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Authors

Graham, Susan M
Nance, Robin M
Chen, Junmei
et al.

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Open Forum Infectious Diseases

MAJOR ARTICLE

Elevated Plasma von Willebrand Factor Levels Are Associated With Subsequent Ischemic Stroke in Persons With Treated HIV Infection

Susan M. Graham,[1,2,3] Robin M. Nance,[2] Junmei Chen,[4] Jennie Le,[4] Dominic W. Chung,[4] Mark M. Wurfel,[2] David L. Tirschwell,[5] Joseph R. Zunt,[1,2,3,5] Christina M. Marra,[2,5] Emily L. Ho,[5] Andrew Huffer,[5] Felicia C. Chow,[6] Jeffrey N. Martin,[7] Alice S. Ryan,[8] Heidi M. Crane,[2,9] José A. López,[4] and W. Conrad Liles[1,2,10,11]

[1]Department of Global Health, University of Washington, Seattle, Washington, USA, [2]Department of Medicine, University of Washington, Seattle, Washington, USA, [3]Department of Epidemiology, University of Washington, Seattle, Washington, USA, [4]Bloodworks Research Institute, Seattle, Washington, USA, [5]Department of Neurology, University of Washington, Seattle, Washington, USA, [6]Departments of Neurology and Medicine, University of California at San Francisco, San Francisco, California, USA, [7]Departments of Epidemiology and Biostatistics, University of California at San Francisco, San Francisco, California, USA, [8]Department of Medicine, University of Maryland, Baltimore, Maryland, USA, [9]Department of Health Services, University of Washington, Seattle, Washington, USA, [10]Department of Pathology, University of Washington, Seattle, Washington, USA, and [11]Department of Pharmacology, University of Washington, Seattle, Washington, USA

Background. We assessed whether key biomarkers of endothelial activation and hemostasis/thrombosis were elevated in individuals receiving effective antiretroviral therapy (ART) in the year before ischemic stroke.

Methods. We conducted a case–control study nested in the CFAR Network of Integrated Clinical Systems cohort, comparing 42 adjudicated cases with ischemic stroke with 83 controls matched for ART regimen. Angiopoietin-1, angiopoietin-2, C-reactive protein, interleukin-6, plasminogen activation inhibitor–1, P-selectin, serum amyloid–A, soluble CD14, ICAM-1, VCAM-1, apolipoprotein A1, ADAMTS13, and von Willebrand factor (VWF) were measured in stored plasma collected before the stroke event. We used conditional logistic regression to identify associations with ischemic stroke, with and without adjustment for Atherosclerotic Cardiovascular Disease (ASCVD) and Veterans Aging Cohort Study (VACS) scores.

Results. After adjustment for age and sex, higher plasma viral load and higher angiopoeitin-2, soluble CD14, and VWF were associated with increased odds of ischemic stroke; higher nadir CD4 count was associated with decreased odds of ischemic stroke. VWF remained associated with subsequent ischemic stroke after adjustment for ASCVD score (adjusted odds, 1.74; 95% CI, 1.01–2.98 per $\log_2$ increment). In a separate model adjusting for VACS score, only VWF (adjusted odds, 1.80; 95% CI, 1.04–3.12 per $\log_2$ increment) was associated with subsequent ischemic stroke. In a sensitivity analysis excluding participants with viral load ≥400 copies/mL, associations between VWF and ischemic stroke were attenuated, with risk estimates ranging from 1.59 to 1.64 per $\log_2$ increment.

Conclusions. Endothelial activation and related release and attachment of VWF may play an important role in ischemic stroke among persons with treated HIV infection.

Keywords. endothelial activation; hemostasis/thrombosis; HIV infection; ischemic stroke; von Willebrand factor.

While antiretroviral therapy (ART) has greatly decreased the risk of opportunistic infections among persons with HIV infection (PWH) and increased survival, there is growing recognition that PWH are at increased risk for cardiovascular disease (CVD), including ischemic stroke [1, 2]. Although the mechanistic pathways linking chronic HIV infection and long-term ART with stroke are not yet clear, mounting evidence points to a key role for chronic activation of inflammatory and hemostatic pathways [3–8]. Even prolonged effective ART may not normalize biomarkers of inflammation and coagulation [3], so a better understanding of the relationship between these pathways and the pathogenesis of HIV-associated CVD risk is urgently needed.

Endothelial activation, in particular, appears to be a critical link between immune activation, inflammation, thrombosis, and CVD in HIV infection [9]. Plasma biomarkers of endothelial activation, including soluble forms of intercellular adhesion molecule–1 (sICAM-1), vascular cell adhesion molecule–1 (sVCAM-1), and E-selectin, as well as the angiopoietin-2 to angiopoietin-1 (ANG-2:ANG-1) ratio, increase soon after HIV-1 acquisition [10], and elevated plasma sVCAM-1 and ANG-2 levels are associated with increased risk of HIV disease progression and death [11, 12]. Because endothelial cells are involved in many critical aspects

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Correspondence: Susan M. Graham, MD, MPH, PhD, HMC Box 359909, 325 Ninth Avenue, Seattle, WA 98104 (grahamsm@uw.edu).

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of vascular biology, including barrier function, immune surveillance, inflammation, blood clotting, and atherosclerosis, ongoing endothelial activation likely accelerates development of CVD. For example, both sICAM-1 and sVCAM-1 have been implicated as biomarkers of symptomatic atherosclerotic plaque [13, 14].

Chronic endothelial activation is also accompanied by increased release and persistent attachment of von Willebrand factor (VWF) to the luminal surface of the endothelium, enabling platelets to adhere to the vessel wall, thereby promoting subclinical microangiopathy [12]. Elevations of VWF, especially of large multimers that represent its most adhesive forms, correlate with disease severity in several inflammatory conditions, including malaria [15], sickle cell disease [16], and prothrombotic conditions such as antiphospholipid syndrome [17]. Acute HIV infection increases plasma VWF levels, which correlate with plasma viral load, disease progression, and death [18–21]. Moreover, elevated plasma VWF levels have been associated with ischemic stroke in both PWH and populations uninfected by HIV [22–25]. We previously reported that total active VWF, a parameter that takes into account the amount and adhesive activity of VWF, and ADAMTS13, the protease that regulates adhesive activity by cleaving VWF, were positively correlated with plasma HIV-1 RNA levels in PWH [12].

The objective of the present study was to determine whether plasma biomarkers of endothelial activation (ANG-1, ANG-2, sICAM-1, sVCAM-1) and hemostasis/thrombosis (VWF antigen, ADAMTS13) are elevated among PWH receiving effective ART before the development of ischemic stroke. Our hypothesis was that endothelial activation and hemostasis/thrombosis biomarkers, including VWF levels, would be elevated in the pre-event plasma of ART-treated PWH who subsequently develop ischemic stroke relative to controls (ie, PWH who did not develop a subsequent stroke) selected from the same cohort who were matched for ART treatment regimen.

METHODS

Study Population

The CFAR Network of Integrated Clinical Systems (CNICS) is a national network of 8 HIV clinical sites that integrates data for ~30 000 participating PWH from electronic health record systems and other sources into a single research database [26]. All patients in care at each site are eligible for CNICS enrollment, which corresponds to the date each patient began HIV care at the participating site. Because CNICS data reflect clinical practice, they are less subject to volunteer and nonresponse biases than data collected in traditional cohort studies [26]. The CNICS Data Management Core has established standards for data terminology, format, verification, and quality assurance for clinical diagnoses, laboratory results, and medication data. Each site received human subjects approval for CNICS [26].

Study Design

A matched case–control design was used to evaluate associations of the biomarkers studied with subsequent ischemic stroke. Cases were CNICS participants who had attained viral suppression (ie, <400 copies/mL) on ART after enrollment, experienced an ischemic stroke ≥6 months after ART initiation, and had stored plasma available within 12 months before the stroke event. We selected controls who had attained viral suppression on ART, had not experienced an ischemic stroke, and had stored plasma available following viral suppression. In addition, controls were matched to cases by ART regimen prescribed on the date of sampling. CNICS participants who had experienced a myocardial infarction after ART initiation were excluded. Two controls were identified for each case; in some cases, controls were used for >1 case. Participants were followed in CNICS between 1997 and 2015.

Primary Outcome

Ischemic stroke events were adjudicated using a state-of-the-art protocol based on Multi-Ethnic Study of Atherosclerosis (MESA) criteria [27, 28]. Briefly, for all potential events, sites assembled de-identified packets with physician notes, radiology reports, and procedure and laboratory results and uploaded them to a central web-based platform for review by 2 neurologists, followed by a third reviewer if discrepancies occurred. Reviewers categorized each stroke as ischemic vs hemorrhagic. Ischemic strokes were further classified by whether a predisposing factor (eg, infection, illicit drug use) was present when the stroke occurred and by ischemic stroke subtype (ie, cardioembolic, large vessel atherosclerosis, or small vessel) [29].

Biomarker Predictors

A 400-μL aliquot of stored plasma from all included participants was shipped from each participating CNICS site to Seattle on dry ice and stored at −80°C before testing. The Meso Scale Discovery (Rockville, MD, USA) immunoassay platform was used to measure concentrations of ANG-1, ANG-2, C-reactive protein (CRP), interleukin 6 (IL-6), plasminogen activation inhibitor–1, P-selectin, serum amyloid A (SAA), soluble CD14, sICAM-1, sVCAM-1, apolipoprotein A1, ADAMTS13, and VWF. Biomarkers were $\log_2$-transformed to normalize skewed data and increase biological relevance, as a $\log_2$ increase corresponds to doubling of concentration (and a $\log_2$ decrease corresponds to a 50% decrease in concentration). Biomarkers of HIV disease status were also evaluated as predictors, including most recent CD4 count and viral load, peak viral load, and CD4 count nadir.

Comorbidity and Health-Related Variables

Variables from the CNICS data repository included demographic characteristics (sex, age, and race/ethnicity) and clinical data including stroke risk factors, vital signs, and

laboratory measures. Hypertension was defined as mean systolic blood pressure (BP) >140 mmHg or diastolic BP >90 mmHg in the previous 6 months or use of antihypertensive drugs [30]. Diabetes was defined as a hemoglobin A1c level >6.5% or use of a diabetes-specific medication such as insulin or a diabetes-related medication frequently but not exclusively used to treat diabetes (eg, biguanides) in the setting of a diabetes diagnosis [31]. To optimize power given the relatively small sample size, calculated risk scores were evaluated as potential confounders in separate models due to the overlap in score components. The Atherosclerotic Cardiovascular Disease (ASCVD) score, which predicts 10-year risk for atherosclerotic CVD [32], is based on sex, age, race, systolic blood pressure, hypertension treatment status, diabetes status, tobacco use, total cholesterol, and high-density lipoproteins. The Veterans Aging Cohort Study (VACS) index, which predicts both all-cause and cardiovascular mortality in PWH [33], is based on sex, age, race, hemoglobin, platelet count, creatinine, aspartate aminotransferase, alanine aminotransferase, hepatitis C status, CD4 count, and plasma viral load.

Data Analysis

To retain analytic power, missing data were imputed before regression analysis using multiple imputation by chained equations. Demographic and clinical data at the time of sample collection were summarized using descriptive statistics. Dot plots were created to visualize differences in the distribution of biomarker levels between stroke cases and matched controls. Pearson correlations were used to evaluate associations between biomarkers, with Bonferroni adjustment due to multiple comparisons. A heat map was generated to show the strength and direction of correlations.

Conditional logistic regression was performed to determine if individual biomarkers were independently associated with ischemic stroke in unadjusted analyses and after adjustment for age and sex (Model 1), for ASCVD score (Model 2), and for VACS score (Model 3). Odds ratios (ORs) from these analyses indicate the increased odds for each $\log_2$ increase in biomarker level, $\log_{10}$ increase in viral load or 100-cell/μL increase in CD4 count. Because some participants had viremia on the date of sampling despite attaining viral suppression after CNICS enrollment, we conducted a sensitivity analysis in which the sample was restricted to those individuals with a plasma viral load <400 copies/mL. The impact of adjustment for years on ART was evaluated for each analysis. Stata, version 14.2 (StataCorps, College Station, TX, USA), was used, and P values <.05 were considered significant.

RESULTS

Study Population

The study population consisted of 42 cases (ie, individuals with a subsequent ischemic stroke event) and 83 matched controls, as 1 control sample had insufficient volume for testing. Table 1 presents demographic and clinical characteristics of cases and controls, indicating which variables are included in the ASCVD and VACS scores. Most stroke cases (30; 71.4%) had no precipitating factor identified, while 7 (16.7%) had an infection such as endocarditis or sepsis and 5 (11.9%) occurred in PWH who used illicit drugs such as cocaine. Stroke subtypes included small vessel (13; 31.0%), other (10; 23.8%), unknown (7; 16.7%), cardioembolic (5; 11.9%), large vessel extracranial atheroembolic (4; 9.5%), and large vessel intracranial aeroembolism (3; 7.1%). The median time on ART (interquartile range [IQR]) was 5.1 (2.3–11.7) years for cases and 5.8 (2.1–11.5) years for controls. For ART, 45.6% of participants were taking a protease inhibitor (PI)–based regimen; 32.8% a nonnucleoside reverse transcriptase inhibitor (NNRTI)–based regimen; 8% a regimen including both PI and NNRTI drugs; 6.4% a "salvage-type" regimen including a PI, NNRTI, and integrase strand transfer inhibitor (INSTI); and 2.4% a triple nucleotide reverse transcriptase inhibitor (NRTI)–based regimen without other classes. Overall, 84% of participants had a viral load <400 copies/mL at the time of sampling; 10 cases and 10 controls had viral loads over this threshold. In general, cases were older, non-Caucasian, and had lower CD4 counts, higher viral loads, and higher ASCVD and VACS scores than controls.

Correlations

Figure 1 presents a heat map of correlations between biomarkers, ASCVD score, and VACS score. Other than expected correlations between CD4 count and viral load measures, correlations that were significant at $P < .01$ after Bonferroni adjustment were the following: ANG-2 and IL-6 ($r = 0.4455$), ANG-2 and VACS score ($r = 0.5449$), IL-6 and SAA ($r = 0.4634$), and VACS score and ASCVD score ($r = 0.4145$). Moderate positive correlations (r between 0.2 and 0.4) were seen between VWF and ASCVD score and between WVF, sCD14, and IL-6, none of which were significant after Bonferroni adjustment.

Regression Analysis

Table 2 presents the results of bivariable and multivariable conditional logistic regression. Figure 2 presents dot plots of biomarker levels in cases and controls that differed at $P < .20$ in unadjusted analysis. In the model adjusted for age and sex only (Model 1), higher plasma viral load and higher ANG-2, sCD14, and VWF were associated with increased odds of ischemic stroke; higher nadir CD4 count was associated with decreased odds of ischemic stroke. In the model adjusted for ASCVD score (Model 2), plasma viral load (adjusted odds ratio [AOR], 2.11; 95% CI, 1.16–3.84 per $\log_{10}$ increment), nadir CD4 count (AOR, 0.65; 95% CI, 0.45–0.95 per 100 cells), ANG-2 (AOR, 2.07; 95% CI, 1.16–3.68 per $\log_2$ increment), and VWF (AOR, 1.74; 95% CI, 1.01–2.98 per $\log_2$ increment) remained associated with ischemic stroke; IL-6 and SAA

Table 1. Characteristics of Cases and Controls at the Time of Sample Collection[a]

Characteristic	No. (%) or Mean (SD)	
	Stroke Cases (n = 42)	Controls (n = 83)
Matching variable		
ART regimen type		
PI	19 (45.2)	38 (45.8)
NNRTI	13 (31.0)	28 (33.7)
NNRTI/PI	4 (9.5)	6 (7.2)
INSTI/NNRTI/PI	3 (7.1)	5 (6.0)
NRTI	1 (2.4)	2 (2.4)
None	2 (4.8)	4 (4.8)
Sociodemographic characteristics		
Male sex[b,c]	32 (76.2)	65 (78.3)
Age at specimen collection, y[b,c]	51.8 (11.0)	45.3 (9.4)
Race[b,c]		
White	9 (21.4)	31 (37.4)
Black	28 (66.7)	44 (53.0)
Hispanic	4 (9.5)	8 (9.6)
Other	1 (2.4)	0
Clinical characteristics		
Diabetes history[b]	12 (28.6)	8 (9.6)
Hypertension history[b]	23 (54.8)	26 (31.3)
Systolic blood pressure[b]	130 (22)	125 (14)
Tobacco use[b]	17 (40.5)	21 (25.3)
Laboratory values		
Hemoglobin[c]	12.7 (2.1)	14.1 (1.9)
Platelets[c]	253 (131)	234 (64)
Creatinine[c]	1.1 (0.5)	1.4 (1.8)
Alanine aminotransaminase[c]	45.5 (53.7)	46.3 (35.0)
Aspartate aminotransferase[c]	42.6 (43.8)	41.8 (32.1)
Total cholesterol[b]	183.0 (62.6)	177.9 (43.5)
High-density lipoprotein[b]	49.3 (17.7)	48.9 (15.6)
Hepatitis C infection status[c]	17 (40.5)	33 (39.8)
HIV-related characteristics		
Years on ART	6.7 (5.5)	7.2 (5.9)
Log_{10} HIV viral load, IU/mL[c]	2.3 (1.3)	1.8 (0.8)
Log_{10} peak HIV viral load, IU/mL	4.9 (1.1)	4.6 (1.1)
CD4 count, cells/μL[c]	418 (332)	516 (305)
Nadir CD4, cells/μL	122 (132)	192 (164)
Viral suppression (<400 copies/mL)	32 (76.2)	73 (88.0)
Composite risk scores		
ASCVD score	0.14 (0.10)	0.06 (0.07)
VACS score	41.3 (21.5)	22.0 (19.5)
Biomarkers	Median (IQR)	
Angiopoietin-1, ng/mL	3.5 (1.7–7.9)	4.2 (1.8–7.9)
Angiopoietin-2, ng/mL	13.8 (8.9–19.6)	9.6 (6.9–14.5)
C-reactive protein, mcg/mL	2.7 (0.7–9.3)	1.6 (0.5–5.7)
Interleukin-6, pg/mL	1.1 (0.6–2.1)	0.6 (0.4–1.5)
Plasma activation inhibitor–1, mcg/mL	0.7 (0.2–1.0)	0.7 (0.4–1.0)
P-selectin, ng/mL	47.9 (31.8–60.1)	44.1 (28.6–58.3)
Soluble ICAM-1, mcg/mL	0.3 (0.3–0.4)	0.3 (0.2–0.4)
Soluble VCAM-1, mcg/mL	0.3 (0.3–0.5)	0.3 (0.3–0.5)
Serum amyloid A, mcg/mL	4.2 (1.7–12.7)	2.6 (1.4–5.3)
Soluble CD14, mcg/mL	2.4 (1.8–3.0)	2.1 (1.8–2.7)
Apolipoprotein A1, mcg/mL	0.09 (0.07–0.13)	0.10 (0.07–0.14)
ADAMTS-13, ng/mL	0.15 (0.10–0.20)	0.14 (0.11–0.17)
Von Willebrand factor, ng/mL	18.0 (12.6–25.5)	14.4 (9.5–19.9)

Abbreviations: ART, antiretroviral therapy; ASCVD, Atherosclerotic Cardiovascular Disease score; CD4, cluster of differentiation 4; CNICS, CFAR Network of Integrated Clinical Systems; INSTI, integrase strand transfer inhibitor; IQR, interquartile range; NNRTI, non-nucleotide reverse transcriptase inhibitor; NRTI, nucleoside/nucleotide reverse transcriptase inhibitor; PI, protease inhibitor; VACS, Veterans Aging Cohort Study.

[a]Missing values were imputed using multiple imputation with chained equations. Imputation included all Table 1 variables, in addition to CNICS site, alcohol use, marijuana use, illicit drug use, coronary artery disease, heart failure, warfarin use, statin use, dyslipidemia, body mass index, glomerular filtration rate, and triglycerides.

[b]ASCVD score component.

[c]VACS score component.

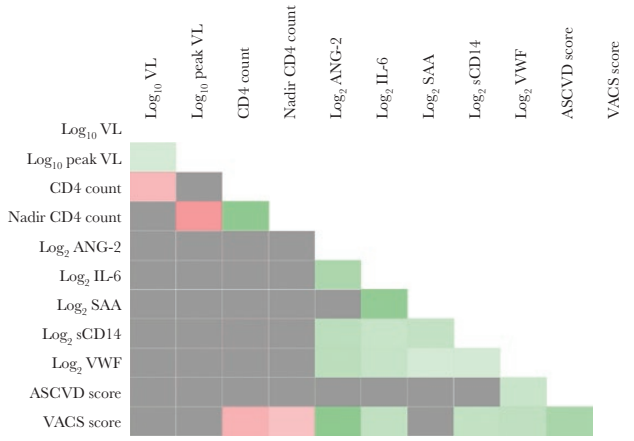

Figure 1. Heat map of correlations between biomarkers, ASCVD score, and VACS score. Biomarkers included are measures for which cases and controls differed in unadjusted conditional logistic regression analysis by $P < .20$. Negative correlations are in red, with a perfect negative correlation (ie, a Pearson coefficient of –1) in dark red. Positive correlations are in green, with a perfect positive correlation (ie, a Pearson coefficient of +1) in dark green. Nonsignificant correlations are in gray. Abbreviations: ANG-2, angiopoietin-2; ASCVD, Atherosclerotic Cardiovascular Disease score; CD4, cluster of differentiation 4; IL-6, interleukin-6; SAA, serum amyloid A; sCD14, soluble CD14; VACS, Veterans Aging Cohort Study; VL, viral load; VWF, von Willibrand factor.

became significant predictors (AOR, 1.77; 95% CI, 1.15–2.73; and AOR, 1.26; 95% CI, 1.01–1.57, respectively), and sCD14 was not significant. After adjustment for VACS score (Model 3), only VWF (AOR, 1.80; 95% CI, 1.04–3.12) was associated with ischemic stroke. These results did not change with adjustment for years on ART.

Sensitivity Analysis

Table 3 presents the results of a sensitivity analysis restricted to the 89 individuals with plasma viral load <400 copies/mL who had at least 1 matched case or control. In this analysis, the association of higher levels of VWF with an increased odds of ischemic stroke was of borderline significance in unadjusted analysis, but was not significant in adjusted models, with or without further adjustment for years on ART.

DISCUSSION

In this case–control study, plasma viral load, ANG-2, sCD14, and VWF levels were all elevated, after adjustment for age and sex, in treated PWH in the 12 months before an ischemic stroke, compared with PWH taking the same ART regimen who did not subsequently develop a stroke. The nadir CD4 count was

Table 2. Associations Between Biomarkers and Ischemic Stroke Using Conditional Logistic Regression With the Imputed Data Set and All Participants Included (n = 125)

Biomarker	OR (95% CI)	*P* Value	Model 1: Adjusted for Age and Sex AOR (95% CI)	*P* Value	Model 2: Adjusted for ASCVD Score AOR (95% CI)	*P* Value	Model 3: Adjusted for VACS Score AOR (95% CI)	*P* Value
Log_{10} viral load	1.84 (1.13–3.01)	.01	2.15 (1.18–3.92)	.01	2.11 (1.16–3.84)	.01	1.29 (0.73–2.27)	.38
Log_{10} peak viral load	1.33 (0.90–1.97)	.16	1.45 (0.94–2.24)	.09	1.33 (0.86–2.07)	.20	1.25 (0.78–2.00)	.35
CD4 count, per 100 cells	0.90 (0.79–1.03)	.12	0.87 (0.75–1.01)	.06	0.87 (0.74–1.02)	.09	1.06 (0.91–1.24)	.43
Nadir CD4 count, per 100 cells	0.67 (0.49–0.94)	.02	0.69 (0.49–0.98)	.04	0.65 (0.45–0.95)	.03	0.84 (0.57–1.24)	.37
Log_2 angiopoietin-1	0.91 (0.68–1.24)	.57	0.92 (0.67–1.28)	.64	0.90 (0.63–1.29)	.57	1.05 (0.74–1.48)	.79
Log_2 angiopoietin-2	1.63 (1.05–2.52)	.03	1.73 (1.05–2.86)	.03	2.07 (1.16–3.68)	.01	0.97 (0.57–1.67)	.92
Log_2 C-reactive protein	1.11 (0.95–1.30)	.20	1.12 (0.93–1.36)	.23	1.14 (0.95–1.37)	.15	1.02 (0.84–1.24)	.85
Log_2 interleukin-6	1.42 (1.02–1.96)	.04	1.36 (0.95–1.94)	.10	1.77 (1.15–2.73)	.01	1.06 (0.71–1.57)	.79
Log_2 plasma activation inhibitor-1	0.79 (0.54–1.13)	.20	0.86 (0.57–1.31)	.49	0.81 (0.51–1.29)	.37	0.93 (0.58–1.51)	.78
Log_2 P-selectin	1.30 (0.69–2.46)	.42	1.16 (0.57–2.35)	.68	0.97 (0.47–2.01)	.94	1.18 (0.55–2.53)	.67
Log_2 soluble ICAM-1	1.49 (0.81–2.75)	.20	1.42 (0.74–2.71)	.29	1.94 (0.91–4.10)	.08	0.94 (0.43–2.05)	.87
Log_2 soluble VCAM-1	1.10 (0.65–1.86)	.73	1.16 (0.67–2.02	.60	1.51 (0.75–3.03)	.24	0.54 (0.26–1.14)	.11
Log_2 serum amyloid A	1.17 (0.97–1.41)	.10	1.20 (0.96–1.48)	.10	1.26 (1.01–1.57)	.04	1.07 (0.86–1.34)	.53
Log_2 soluble CD14	1.91 (0.84–4.35)	.13	2.57 (1.01–6.49)	.05	2.69 (0.92–7.85)	.07	0.86 (0.32–2.33)	.77
Log_2 apolipoprotein A1	0.75 (0.33–1.71)	.50	0.78 (0.31–1.93)	.59	0.77 (0.29–2.04)	.60	0.62 (0.22–1.77)	.37
Log_2 ADAMTS-13	1.37 (0.66–2.84)	.39	1.59 (0.72–3.52)	.26	2.25 (0.86–5.86)	.10	1.48 (0.60–3.64)	.40
Log_2 von Willebrand factor	1.75 (1.09–2.79)	.02	1.75 (1.03–2.97)	.04	1.74 (1.01–2.98)	.05	1.80 (1.04–3.12)	.04

Abbreviations: AOR, adjusted odds ratio; ASCVD, Atherosclerotic Cardiovascular Disease score; CD4, cluster of differentiation 4; OR, odds ratio; VACS, Veterans Aging Cohort Study.

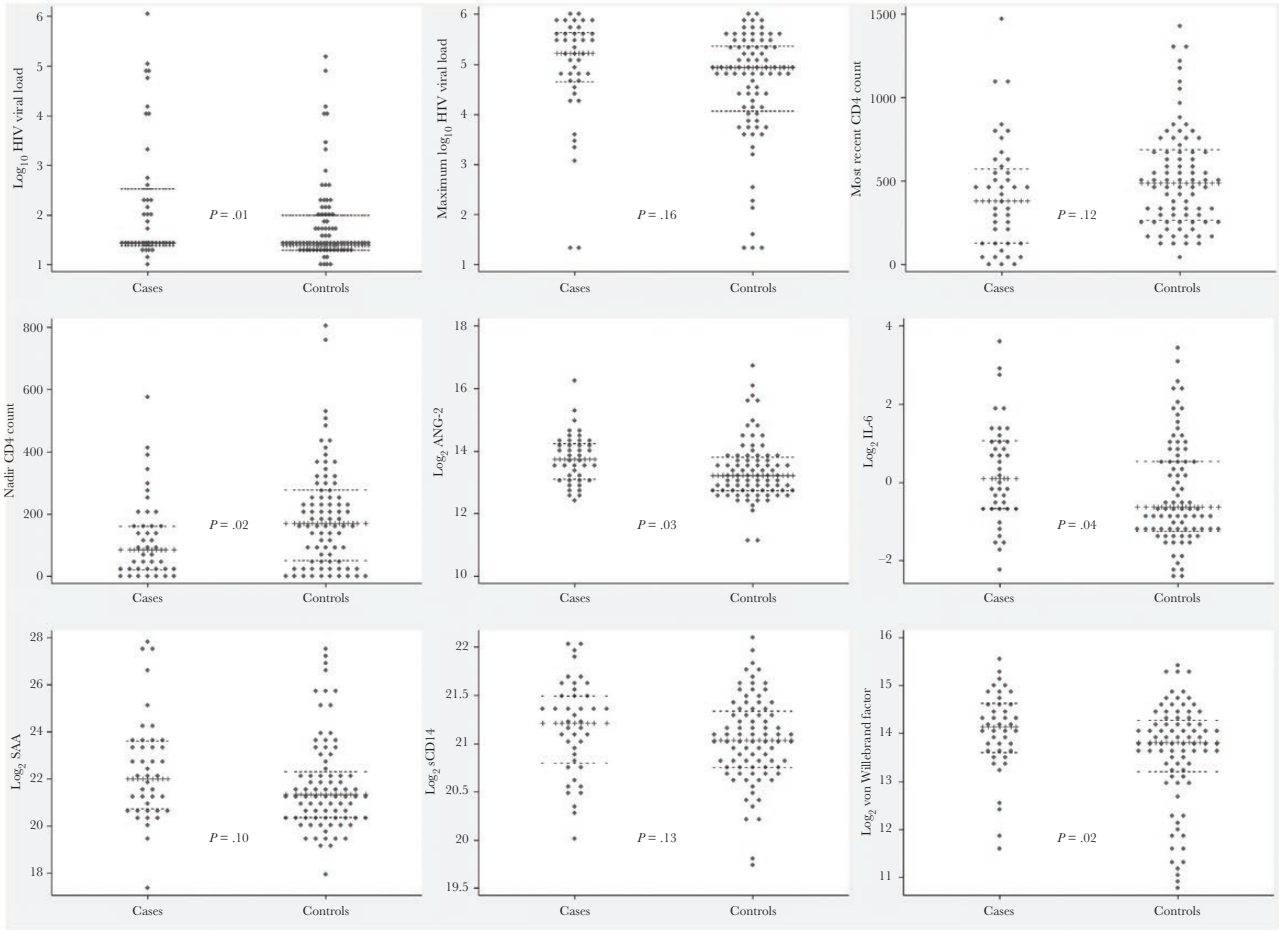

Figure 2. Dot plots of biomarker levels in cases and controls. Individual data points are graphed with gray circles, a horizontal line of black Xs indicates the median, and horizontal dashed lines indicate the upper and lower quartiles. Biomarkers presented are measures for which cases and controls differed in unadjusted conditional logistic regression analysis by $P < .20$; P values are also presented. Abbreviations: ANG-2, angiopoietin-2; CD4, cluster of differentiation 4; IL-6, interleukin-6; SAA, serum amyloid A; sCD14, soluble CD14.

also significantly lower among those who experienced a stroke. While plasma viral load, nadir CD4 count, ANG-2, IL-6, SAA, and VWF were all independently associated with ischemic stroke after adjustment for ASCVD score (a measure based on traditional CVD risk factors), only VWF was associated with ischemic stroke after adjustment for VACS score, a measure that incorporates viral load and CD4 count. In a sensitivity analysis restricted to 89 participants with a plasma viral load <400 copies/mL at the time of sampling, the association between VWF and ischemic stroke was attenuated (risk estimates ranging from 1.59 to 1.64 instead of 1.74 to 1.80), suggesting that VWF as a risk factor may be weaker when viral load is suppressed to very low levels; however, this analysis lacked sufficient power.

PWH have been found to be at increased risk for CVD events, despite effective ART [3, 4, 34–36]. The SMART trial found an increased risk of CVD events in patients undergoing CD4-guided intermittent ART compared with patients on continuous ART, accompanied by increases in the inflammatory and hemostatic biomarkers IL-6 and D-dimer [5, 6, 37, 38]. In addition, the SMART study and subsequent cohort studies demonstrated a ~40% increase in the risk of ischemic stroke in PWH compared with uninfected individuals [3, 4]. Notably, although an increasing burden of morbidity and mortality due to stroke is likely as PWH age, ischemic stroke risk may be highest in younger PWH compared with age-matched controls. In a population-based study in Taiwan, HIV infection was associated with an elevated risk of developing any stroke (adjusted hazard ratio [AHR], 1.57; 95% CI, 1.15–2.14) and specifically ischemic stroke (AHR, 1.91; 95% CI, 1.25–2.91) in patients 45 years of age and younger, but no association was observed in older age groups [39]. While some data suggest that the newer INSTI-based regimens may be associated with a lower risk of ischemic stroke than older NNRTI- and PI-based regimens [40], our study population included only 8 participants (6.4%; 3 cases and 5 controls) with regimens including the first approved INSTI (ie, raltegravir), and our method of matching by regimen precluded analysis of regimen type as a predictor.

The evidence supporting an increased risk of CVD in PWH has raised the question of whether adjunctive anti-inflammatory

Table 3. Associations Between Biomarkers and Ischemic Stroke Using Conditional Logistic Regression With the Imputed Data Set, Restricted to Matched Cases and Controls Whose Viral Load Was <400 Copies/mL (n = 89)

Biomarker	OR (95% CI)	*P* Value	Model 1: Adjusted for Age and Sex, AOR (95% CI)	*P* Value	Model 2: Adjusted for ASCVD Score, AOR (95% CI)	*P* Value	Model 3: Adjusted for VACS Score, AOR (95% CI)	*P* Value
Log_{10} viral load	2.79 (0.65–11.9)	.17	3.56 (0.62–20.4)	.16	2.74 (0.36–21.2)	.33	1.34 (0.24–7.38)	.74
Log_{10} peak viral load	1.10 (0.72–1.68)	.66	1.18 (0.75–1.86)	.48	0.90 (0.51–1.61)	.73	1.15 (0.71–1.86)	.56
CD4 count, per 100 cells	0.98 (0.85–1.14)	.83	0.97 (0.82–1.13)	.66	0.98 (0.81–1.18)	.83	1.12 (0.93–1.33)	.23
Nadir CD4 count, per 100 cells	0.74 (0.50–1.10)	.14	0.77 (0.51–1.15)	.21	0.78 (0.49–1.25)	.30	0.83 (0.54–1.29)	.41
Log_2 angiopoietin-1	0.90 (0.64–1.27)	.55	0.93 (0.64–1.36)	.72	0.87 (0.56 –1.36)	.54	1.05 (0.72–1.54)	.80
Log_2 angiopoietin-2	1.33 (0.85–2.06)	.21	1.46 (0.87–2.46)	.15	1.72 (0.96–3.10)	.07	0.95 (0.57–1.60)	.86
Log_2 C-reactive protein	1.13 (0.92–1.39)	.25	1.18 (0.92–1.52)	.20	1.15 (0.89–1.48)	.28	1.08 (0.85–1.38)	.51
Log_2 interleukin-6	1.16 (0.81–1.67)	.41	1.15 (0.77–1.72)	.49	1.31 (0.79–2.18)	.30	0.96 (0.63–1.48)	.86
Log_2 plasma activation inhibitor–1	0.73 (0.46–1.15)	.17	0.81 (0.48–1.37)	.44	0.70 (0.37–1.34)	.29	0.92 (0.52–1.63)	.79
Log_2 P-selectin	1.19 (0.58–2.46)	.64	0.97 (0.43–2.19)	.94	0.72 (0.28–1.88)	.50	1.06 (0.48–2.34)	.89
Log_2 soluble ICAM-1	1.30 (0.58–2.90)	.52	1.19 (0.50–2.88)	.69	1.48 (0.53–4.15)	.46	0.98 (0.36–2.64)	.97
Log_2 soluble VCAM-1	0.98 (0.51–1.91)	.96	1.00 (0.49–2.07)	.99	1.24 (0.54–2.84)	.61	0.41 (0.15–1.11)	.08
Log_2 serum amyloid A	1.09 (0.86–1.40)	.47	1.15 (0.86–1.54)	.36	1.15 (0.86–1.55)	.34	1.05 (0.80–1.38)	.71
Log_2 soluble CD14	1.35 (0.52–3.49)	.54	1.89 (0.61–5.88)	.27	1.84 (0.51–6.65)	.35	0.60 (0.18–2.00)	.40
Log_2 apolipoprotein 1	0.77 (0.27–2.17)	.62	0.73 (0.23–2.35)	.60	0.93 (0.24–3.57)	.92	0.58 (0.16–2.02)	.39
Log_2 ADAMTS-13	1.29 (0.55–3.02)	.56	1.41 (0.55–3.62)	.47	2.16 (0.63–7.36)	.22	1.62 (0.60–4.38)	.35
Log_2 von Willebrand factor	1.61 (0.99–2.62)	.06	1.60 (0.90–2.82)	.11	1.64 (0.88–3.05)	.12	1.59 (0.93–2.74)	.09

Abbreviations: AOR, adjusted odds ratio; ASCVD, Atherosclerotic Cardiovascular Disease score; CD4, cluster of differentiation 4; OR, odds ratio; VACS, Veterans Aging Cohort Study.

treatments should be combined with ART to decrease mortality [6, 41, 42]. One mechanism for inflammation in HIV relates to the death of abortively infected CD4 T cells through pyroptosis, a highly inflammatory process that leads to release of intracellular content from dying cells [43]. This content stimulates the danger-associated innate immune response, which activates endothelial cells and triggers the release of inflammatory mediators—a process that persists even after virologic suppression by ART [11]. Activated endothelial cells express adhesion receptors for leukocytes, the first step in leukocyte egress from the blood, and release VWF from their storage granules (Weibel-Palade bodies), which allows platelets to adhere to the endothelial surface [44]. These events favor pathology that accelerates CVD [45]. In large vessels, leukocyte and platelet adhesion are both early steps in the development of atherosclerosis [46]. In small vessels, adherent platelets form aggregates that can occlude the vessels and produce microinfarcts [47]. Similar to our findings, a prior case–control study demonstrated that levels of VWF were elevated in young PWH with stroke, compared with both uninfected patients and PWH without stroke [25]. Samples in that study were collected after the event, and low levels of ADAMTS13 were also associated with stroke [25]. In contrast, we did not find any significant differences in ADAMTS13 between cases and controls in our study, in which samples were collected in the 12 months before the stroke had occurred.

This study has a number of limitations. First, the number of cases was small, a consequence of sample identification during the first year of stroke adjudication for CNICS. Because of this small sample size, we included 12 cases (28.6% of the total) for which a potential precipitating factor—either infection or ongoing drug use—was identified. Second, study participants were followed before modern INSTI regimens were available; this is reflected in the relatively low rates of viral suppression. Third, in open-cohort studies with replacement of participants who do not return, a difference in participants who are retained from those lost to follow-up can be a limitation. Fortunately, loss to follow-up in CNICS is relatively uncommon, at <10% [26]. Fourth, data on traditional CVD risk factors, such as body mass index, systolic blood pressure, and lipid values, were missing for up to 22% of participants, potentially limiting our ability to adjust for these factors. However, by creating multiple predictions for each missing value, multiple imputation with chained equations takes into account the data uncertainties, yielding accurate standard errors [48]. Finally, we did not have complete information on physical activity levels or use of some medications, such

as aspirin and hormone therapies, that may have affected CVD risk in the sample. Nevertheless, we were able to adjust for comprehensive and validated predictors of stroke risk, including the ASCVD score, the most commonly used assessment of CVD risk [32]. While the generalizability of our results to PWH not meeting our inclusion criteria, especially those not taking ART or in the early months of treatment, may be limited, the strengths of this study include rigorously adjudicated clinical outcomes in a prospective cohort study; matching of cases and controls by ART regimen, an important potential confounder; and the collection of plasma before the stroke event.

In conclusion, our study demonstrated that plasma VWF levels were elevated in treated PWH in the 12 months preceding an ischemic stroke event, compared with PWH who were taking the same regimen who did not experience a stroke. This association persisted after adjustment for traditional CVD risk factors and for VACS score, which includes CD4 count and plasma viral load. Assessment of circulating levels of VWF may identify subgroups of PWH with increased risk for development of adverse CVD events, including stroke. Further work to validate these findings in other clinical cohorts and to investigate potential interventions, such as the use of antiplatelet medications (eg, acetylsalicylic acid [aspirin], clopidogrel, and dipyridamole), as primary prophylaxis to reduce CVD risk in PWH with elevated VWF levels is warranted.

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