Is arterial stiffness in HIV-infected individuals associated with HIV-related factors?

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Abstract

We investigated the association between pulse wave velocity (PWV) and HIV infection, antiretroviral treatment-related characteristics, viral load, immune status, and metabolic changes in a cross-sectional study nested in a cohort of HIV/AIDS patients who have been followed for metabolic and cardiovascular changes since 2007. The study included patients recruited from the cohort (N = 261) and a comparison group (N = 82) of uninfected individuals, all enrolled from April to November 2009. Aortic stiffness was estimated using the carotid-femoral PWV (Complior-Artech, Paris, France). The groups were similar with respect to age, metabolic syndrome, diabetes mellitus, Framingham score, and use of antihypertensive and hypolipidemic medications. Hypertension was more frequent among the controls. Individuals with HIV had higher triglyceride, glucose and HDL cholesterol levels. Among individuals with HIV/AIDS, those with a nadir CD4⁺ T-cell count <200 cells/mm³ had a higher PWV (P = 0.01). There was no statistically significant difference when subjects were stratified by gender. Heart rate, age, male gender, and blood pressure were independently correlated with PWV. Nadir CD4⁺ T-cell count did not remain in the final model. There was no significance difference in PWV between HIV-infected individuals and uninfected controls. PWV was correlated with age, gender, and blood pressure across the entire population and among those infected with HIV. We recommend cohort studies to further explore the association between inflammation related to HIV infection and/or immune reconstitution and antiretroviral use and PWV.

Key words: HIV; AIDS; Antiretroviral therapy; Arterial stiffness; Pulse wave velocity

Introduction

The typical course of HIV infection has changed since 1995, when the availability of antiretroviral therapy (ART) resulted in a marked reduction in mortality rate (1). Since then, treatment-related toxicities have been identified, including an array of metabolic and body composition changes (2). Moreover the prevalence of well-established risk factors for cardiovascular disease (CVD) such as smoking, illicit drug use, dyslipidemia, and diabetes was found to be higher among HIV-infected individuals than age-matched controls. In addition, other possible factors contributing to CVD were recognized in this population such as lipodystrophy (LD), HIV-related immunodeficiency and immune activation (3,4).

A number of studies have suggested that cumulative ART is associated with a higher risk of myocardial infarction (3,5-8). Alternatively, interruption of ART was also found to be associated with increased cardiovascular risk when compared to continuous therapy (9). However, there is speculation about whether the risk assigned to this population is due to a higher prevalence of traditional cardiovascular factors or to their association with specific variables related to HIV infection itself.

Several methods have been used to assess subclinical atherosclerosis, such as tests to detect the functional capac-
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The present study, carried out at two HIV/AIDS referral centers in Brazil, was to investigate the association between PWV and HIV infection and, in HIV-infected individuals, the association between PWV and ARV treatment-related characteristics, viral load, immune status, and metabolic changes.

Material and Methods

Study population
This was a cross-sectional study (nested in a cohort) conducted from April to November 2009. The study included male and female patients treated at HIV/AIDS outpatient clinics in Oswaldo Cruz and Correa Picanço Hospitals. Participants were recruited from a cohort that had been followed for metabolic and cardiovascular changes since 2007.

Individuals attending other specialty outpatient clinics at Oswaldo Cruz University Hospital, such as dermatology, odontology, ophthalmology, and general infectious disease, were asked to participate in the study as the control group. Patients were approached by interviewers during routine medical visits.

Inclusion criteria were being older than 18 years of age and having a negative test for HIV in the previous 6 months in the case of controls; patients with clinical signs of active infection or who had been hospitalized during the past three months were excluded. Pregnant women were also excluded. This study was approved by the Research Ethics Committee of Hospital Universitário Oswaldo Cruz (protocol No. 127/2006) and all subjects gave written informed consent to participate.

Data collection
After giving informed consent, the participants filled a standardized questionnaire and had their blood pressure, heart rate and anthropometric measurements taken under standard conditions. We recorded data on smoking, illicit drug use, history of CVD, hyperlipidemia, and use of anti-hypertensive and/or hypolipidemic agents.

Detailed information about ARV therapy, CD4+ T-cell count, viral load, and a history of AIDS-defining illnesses were obtained from the medical records. Blood samples were collected after a 12-h fast and tested for CD4+ T-cell count (flow cytometry, FacsCalibur three colors, Becton Dickinson, USA), viral load (enzymatic colorimetric method, Cobas Integra 400 II, Roche Diagnostics, Germany), total cholesterol, high-density cholesterol (HDL), triglycerides, and glucose (enzymatic method, Cobas Mira, Roche Diagnostics). Low-density cholesterol (LDL) was calculated indirectly using the Friedewald formula.

Metabolic syndrome was defined according to the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (18). Hypertension was considered when the average of two measurements at different times of systolic blood pressure was equal to or greater than 140 mmHg and/or diastolic blood pressure was 90 mmHg or greater, or by self-reported treatment with medications. Diabetes mellitus was defined by fasting glucose ≥126 mg/dL or current treatment. Subjects were classified according to body mass index based on measured weight and height, as normal (≤25 kg/m²), overweight (25-29.9 kg/m²), and obese (≥30 kg/m²). The Framingham risk score, validated in the general population as a measure of coronary heart disease risk, was calculated (19).

To assess the patient’s perception of changes in body fat distribution after starting ARV therapy, the participants answered a specific standardized questionnaire, which was constructed based on the findings of other studies (20,21) that employed subjective criteria to define lipodystrophy (available from the author). Aortic stiffness was estimated by automatic and noninvasive measurement of carotid-femoral PWV according to the recommendations on procedures for the use of arterial stiffness in clinical practice (22).

The PWV measurements were performed by the same trained technician. The intraclass correlation coefficient, based on duplicate measurements taken from 20 participants, was 0.975. For the PWV measurement, we placed two transducers (TY-306, Fukuda Denshi Co., Japan) on the skin points of palpation of the right common carotid artery at the base of the neck and the right femoral artery at the groin, linked to an automatic processor from Complior (Artech, France). PWV was calculated as the distance between recording sites measured over the surface of the body, divided by the time interval between the feet of the pressure waves. The average of 10 different cardiac cycles at each site was used for analysis.

Statistical analysis
The analysis was performed in two stages. In the first stage, we compared the characteristics of HIV-infected individuals and controls and tested the association between HIV infection and PWV, with adjustment for the effect of other variables.
In the second stage, we included only HIV-infected individuals and tested the association between the characteristics of this cohort and PWV. Continuous variables are reported as means ± SD (or median and inter-quartile range, when appropriate) and categorical variables are reported as percentage. To test the statistical significance of differences between means, the continuous variables displaying normal distribution and homogeneity of variance were analyzed using parametric tests, i.e., the Student t-test and analysis of variance (ANOVA). The non-parametric Mann-Whitney and Kruskal-Wallis tests were for variables that did not have a normal distribution. The chi-square test was used to compare categorical variables. Variables that did not show a normal distribution were log-transformed to calculate the correlation coefficient. For PWV, the most appropriate transformation was inverse PWV.

We used linear regression models to test the association between PWV and all independent variables and Pearson correlation coefficients to assess the correlation between nadir CD4+ T-cell count and PWV.

Finally, all variables that showed a statistically significant association with PWV were included in the multiple regression model. We used a forward regression method for inclusion of variables in the model. Variables that exhibited a P value of less than 0.05 in their correlation with PWV remained in the final model. Statistical significance was defined as P < 0.05. Data analysis was performed using the STATA 10.0 software (USA).

Results

Characteristics of the study population

Arterial stiffness was determined in 343 individuals, 261 HIV-infected and 82 uninfected controls. The two groups were similar with respect to age, prevalence of metabolic syndrome, diabetes mellitus, and risk of coronary artery disease (CAD) (as assessed by the Framingham score) and the use of antihypertensive and hypolipidemic medications. The age of the participants ranged from 20 to 70 years, with a median of 43 years for the entire study population.

Forty-nine percent of HIV-infected individuals had hypertriglyceridemia, in contrast to 25% of the controls. Twenty-two percent of HIV-infected individuals had an elevated blood glucose level and/or diabetes compared to only 10% of controls. Low serum levels of HDL cholesterol were found in 62% of individuals with HIV and in 43% of controls. The clinical and laboratory characteristics of the participants are shown in Table 1.

| Table 1. Clinical and laboratory characteristics of the study population. |
|---------------------------------------------------------------|-----------|
| HIV-infected patients (N = 261)                               | Control subjects (N = 82) |
| Age (years)                                                  | 42 ± 8    | 42 ± 11 |
| Males                                                        | 57.8% (151)| 32.9% (27) |
| Ethnic group                                                 |           |          |
| White                                                        | 28% (73) | 24% (19) |
| Asian                                                        | 69% (160)| 74% (59) |
| Black/Mixed                                                  | 0.7% (2) | 2.5% (2) |
| Indigenous                                                   | 2% (6)   | 0%       |
| Systolic blood pressure (mmHg)                               | 122 ± 11 (261) | 124 ± 13 (82) |
| Diastolic blood pressure (mmHg)                              | 77 ± 8 (261) | 80 ± 10 (82) |
| Heart rate (bpm)                                             | 75 ± 11 (261) | 72 ± 10 (82) |
| Total cholesterol (mg/dL)                                    | 189 ± 49 (258) | 184 ± 43 (65) |
| Low-density lipoprotein (mg/dL)                              | 109 ± 42 (256) | 107 ± 38 (61) |
| High-density lipoprotein (mg/dL)                             | 40* (35-48) (256) | 48 (39-62) (74) |
| Triglycerides (mg/dL)                                        | 145* (99-216) (259) | 105 (83-149) (73) |
| Glucose (mg/dL)                                              | 89* (82-98) (257) | 81 (74-89) (77) |
| Metabolic syndrome                                           | 28% (258) | 23% (79) |
| Hypertension                                                 | 20%* (261) | 32% (82) |
| Diabetes                                                     | 2% (255) | 1% (77) |
| Smoking                                                      | 23%* (261) | 6% (81) |
| Illicit drug use                                             | 20%* (261) | 2% (81) |
| Body mass index (kg/m²)                                      | 24 ± 4* (260) | 27 ± 4 (82) |
| Use of antihypertensives                                     | 5% (261) | 6% (82) |
| Use of hypolipidemics                                       | 3% (261) | 6% (82) |
| Framingham score <10%                                        | 85% (209) | 84% (59) |
| Pulse wave velocity (m/s)                                   | 7.85 ± 1.50 (261) | 7.75 ± 1.54 (82) |

Data are reported as means ± SD or median and inter-quartile range. *P < 0.05 compared to control group (Student t-test, Mann-Whitney test and chi-square test).
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The use of ARV, NRTI, NNRTI, and the length of PI were the only variables for which ARV treatment was correlated with higher PWV measurements. Information regarding antiretroviral treatment is outlined in Table 4. These factors were no longer statistically significant after adjusting for age in the multivariate analysis model. In the multiple regression model (Table 5), heart rate, age, male gender, and systolic and diastolic blood pressures were independently correlated with PWV.

**Discussion**

This study compared treated and untreated HIV-infected individuals to uninfected controls and found similar carotid-femoral PWV measures for the two groups. Individuals with HIV had a high frequency of elevated levels of serum triglycerides and low levels of serum HDL cholesterol. The frequency of hypertension was higher in the uninfected controls (32\% vs 20\%, P = 0.02) and the frequency in HIV-infected patients was similar to previously reported values (23). Since the carotid-femoral segment of the vasculature is highly sensitive to age-related changes and increases in blood pressure (24), the higher prevalence of hypertension in the control group could explain, at least in part, the absence of association of PWV and HIV status. Even after adjusting for systolic or diastolic blood pressure in the multivariate analysis, the results remained unchanged.

Systolic and diastolic blood pressure, age, male gender, and heart rate were significant predictors of PWV, findings highly consistent with those of studies conducted on unin-

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**Table 2. Clinical and laboratory determinants of pulse wave velocity (m/s) of 343 participants.**

|                              | Univariate model |                           | Multivariate model (adjusted for clinical and HIV-related covariates) |   |
|------------------------------|------------------|---------------------------|---------------------------------------------------------------------|---|
|                              | \( \beta \)   | P          | \( \beta \)   | P          |
| **Clinical**                 |                 |             |                 |             |
| HIV-infected                 | -0.0021         | 0.483       | -0.0013         | 0.575       |
| Age (years)                  | -0.0013         | 0.000       | -0.0011         | 0.000       |
| Males                        | -0.0104         | 0.000       | -0.0096         | 0.000       |
| Ethnic group: White (%)      | 0.0015          | 0.705       | -              |             |
| Smoking (%)                  | -0.0030         | 0.248       | -              |             |
| Illicit drug use (%)         | 0.0045          | 0.203       | -              |             |
| Antihypertensive medication use (%) | 0.00003      | 0.997       | -              |             |
| Systolic blood pressure (mmHg) | -0.0965     | 0.000       | -0.0308         | 0.029       |
| Diastolic blood pressure (mmHg) | -0.0857    | 0.000       | -0.0447         | 0.000       |
| Body mass index (kg/m²)      | -0.0053         | 0.491       | -              |             |
| Heart rate (bpm)             | -0.0262         | 0.003       | -0.0190         | 0.005       |
| **Laboratory**               |                 |             |                 |             |
| Low-density lipoprotein <160 (mg/dL) | 0.0058      | 0.183       | -              |             |
| Total cholesterol >200 (mg/dL) | -0.0030     | 0.271       | -              |             |
| Triglycerides (mg/dL)        | -0.0088         | 0.001       | -              |             |
| Glycemia >100 (mg/dL)        | -0.0090         | 0.006       | -              |             |

Linear regression models were used for statistical analysis.
The independent association between PWV and heart rate has been reported (25). Heart rate may be a confounding factor that should be incorporated into any analysis related to PWV (26).

Despite the small number of diabetics, we failed to detect consistent associations between diabetes mellitus and PWV. The same applies to smoking, total cholesterol and fractions and triglycerides. According to a recent systematic review, age and blood pressure were significantly and independently associated with PWV in 91 and 90% of studies, respectively (26). In the same study, the presence of diabetes mellitus was associated with PWV in 52% of studies and smoking in 14%. Five percent of studies found a significant association with total cholesterol and LDL cholesterol, and HDL was significantly associated in 11% of studies. Only 3% reported a significant association with triglyceride levels.

Preliminary studies that assessed arterial stiffness in the HIV population detected increased arterial stiffness in HIV-infected individuals compared to uninfected controls (27,28). Considering HIV treatment and the role of ARV drugs in the development of arterial stiffness, the use of ARV (29,30) and its duration (27,29,31,32) as well as the use of PI (31,32) have been associated with arterial stiffness. However, the majority of these studies were characterized by their relatively small sample size, ranging from 32 to 56 individuals, compromising their ability to determine the role of HIV-related variables in arterial stiffness. Furthermore, the interpretation of prior studies is not easy on account of differences in the profile of participants and in the methods used to assess arterial stiffness as well as due to the presence of confounding variables including traditional cardiovascular risk factors, use of distinct ARV combinations, and duration of HIV infection.

Furthermore, in 2009 van Vonderen et al. (33) observed increased stiffness in the femoral artery 24 months after the initiation of ARV, with no changes in systemic arterial stiffness assessed by aortic augmentation index. Indeed, the femoral segment of the vasculature, which consists mostly of smooth muscle cells, is characterized by greater rigidity and sensitivity to vasoactive substances, especially those of endothelial origin (24). The muscular femoral artery is also more susceptible to the effects of fat accumulation, as well as glucose intolerance and metabolic syndrome (34). In contrast, there was a reduction in the levels of endothelial adhesion molecules (ICAM-1 and VCAM-1) and levels of

| Variable                              | PWV (m/s) | P   | HIV-infected patients, N (%) |
|---------------------------------------|-----------|-----|------------------------------|
| Recent CD4+ T-cell count              |           |     |                              |
| <200 cells/mm³                        | 7.75 ± 1.51 | 0.75 | 26 (10%)                     |
| ≥200 cells/mm³                       | 7.85 ± 1.50 | 0.23 | 233 (90%)                    |
| Nadir CD4+ T-cell count               |           |     |                              |
| <200 cells/mm³                        | 8.01 ± 1.53 | 0.01 | 150 (57%)                    |
| ≥200 cells/mm³                       | 7.62 ± 1.40 |     | 110 (43%)                    |
| Recent viral load, log₁₀ copies/mL    |           |     |                              |
| <4.0                                  | 7.84 ± 1.50 | 0.94 | 224 (88%)                    |
| 4.0-5.0                               | 7.97 ± 1.64 | 0.23 | 23 (9%)                      |
| >5.0                                  | 7.78 ± 0.76 | 8 (3%)                   |
| Maximum viral load, log₁₀ copies/mL   |           |     |                              |
| <4.0                                  | 7.87 ± 1.57 | 0.11 | 59 (23%)                     |
| 4.0-5.0                               | 7.66 ± 1.49 | 87 (33%)               |
| >5.0                                  | 7.98 ± 1.45 | 114 (44%)                  |
| Length of HIV infection               |           |     |                              |
| <5 years                              | 7.75 ± 1.50 | 0.30 | 81 (31%)                     |
| ≥5 years                              | 7.89 ± 1.48 | 180 (69%)                  |
| Lipodystrophy                         |           |     |                              |
| Yes                                   | 7.96 ± 1.42 | 0.38 | 123 (53%)                    |
| No                                    | 7.86 ± 1.59 | 110 (47%)                  |
| Metabolic syndrome                    |           |     |                              |
| Yes                                   | 8.07 ± 1.38 | 0.04 | 78 (30%)                     |
| No                                    | 7.76 ± 1.52 | 180 (70%)                  |

Data are reported as means ± SD. Student t-test, one-way ANOVA, Mann-Whitney test, and Kruskal-Wallis test were used for statistical analysis.
von Willebrand factor (33).

In another study by the same investigators, 77 HIV-infected men [55 on highly active ARV therapy (HAART) and 22 treatment-naive individuals] were found to have similar PWV compared to uninfected controls (29).

A recent study compared measures of arterial stiffness using radial artery tonometry in 276 HIV-infected and 67 HIV-uninfected Rwandan women. They reported that HIV infection was not associated with greater arterial wave reflection in women with little exposure to ARV therapy and without other CVD risk factors (35). Thus, the lack of association between arterial stiffness and HIV status in our study is not surprising.

Among those infected with HIV, 89% were taking ARV therapy. The frequency of NNRTI use (66%) was similar to the PI use (57%). The average time from the diagnosis of HIV infection to being enrolled in the study was 8 years. ARV therapy-related characteristics did not appear to be associated with arterial stiffness in adjusted analyses.

LD was present in 53% of individuals using ARV therapy, in agreement with previous studies (36,37). We did not observe an association between LD and arterial stiffness, as also reported by others (29,31).

In addition, 76% of the individuals had an undetectable viral load (<50 copies/mL), and 89% had a recent CD4+ T-cell count equal to or greater than 200 cells/mm³. Data, besides reflecting the prevalent use of ARV drugs, may also suggest that the inflammatory effects of HIV on the vasculature were attenuated and, therefore, altered the pathogenic process, which contributes to CVD by means unrelated to the metabolic pathway. Endothelial activation and dysfunction have been proposed as plausible links between HIV infection and atherosclerosis (38,39).

Among HIV-infected patients, a recent CD4+ T-cell count was not significantly correlated with PWV. There were also no significant differences in PWV between subjects with CD4 count <200 and those with CD4 counts ≥200 cells/mm³, as well as with the threshold of 350 cells/mm³.

Finally, the nadir CD4+ T-cell count <200 cells/mm³ was associated with PWV, but after adjustment for clinical and HIV-related covariates it was no longer associated. Previous studies have shown conflicting results, with some reports of an association between a lower nadir CD4+ T-cell count and arterial stiffness (32,40) and other reports of no association (31). The results of a more recent trial showed that the nadir CD4+ T-cell count, as represented by a nadir CD4+ T-cell count below 350 cells/mm³, is a predictor of PWV (40). The association appeared to be independent of other factors known to influence measures of arterial stiffness such as age, blood pressure, diabetes mellitus, hypercholesterolemia, and HAART duration (40). Our study differs from the latter study because our sample included both genders and we used a different threshold for nadir CD4+ T-cell count. However, when the analysis was stratified by gender there was no association between nadir CD4+ T-cell count and PWV either for men (N = 151) or for women (N = 110), even in univariate analysis.

Our study has limitations common to a cross-sectional observational study and, as such, it is subject to potential selection biases and limitations in establishing causality. On the other hand, this is one of the largest sample sizes investigated in studies published on this issue. The novel

| Table 4. Pulse wave velocity (PWV) values in relation to characteristics associated with antiretroviral therapy. |
|---------------------------------------------------------------|
| **ARV use** | **PWV (m/s)** | **P** | **HIV-infected patients** |
|---|---|---|---|
| Yes | 7.92 ± 1.50 | 0.03 | 233 (89%) |
| No | 7.26 ± 1.25 | | 28 (11%) |
| PI use | | | |
| Yes | 7.96 ± 1.56 | 0.17 | 150 (57%) |
| No | 7.68 ± 1.36 | | 111 (43%) |
| NRTI use | | | |
| Yes | 7.92 ± 1.50 | 0.04 | 229 (88%) |
| No | 7.34 ± 1.25 | | 32 (12%) |
| NNRTI use | | | |
| Yes | 8.01 ± 1.53 | 0.01 | 173 (66%) |
| No | 7.51 ± 1.34 | | 88 (34%) |
| Time on ARV | | | |
| <2 years | 7.70 ± 1.50 | 0.27 | 55 (24%) |
| 2-4 years | 7.76 ± 1.30 | | 44 (19%) |
| >4 years | 8.05 ± 1.55 | | 134 (58%) |
| PI cumulative duration | | | |
| <2 years | 7.67 ± 1.51 | 0.02 | 54 (36%) |
| 2-4 years | 7.68 ± 1.49 | | 38 (25%) |
| >4 years | 8.41 ± 1.58 | | 58 (39%) |
| NRTI cumulative duration | | | |
| <2 years | 7.72 ± 1.52 | 0.21 | 54 (24%) |
| 2-4 years | 7.69 ± 1.27 | | 44 (19%) |
| >4 years | 8.07 ± 1.57 | | 131 (57%) |
| NNRTI cumulative duration | | | |
| <2 years | 7.90 ± 1.56 | 0.48 | 61 (35%) |
| 2-4 years | 6.33 ± 1.80 | | 30 (17%) |
| >4 years | 7.97 ± 1.40 | | 82 (48%) |

Data are reported as means ± SD or number with percent in parentheses. ARV = antiretroviral therapy; PI = protease inhibitor; NRTI = nucleoside analogue reverse transcriptase inhibitor; NNRTI = non-nucleoside analogue reverse transcriptase inhibitor. Student t-test, one-way ANOVA, Mann-Whitney test, and Kruskal-Wallis test were used for statistical analysis.
aspect of the present study is that it included treated and untreated HIV-infected patients of both genders and especially outpatient clinic subjects as controls. The sample size is also the strength of the study, which is the largest study examining arterial stiffness to date. The participants were recruited from a longitudinal cohort, and thus may provide important initial evidence addressing the impact of HIV on cardiovascular risk in ongoing follow-up studies.

The present results indicate that HIV infection was not associated with increased PWV. On the other hand, age, gender and blood pressure were strongly associated with PWV. The contribution of other cardiovascular risk factors was found to be non-significant. Our findings do not provide evidence of an association between HIV-related characteristics and PWV. However, due to common limitations in cross-sectional studies, we would recommend additional cohort studies to further explore the association between inflammation related to HIV infection and/or immune reconstitution and ARV use and PWV.

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Table 5. Clinical, laboratory, HIV- and ARV-related determinants of pulse wave velocity (m/s) of 261 HIV-infected individuals.

| Clinical                        | Univariate model | Multivariate model (adjusted for clinical and HIV-related covariates) |
|---------------------------------|------------------|---------------------------------------------------------------------|
|                                  | β                | P         | β        | P         |
| Age                             | -0.0012          | 0.000     | -0.0010  | 0.000     |
| Men                             | -0.0125          | 0.000     | -0.0094  | 0.000     |
| Ethnic group: White (%)         | -0.0002          | 0.963     | -        | -         |
| Smoking (%)                     | -0.0024          | 0.398     | -        | -         |
| Illicit drug use (%)            | 0.0047           | 0.191     | -        | -         |
| Antihypertensive medication use (%) | -0.0007         | 0.913     | -        | -         |
| Systolic blood pressure (mmHg)  | -0.0925          | 0.000     | -0.0324  | 0.051     |
| Diastolic blood pressure (mmHg) | -0.0081          | 0.000     | -0.0438  | 0.003     |
| Body mass index (kg/m²)         | 0.0020           | 0.819     | -        | -         |
| Heart rate (bpm)                | -0.0275          | 0.005     | -0.0147  | 0.065     |

| Laboratory                      |                  |           |          |           |
| Low-density lipoprotein <160 (mg/dL) | 0.0040         | 0.407     | -        | -         |
| Total cholesterol >200 (mg/dL)  | 0.0003           | 0.905     | -        | -         |
| Triglycerides (mg/dL)           | -0.0002          | 0.016     | -        | -         |
| Glycemia >100 (mg/dL)           | 0.0061           | 0.081     | -        | -         |

| HIV-related                     |                  |           |          |           |
| Length of HIV infection ≥5 years| -0.0029          | 0.358     | -        | -         |
| ARV use                         | -0.0105          | 0.024     | -        | -         |
| Time on ARV >5 years            | -0.0126          | 0.010     | -        | -         |
| Nadir CD4+ T-cell count <200 cells/mm³ | -0.0058       | 0.047     | -        | -         |
| Recent CD4+ T-cell count <200 cells/mm³ | 0.0016       | 0.730     | -        | -         |
| Current detected viral load (log₁₀ cells/mm³) | -0.0026      | 0.416     | -        | -         |
| Maximum viral load (>5.0 log₁₀ copies/mL) | -0.0043      | 0.143     | -        | -         |

| ARV-related                     |                  |           |          |           |
| PI use                          | -0.0038          | 0.190     | -        | -         |
| NNRTI use                       | -0.0082          | 0.007     | -        | -         |
| D4T use                         | -0.0057          | 0.103     | -        | -         |
| TDF use                         | -0.0031          | 0.358     | -        | -         |
| EFV use                         | 0.0010           | 0.719     | -        | -         |
| ABC use                         | 0.0184           | 0.080     | -        | -         |

ARV = antiretroviral therapy; PI = protease inhibitor; NNRTI = non-nucleoside analogue reverse transcriptase inhibitor; D4T = stavudine; TDF = tenofovir; EFZ = efavirenz; ABC = abacavir. Linear regression models were used for statistical analysis.
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