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The association of nadir CD4-T cell count and endothelial dysfunction in a healthy HIV cohort without major cardiovascular risk factors

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Abstract

Objectives: HIV-infected population may have increased risk of cardiovascular disease. The prevalence of traditional cardiovascular disease risk factors such as hypertension, diabetes and dyslipidemia in HIV-infected individuals has made it difficult to assess the direct effects of HIV and immune factors on endothelial dysfunction and associated increased risk of atherosclerosis. The purpose of this study was to investigate indicators of endothelial dysfunction in an HIV cohort without hypertension and diabetes.

Methods: We studied 19 HIV-infected patients between the ages of 25–76 years old with effectively suppressed viral load and without diagnosis of hypertension or diabetes. Endothelial function was measured by digital thermal monitoring of vascular reactivity using the VENDYS technique. Endothelial function was reported as vascular reactivity index. Systolic blood pressure and diastolic blood pressure at the time of VENDYS test were measured and latest lipid panels were recorded. The association between vascular reactivity index and CD4-T cells count, different antiretroviral therapy types (non-nucleoside reverse transcriptase, nucleoside reverse transcriptase, protease inhibitors, integrase inhibitors), vitamins use, systolic blood pressure, diastolic blood pressure, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol was investigated.

Results: Mean vascular reactivity index was 1.87 ± 0.53. Vascular reactivity index, marker of endothelial dysfunction, showed a significant correlation with lower nadir CD4 count ($p = 0.003$) as well as low-density lipoprotein cholesterol ($p = 0.02$). No additional significant correlation between vascular reactivity index and the rest of the investigated variables was found.

Conclusion: Vascular reactivity index, a clinical predictor of endothelial dysfunction, is associated with lower nadir CD4-T cell and low-density lipoprotein cholesterol in HIV-infected men with no history of hypertension or diabetes and before clinical evidence of cardiovascular disease.

Keywords

HIV, nadir CD4-T cell count, endothelial dysfunction, VENDYS

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Introduction

The advent of effective antiretroviral therapy (ART) has shifted the types of cardiovascular disease (CVD) in HIV population from pericardial effusion and dilated cardiomyopathy to atherosclerosis and heart failure.[1] Increased rates of early atherosclerosis and coronary artery disease (CAD) in HIV population have been demonstrated in several studies.[2–4] The underlying mechanisms of HIV-associated atherosclerosis is not well established. Chronic inflammation, hypercoagulability and platelet activation all contribute to endothelial dysfunction, and may be a link between HIV and its associated atherosclerosis.[5] Factors with high predictive value for endothelial dysfunction can help to identify individuals at risk. In one study, for example, the association between HIV and progression of atherosclerosis in HIV-infected patients was shown by the increased carotid intima-media thickness (IMT) assessed by ultrasound.[4] In the present study, we hypothesized that nadir CD4-T cells can be a reliable indicator of peripheral endothelial dysfunction.

The most commonly employed rating scale for assessing severity of initial infection in HIV is based upon the nadir CD4 count prior to viral suppression. One previous study by Ho et al.[6] showed an association between lower nadir CD4 count and endothelial dysfunction as indicated by reduced brachial artery flow-mediated vasodilation after a brief ischemic period induced by inflation of a blood pressure cuff. This association has not been consistently observed in other studies.[7] The study by Ho et al.[6] conducted on endothelial function in HIV has included heterogeneous cohorts where the effects of HIV-related conditions might be significant contributors to poor health outcomes. Many other studies have included individuals whose HIV was not yet virally suppressed or who had confounding risk factors such as diabetes, hypertension and dyslipidemia.[8] Some studies have determined that an increased prevalence of these risk factors, and not HIV itself, may underlie increased cardiovascular risk.[9] There is a lack of knowledge about the relationship between vascular function and CD4 nadir in patients with HIV who lack other cardiovascular risk factors. Therefore, we investigated the presence of such association in an HIV cohort with long-term effective viral suppression and without hypertension and diabetes.

Methods

Study subjects and data

We conducted a retrospective cohort study in 19 HIV-infected patients with undetectable plasma HIV RNA levels and without hypertension or diabetes. The study subjects were selected from Phil Simon HIV clinic at Huntington Hospital, Pasadena, CA. None of the individuals had documented history of myocardial infarction, angina, stroke, transient ischemic attack, history of an invasive procedure for CVD such as coronary artery bypass graft or angioplasty. The protocol and consent were approved by Quorum Review (now Advarra) Institutional Review Board. Written informed consent was obtained from all participants.

The following data were collected: nadir CD4-T cell counts, current CD4-T cell count, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), systolic blood pressure (SBP) and diastolic blood pressure (DBP), ART types (non-nucleoside reverse transcriptase (NNRTI), nucleoside reverse transcriptase (NRTI), protease inhibitor (PI), integrase inhibitor (INI)), use of vitamins, sex, age and body mass index (BMI). Diabetes mellitus was defined as fasting glucose $\geqslant$126 mg/dL or the use of hypoglycemic medications. Resting blood pressure was measured in the seated position. Hypertension was defined as a SBP $\geqslant$140 mmHg, DBP $\geqslant$90 mmHg or use of medication for treatment of hypertension. BMI was calculated as weight (kg)/(height (m))^2. CD4-T cell measurements were obtained from Lymphocytes Subset Panel 4 that were mostly done at Quest Diagnostic Laboratories. Quest Diagnostic Laboratories uses flow cytometry (FCM) as its methodology. FCM includes enumeration of mature T cells (CD3), helper T cells (CD4) and suppressor T cells (CD8) to ensure all major T cell subsets are accounted for (the sum of helper CD4 and suppressor CD8-T cells is roughly close to the total number of CD3 positive T cells). This ensures that the absolute CD4 is not artificially decreased due to sample degradation or another artifact. Patients were not required to fast prior to this test. The research participants' medications are listed in Table S4 in Supplemental Materials.

Measurement of endothelial function

Vascular reactivity index (VRI), a marker for vascular endothelial function, was measured using digital thermal monitoring (DTM) via the VENDYS technique. All 19 patients had VENDYS test between August till October 2017. All DTM tests were performed using a VENDYS-II System (Endothelix, Houston, TX), that fully automates the arm-cuff reactive hyperemia protocol. VENDYS is a non-invasive test that monitors index fingertip temperature of the right and left hands before, during and after 5 min of suprasystolic right brachial artery occlusion. The finger temperature in the occluded arm falls during occlusion and rebounds after releasing the cuff. VENDYS uses temperature rebound in response to hyperemic blood flow and vasodilation to the forearm following occlusion of the brachial artery. The higher and faster the temperature rebound the better the endothelial function and vascular health.[10] Blood pressure cuffs were placed on one of the subject's upper arms and VENDYS skin temperature sensors were affixed to the subject's right and left index fingers. Following a 5-min period of patient and temperature stabilization, a 5-min brachial artery cuff occlusion (inflated to 30 mmHg above SBP) was performed in the right arm. After the cuff was released, hyperemic blood flow to the forearm and hand was restored

(Supplementary Figure 2). This resulted in temperature rebound in the fingertip that is directly related to the subject's hyperemic blood flow and subsequent flow-mediated macro- and microvascular dilation, a measure of endothelial function and vascular reactivity (Supplementary Figure 3). One of the common primary endpoints for the VENDYS study is VRI which is vascular reactivity index calculated based on normalized temperature rebound. This technique has been validated in numerous studies.[11–14] To prevent a preconditioning effect (which is known to occur with repeat flow-mediated vasodilation testing), the test was only performed once for each subject.

Statistical analysis

The distributions of all recorded variables including age, sex, LDL-C, HDL-C, SBP, DBP, CD4-T cell nadir, CD4-T cell count, viral load, and ART types and non-ART medications were examined. Based on their distributions, both CD4 and nadir CD4 cell counts were log transformed. One data point for HDL-C (HDL=230) was eliminated as an outlier. Preliminary univariate analyses were used to examine association of VRI with the above variables (Spearman correlations for continuous variables; Wilcoxon non-parametric tests for categorical variables). Based on these analyses, variables that were associated with VRI with a significance level of at least $p=0.1$ were entered into a multiple regression model, to determine which variables are independently predictive of VRI. Variables from the multiple regression model were considered to be statistically significant at $p<0.05$, two-tailed. Analyses were conducted using SAS v9.4.

Results

The 19 HIV-infected patients with undetectable viral load were studied. In total, 18 patients were on ART and 1 patient had undetectable viral load without treatment (elite controller). Key characteristics included mean age 48 ± 10 years; 85% men, 15% women; 10 Caucasians, 6 Asian Americans; 2 Hispanics and 1 African American; BMI $25 \pm 3\,kg/m^2$; mean SBP 118 ± 11 mmHg, DBP 71 ± 10 mmHg; HDL-C 58 ± 45 mg/dL, LDL-C 108 ± 34 mg/dL. Disease status characteristics included a nadir CD4-T cells of 391 ± 321 cells/mm^3 and current CD4-T cell count of 593 ± 336 cells/mm^3. In total, 6 out of 19 study subjects had nadir CD4 count <200 cells/μL.

VRI was 1.87 ± 0.53. In general, normal values are 2.0 or greater. Significant association between VRI and nadir CD4 count was found ($p=0.003$) (Figure 1). The equation for linear fit between VRI versus nadir CD4 count was calculated as $VRI = 1.45 + 0.001 \times CD4\ nadir$. R-value was 0.64. Lower CD4 nadir was associated with lower VRI, a marker of endothelial dysfunction. VRI was 1.5 ± 0.6 in patients with CD4 nadir <200 cells/μL and 2.0 ± 0.4 in those with CD4 nadir >200 cells/μL. Patients with CD4 nadir <200 had lower VRI and thus worse endothelial dysfunction compared to those with CD4 nadir >200 cells/μL ($p=0.04$). In addition, LDL-C was also found to be directly correlated with VRI and thus endothelial function ($p=0.02$).

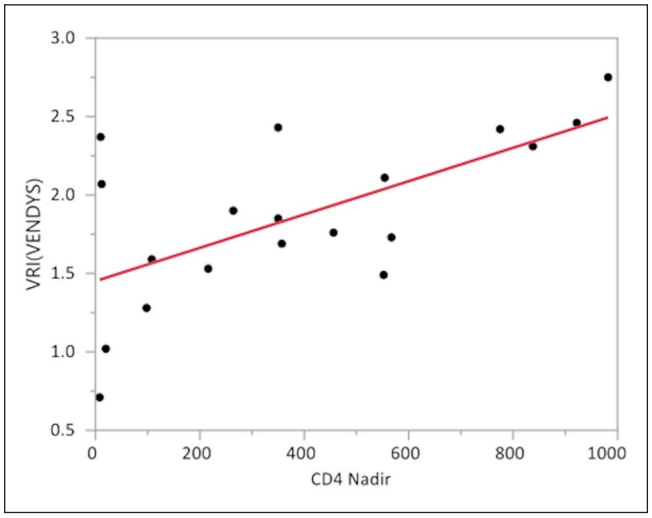

Figure 1. Association of vascular reactivity index with nadir CD4-T cell count ($p=0.003$). VRI < 1, VRI < 2 and VRI > 2 determines poor, intermediate and good endothelial function, respectively.

There was no significant association between VRI and ART types (NRTI=0.78, NNRTI=0.35, PI=0.68, INI=0.9) or the use of vitamin. In addition, no significant association between VRI and other studied variables, HDL-C ($p=0.7$), SBP ($p=0.17$), DBP ($p=0.13$) could be found.

Discussion

Endothelial dysfunction is a significant factor in the development and progression of subclinical atherosclerosis.[15–17] Advanced endothelial dysfunction correlates with future cardiovascular events in high-risk patients.[18] Thrombosis, leukocyte adhesion and smooth muscle proliferation play an important role in endothelial dysfunction and subsequent development and progression of atherosclerosis. The increased endothelial dysfunction has often been attributed to common atherosclerotic risk factors such as hypertension, diabetes and dyslipidemia that are prevalent in HIV cohort. Some studies showed worse endothelial dysfunction with higher viral load.[19,20] The diagram of the association between HIV and endothelial dysfunction and subsequent atherosclerosis is shown in Supplementary Figure 1. The HIV cohort in our study did not have hypertension or diabetes and all had undetectable HIV RNA viral load. The goal of our study was to investigate factors that are independently associated with endothelial dysfunction in an otherwise healthy cohort with long-term effective viral suppression

and without confounding from hypertension, diabetes and in the absence of any clinical sign of CVD.

In our study, we found that lower nadir CD4+ T cell count was independently a strong indicator of endothelial dysfunction as measured by VENDYS technique as shown in Figure 1. Several other studies have also investigated the association between CD4 count and endothelial dysfunction. In one study of 74 HIV-infected cohorts with undetectable viral load and no known CVD, CD4 less than 350 cells/mm^3 was shown to strongly correlate with endothelial dysfunction.[8] In another study by Post et al.,[21] coronary artery stenosis greater than 50% was shown to be associated with lower nadir CD4+ T cell and longer treatment with ART in HIV-infected men. Similarly, low CD4 count was associated with CVD despite being on ART.[22] In almost all of the studies, the patients had comorbidities, while in our study, the comorbidities of hypertension and diabetes were specifically not included.

The association between low nadir CD4-T cell count and accelerated endothelial dysfunction is not well understood. Advanced HIV disease is associated with T-cell proliferation, heightened T-cell activation and high levels of inflammatory markers. T-cell activation is in turn associated with lower CD4-T cell count.[23] Moreover, chronic inflammatory state was suggested to have a role in accelerating the rate of cholesterol plaque erosion and rupture. Factors that preferentially differentiate macrophages into subtypes that increase cholesterol accumulation and plaque instability (M1-type macrophages) were also found to be elevated in HIV.[24] The increase in such inflammatory cells and pro-inflammatory cytokines are markers for more advanced HIV disease and likely correlate to lower nadir CD4-T. Our finding suggests that the endothelial damage that occurs at the time of diagnosis remains a significant risk factor for potential future CVD despite recovery of CD4-T cells following initiation of treatment. In other words, each subject's current immunologic state as assessed by their latest CD4-T cell count does not eliminate the degree of endothelial damage that occurred prior to initiation of ART. Furthermore, the severity of endothelial dysfunction was more pronounced in subjects with nadir CD-4 cell count <200 cells/μL. This is consistent with higher rates of thromboembolic events found in patients with a CD4 count <200/mm^3 compared to those with a CD4 >200/mm^3 in a study by Saif et al.[25]

An interesting finding in our study was the independent and direct association between endothelial function VRI with LDL-C levels (Figure 2). Available information on the effects of HIV disease and its treatment on serum lipids is limited. This is in part due to the difficulty to separate the effects of HIV disease itself from the effects of ART. In one study, El-Sadr et al. noted that HIV seroconversion was associated with a decrease in total, HDL and LDL cholesterol levels from pre-seroconversion values and higher HIV RNA level was associated with lower levels of LDL-C.[26] In another study by Floris-Moore et al. among HIV-infected

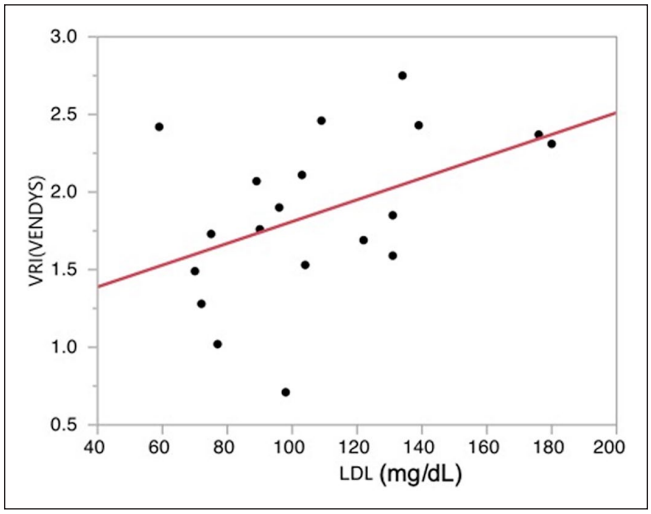

Figure 2. Association of vascular reactivity index with LDL-C ($p = 0.02$). VRI < 1, VRI < 2 and VRI > 2 determines poor, intermediate and good endothelial function, respectively.

women, after adjustment for ART use, women with CD4 lymphocyte count >500 cells/μL had LDL-C higher, on average, than women with a CD4 count <200 cells/μL.[27]

There are many structural and functional evaluations of the endothelium for early diagnosis of CVD in its asymptomatic stages. One of the strengths of our study is the use of VENDYS technique to measure vascular reactivity that can be used as a marker of endothelial function. VRI > 2 correlates to good, $1 <$ VRI < 2 correlates to intermediate and VRI < 1 correlates with poor endothelial function. Significant association between poor VRI and high Framingham Risk Score and also high coronary calcium score have been shown in previous studies.[28] Temperature rebound was shown to have the best correlation with the level of reactive hyperemia with good sensitivity to predict the status of individual's subclinical atherosclerotic disease.[11,29] In our study, the assessment of endothelial dysfunction is better correlated with the majority of the body's microvascular system compared to other techniques such as ultrasound imaging of brachial flow-mediated dilatation (FMD), that is only a measure of vascular reactivity of the conduit arteries in which the measurement is performed.

One of the limitations of our study is the small sample size, which may limit our ability to examine covariates impacting the association of endothelial function and CD4 cell count. This may also limit our statistical power. Our study was not designed for sex differences, and we had predominantly male patients in our cohort. In addition, our study was not designed for, and analyses were not adjusted for demographics (race/ethnicity). Another limitation of our study was the lack of information regarding duration of ART and duration of time from ART initiation until effective viral suppression for all the patients and thus, our results must be interpreted with caution. Finally, the observational nature of our study

addresses only associations and limits any inferences regarding causality. Further studies need to be done to investigate the initiation of ART or statins at higher CD4-T cell counts and endothelial function in HIV-infected population. Despite these limitations, the strengths of the study were a sample of HIV-infected individuals who have undetectable viral load and accurate assessment of endothelial function.

In conclusion, lower nadir CD4-T cell count was independently associated with worse endothelial function in HIV-infected patients with effectively suppressed viral load without hypertension or diabetes and before clinical evidence of CVD. This indicates current immunologic state as assessed by their latest CD4-T cell count does not eliminate the degree of endothelial damage that occurred prior to initiation of ART.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

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Informed consent

Written informed consent was obtained from all subjects before the study.

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Supplemental material

Supplemental material for this article is available online.

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