Carotid atherosclerosis in virologically suppressed HIV-patients: comparison with a healthy sample and prediction by cardiovascular risk equations

Short title: Carotid plaque in supressed HIV-patients

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ABSTRACT

Objectives: to compare the prevalence of carotid atherosclerosis in virologically suppressed HIV-patients with that of a community sample; to evaluate the capacity of various cardiovascular risk (CVR) equations for predicting carotid atherosclerosis.

Methods: Cross-sectional study with two randomly selected groups: HIV-patients from an HIV Unit and a control group drawn from the community. Participants were matched by age (30-80 years) and sex without history of cardiovascular disease. Carotid plaque, common carotid intima-media thickness (cc-IMT), and subclinical atherosclerosis (carotid plaque and/or cc-IMT>75th percentile) were assessed by carotid ultrasound. The SCORE, Framingham, REGICOR, reduced D:A:D, and COMVIH equations were applied; their ability to predict carotid plaque was compared using the area under curve (AUC).

Results: Each group included 379 subjects (77.8% men, age 49.7 years). Duration of antiretroviral therapy 15.5 years. There were no differences between the groups for carotid plaque (HIV 33.2%, control 31.3%), mean cc-IMT (HIV 0.63 mm, control 0.61 mm), or subclinical atherosclerosis (HIV 42.9%, control 47.9%). Thymidine analogues were independently associated with subclinical atherosclerosis in HIV-infected patients. CVR equations revealed AUCs between 0.715 and 0.807 for prediction of carotid plaque; prediction was better in the control group and did not improve when HIV-adapted scales were used.

Conclusions: The features of carotid atherosclerosis did not differ between the HIV-infected and the control group, although CVR equations were more predictive for carotid plaque in controls than in HIV-infected patients. HIV-specific equations did not improve prediction.

Key words: Subclinical atherosclerosis, carotid plaque, carotid intima-media thickness, cardiovascular risk equations, HIV infection

INTRODUCTION

Since the introduction of combined antiretroviral therapy (ART) in 1996, the global mortality of people living with HIV (PLWH) has decreased, mainly AIDS-related deaths, although there has been a proportional increase in deaths from non–AIDS-related disorders such as non–AIDS-related cancer, cardiovascular disease (CVD), and liver disease (1). A recent meta-analysis reported that the risk of CVD was 2-fold higher in PLWH than in the general population, as is the case with diabetes mellitus, hypertension, and smoking (2,3). Moreover, CVD appears earlier in PLWH than in age-matched noninfected individuals (4). Therefore, it is of pivotal importance to accurately detect HIV-infected patients at risk of developing CVD in order to apply preventive measures.

The cardiovascular risk (CVR) prediction equations used in the general population are usually the first approach when assessing CVR in PLWH, although they have generally underestimated CVR in this population (5-7). The data collection on adverse effects of anti-HIV drugs (D:A:D) study developed a CVR equation including CD4 count and ART in the model (8), although results for improvement in the prediction of CVR are discordant (6,7,9). The COMVIH equation, which is based on the Framingham equation, was recently calibrated to evaluate CVR in the Spanish PLWH population (10).

Carotid plaque and carotid intima-media thickness (c-IMT) are markers of subclinical atherosclerosis that are evaluated using carotid ultrasound. Both predict future cardiovascular events and have been used as surrogate markers of CVD in clinical trials (11). Studies report contradictory data on whether PLWH have an increased burden of subclinical atherosclerosis. Some found a higher c-IMT and/or occurrence of carotid plaque in PLWH than in non-PLWH (12,13), whereas others did not (14,15). However, there is notable heterogeneity regarding viral control, use of ART, and carotid measurement methods.

In general population, assessment of carotid plaque has improved prediction of CVR compared with traditional risk factors and could reclassify patients to a higher CVR (16,17). An

underestimation of carotid plaque and/or c-IMT by various CVR equations has been reported in PLWH, although data are scarce (9,18,19).

Our main objective was to assess the prevalence of carotid plaque in a population of contemporary, ART-experienced and virologically suppressed HIV-infected patients and to compare it with that of age- and sex-matched controls. The contribution of traditional and HIV-specific risk factors to carotid atherosclerosis was also analyzed. The second objective was to determine the predictive value of various CVR equations in detecting atherosclerotic carotid features in both groups. Knowledge of factors related to carotid atherosclerosis and improvement in prediction of CVR in PLWH would enable us to identify subjects who would benefit most from antiatherogenic therapies.

METHODS

Study design and patient population

The study was observational and cross-sectional and included 2 groups (HIV-infected patients and controls) matched 1:1 by sex and age (range ±5 years). HIV-infected patients were randomly selected from outpatients monitored at the HIV and STD Unit of Bellvitge University Hospital (Hospitalet de Llobregat, Spain) from 2014 to 2016. Controls came from a community–based sample randomly selected in Girona (Spain) in 2016. All participants were aged 30 to 80 years and had no personal history of CVD (acute myocardial infarction, angina, coronary angioplasty or bypass, ischemic stroke, transient ischemic attack and carotid endarterectomy), active infection, inflammatory disease or active cancer. HIV-infected patients had an undetectable viral load for at least the previous 6 months. The study was approved by the Institutional Review Boards of Bellvitge University Hospital and Parc de Salut Mar (CEIC-PSMAR, #2014/5815/I). The latter provided data for individuals from Girona. All participants gave their written informed consent.

Clinical assessment

The variables recorded were smoking, diabetes mellitus (hemoglobin A1c ≥6.5% and/or nonfasting blood glucose ≥11.1 mmol/L or fasting blood glucose ≥7.0 mmol/L, and/or antidiabetic medication), hypertension (systolic/diastolic blood pressure ≥140/90 mm Hg and/or on antihypertensive medication), and current treatment with lipid-lowering drugs. Anthropometric measures (height, weight, and waist and hip circumferences) were recorded. Variables associated with HIV infection were collected in the HIV-infected group.

Five equations to predict CVR were applied. Three were designed for the general population: Framingham and REGICOR (Framingham calibrated and validated for the Spanish population), both of which estimate the risk of coronary heart disease events within the next 10 years, and the Systematic Coronary Risk Evaluation (SCORE) which estimates the risk of fatal cardiovascular events within the next 10 years (20-22). The other 2 equations were calibrated to the HIV-infected population: COMVIH (Framingham score calibrated to Spanish PLWH) and the reduced D:A:D, both of which estimate coronary heart disease events within the next 10 years (10,23). The CVR equations categories are defined in Figure 1.

Laboratory methods

Venous blood samples were collected after an overnight fast and centrifuged. Serum/plasma aliquots were stored at −80ºC to analyze cardiovascular biomarkers. Total cholesterol (TC), triglycerides, and glucose were measured after sampling by standard enzymatic methods: high-density-lipoprotein cholesterol (HDL-c) was measured using a homogeneous direct method, and low-density-lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula when triglyceride concentrations were <300 mg/dL.

Cardiovascular biomarkers were measured only in the HIV-infected group in a central laboratory in Barcelona (Biochemistry Department, IIB-Sant Pau Hospital). Lipoprotein-associated phospholipase A2 activity was measured in serum using 2-thio-PAF (Cayman Chemical Company, Ann Arbor, Michigan, USA). High-sensitivity C-reactive protein (hs-CRP) was

quantified using immunocolorimetry (Roche Diagnostics, Basel, Switzerland) in a Cobas c501/6000 autoanalyzer. Interleukin-6 (IL-6, eBioscience Thermo Fisher, Waltham, MA, USA) and soluble clusters of differentiation 14 (sCD14, Hycult Biotech, Uden, Netherland) and 163 (sCD163, R&D Systems, Minneapolis, Minnesota, USA), were measured using semiautomated ELISA. D-Dimer and soluble vascular cell adhesion molecule (sVCAM) were measured using a multiplex analysis in a Luminex system (Milliplex, HCVD2MAG-67K-02, Merck-Millipore, Darmstadt, Germany).

Carotid ultrasound

High-resolution B-mode ultrasound imaging of the carotid arteries was performed using a DP-50/DP50T Digital Ultrasonic Diagnostic Imaging System Mindray (Nanshan, Shenzhen, China) in the HIV-group and UF-870 machine LA38 linear array transducer (Fukuda Denshi, Japan) in the control-group. The intraclass correlation coefficient between the observers in both centers was >80%. Screening for carotid plaque was performed in the common, bulb, and internal carotid segments. Carotid plaque was defined as a c-IMT value >1.5 mm. Other markers of carotid atherosclerosis were: common c-IMT (cc-IMT) and subclinical atherosclerosis. The cc-IMT value was the mean of the measures obtained at 1 cm from the left and right far wall (plaque-free) of the distal cc-IMT, a value >75th percentile for a reference population was considered pathologic. Subclinical atherosclerosis was defined as the presence of a carotid plaque and/or a cc-IMT >75th percentile for a reference population (24,25).

Statistical analysis

Our sample size enabled the detection of an odds ratio (OR) ≥1.6 for carotid plaque, assuming a prevalence of 25% (26) and accepting an α-error of 0.05 and a β-error of 0.2 in a 2-sided test.

Normality of distributions was checked using normal Q-Q plots. Continuous variables were expressed as mean (standard deviation, SD) when normally distributed and median (interquartile range, IQR) when not; categorical variables were shown as percentages. The *t* test or Mann-Whitney test was used as appropriate to compare continuous variables between groups. Qualitative variables were compared using the chi-square or Fisher exact test. The Pearson correlation coefficient was calculated to estimate the strength of the association between continuous variables. Logistic regression models to predict carotid plaque and subclinical atherosclerosis were adjusted for variables that presented a significant univariate association with the variable HIV or non-HIV or with the dependent variable (e.g. carotid plaque or subclinical atherosclerosis). The stepwise Akaike information criterion was used to choose the variables to be included in the models. The predictive performance of each CVR equation for detecting carotid plaque or subclinical atherosclerosis was based on the area under the receiving operating characteristic curve (AUC) (moderate, 0.7-0.8; good, 0.8-0-9). The AUC was compared between the control and HIV-infected group using the DeLong test for independent samples. All the statistical analyses were performed using R (R Foundation for Statistical Computing, Vienna, Austria; V.3.6.0).

RESULTS

General characteristics of participants

We assessed 1124 individuals, 450 with HIV-infection and 674 from the community. After matching by sex and age, we obtained 379 participants per group. The characteristics of both groups are described in Table 1. Mean age was 49.7 (9.5) years, and 590 (77.8%) were men. HIV-infected patients were more frequently smokers and had a lower body mass index and higher waist-to-hip ratio. They also had lower concentrations of TC and LDL-c but increased triglycerides ($p \leq 0.001$ in all cases). Lipid-lowering therapies were three times more frequent in the HIV-infected group (24.6% vs. 7.12%).

HIV-specific variables are shown in Table 1. Two participants were elite controllers (sustained undetectable viral load without combined ART).

Carotid ultrasound

Carotid plaques were equally frequent in both groups: 31.3% in controls and 33.2% in HIV-infected patients (p=0.636). The occurrence of carotid plaque increased with age (p<0.001), with no differences between groups, and was more frequent in men (30%) than in women (21.6%) (p=0.001) (Figure 2). The carotid bulb was the most common location for plaque in both groups, although it was more frequently observed in the HIV-infected group than in controls (32.1% vs. 23.9%; p=0.016). In contrast, internal carotid plaques were more frequent in controls (14% vs. 8.6%; p=0.020).

Mean cc-IMT was similar in controls and HIV-infected patients (0.61±0.11 mm and 0.63±0.13 mm, respectively; p=0.117); as for carotid plaque, there was a similar age-related increase in both groups (Figure 2). The percentage of participants with cc-IMT>75th percentile was 23.2% and 20.7% in the control and HIV-infected groups, respectively (p=0.520). Subclinical atherosclerosis was detected in 47.9% of controls and 42.4% of patients (p=0.148).

Figure 3 shows the adjusted OR for carotid plaque, cc-IMT>75th percentile, and subclinical atherosclerosis. None of the ORs analyzed, except for that of carotid bulb plaque (p=0.014), was significantly different between controls and HIV-infected group.

In the HIV-infected group, cc-IMT was positively but weakly associated with the duration of HIV infection (r= 0.21; p<0.001) and combined ART (r=0.28; p<0.001), body mass index (r=0.18, p=0.001), waist-to-hip ratio (r=0.30; p<0.001), glycemia (r=0.13; p=0.011), and TC (r=0.12; p=0.018). Negative correlations were found with CD4 count (r=0.13; p=0.013) and HIV viral load zenith (r=0.16; p=0.002).

Table 2 shows the univariate analysis of the variables associated with the occurrence of carotid

plaque. Not surprisingly, worse anthropometric variables, hypertension, dyslipidemia, and diabetes were more frequent in participants with plaque in both groups. In addition, several HIV-specific factors were also associated with carotid plaque. Plasma levels of sCD163, IL-6, D-dimer and sVCAM were significantly higher in HIV-infected patients with carotid plaque than in those without carotid plaque (table 2).

In the multivariate analyses, several traditional CVR factors such as age, smoking status, and TC were independently associated with carotid plaque and subclinical atherosclerosis in both groups; anthropometric variables in the control group and hypertension in the HIV-infected group were correlated with carotid plaque. Duration of therapy with thymidine analogues was associated with subclinical atherosclerosis in the HIV-infected group (Table 3).

Cardiovascular risk equations

All three CVR equations for the general population (Framingham, REGICOR, SCORE) showed that HIV-infected patients have a slight but significantly higher risk of future severe cardiovascular outcomes than controls ($p \leq 0.001$) (Table 1). However, when controls and HIV-infected patients were stratified by risk category (Figure 1), there were no significant differences between them in the percentage of controls or HIV-infected patients in different risk categories for Framingham ($p=0.843$), REGICOR ($p=0.375$), or SCORE ($p=1.000$). We found that the COMVIH equation in the HIV-infected group and the Framingham equation in both groups classified a higher percentage of participants in the moderate-high CVR categories (Figure 1).

CVR equations were highly correlated with cc-IMT (correlation coefficients 0.45 to 0.50; $p<0.001$ for all).

The relationship between CVR equations and carotid plaque is shown in figure 3. The presence of carotid plaque increased alongside the increase in risk category, with no significant differences according to HIV serostatus. Among participants classified as low-risk by the five equations, 21%

to 27% had carotid plaque. This percentage increased from 41% to 76% in the moderate risk category.

We calculated the predictive performance of the CVR equations for detecting carotid plaque and subclinical atherosclerosis. For carotid plaque, the AUC in the control group and HIV-infected group was 0.798 (95% confidence interval (CI), 0.751;0.845) and 0.715 (95% CI, 0.655;0.775) respectively for Framingham equation. The AUC for the SCORE equation was 0.807 (95% CI, 0.761;0.854) for the control group and 0.788 (95% CI, 0.740;0.836) for the HIV-infected group. Finally, the AUC for REGICOR equation was 0.802 (95% CI, 0.756;0.849) for the control group and 0.720 [95% CI, 0.661;0.779) for the HIV-infected group. The AUC was significantly higher in the control group for the Framingham (p=0.032) and REGICOR (p=0.032) equations. Predictive performance did not improve when HIV-adapted scales were used (0.721 (95% CI, 0.662;0.780) for COMVIH and 0.764 (95% CI, 0.710;0.819) for reduced D:A:D). The analysis of subclinical atherosclerosis did not improve predictive performance (AUC, 0.688-0.726).

DISCUSSION

The main findings of our study were as follows. First, the occurrence of carotid atherosclerosis measured by carotid plaque and cc-IMT did not differ between virologically suppressed, highly ART-experienced, HIV-infected patients and age- and sex-matched community controls. Second, traditional risk factors were closely associated with carotid atherosclerosis in HIV-infected patients and controls, although the duration of thymidine analogues was also significantly associated with carotid atherosclerosis. Third, the risk of adverse cardiovascular outcomes predicted by equations was similar in HIV-infected patients and controls. However, the Framingham and REGICOR equations were better predictors of carotid plaque in controls than in HIV-infected patients. Fourth, HIV-specific CVR equations did not improve prediction of carotid plaque.

While carotid plaques were highly prevalent in the HIV-infected group, prevalence has been

reported to vary widely in PLWH from 5% (in a less ART-experienced cohort) to 65% (9,14,27-29). We found the prevalence of carotid plaques to be similar in both patients and controls, although localization in the carotid bulb was more prevalent in HIV-infected patients. Data on the effect of HIV infection on carotid plaque are heterogeneous. No effect was observed in some cohorts (15), whereas a higher prevalence and incidence of new plaques, mainly in the carotid bulb, was observed in others (28,29). Low endothelial shear stress in the carotid bulb is associated with the stimulation of a proinflammatory phenotype at the molecular level (30). Additionally, some degree of inflammation and immune activation remains in virologically suppressed HIV-infected patients (31), and this may trigger or accelerate atherogenesis in this susceptible region. Similarly, we found an increased plasma concentration of several inflammation and immune activation biomarkers in HIV-infected patients with carotid plaque, thus reinforcing their probable role in the development of carotid atherosclerosis in PLWH.

In our study, other markers of carotid atherosclerosis, such as cc-IMT and subclinical atherosclerosis, did not differ between HIV-infected patients and controls. With regard to c-IMT, previous studies comparing HIV and non–HIV-infected subjects have shown discordant results (12-15). In a large pooled cohort comprising around 1600 HIV-infected and 600 non–HIV-infected participants, Hanna et al (15) did not find differences or even a trend toward a lower cc-IMT in HIV-infected patients aged 50-75 years. The variability in the methods used for measurement of c-IMT and in the use of ART or viral control in tested subjects may explain the discrepancies between studies.

Consistent with previous studies carried out in HIV-infected patients (13-15,32), we found that traditional CVR factors were the major determinants of carotid atherosclerosis. A similar age-related increase in the frequency of carotid atherosclerosis was found in controls and HIV-infected patients, suggesting that atherosclerosis was poorly related to HIV-associated factors. However, duration of thymidine analogues was independently associated with subclinical atherosclerosis. Data from our univariate analysis also suggest that the longer experience in the use of old ART drugs (eg, thymidine analogues and old protease inhibitors), which are highly

related with metabolic toxicity (33), may have contributed to development of atherosclerosis in the HIV-infected group.

In our sample, the Framingham and COMVIH equations classified a higher percentage of participants in the moderate or high CVR categories. Overestimation resulting from the Framingham equation has previously been reported in PLWH and in the general population (34-37). However, for the first time, the COMVIH equation has been compared with other CVR equations. A prospective follow-up with clinical endpoints would be necessary to validate these data.

Consistent with data from the general population (26,38), we found carotid atherosclerosis in a high percentage of HIV-infected patients and controls classified as low-moderate CVR categories by the equations tested, with no differences between the groups. However, few data have been reported on the accuracy of CVR equations for predicting carotid atherosclerosis (measured as carotid plaque or c-IMT) in PLWH (9,18,19). In our study, the predictive performance of CVR equations for carotid plaque in HIV-infected patients was moderate, with no improvement when HIV-specific equations were applied. Moreover, we observed poorer performance for the Framingham and REGICOR equations in HIV-infected patients. Kapelios et al (18) reported better performance for the Framingham equation in PLWH than we observed, although when both carotid and femoral plaques were analyzed together. In addition, the D:A:D equation did not improve prediction of carotid/femoral plaque (18).

Our study has several limitations. Given its cross-sectional design, we are unable to establish causal associations. We described carotid plaque categorically, without taking into account morphology, burden, or composition, all of which have been shown to improve prediction of CVR (16). The CVR equations we tested have been validated for prediction of major or fatal cardiovascular events, but not for carotid plaque; therefore, conclusions regarding the usefulness of the equations should be interpreted with caution. There are several variables related with HIV but also non related, such as illicit drug use or CV biomarkers, only available in the HIV-group

that can be associated to carotid atherosclerosis, and prevent us to compare its relation with carotid atherosclerosis in both samples. Finally, since the HIV-infected patients in our study were very ART-experienced, our results cannot be generalized to recently infected patients or those with little ART experience. The strengths of our study are its wide and well-characterized HIV-infected sample, the age- and gender-matched community sample, and the prospective collection of all the variables analyzed.

In conclusion, in a contemporary and highly ART-experienced population we did not find differences in any markers of carotid atherosclerosis compared with a matched community sample. The Framingham and REGICOR equations were better predictors of carotid plaque in controls than in HIV-infected patients and HIV-adapted CVR equations did not improve prediction of carotid atherosclerosis.

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Conflict of interest

M Saumoy: has received financial compensation for educational activities from Gilead Sciences and Janssen-Cilag.

A. Imaz and J Tiraboschi: have received financial compensation for lectures, advisory boards, and educational activities, as well as research funding from AbbVie, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcare.

D. Podzamczer: has received research grants and/or honoraria for advisories and/or conferences from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcar.

The rest of authors have nothing to declare.

Author's contributions

M. Saumoy, S. Di Yacovo and D. Podzamczer contributed to the design of the work.

M. Saumoy, S. Di Yacovo, A. Imaz, J.M. Tiraboschi, B. García, M. Grau contributed to the acquisition and analysis of data for the work.

M. Saumoy and J.M. Valdivielso : performed carotid ultrasound.

J.L. Sánchez-Quesada, S. Benitez and J. Ordoñez-Llanos designed and conducted the laboratory studies.

S. Pérez and I. Subirana performed the data analyses.

M. Saumoy, S. Di Yacovo, M. Grau and D. Podzamczer drafted the manuscript.

J. Ordoñez-Llanos critically revised the manuscript.

All gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy

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TABLE

Table 1. Characteristics of participants.

	Control group N=379	**HIV-infected group N=379**	p-value
	N=379		
Sex, men, n (%)	295 (77.8)	295 (77.8)	0.985
Age (years)	49.7 (9.59)	49.7 (9.46)	0.858
Smoking status			<0.001
Never smoker, n (%)	156 (41.2%)	82 (21.6%)	
Former smoker, n (%)	136 (35.9%	98 (25.9%)	
Current smoker, n (%)	87 (23.0%)	199 (52.5%)	
Body mass index (kg/m2)	26.7 (4.05)	25.6 (5.21)	0.001
Waist-to-hip circumference ratio	0.94 (0.09)	0.96 (0.07)	<0.001
Hypertension, n (%)	98 (25.9%)	86 (22.7%)	0.351
Diabetes mellitus, n (%)	25 (6.60%)	26 (6.86%)	1.000
Glycaemia (mg/dL)	93 [87-100]	93.6 [83.6-101]	0.934
Lipids and lipoproteins			
Total cholesterol (mg/dL)	209 (38.2)	183 (38.0)	<0.001
Total cholesterol ≥200 (mg/dL), n (%)	207 (55.3%)	104 (28.7%)	<0.001
LDL-c (mg/dL)	135 (37.9)	106 (32.4)	<0.001
LDL-c ≥115 (mg/dL), n (%)	264 (70.6%)	49 (31.2%)	<0.001
HDL-c (mg/dL)	52.4 (11.5)	52.0 (19.1)	0.750
HDL-c ≤45 (mg/dL) (women) ≤40 (mg/dL) (men), n (%)	119 (31.8%)	123 (38.0%)	0.105
Triglycerides (mg/dL)	87.0 [63.0;115]	118 [85.0;165]	<0.001
Triglycerides ≥150 (mg/dL), n (%)	51(13.6%)	116 (31.5%)	<0.001
Lipid lowering therapy	27 (7.12%)	93 (24.6%)	<0.001
Framingham equation (10-year risk of MI or death)	6.48 [3.53;11.6]	7.52 [4.84;12.2]	0.001
REGICOR equation (10-year risk of fatal and non-fatal MI, silent MI or angina)	2.16 [1.21; 4.06]	2.67 [1.68; 4.14]	0.001
SCORE equation (10-year risk of fatal CVD)	0.26 [0.10; 0.88]	0.40 [0.17; 0.95]	<0.001
COMVIH equation (10-year risk of fatal and non-fatal MI, silent MI or angina)	-	4.74 [3.01; 7.32]	

Reduced D:A:D equation (10-year risk of CVD)	-	6.61 [3.64; 11.3]
HIV-specific factors		
Acquisition of HIV infection, n (%)		
Injecting drug use		127 (33.5%)
Heterosexual		129 (34%)
Men who have sex with men		108 (28.5%)
Other/unknown		15 (3.9%)
Current or former cocaine use, n (%)		84 (22%)
Current or former heroin use, n (%)		72 (19%)
Nadir $CD4^+$ T (cells/µL)		341 (215)
Current $CD4^+$ T (cells/µL)		718 (338)
CD4/CD8 ratio		0.86 (0.50)
AIDS, n (%)		120 (31.7%)
HIV viral load zenith (ln)		4.77 (0.99)
HCV positive serology, n (%)		146 (38.5%)
Duration of HIV infection (years)		16.3 [9.57;22.5]
Duration of combined ART(years)		15.5 [8.60;19.7]
Current combined ART, n (%)		
NNRTI/Protease Inhibitors/Integrase Inhibitors		243(64.1%)/112(29.6%)/80(21.1%)
Abacavir/Tenofovir disoproxil fumarate		154(40.6%)/127 (33.5%)
Use of abacavir at any point, n (%)		224 (59.1%)
Duration of thymidine analogues (years)		6.9 (5.5)
Use of thymidine analogues at any point, n (%)		247 (65.2%)
Cardiovascular biomarkers		
hs-CRP (mg/L)		4.31 (10.9)
sCD163 (ng/mL)		310 [206;437]
sCD14 (µg/mL)		1.35 [0.95;1.71]
Interleukin-6 (pg/mL)		1.11 [0.43;2.02]
D-dimer (ng/mL)		14.4 [9.70;24.1]
sVCAM (ng/mL)		855 [680;1060]
Total Lp-PLA2 activity ($\mu mol/min^{-1}*mL$)		20.6 [17.5;24.2]

Data are presented as number (percentage), mean (standard deviation) or median [interquartile range].

Abbreviations: AIDS, acquired immune deficiency syndrome; ART, antiretroviral therapy; HCV, hepatitis C virus; HDL-c, high-density lipoprotein cholesterol; hs-CRP, high sensitivity C-reactive protein; LDL-c, low-density-lipoprotein cholesterol; Lp-PLA2: Lipoprotein-associated phospholipase A2; NNRTI, non-nucleoside reverse transcriptase inhibitor; MI, myocardial infarction; sCD163 and sCD14, soluble forms of clusters of differentiation 163 and 14; s-VCAM, soluble vascular cell adhesion molecule.

Table 2. Univariate analyses of variables associated with carotid plaque in the control group and in the HIV-infected group (only statistically significant variables are shown).

	Control group			**HIV-infected group**		
	No plaque	**Plaque**	**p-value**	**No plaque**	**Plaque**	**p-value**
	N=255	*N=116*		*N=250*	*N=124*	
Smoking status			<0.001			0.007
Never smoker, n (%)	112 (50.2%)	27 (23.3%)		66 (26.4%)	16 (12.9%)	
Former smoker, n (%)	77 (30.2%)	56 (48.3%)		56 (22.4%)	39 (31.5%)	
Current smoker, n (%)	50 (19.6%)	33 (28.4%)		128 (51.2%)	69 (55.6%)	

Body mass index (kg/m^2)	26.1 (3.88)	28.1 (4.09)	<0.001	25.2 (4.16)	26.0 (6.45)	0.241
Waist-to-hip circumference	0.91 (0.09)	0.98 (0.09)	<0.001	0.95 (0.07)	0.98 (0.07)	<0.001
Hypertension	49 (19.2%)	46 (39.7%)	<0.001	34 (13.6%)	52 (41.9%)	<0.001
Total cholesterol (mg/dL)	205 (37)	220 (39.5)	0.001	181 (37.3)	189 (38.1)	0.052
LDL-c (mg/dL)	130 (35.9)	145 (40.9)	0.001	109 (30.4)	100 (36.3)	0.178
Triglycerides (mg/dL)	80 [58;112]	96.5 [76.8;126]	<0.001	112 [82.3;154]	129 [93.8;198]	0.002
Diabetes mellitus, n (%)	9 (3.53%)	16 (13.8%)	0.001	10 (4.00%)	16 (12.9%)	0.003
Lipid lowering therapy, n (%)	11 (4.31%)	16 (13.8%)	0.002	38 (15.2%) 27 (10.8%)	55 (44.7%) 46 (37.1%)	<0.001 <0.001
HIV-specific factors						
Injecting drug use				74 (29.6%)	52 (41.9%)	0.024
Current CD4+ T (cells/µL)				746 (330)	664 (352)	0.031
HIV viral load zenith (ln)				4.86 (0.94)	4.58 (1.08)	0.015
HCV positive serology, n (%)				86 (34.4%)	59 (47.6%)	0.019
Duration of HIV infection (years)				14.2 [8.15;21.1]	19.8 [13.5;25.3]	<0.001
Duration of combined ART (years)				13.1 [7.55;18.6]	18.9 [13.8;22.3]	<0.001
Use of thymidine analogues, n (%)[1]				148 (59.2%)	96 (77.4%)	0.001
Duration of thymidine analogues (years)				4.84 [2.59;8.15]	7.15 [2.96;12.0]	0.001
Use of protease inhibitors, n (%)[1]				169 (67.6)	102(82.3%)	0.004
Cardiovascular biomarkers						
sCD163 (ng/mL)				291 [180;403]	357 [252;517]	0.001
Interleukin-6 (pg/mL)				0.92 [0.43;1.84]	1.32 [0.65;2.27]	0.020
D-dimer (ng/mL)				13.6 [9.38;20.6]	19.2 [10.6;33.7]	0.002
sVCAM (ng/mL)				820 [670;1020]	920 [750;1140]	0.012

Data are presented as number (percentage), mean (standard deviation), or median [interquartile range].

Abbreviations: ART, antiretroviral therapy; HCV, hepatitis C virus; LDL-c, low-density-lipoprotein cholesterol; sVCAM, soluble vascular cell adhesion molecule-1.

[1] Used at any point.

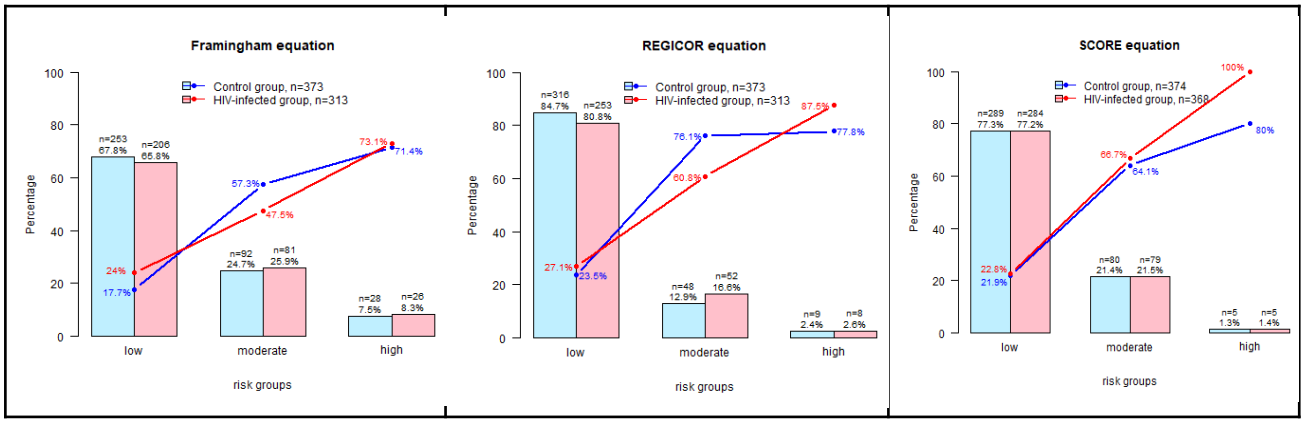
Table 3. Multivariate analyses of the presence of carotid plaque and subclinical atherosclerosis in the control and HIV-infected groups (only significant associations are shown).

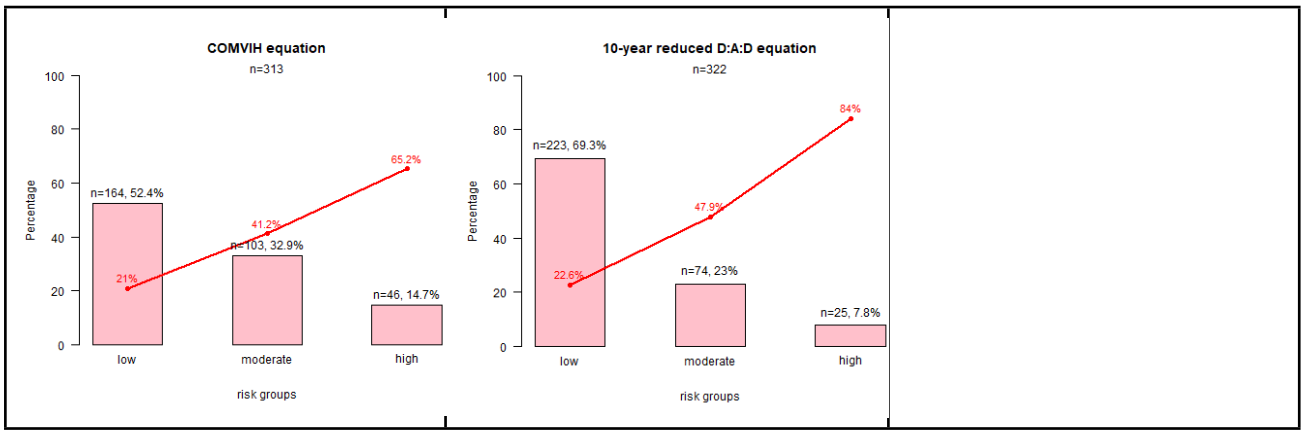
	Carotid plaque		**Subclinical atherosclerosis**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age				
Control group	1.096 (1.064; 1.132)	<0.001	1.049 (1.023; 1.077)	<0.001
HIV-infected group	1.057 (1.016; 1.100)	0.006	1.042 (1.008; 1.079)	0.018
Gender (female)				
Control group	--	--	--	--
HIV-infected group	--	--	--	--
Ever smoker				
Control group	2.972 (1.696;5.351)	<0.001	1.806 (1.137; 2.883)	0.013
HIV-infected group	2.611 (1.083;6.736)	0.038	--	--
Total cholesterol (x10 mg/dL)				
Control group	1.078 (1.009; 1.155)	0.028	1.088 (1.024; 1.158)	0.007
HIV-infected group	1.087 (1.003 ;1.183)	0.047	1.105 (1.024; 1.197)	0.012
Body mass index				
Control group	--	--	1.129 (1.064;1.202)	<0.001
Waist-to-hip circumference (0.1 units)				
Control group	1.636 (1.192;2.277)	0.003	--	--
Hypertension				
HIV-infected group	2.988 (1.518; 5.984)	0.002	2.340 (1.214; 4.589)	0.003
Duration of thymidine analogues		--		
HIV-infected group	--	--	1.064 (1.010;1.124)	0.023

Variables included in the multivariate analyses by the stepwise Akaike information criterion. **Control group**: smoking status, age, sex, body mass index, waist-to-hip circumference, total cholesterol, triglycerides, diabetes, hypertension, and lipid-lowering therapy. **HIV-infected group**: smoking status, age, sex, waist-to-hip circumference, total cholesterol, triglycerides, diabetes, hypertension, lipid-lowering therapy, injection drug use, current CD4, HIV zenith (log), HCV-positive serology, duration of combined ART, duration of thymidine analogues, use of protease inhibitors, sCD163, interleukin 6, D-dimer, and sVCAM.

FIGURES

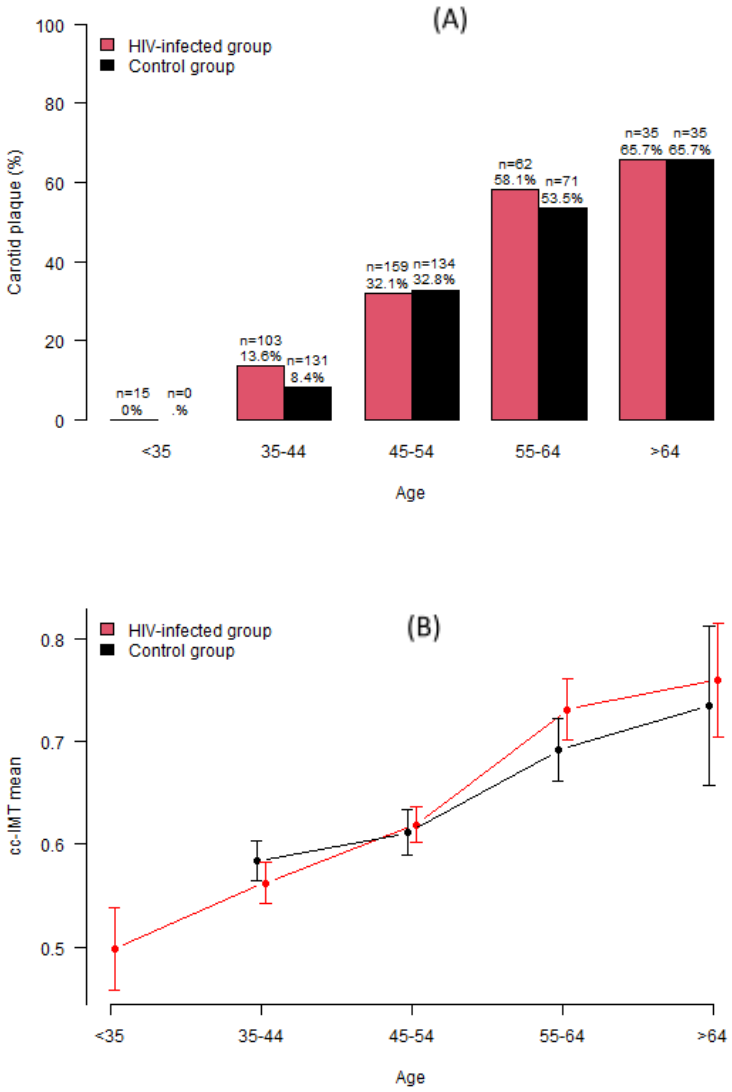
Figure 1. Relationship between cardiovascular risk equations and the presence of carotid plaque in the HIV-infected group and the control group





The columns show the percentage of participants in each cardiovascular risk category; the lines show the percentage of participants with carotid plaque in each cardiovascular risk category. Framingham categories were classified as low (<10%), moderate (10-20%), and high (<20%); REGICOR as low (<5%), moderate (5-10%), and high (>10%); SCORE as low (<1%), moderate (1-5%), and high (>5%); COMVIH as low (<5%), moderate (5-10%), and high (10%); D:A:D as low (<10%), moderate (10-20%), and high (>20%).

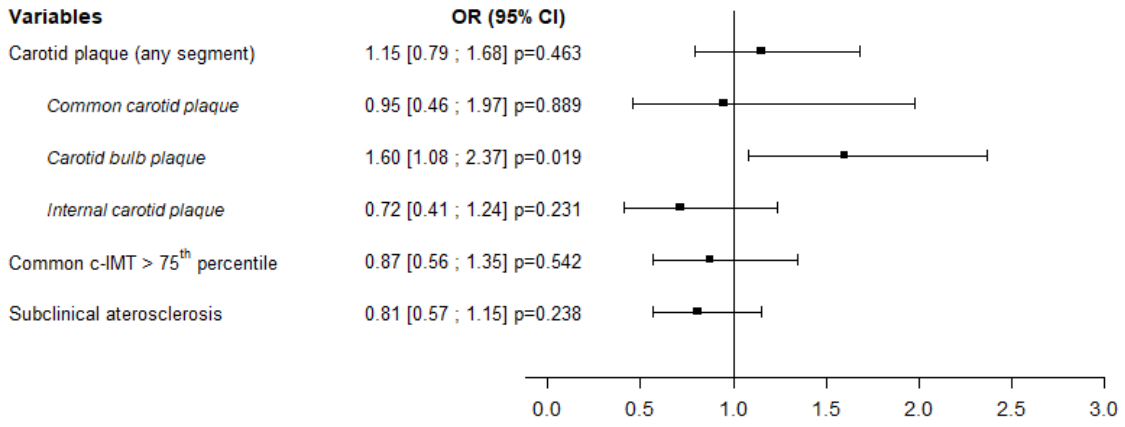
Figure 2. Percentage of participants with carotid plaque (A) and mean common carotid-intima media thickness (B) in the HIV-infected group and control group according to age strata.



No statistically significant differences were found between HIV-infected patients and controls for any age strata for plaque or cc-IMT. The n in the columns of Figure A refers to the number of participants in each age stratum

Abbreviation: cc-IMT, common carotid intima-media thickness

Figure 3. Adjusted* odds ratio (OR) for carotid plaque, subclinical atherosclerosis, and common c-IMT > 75th percentile in the HIV-infected group compared with the control group.



Subclinical atherosclerosis was defined as the presence of a carotid plaque and/or a common c-IMT > 75th percentile of a reference population.

*The odds ratio was adjusted for the following variables according to the stepwise Akaike information criterion: **Carotid plaque (any segment)** (ever smoker, body mass index, hypertension, diabetes, and total cholesterol), **Common carotid plaque** (ever smoker and hypertension), **Carotid bulb plaque** (ever smoker, body mass index, hypertension, diabetes, and total cholesterol), **Internal carotid plaque** (ever smoker, body mass index, hypertension, diabetes, and total cholesterol), **Common c-IMT > 75th percentile** (body mass index, hypertension, total cholesterol, and HDL cholesterol), **Subclinical atherosclerosis** (ever smoker, body mass index, hypertension, diabetes, and total cholesterol). It is a descriptive table, comparison must be done with caution as covariates included in the analyses and prevalence of the outcomes are different.

Abbreviation: c-IMT, common carotid intima-media thickness