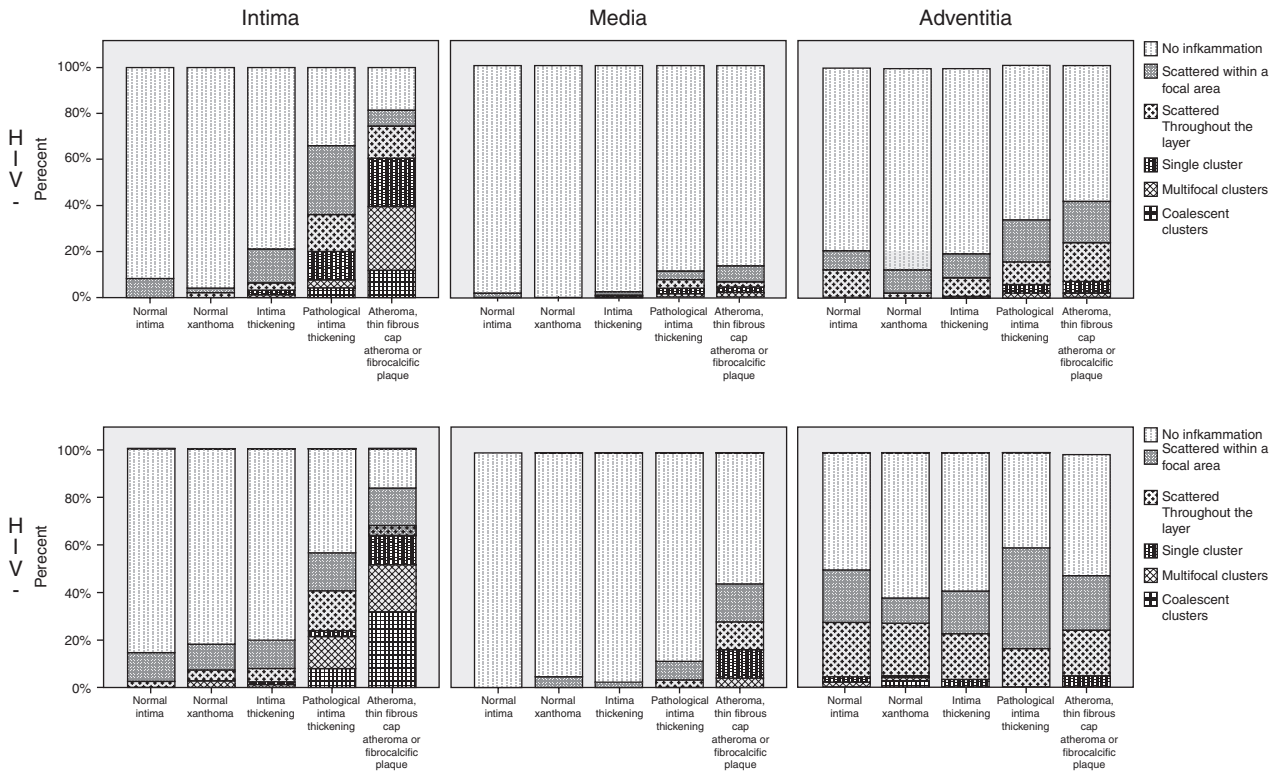

Fig. 2. Progressive ILAA was often accompanied by greater degrees of inflammation in the intima and the media, with no apparent difference by HIV status. In brain arteries from HIV− cases, progressive ILAA appeared also as the main driver on adventitia inflammation, whereas in HIV+ cases, not only was adventitia inflammation more frequent, but it appeared less influenced by ILAA. ILAA, intracranial large artery atherosclerosis.

Table 2. Association between inflammation and brain arterial characteristics.

		Z-score for lumen-to-wall ratio[a] Standardized β coefficient	Z-score for media thickness[a] Standardized β coefficient	Intracranial large artery atherosclerosis[a] Standardized β coefficient	Dolichoectasia[a] Standardized β coefficient
All cases					
	Any inflammation	−0.13 ± 0.01 $P < 0.001$	−0.07 ± 0.02 $P = 0.001$	0.31 ± 0.03 $P < 0.001$	−0.52 ± 0.22 $P = 0.02$
	Intima inflammation	−0.24 ± 0.02 $P = <0.001$	−0.09 ± 0.03 $P = <0.001$	0.66 ± 0.06 $P < 0.001$	−0.76 ± 0.26 $P = 0.003$
	Adventitia inflammation	0.02 ± 0.03 $P = 0.66$	−0.09 ± 0.04 $P = 0.01$	−0.04 ± 0.10 $P = 0.66$	−0.29 ± 0.24 $P = 0.23$
HIV− cases					
	Any inflammation	−0.14 ± 0.02 (<0.001)	−0.09 ± 0.02 (<0.001)	0.28 ± 0.03 $P < 0.001$	−0.54 ± 0.19 $P = 0.006$
	Intima inflammation	−0.24 ± 0.02 $P < 0.001$	−0.13 ± 0.03 $P < 0.001$	0.60 ± 0.08 $P < 0.001$	−0.94 ± 0.32 $P = 0.004$
	Adventitia inflammation	0.01 ± 0.01 $P = 0.88$	−0.07 ± 0.06 $P = 0.24$	0.06 ± 0.12 $P = 0.63$	−0.20 ± 0.26 $P = 0.45$
HIV+ cases					
	Any inflammation	−0.13 ± 0.02 $P < 0.001$	−0.05 ± 0.02 $P = 0.007$	0.35 ± 0.04 $P < 0.001$	−0.46 ± 0.19 $P = 0.01$
	Intima inflammation	−0.26 ± 0.03 $P = 0.001$	−0.04 ± 0.03 $P = 0.19$	0.66 ± 0.10 $P < 0.001$	−0.64 ± 0.33 $P = 0.06$
	Adventitia inflammation	0.03 ± 0.04 $P = 0.56$	−0.09 ± 0.04 $P = 0.05$	−0.12 ± 0.21 $P = 0.21$	−0.36 ± 0.28 $P = 0.19$

[a]All estimates are adjusted for age, sex, race, ethnicity, vascular risk factors, cocaine use, latent varicella zoster virus, and country of origin.

Table 3. Associations between demographic, clinical, and pathological variables with arterial inflammation by HIV status.

		HIV−		HIV+	
		Intima inflammation Standardized β coefficient	Adventitial inflammation Standardized β coefficient	Intima inflammation Standardized β coefficient	Adventitial inflammation Standardized β coefficient
Age (years)		−0.01 ± 0.01	−0.01 ± 0.01	0.05 ± 0.01[a]	−0.01 ± 0.01
Male sex		−0.08 ± 0.25	0.01 ± 0.41	−0.35 ± 0.33	−0.34 ± 0.25
Ethnicity	Non-Hispanic white	Reference	Reference	Reference	Reference
	African-American or black	−1.30 ± 0.50[a]	−0.61 ± 0.73	0.39 ± 0.37	−0.22 ± 0.30
	Hispanic	−0.03 ± 0.25	0.87 ± 0.44	−0.29 ± 0.41	−0.29 V 0.40
Hypertension		0.52 ± 0.26[a]	0.17 ± 0.39	0.11 ± 0.40	−0.13 ± 0.25
Diabetes		−0.02 ± 0.27	−0.71 ± 0.55	−0.03 ± 0.32	−0.14 ± 0.28
Dyslipidemia		0.14 ± 0.34	0.18 ± 0.34	−0.55 ± 0.31	0.23 ± 0.28
Smoking		−0.43 ± 0.29	−0.45 ± 0.49	0.26 ± 0.22	0.01 ± 0.01
Cocaine use		0.22 ± 0.49	0.44 ± 0.53	−0.60 ± 0.25	0.17 ± 0.25
Latent VZV infection		0.31 ± 0.19	0.14 ± 0.18	0.03 ± 0.27	0.11 ± 0.09
Intracranial large artery atherosclerosis		1.54 ± 0.19[a]	1.13 ± 0.32[a]	0.89 ± 0.26[a]	0.51 ± 0.34
Any prior opportunistic infections		NA	NA	0.15 ± 0.27	0.44 ± 0.38
$CD4^+$ cell count <200 cells/μl at death		NA	NA	−0.04 ± 0.42	−0.27 ± 0.39
$CD4^+$ cell count nadir		NA	NA	−0.001 ± 0.001	0.001 ± 0.001
Viral load (per 50 000 copies/ml at death		NA	NA	−0.001 ± 0.001	0.03 ± 0.02
Number of years with HIV		NA	NA	0.004 ± 0.031	−0.005 ± 0.027
Number of visits with <100 cells/μl $CD4^+$ cell counts		NA	NA	0.008 ± 0.047	0.06 ± 0.02[a]
cART at death		NA	NA	0.12 ± 0.45	0.72 ± 0.33[a]

cART, combined antiretroviral therapy; NA, not applicable; VZV, varicella zoster virus. All estimates are adjusted for interadventitial diameter, artery location (proximal versus distal segments of the internal carotid, middle cerebral, posterior cerebral, etc.), country of origin, and all the variables listed in this table used in the same model.
[a]*P* value ≤0.05, a negative standardized β coefficient implies that the variable is less likely to occur in those with HIV.

however, varied by HIV status. Although intima inflammation was associated with a thinner media in HIV− cases only ($B=-0.13$, $P<0.001$), adventitial inflammation was associated with a thinner media in HIV+ cases ($B=-0.09$, $P=0.05$), but not in those without HIV. Adventitia inflammation was not associated with dolichoectasia or with a higher LWR, but adventitial inflammation in arteries with a media thickness in the 5th percentile was associated with dolichoectasia ($B=0.84$, $P=0.02$) in HIV+ cases but not in HIV− cases ($B=-19.9$, $P=0.99$).

Predictors of arterial inflammation by HIV status

In arteries from HIV− cases, intima inflammation was associated with hypertension ($B=0.52$, $P=0.05$) and ILAA ($B=1.54$, $P<0.001$) (Table 3). ILAA was also associated with adventitial inflammation ($B=1.13$, $P<0.001$) in this group. In arteries from HIV+ cases, older age ($B=0.05$/year, $P=<0.001$) and ILAA ($B=0.89$, $P=<0.001$) were associated with intima inflammation, whereas the use of cART at death ($B=0.72$, $P=0.03$) and a higher number of visits with $CD4^+$ cell counts <100 ($B=0.06$/visit, $P=0.03$) were associated with adventitial inflammation.

In post hoc analysis in HIV+ cases, adventitial inflammation was associated with cART use at death in those with prior opportunistic infections ($B=0.60$, $P=0.04$), with higher viral loads at death in those with longer HIV infection duration ($B=0.003$, $P=0.03$) or higher viral loads at death in those with chronic severe immunosuppression (i.e. greater number of premortem visits with <100 $CD4^+$ cell counts, $B=0.007$, $P=0.04$). There was no association between HIV-related variables with intima inflammation independent of atherosclerosis.

Discussion

Arterial inflammation in brain arteries was observed frequently among cases with and without HIV. In this sample, HIV+ cases had a higher inflammation score than HIV− cases, but this difference was mostly driven by a significant difference in adventitial inflammation in HIV+ cases. Intima inflammation was progressively more intense and widespread as the atherosclerotic phenotype evolved, but this effect was independent of HIV status. Inflammation in the media was typically observed in the context of intima inflammation. Among HIV− cases but not in HIV+ cases, intima inflammation was also the most important predictor of adventitial inflammation. Consequently, brain arteries in HIV+ cases appeared to have an enhanced susceptibility to develop adventitial inflammation compared with HIV− cases, independent of atherosclerosis or intimal inflammation.

The strong association noted between HIV and adventitial inflammation is of great interest. Contrary to what was seen in HIV− cases, adventitial inflammation in HIV+ cases was not entirely explained by associations with intimal inflammation because of atherosclerosis (Fig. 2). Adventitial inflammation is frequently reported in animal and human arterial specimens from HIV+ cases with 'HIV vasculopathy' [13,22,23]. HIV vasculopathy is characterized by a thin arterial wall, an atrophic media, and intimal fibrosis, but no atheromas, and thus is not typically considered to be atherosclerotic [24–26]. The source of inflammatory cells in the adventitia of those with HIV is not clear. Because the brain arteries are wrapped by the leptomeninges as they enter the skull, an association with aseptic meningitis and adventitial inflammation seems plausible. Aseptic meningitis is observed in about a fifth of patients with HIV in samples with a preponderance of immunosuppressed patients, and it is observed more frequently in brain specimens with evidence of parenchymal HIV brain disorder [4,27]. The association of adventitial inflammation as a predictor of dolichoectasia in arteries with a thin media further supports the notion that HIV vasculopathy is a form of pathological brain arterial remodeling observed predominantly in HIV cases with persistent immunosuppression, in which adventitial inflammation may be either a surrogate of immunosuppression per se or a marker of the underlying pathophysiology leading to thinning of the media and subsequent arterial dilatation. We considered latent VZV as a potential antigenic stimulus in the arterial wall given evidence that latent VZV is frequently found in cases with HIV, but adjusting for latent VZV did not change the association between HIV infection and inflammation [28,29]. Infection of the arterial wall by HIV is, in our view, a plausible hypothesis that fits well the pattern and associations reported between HIV and brain arterial remodeling; however, it is a hypothesis that remains to be tested.

Atherosclerosis has long been considered an inflammatory disease [30,31]. Macrophages are usually observed in coronary atherosclerosis and heavy inflammatory infiltrates in these plaques are considered a feature of vulnerability to rupture [32]. There is substantial evidence in HIV models of atherosclerosis (animal and human) that inflammation might also play an important role in the pathophysiology of vascular disease. For example, studies have demonstrated an increased expression of chemoattractant proteins in endothelial and other vascular cells (e.g. smooth muscle cells) upon exposure to HIV viral proteins like tat or p24 [11,22,33]. Not only is chemoattraction increased, but there is also a substantial increment in adhesive proteins in the endothelial surface, like vascular cell adhesion molecule-1 and endothelial-leukocyte adhesion molecule 1, that can facilitate the recruitment and transit of inflammatory cells into the arterial wall [34,35]. Given these data, it is not surprising that inflammatory cells (macrophages and/or

lymphocytes) are frequently reported in the arterial wall of in-vitro and in-vivo specimens with HIV with atherosclerosis [22,33].

The results presented here confirm that intima inflammation rises and spreads out to the media as ILAA severity increases. The drivers of intima inflammation in atherosclerosis are beyond the scope of this discussion, but it is worth mentioning that among HIV+ cases, older age and ILAA were the most important determinants of intima inflammation. In a prior report including the specimens used for this study, we reported that the most important predictors of ILAA in HIV+ cases were diabetes, older age, low nadir $CD4^+$ cell count, and higher $CD4^+$ cell counts at death [36]. Contextualizing both studies, we conclude that ILAA is the most important predictor of intimal inflammation irrespective of HIV, and that among those with HIV; a higher $CD4^+$ cell count may have enhanced the capability of the host to mount an inflammatory response against the arterial wall. This interpretation fits well with clinical evidence of increased aortic inflammation characterized by higher fludeoxyglucose uptake and increased soluble $CD163^+$ (a marker of monocyte/macrophage activation) in those with HIV compared with control groups matched by traditional risk factors [12]. The arterial inflammatory process in HIV appears to be systemic, as evidenced by a significant increase in serum markers of endothelial activation in patients with HIV compared with uninfected controls, only partially blunted after initiation of cART [37]. Whether the antigenic stimulus for intimal inflammation differs by HIV status is still a matter of research, but understanding the mechanisms of ILAA is important, as ILAA accounts for up to 42% of strokes in HIV patients [7].

The results presented here need to be framed in the context of several limitations, including the biases referable to autopsy samples, the lack of a homogenous sample for HIV− cases with the inclusion of non-US cases, the inherent error and lack of precision attributable to immunohistochemistry, and the lack of other markers of inflammation that may have revealed a richer description of the complex immunological cascade likely present in these arterial specimens. Additionally, $CD68^+$ antibody can cross-react with fibroblast and monocytes, but the reproduction of well known relationship between inflammation and atherosclerosis makes us believe that the cross-reaction with other cells is unlikely to reduce the significance of these findings [38]. Our study, however, has several strengths, including the relatively large sample size, the systematic processing of the specimens with reliable methods used to quantify and describe the arterial phenotypes and intensity of staining, the longitudinal data among HIV cases, as well as the comparable groups by age and sex.

We report here an association between HIV infection and inflammation in brain large arteries. Although intimal inflammation is closely linked to ILAA independent of HIV, adventitial inflammation was strongly associated with HIV and it was predictive of a thinner media and dolichoectasia. These results support a role for adventitia inflammation in the pathogenesis of HIV vasculopathy. Further understanding of the drivers of arterial inflammation may offer the opportunity to conceive novel therapies that may tackle the rising incidence of cerebrovascular disease in the HIV population.

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Conflicts of interest

J.G. has received honoraria from Prophase outside the submitted work, currently receiving a grant from the American Heart Association (13CRP14800040) and has one grant submitted for review at the Campbell Foundation related to the submitted work; K.M.: none; and M.G.: none. M.S.V.E. receives compensation for providing consultative services for Biogen IDEC, Biotelemetry/Cardionet, BMS-Pfizer Partnership, Boehringer-Ingelheim, Daiichi-Sankyo, and Janssen Pharmaceuticals; receives research support from diaDexus, Inc., and the NIH/NINDS; has given expert legal opinions on behalf of Merck/Organon (NuvaRing and stroke litigation); and serves on the National, Founders Affiliate, and New York City chapter boards of the American Heart Association/American Stroke Association. He receives royalties from UpToDate for chapters related to stroke. J.G.: none. R.M. is receiving NIH funding, royalties for On Call Neurology from Elsevier, Editorial Boards Stroke, Neurology, Annals of Neurology, JAMA Neurology. S.M. is currently receiving grants from the NIH (R25MH080663 and U24MH100931).

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