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BRIEF REPORT

Inflammation Associates With Impaired Small Arterial Elasticity Early in HIV Disease

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We estimated small arterial elasticity and used linear regression to evaluate its association with inflammatory biomarkers among antiretroviral therapy–naïve, HIV-positive patients with high CD4+ counts. After adjustment, high-sensitivity C-reactive protein and interleukin-6 were inversely associated with small arterial elasticity. These data suggest that systemic inflammation may contribute to vascular dysfunction even in very early HIV disease.

Keywords. arterial elasticity; cardiovascular disease; HIV infection; systemic inflammation; vascular dysfunction.

HIV infection is associated with higher risk of cardiovascular disease (CVD) [1], which is a leading cause of morbidity and mortality in the era of effective antiretroviral therapy (ART) [2]. HIV-associated factors that have been suggested to contribute to higher risk of CVD include immune activation, chronic inflammation, and activation of coagulation pathways [3, 4].

Small arterial elasticity (SAE) is an established measure of microvascular (dys)function [5, 6] associated with future CVD events in the general population [7] and is impaired in HIV infection [8]. Clinical hypertension leads to impaired arterial elasticity and vice versa. However, arterial elasticity impairment typically precedes changes in blood pressure (BP) [9], predicts incident hypertension [10], and is responsive to treatment [11].

The Strategic Timing of AntiRetroviral Treatment (START) Trial is an international randomized trial investigating the optimal timing for ART initiation and represents a unique asymptomatic HIV-positive, ART-naïve population with preserved immunity (CD4+ counts >500 cells/μL) and global distribution [12]. Biomarker levels were assessed in START reflective of systemic inflammation (high-sensitivity C-reactive protein [hsCRP], interleukin-6 [IL-6], and serum amyloid A [SAA]), adaptive immune activation (interleukin-27 [IL-27]), vascular injury (soluble intercellular adhesion molecule-1 [sICAM-1] and soluble vascular adhesion molecule-1 [sVCAM-1]), and coagulation (D-dimer). The START Arterial Elasticity substudy co-enrolled and consented START participants in 10 countries over 6 continents, collecting data on arterial elasticity [6]. The aim of this study was to explore cross-sectional baseline associations between the biomarkers noted above and measures of small arterial elasticity in this START substudy population. Evaluating these associations could inform future research strategies and potentially contribute to our understanding of early HIV-related CVD pathogenesis apart from the complex effects of ART.

METHODS

Study Design

The International Network for Strategic Initiatives in Global HIV Trials (INSIGHT) START trial is an international randomized clinical trial comparing immediate vs deferred initiation of ART, and the design and primary results have been reported [12]. Participants at entry in START were HIV-positive and ART-naïve with no prior AIDS event and CD4+ counts >500 cells/μL.

The Arterial Elasticity substudy co-enrolled START participants to ascertain ancillary measurements of SAE, estimated via BP waveform analysis. Clinical and plasma biomarker data were collected as part of the main START trial [12, 13]. The study presented here is of a cross-sectional design among persons at entry into the START Arterial Elasticity substudy.

Arterial Elasticity Measurement

Participants were asked to refrain from caffeine, nicotine, alcohol, antihistamines, and nonsteroidal anti-inflammatory medications for 8 hours before the study visit. The HDI/PulseWave CR-2000 (Hypertension Diagnostics, Inc., Eagan, MN) was used to estimate the radial artery BP waveform. A tonometer was placed over the radial artery of the participant's dominant arm to record the BP contour while an oscillatory BP measurement

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was taken at the brachial artery of the contralateral arm. As previously described, arterial elasticity was then estimated using a modified Windkessel model [5–7].

Biomarker Measurements

Seven plasma biomarkers were measured: D-dimer, IL-6, IL-27, hsCRP, SAA, sICAM-1, and sVCAM-1. Plasma was stored at −70°C after isolation from nonfasting blood collected in EDTA tubes. D-dimer was measured using an enzyme-linked fluorescence assay (VIDAS bioMerieux), IL-6 using a high-sensitivity enzyme-linked immunosorbent assay (R&D Systems), and IL-27 using an electro-chemiluminescence (ECL) immunoassay (Meso Scale Diagnostics). A multiplex ECL was used to measure hsCRP, SAA, sICAM-1, and sVCAM-1 concurrently (Vascular Injury Panel 2, Meso Scale Diagnostics). These methods are consistent with those used in previous work [13].

Statistical Methods

The START Arterial Elasticity substudy co-enrolled 337 participants [6]. Excluded from analyses were those with HIV-negative testing (n = 1), prior CVD (n = 4), missing waveform measurements (n = 4), or missing biomarker measurements (n = 2), resulting in a final sample size of 326.

SAE was approximately normally distributed, and crude associations were all reasonably linear; no continuous variables were therefore categorized. Biomarkers were transformed on a $\log_2$ scale for all analyses to aid interpretation of model output. Specifically, regression coefficients for biomarker predictors will estimate the difference in SAE per $\log_2$-unit increment in, or 2-fold higher, biomarker level.

SAE was modeled using linear regression. Fully adjusted models included predictors for sex, age, race/ethnicity, CD4+ count (cells/μL), HIV viral load ($\log_{10}$ RNA copies/mL), current smoking, hypertension (defined as systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of BP-lowering therapy), body mass index (BMI; kg/m^2), high-density lipoprotein cholesterol (HDL-c; mg/dL), and total cholesterol (mg/dL). Other relevant factors, such as hepatitis B or C diagnosis, diabetes, and use of lipid-lowering therapy, occurred too infrequently in this sample to include. Finally, because these biomarkers do not exist in isolation in vivo, an additional model was fit with all biomarkers included simultaneously, controlling for the same set of covariates in the fully adjusted models described above.

Coefficients of determination (R^2) were used to assess model fit, interpreted as the proportion of the variance in SAE explained by the predictors in the model. All analyses were conducted using SAS, version 9.4, and a 2-sided type I error probability of .05.

RESULTS

Baseline demographic, clinical characteristics, and their associations with SAE in this study have been presented previously [6]. This sample was both young and diverse, with a median (interquartile range [IQR]) age of 33 (28–41) years and 34% white, 24% black, and 37% Asian self-identified race/ethnicity. Seventy percent of participants were male, and 29% were self-reported current smokers. The median (IQR) CD4+ count was 625 (562–728) cells/μL, and the HIV viral load was 4.2 (3.7–4.7) $\log_{10}$ RNA copies/mL. The overall mean (SD) SAE was 7.4 (3.0) mL/mmHg×100.

Table 1 presents 15 linear regression models: 7 univariable models, 7 fully adjusted multivariable models assessing biomarkers individually, and 1 fully adjusted multivariable model assessing all biomarkers simultaneously. In unadjusted models, SAE was associated with $\log_2$-hsCRP ($ß$ = −0.18, P = .04), $\log_2$-IL-6 ($ß$ = −0.49, P = .006), $\log_2$-sICAM-1 ($ß$ = −0.59, P = .05), and $\log_2$-D-dimer ($ß$ = −0.99, P < .001). After full adjustment for age, sex, race/ethnicity, CD4+ count, HIV viral load, smoking, hypertension, BMI, HDL-c, and total cholesterol, $\log_2$-hsCRP ($ß$ = −0.18, P = .03) and $\log_2$-IL-6 ($ß$ = −0.56, P < .001) had independent associations with SAE at baseline. The confounding effects of age and sex largely account for the

Table 1. Associations of Baseline Biomarker Levels and Small Arterial Elasticity Evaluated via Linear Regression

	Univariable Models		Multivariable Model,[a] Markers Studied Individually		Multivariable Model,[a] Markers Studied Simultaneously	
Biomarker, $\log_2$	$ß$ (SE)	*P* Value	$ß$ (SE)	*P* Value	$ß$ (SE)	*P* Value
D-dimer, μg/mL	−0.99 (0.19)	<.001	−0.07 (0.20)	.74	0.18 (0.21)	.40
hsCRP, μg/mL	−0.18 (0.09)	.04	−0.18 (0.08)	.03	−0.08 (0.13)	.51
IL-6, pg/mL	−0.49 (0.18)	.006	−0.56 (0.16)	<.001	−0.52 (0.18)	.003
SAA, μg/mL	−0.14 (0.11)	.21	−0.13 (0.09)	.18	0.07 (0.14)	.63
IL-27, pg/mL	0.04 (0.10)	.66	−0.06 (0.09)	.49	−0.04 (0.09)	.61
sICAM-1, μg/mL	−0.59 (0.29)	.05	−0.45 (0.26)	.09	−0.70 (0.42)	.10
sVCAM-1, μg/mL	0.07 (0.31)	.82	−0.14 (0.28)	.63	0.57 (0.44)	.20

Regression coefficient ($ß$) estimates the difference in small arterial elasticity per $\log_2$-unit increment in (or 2-fold higher) biomarker level.

Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; IL-27, interleukin-27; SAA, serum amyloid A; SE, standard error of $ß$ estimate; sICAM-1, soluble intercellular adhesion molecule–1; sVCAM-1, soluble vascular adhesion molecule–1.

[a]Adjusted for sex at birth, age, race/ethnicity, CD4+ count, HIV viral load, smoking, hypertension (systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg, or use of BP-lowering therapy), body mass index, high-density lipoprotein cholesterol, and total cholesterol.

substantial difference in coefficient estimates observed for $\log_2$-D-dimer in the univariable vs fully adjusted models.

When biomarkers were considered simultaneously, only $\log_2$-IL-6 (β = –0.52, P = .003) had an association with SAE independent of the other biomarkers and demographic and clinical characteristics. That is, holding demographics, clinical characteristics, and other measured biomarkers constant, there was, on average, a 0.52-unit decrement in SAE per 2-fold higher IL-6. Overall, this final model fit well (R^2 = 0.368).

DISCUSSION

We explored associations between levels of inflammatory biomarkers and small arterial elasticity—which reflects early CVD pathogenesis—in an asymptomatic HIV-positive, ART-naïve population with high CD4+ counts. When individually evaluated, participants with higher levels of 2 biomarkers of systemic inflammation (IL-6 and hsCRP) were found to have significantly more impaired (lower) SAE after adjustment for traditional CVD risk factors. When all biomarkers were evaluated simultaneously, however, only the association between SAE and IL-6 persisted after full adjustment.

Inflammatory pathways contribute to CVD risk not only via their role in the initiation, progression, and rupture of atherosclerotic plaques [14] but also via blood pressure elevation and hypertension [15]. Our study population had a very short duration of HIV diagnosis (median, 1 year) and was also largely normotensive (87%) without known CHD. HIV infection is characterized by higher levels of IL-6 and hsCRP compared with uninfected controls, even after viral suppression [4]. Given this, hypertension may become an important manifestation of HIV-associated CVD. A recent study using insurance claims data supports this, suggesting that hypertension prevalence ranges from 25% to 65% among HIV-positive persons [16]. These observations, combined with new guidance on the diagnosis and management of hypertension [17], suggest that BP management may require increased priority in HIV clinical practice.

There are limitations to this study. First, analyses are cross-sectional. There is therefore temporal ambiguity of these relationships, and no causality can be inferred from the results. Additionally, though SAE is an established measure of microvascular (dys)function, there are presently no well-validated reference values. We did, however, make an informal comparison with participants of similar age in the general population cohort CARDIA (mean [SD] SAE, 8.1 [2.7] mL/mmHg×100; n = 1250), which suggested that values in this HIV-positive study population may be low (P. Schreiner, April 2018, personal communication).

Statistical limitations include relatively narrow biomarker interquartile ranges, limiting prediction power and possibly threatening validity. These were also exploratory analyses, leading to use of several modeling approaches and inflation of type I error. Overadjustment may be of concern with this sample size, particularly in fully adjusted models; however, the large R^2 observed in the simultaneously adjusted model suggests good model fit.

Caution should be taken in generalizing results to other HIV-positive populations, such as those who have initiated ART or are older with more substantial vascular disease. We are unable to determine whether inflammation continues to be associated with impaired arterial elasticity once viral suppression is achieved on long-standing continuous ART.

In summary, this study demonstrated a significant cross-sectional association between higher levels of systemic inflammation and impaired small arterial elasticity in an HIV-positive, ART-naïve population with preserved immunity and at relatively low risk for both AIDS and CVD. These findings support the relevance of IL-6 as a potential contributor to HIV-associated CVD—including hypertension—and have implications for targeted prevention strategies.

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