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BRIEF REPORT

Association Between the Development of Subclinical Cardiovascular Disease and Human Immunodeficiency Virus (HIV) Reservoir Markers in People With HIV on Suppressive Antiretroviral Therapy

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We report that people with human immunodeficiency virus (HIV) diagnosed with coronary artery atherosclerotic plaques display higher levels of HIV DNA compared with those without atherosclerotic plaques. In a multivariable prediction model that included 27 traditional and HIV-related risk factors, measures of HIV DNA were among the most important predictors of atherosclerotic plaque formation.

Keywords. HIV reservoir; cardiovascular diseases; HIV DNA; atherosclerotic plaque; aging.

Modern combination antiretroviral therapies (ARTs) greatly increased the life expectancy of people with HIV (PWH). As such, approximately one-half of individuals infected with HIV in the United States are more than 50 years old [1]. This observed demographic shift has led to a raise in age-related comorbidities including cardiovascular disease (CVD) [2]. In addition, PWH are at higher risk of experiencing myocardial infarction compared with individuals without HIV, even in the setting of effective ART [3]. Although some traditional risk factors for CVD are more prevalent in those with HIV, they do not fully account for the disparity between the 2 groups [4]. In PWH receiving ART, HIV persists in viral reservoirs from which viral replication reignites during treatment interruption. A larger size of the HIV reservoir has been associated with heightened levels of immune activation in some [5] but not all studies [6]. Because inflammation is a critical factor in the development of atherosclerosis, we hypothesized that the size of the HIV reservoir is associated with the development of CVD in PWH [7, 8].

METHODS

Study Participants and Computed Tomography Scan

Samples from 59 PWH enrolled in the cardiovascular imaging substudy of the Canadian HIV and Aging Cohort Study were used [9]. Participants were older than 40 years of age, had no history of overt cardiovascular disease, as ascertained by chart review and self-report, and a Framingham Risk score for overt CVD at 10 years ranging from 5% to 20%. All participants were male and had <40 HIV RNA copies/mL at the time of sampling. Blood was collected from all participants and peripheral blood mononuclear cells were isolated by Ficoll gradient and stored in liquid nitrogen until use. The total atherosclerotic plaque volume in the coronary artery was assessed by cardiac contrast-enhanced computed tomography scan. Participants with a measurable coronary artery plaque of any size were considered to have subclinical CVD [10].

Quantification of HIV DNA and Cell-associated RNA

CD4+ T cells were isolated from peripheral blood mononuclear cells by negative magnetic selection using the EasySep Human CD4+ T-cell Enrichment Kit (StemCell). HIV DNA and RNA were coextracted using the AllPrep DNA/RNA Mini Kit (Qiagen). Total (LTR-gag) and integrated (Alu-LTR) HIV genomes were quantified using ultrasensitive nested polymerase chain reaction as previously described [11]. Cell-associated LTR-gag RNA was measured by ultrasensitive seminested real-time reverse transcription polymerase chain reaction [12]. Results were expressed as HIV DNA copies per million CD4+ T cells and HIV RNA copies per proviral genome. Sequences of primers and probes are indicated in Supplementary Table 1.

Statistical Analysis

Differences in each HIV-related risk factor between individuals with or without CVD were assessed with nonparametric Mann-Whitney test, performed with GraphPad Prism 8 (GraphPad software, San Diego, CA). To assess the effect of potential confounding factors on the association between atherosclerotic plaques and measures of HIV reservoir, logistic regression analyses were implemented in R (https://cran.r-project.org). Then, multivariate prediction of presence/absence

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of atherosclerotic plaques was performed using a random forest classification model [13], as implemented in the ranger R package [14]. All statistical analyses were performed using the statistical package R version 4.0.1. Complete descriptions of statistical analyses are presented as Supplementary Material.

RESULTS

To assess possible associations between markers of HIV persistence and the presence of subclinical CVD, we measured the levels of total and integrated HIV DNA as well as cell-associated HIV RNA in isolated CD4+ cells from PWH on suppressive ART, with and without atherosclerotic plaques. Participants with atherosclerotic plaques (CVD+) displayed significantly higher frequencies of CD4+ T cells harboring total (median = CVD−; 234.7, CVD+; 627.3; $P = .0087$) and integrated HIV DNA (median = CVD−; 89.7, CVD+; 281.3; $P = .0116$) compared with those who had no plaques (CVD−) (Figure 1). Of note, when the total plaque volume for participants with detectable plaques was assessed as a continuous variable (n = 37), there was no significant correlation with these markers of HIV reservoir (data not shown). The residual activity of the reservoir, as assessed by the total/integrated HIV DNA ratio as well as by cell-associated HIV RNA levels, was not associated with the presence of CVD (Supplementary Figure 1).

To identify possible confounding factors that could underlie these associations, we first compared 28 clinical parameters between the CVD+ and CVD− groups. Participants with atherosclerotic plaques were slightly older, with a modest but significantly longer duration of HIV infection and ART history. They were also treated longer with protease inhibitors, had higher low-density lipoprotein (LDL) levels and a greater exposure to tobacco (Supplementary Table 2). Using a logistic regression prediction model with CVD risk factors, including inflammation markers and HIV-associated variables, we observed that the duration of HIV infection, the age of the participants, the time under protease inhibitor treatment, the nadir CD4 count, and the presence of hepatitis C virus co-infection were confounders of these associations, as shown by a >10% variation in the regression coefficient of both reservoir measures when 1 of these factors was included. Although the associations were attenuated, and adjusted confidence intervals included the null value, the direction of the effect size remained. There was a similar observation for exposure to tobacco when analyzed with integrated HIV DNA. In contrast, this modulation of the reservoir's coefficient was not observed when the usage of statin, the levels of LDL or high-sensitivity C-reactive protein and duration of nontreated infection were included in the model (Supplementary Table 3). This suggest that these factors were not confounders of the association between reservoir size and presence of coronary artery plaque.

To identify the relative importance of HIV reservoir markers in predicting the CVD status outcome, total and integrated HIV DNA were analyzed with clinically relevant factors in a multivariable prediction model of atherosclerotic plaques formation using random forest [14] in combination with a variable importance test [15]. We found that both measures of HIV DNA were among the top significant important predictors, along with exposure to tobacco and time under protease inhibitors (Supplementary Figure 2*A* and 2*B*). To a lesser extent, the duration of HIV infection, the duration of ART, and LDL levels were also identified as important contributors to the prediction model.

DISCUSSION

We assessed the association between subclinical CVD and markers of HIV persistence in PWH on suppressive ART. In this small study of 59 participants, individuals with coronary artery atherosclerotic plaques had significantly higher levels

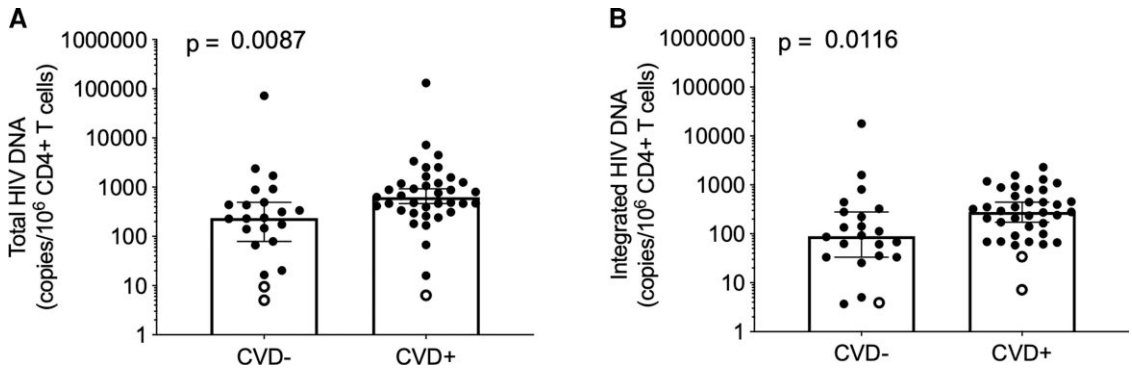

Figure 1. Association between the presence of atherosclerotic plaque and HIV DNA levels. Frequencies of CD4+ T cells harboring total HIV DNA (*A*) and integrated HIV DNA (*B*) in PWH presenting atherosclerotic plaques (CVD+, n = 37) or not (CVD−, n = 22) in the coronary artery. Samples with no detectable HIV DNA are represented by open symbols and are plotted at the limit of detection of the assay calculated from the cell input in each sample. *P* values were obtained using a nonparametric Mann-Whitney test. CVD, cardiovascular disease; HIV, human immunodeficiency virus; PWH, people with human immunodeficiency virus.

of total and integrated HIV DNA compared with those without plaques. Our results are in agreement with those by McLaughlin et al., who recently reported that increases in HIV DNA levels were associated with increased risk of incident plaque [8]. We and others previously reported that the size of the pool of cells carrying HIV DNA in virally suppressed individuals, even after many years on ART, is primarily driven by the frequency of infected cells at the time of ART initiation [16]. This suggests that the association between HIV reservoir size and the presence of atherosclerotic plaques during therapy may be a long-term consequence of heightened levels of viral replication before ART initiation. This could result in higher and more sustained levels of systemic activation leading to irreversible damage to blood vessel's walls and to the development of atherosclerotic plaques later in the course of the infection. Longitudinal measures of immune activation and HIV reservoir markers since the time of ART initiation in combination with a monitoring of atherosclerotic plaques formation would untangle the contribution of inflammation caused by viral replication in our observations.

In this study, the duration of HIV infection and the time under ART, particularly the time under protease inhibitor, were higher in people with atherosclerotic plaques. This observation might be driven by the increased dyslipidemia caused by this class of antiretroviral drug, which is associated with a higher predisposition to cardiovascular events [17]. Although the duration of HIV infection was in the top 4 contributors to our prediction models, we observed that the size of the HIV reservoir was similarly important as exposure to tobacco and time under protease inhibitors (well-known predictors of CVD) in its ability to predict subclinical CVD. Unlike the duration of HIV infection, which is unknown or inaccurate for many PWH, HIV DNA is easy to measure precisely and contributes to a better prediction of the outcome when assessed with traditional risk factors. Collectively, our results indicate that HIV reservoir measures are not strong predictors of CVD when considered individually, but were among the top significant predictors in a multivariate model including many traditional and HIV-related risk factors. Aside from the relatively small number of participants, a limitation of our study is that measures of total and integrated HIV DNA largely overestimate the size of the genetically intact and replication competent HIV reservoir. Nonetheless, our findings fuel the conversation surrounding the addition of the quantification of HIV reservoir in the standard of care, considering that this technology is easily transferable and increasingly accessible. Prospective and mechanistic studies are warranted to assess the clinical relevance of such markers and to elucidate the interplay between HIV reservoir and the development of CVD.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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