DOI: 10.1111/eci.12780

ORIGINAL ARTICLE

Subclinical atherosclerosis in low Framingham risk HIV patients

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ABSTRACT

Background Pathogenesis of atherosclerosis is complex, and differences between HIV-infected patients and general population cannot be completely explained by the higher prevalence of traditional cardiovascular risk factors. We aimed to analyse the association between inflammation and subclinical atherosclerosis in HIV patients with low Framingham risk score.

Materials and methods Case–control study. Setting: Outpatient Infectious Diseases clinic in a university hospital. Subjects: HIV-1-infected patients aged > 35 years receiving antiretroviral treatment with viral load < 50 copies/mL and Framingham risk score < 10%. Exclusion criteria: inflammatory diseases; dyslipidaemia requiring statins; smoking > 5 cigarettes/day; diabetes; hypertension; vascular diseases. Main outcome**:** subclinical atherosclerosis determined by ultrasonography: common carotid intima-media thickness greater than 0·8 mm or carotid plaque presence. Explanatory variables**:** ribosomal bacterial DNA (rDNA), sCD14, interleukin-6 (IL-6) and TNF-α.

Results Eighty-four patients were included, 75% male, mean age 42 years and mean CD4+ cells 657 ± 215/mm^3. Median Framingham risk score was 1% at 10 years (percentile 25–75: 0·5–4%). Eighteen patients (21%) had subclinical atherosclerosis; the associated factors were older age (P = 0·001), waist–hip ratio (P = 0·01), time from HIV diagnosis (P = 0·02), rDNA (P = 0·04) and IL-6 (P = 0·01). In multivariate analysis, OR for subclinical atherosclerosis was 7 (95% CI, 1.3–40, P = 0.02) and 9 (95% CI, 1.0–85, P = 0.04) for patients older than 44 years and IL-6 > 6·6 pg/mL, respectively.

Conclusions Well-controlled HIV patients with low Framingham risk score have a high prevalence of subclinical carotid atherosclerosis, and the main risk factors are age and inflammation. These patients are not receiving primary prophylaxis for cardiovascular events according to current guidelines.

Keywords HIV, inflammation, intima-media thickness, microbial translocation, subclinical atherosclerosis.

Eur J Clin Invest 2017; 47 (8): 591–599

Introduction

HIV-infected patients have an increased atherosclerosis incidence and cardiovascular morbidity. Pathogenesis of atherosclerosis is complex, and differences between HIV-infected patients and general population are not completely explained by a higher prevalence of traditional cardiovascular risk factors (CVRF) such as hypertension, diabetes mellitus, smoking and dyslipidaemia [1].

Atherosclerosis behaves as a chronic inflammatory disease, and there are strong data supporting a relationship between inflammation (measured through surrogate markers) and development of cardiovascular events. The role of inflammation can to be even more important in HIV patients, as many inflammatory markers, such as interleukin-6 (IL-6), is higher in HIV infection and do not normalize with antiretroviral therapy (ART) [2,3]. Prospective observational studies and clinical trials support this association [4]. The origin of this inflammatory state is probably multifactorial, but microbial translocation (MT) could be the main pathogenic mechanism [5]. MT, consisting on bacterial products passage through the intestinal wall, can be assessed with some plasma markers such as bacterial ribosomal DNA (rDNA) or soluble CD14 (sCD14) [6,7].

Well-controlled HIV-infected patients without CVRF seem to have an increased risk of cardiovascular events. It has been hypothesized that inflammation could be the main pathogenic mechanism of atherosclerosis in these patients. To clarify that question, we have studied a group of HIV patients with very

low cardiovascular risk, measured with the Framingham score, to determine the subclinical carotid atherosclerosis prevalence and its relationship with inflammatory and MT markers. Carotid ultrasonography is a technique easy to perform, quick and innocuous and can show up the presence of carotid plaques or intima-media thickening, which have been associated with coronary atherosclerosis and could predict cardiovascular events even in patients with low Framingham risk [8].

Material and methods

Design

Case–control study. Reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines (Simera *et al.* January 2010 issue of EJCI).

Study subjects

Study subjects were adults over 35 years with HIV infection receiving treatment and with HIV-viral load (VL) < 50 copies/mL at least for 1 year.

Exclusion criteria were as follows: poor adherence to ART (self-reported intake < 95% of the planned dose in the last 15 days), chronic viral hepatitis, alcohol consumption > 30 g/day, active illegal drug consumption or current smoking (more than 5 cigarettes/day), inflammatory diseases of any aetiology, active cancer, active infection, antibiotics intake or gastrointestinal bleeding within the last month, diabetes mellitus (fasting glucose > 126 mg/dL or HbA1c > 6·5%), high blood pressure (HBP), dyslipidaemia requiring statins, renal impairment (estimated glomerular filtrate < 60 mL/min), known cardiovascular disease, dementia or any other central nervous system disease, severe psychiatric illness. The study was approved by the local Ethics Committee, and patients signed an informed consent.

End point

Subclinical atherosclerosis, defined as the presence of carotid plaque or common carotid intima-media thickness (CIMT) > 0·800 mm.

Main explanatory variables

Markers of MT (rDNA, sCD14) and inflammation (IL-6 and TNF-α) in plasma.

Other studied variables

Sociodemographic: age, sex, risk factor for HIV acquisition, nationality, race. HIV-related variables: year of diagnosis, CDC stage, CD4+ and CD8+ lymphocytes, HIV VL, current ART. Physical examination: weight, height, body mass index (BMI), waist–hip ratio, blood pressure. Vascular risk factors: total and HDL cholesterol; abnormal fast glucose, defined by fasting glucose 106–125 mg/dL or HbA1c 5·7–6%; smoking; sedentarism (exercise < 3 h/week); family history of cardiovascular disease; and 25-OH-vitamin D levels. Framingham vascular risk was calculated with the data of age, sex, total cholesterol, HDL, smoking and systolic blood pressure (http://cvdrisk.nhlbi.nih.gov).

Data collection

Clinical data were obtained from the medical records and by personal interview. A fasting blood sample was obtained for blood count, biochemistry, CD4+ and CD8+ cells, HIV-VL, 25-OH-vitamin D and markers of MT and inflammation.

Carotid ultrasonography was performed by a single sonographer certified by the Spanish Society of Neurology, blind to clinical and analytical data, using a CV 50 PHILIPS ultrasound device with a high-frequency linear probe (L12-3), at the maximum frequency in the resolution setting, with depth of field between 3 and 4 and single focus. Patient was placed in supine with the neck extended and rotated 45 degrees. CIMT was measured twice in both common carotids. Measurement was performed during the diastolic phase, in the posterior wall of the common carotid, one centimetre below carotid bifurcation. QLAB software vs 8.1 takes multiple automatic measurements of the selected segment and obtains the mean value. The value reported as 'CIMT' for each patient was the average of mean CIMT values. The presence of plaques was determined in carotid (common and internal) and carotid bulb. These segments were scanned in several views (anterior, lateral and posterior) to identify plaques defined as follows: focal wall thickening 50% or greater than the thickness of the surrounding wall; focal wall thickening penetrating at least 0·5 mm in the artery lumen; a localized area with CIMT > 1·5 mm, which enters the lumen and is clearly distinguished from the surrounding area [9].

Bacterial DNA (rDNA) was amplified by PCR of the 16S ribosomal gene as previously described [10]. Plasma levels of sCD14, TNF-α and IL-6 were determined by ELISA following the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). HIV-VL was determined by ultrasensitive PCR (COBAS AmpliPrep/COBAS TaqMan HIV-1 test vs 2.0; Roche Diagnostics, Pleasanton, CA, USA).

Data analysis

Qualitative variables are expressed as absolute frequencies and percentages. Parametric variables are expressed as means ± standard deviation (SD) and nonparametric variables as medians and percentiles 25–75 ($P_{25–75}$).

The population was analysed in accordance with the presence of carotid atherosclerosis. To quantify the association between explanatory variables and subclinical atherosclerosis, quantitative variables were turned on dichotomic variables using the cut-offs that best discriminate between groups.

Chi-squared test was used, and the odds ratio (OR) with its 95% confidence interval (95% CI) was calculated. Multivariate, unconditional logistic regression analysis was performed to identify factors independently associated with subclinical atherosclerosis. First model included age, sex, CVRF and all variables showing $P < 0{\cdot}20$ in the univariate model, with the exception of biomarkers. A second model was performed including biomarkers. In the third model, Framingham risk score was included instead of component variables (age, sex, total cholesterol, HDL, smoking and systolic blood pressure).

To study the association between the qualitative variables and CIMT, the Student *t*-test or Mann–Whitney *U*-test was used. To study correlations between quantitative variables and CIMT, the Pearson and Spearman tests were used as appropriate. Linear regression analysis was used to identify factors independently associated with CIMT, including all variables yielding $P < 0{\cdot}20$ in the bivariate analysis and those considered clinically relevant.

In all cases, a *P*-value of $< 0{\cdot}05$ was considered statistically significant. The SPSS version 19.1 statistical package (SPSS Inc., Chicago, IL, USA) was used.

Results

Characteristics of patients

Eighty-four patients were included in this study (Table 1). Overall, mean age was 42 ± 7 years, and the majority of participants were men (75%) and MSM (57%). All participants were receiving ART and 92% had VL < 50 copies/mL, with a mean CD4+ T-cell count of 657 ± 217 cell/mm^3.

Median Framingham risk score was very low, 1% at 10 years ($P_{25–75}$ 0·5–4 years), because most CVRF were exclusion criteria (HBP, dyslipidaemia requiring statins, diabetes and smoking > 5 cigarettes/day). Mean BMI was 25·3 ± 3 kg/m^2, mean total cholesterol was 182 ± 36 mg/dL, 51% were smokers (12% current smokers), and 49% had family history of cardiovascular disease. Among smokers, median pack-years was 8 ($P_{25–75}$ 4–21).

Ultrasonography showed carotid plaque in 18 patients (21%) and the mean CIMT was 0·5595 ± 0·101 mm. CIMT was higher in patients with plaque (0·6070 vs. 0·5470 mm, $P = 0{\cdot}03$). Only three patients had CIMT > 0·8 mm, and all three had carotid plaque, so increased CIMT was only found in patients with carotid plaque.

Biomarkers results are shown in Table 1. Prevalence of MT (rDNA) was 41%, mean value of sCD14 was 13·1 ± 3·8 ng/mL and median value of IL-6 and TNF 6·6 ($P_{25–75}$ 4·2–34) and 76 ($P_{25–75}$ 43–78) pg/mL, respectively.

Factors associated with subclinical atherosclerosis

Table 1 shows the different parameters related to the presence of subclinical atherosclerosis. The variables that were significantly ($P < 0{\cdot}05$) in univariate analysis included older age, waist–hip ratio, time from HIV diagnosis, rDNA and IL-6 levels. There were nonsignificant trends to the association between subclinical atherosclerosis and male ($P = 0{\cdot}13$), smoking ($P = 0{\cdot}14$), family history of cardiovascular disease ($P = 0{\cdot}09$) and low CD4+ cell count ($P = 0{\cdot}13$). Proportion of participants receiving protease inhibitors was similar between groups.

Table 2 shows the correlation between the different variables and subclinical atherosclerosis. The first model of multivariate analysis including age, sex, CVRF and variables with $P < 0{\cdot}20$ in univariate analyses showed that only age was significatively associated with subclinical atherosclerosis (OR 5·2 for age > 44 years, $P = 0{\cdot}03$). In the second model, that includes biomarkers, only age and IL-6 levels remained significatively associated with subclinical atherosclerosis, and odds ratios for age > 44 years and IL-6 > 6·6 pg/mL were 7 (95% CI 1·3–40, $P = 0{\cdot}02$) and 9 (95% CI 1–85, $P = 0{\cdot}04$), respectively. When IL-6 was not included in the model, rDNA was significative ($P = 0{\cdot}03$) along with age ($P = 0{\cdot}02$) (data not shown). In the third multivariate model including Framingham risk score, waist–hip ratio ($P = 0{\cdot}03$), time from HIV diagnosis ($P = 0{\cdot}03$) and IL-6 ($P = 0{\cdot}05$) levels remained significantly associated with subclinical atherosclerosis (data not shown in the table).

Framingham risk score was higher in patients with subclinical atherosclerosis (median 5% vs. 1%, $P = 0{\cdot}001$).

Factors associated with carotid intima-media thickness

Variables that were significatively associated with higher CIMT in univariate analysis were older age ($P = 0{\cdot}01$), blood pressure ($P = 0{\cdot}05$) and time from HIV diagnosis ($P = 0{\cdot}04$) (Table 3). Correlation coefficients between age and time from HIV diagnosis and CIMT were 0·31 (95% CI 0·12–0·52) and 0·24 (95% CI 0·02–0·44), respectively. MT and inflammatory markers were not associated with CIMT. There was a nonsignificant trend to the association between CIMT and male sex ($P = 0{\cdot}09$) and low CD4+/CD8+ ratio ($P = 0{\cdot}14$). When waist–hip ratio was managed as a quantitative variable, there was a significative correlation with CIMT (0·22, 95% CI 0·01–0·47, $P = 0{\cdot}05$).

Multivariate analysis including age, sex, CVRF and significative variables in the univariate analysis showed that only age remained significatively associated with CIMT (p = 0·02).

Factors associated with rDNA and IL-6 levels

As rDNA and sCD14 were associated with subclinical atherosclerosis, determination of possible confounding factors that could impact on them is important.

There was no significant difference between basal characteristics of patients (sociodemographic, vascular risk factors and HIV-related variables) according to the rDNA presence or

Table 1 Characteristics of patients and risk factors for subclinical atherosclerosis in HIV patients with low Framingham risk (univariate analysis)

Variable	Subclinical atherosclerosis			
	Total *N* = 84	Yes *N* = 18	No *N* = 66	*P*-value
Sociodemographic variables				
Age ≥ 44 (years)* (%)	42 (50)	15 (83)	27 (41)	0·001
Sex (male) (%)	63 (75)	16 (90)	47 (71)	0·13
Risk factor for HIV (%)				0·88
Homosexual	48 (57)	10 (56)	38 (58)	
Heterosexual	29 (35)	6 (33)	23 (35)	
Intravenous drug use	5 (6)	2 (11)	3 (5)	
Origin country: Spain	73 (87)	17 (94)	56 (85)	0·29
Vascular risk factors (%)				
BMI ≥ 25 kg/m^2	42 (50)	9 (50)	33 (50)	1·0
Waist–hip ratio ≥ 0·92*	39 (46)	13 (72)	26 (39)	0·01
SBP ≥ 133 or DBP ≥ 80 mmHg†	24 (29)	8 (44)	16 (24)	0·09
25-OH-Vitamin D < 30 ng/mL	76 (91)	17 (94)	59 (89)	0·52
Total cholesterol > 200 mg/dL	26 (31)	6 (33)	20 (30)	0·81
Abnormal fasting glucose	2 (2)	0 (0)	2 (3)	NC
Smoking	43 (51)	12 (67)	31 (47)	0·14
Current smokers	10 (12)	4 (22)	6 (9)	
Former smokers	33 (39)	8 (44)	25 (38)	
Family history of cardiovascular disease	41 (49)	12 (67)	29 (44)	0·09
Sedentarism	49 (58)	12 (67)	37 (56)	0·42
HIV-related variables (%)				
C stage	16 (19)	4 (22)	12 (18)	0·69
CD4+ nadir ≤ 122/μL‡	21 (25)	7 (39)	14 (21)	0·13
Current CD4+ ≤ 515/μL‡	21 (25)	7 (39)	14 (21)	0·13
CD4+/CD8+ rate < 0·67‡	21 (25)	6 (33)	15 (23)	0·36
Time from HIV diagnosis ≥ 12 years*	40 (48)	13 (72)	27 (41)	0·02
Antiretroviral treatment (%)				0·30
Non-nucleoside transcriptase inhibitors	58 (69)	15 (83)	43 (65)	
Protease inhibitors	22 (26)	3 (18)	19 (29)	
Integrase inhibitors	17 (20)	6 (33)	11 (17)	
Other				
Framingham risk score, median ($P_{25–75}$)	1 (0·5–4)	5 (1–7)	1 (0·5–3)	0·001
CIMT, mean (SD)	0·5595 (0·101)	0·607 (0·17)	0·547 (0·07)	0·03
Biomarkers (%)				
rDNA	34 (41)	11 (61)	23 (35)	0·04
sCD14 ≥ 12·7*, ng/mL	42 (50)	10 (56)	32 (49)	0·60
IL-6 ≥ 6·6*, pg/mL	42 (50)	14 (78)	28 (42)	0·01
TNF-α ≥ 76*, pg/mL	43 (51)	9 (50)	34 (52)	0·91

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NC, not calculable; CIMT, carotid intima-media thickness.
*Median value.
†Percentile 75 value.
‡Percentile 25 value.

the IL-6 level, with the exception of a lower prevalence of overweight patients in the group of patients with rDNA (35% vs. 60%, $P = 0·03$), and a higher number of patients receiving PI in the group of patients with IL-6 < 6·6 pg/mL (35% vs. 17%, $P = 0·06$).

Figure 1 shows the relationship between the rDNA presence or the IL-6 level. Patients with MT (rDNA) had a median IL-6 of 34 pg/mL ($P_{25–75}$, 17–51), whereas patients without MT had median IL-6 of 4·94 pg/mL ($P_{25–75}$, 2·4–6·2, $P = 0·001$).

Discussion

Our study shows that 20% of well-controlled HIV patients with a very low Framingham risk (1% at 10 years) have subclinical carotid atherosclerosis, a systemic atherosclerosis marker and cardiovascular risk. The two main risk factors have been age and inflammation (IL-6).

Cohort studies have suggested that cardiovascular diseases are more common in people living with HIV infection, and this cannot be completely explained by a higher prevalence of traditional CVRF such as HBP, diabetes, smoking or dyslipidaemia [1]. There seem to be some factors directly related with HIV (such as low-level HIV replication) or indirectly related (such as MT) play a role. Anyway, inflammation is suspected as the main mechanism underlying the atherosclerosis development, as in general population.

To better define the inflammation impact on atherosclerosis development in HIV-infected patients, clinical studies should now focus on low cardiovascular risk patients, and confounding variables have to be controlled as strictly as possible. In unselected HIV patients, CIMT is strongly associated with traditional CVRF [11]. Patients followed in our clinic have a high CVRF, hepatitis C, drug use and smoking prevalence, so recruitment was difficult, but in the end, we were able to

Table 2 Quantification of the association between risk factors and subclinical atherosclerosis. Univariate and multivariate analysis

	Univariate		Multivariate (excluding biomarkers)		Multivariate	
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Biomarkers						
rDNA	2·9 (1–8·6)	0·04	–		1·1 (0·2–7·8)	0·95
IL-6 ≥ 6·6 pg/mL*	4·8 (1·4–16·0)	0·01	–		9·3 (1·0–85)	0·049
Sociodemographic variables						
Age ≥ 44 years*	7·2 (1·9–27·4)	0·001	5·2 (1·0–25)	0·03	7·3 (1·3–40)	0·02
Sex (male)	3·2 (0·6–15·4)	0·13	2·0 (0·3–13)	0·47	1·4 (0·2–11)	0·72
Vascular risk factors						
BMI ≥ 25 kg/m^2	1 (0·4–2·8)	1·0	0·5 (0·1–2·5)	0·41	0·5 (0·1–2·5)	0·36
Waist–hip ratio ≥ 0·92*	4 (1·3–12·6)	0·01	3·6 (0·7–18)	0·12	5·1 (0·8–32)	0·08
SBP ≥ 133 or DBP ≥ 80 mmHg†	2·5 (0·8–7·4)	0·09	1·2 (0·2–6·0)	0·85	1·6 (0·2–10)	0·65
Total cholesterol > 200 mg/dL	1·2 (0·4–3·5)	0·81	0·5 (0·1–2·1)	0·35	0·6 (0·1–3·4)	0·56
Smoking (current or previous)	2·3 (0·8–6·7)	0·14	2·5 (0·6–11)	0·22	2·1 (0·4–12)	0·42
Family history of cardiovascular disease	2·6 (0·9–7·6)	0·09	1·0 (0·2–4·3)	0·96	0·7 (0·1–3·7)	0·67
Sedentarism	1·6 (0·5–4·7)	0·42	1·0 (0·2–5·3)	0·96	0·8 (0·1–5·3)	0·83
HIV-related variables						
CD4+ nadir ≤ 122/μL‡	2·4 (0·8–7·2)	0·13	1·0 (0·3–4·9)	0·9	0·9 (0·1–5·4)	0·89
Current CD4+ ≤ 515/μL‡	2·4 (0·8–7·2)	0·13	2·9 (0·5–16)	0·21	3·0 (0·4–21)	0·27
Time from HIV diagnosis ≥ 12 years*	3·8 (1·2–11·8)	0·02	3·0 (0·5–17)	0·20	0·7 (0·1–3·7)	0·67

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Reference value: median.
†Reference value: percentile 75.
‡Reference value: percentile 25.

include a group of middle-aged patients with a median 1% 10-year Framingham risk score and lower than 8% in all cases. Mild smoking and hypercholesterolaemia were allowed to get a significant sample. Additionally, we tried to control for other confounders by excluding patients with chronic hepatitis, renal failure (estimated glomerular filtrate < 60 mL/min) and drug intake (including statins), and recording variables that may influence biomarkers levels as physical activity or weight.

Given the restrictive inclusion criteria, we have found lower CIMT values than other studies [11–20], and only in three patients were above 0·8 mm, the usual cut-off for the definition of 'pathological' CIMT. More surprising was the finding of a atherosclerotic plaque prevalence of 21% in these low-risk patients. This result is of great importance because the presence of plaque independently predicts the risk of cardiovascular events. In the general population, patients with low Framingham score without carotid plaque have an almost nil cardiovascular risk. However, for low Framingham risk patients with carotid plaque, cardiovascular risk is 10% at 8 years, a value that would put them close to medium Framingham risk [8]. Recent data on HIV patients with asymptomatic carotid plaque suggest that this risk could be even greater than 10% and at a shorter term (Janjua *et al.*, CROI 2016, abstract 640), and the reason could be that atherosclerotic plaques associated with HIV infection are more frequently 'vulnerable', that is, noncalcified, softer and more prone to rupture [21].

Current guidelines for CVRF management of patients with HIV establish strategies primarily based on cardiovascular risk estimated by equations such as the Framingham risk score and ACCC/AHA CVD risk calculator [22]. The point is that these scores may underestimate the real risk in the HIV population as has been suggested by cohort studies [23], and some patients currently considered as low risk have actually a moderate risk and should be considered for a more aggressive control of

Table 3 Risk factors for increased carotid intima-media thickness. Univariate analysis

	Mean (SD), mm	*P*
Biomarkers		
rDNA positive		
Yes	0·569 (0·13)	0·47
No	0·552 (0·08)	
sCD14 ≥ 12·7 ng/mL*		
Yes	0·561 (0·09)	0·87
No	0·558 (0·11)	
IL-6 ≥ 6·6 pg/mL*		
Yes	0·566 (0·12)	0·54
No	0·553 (0·08)	
TNF-α ≥ 76 pg/mL*		
Yes	0·572 (0·12)	0·27
No	0·547 (0·08)	
Sociodemographic characteristics		
Age ≥ 44 years*		
Yes	0·588 (0·12)	0·01
No	0·531 (0·07)	
Sex (male)		
Yes	0·570 (0·11)	0·09
No	0·527 (0·07)	
Homosexual		
Yes	0·565 (0·08)	0·55
No	0·552 (0·13)	
Vascular risk factors		
BMI ≥ 25 kg/m^2		
Yes	0·567 (0·09)	0·52
No	0·552 (0·11)	
Waist–hip ratio ≥ 0·92*		
Yes	0·570 (0·09)	0·39
No	0·551 (0·11)	
SBP ≥ 133 mmHg or DBP ≥ 80 mmHg†		
Yes	0·594 (0·10)	0·05
No	0·546 (0·10)	
25-OH-Vitamin D < 30 ng/mL		
Yes	0·560 (0·10)	0·80
No	0·551 (0·09)	
Total cholesterol > 200 mg/dL		
Yes	0·579 (0·14)	0·25
No	0·551 (0·08)	
Abnormal fasting glucose		
Yes	0·580 (0·02)	0·77
No	0·559 (0·10)	
Smoking (current or previous)		
Yes	0·561 (0·12)	0·91
No	0·558 (0·08)	
Family history of cardiovascular disease		
Yes	0·553 (0·11)	0·55
No	0·566 (0·09)	
Sedentarism		
Yes	0·560 (0·11)	0·96
No	0·559 (0·09)	
HIV-related variables		
C stage		
Yes	0·586 (0·09)	0·25
No	0·553 (0·10)	
CD4+ nadir ≤ 122/μL‡		
Yes	0·584 (0·14)	0·21
No	0·552 (0·09)	
Current CD4+ ≤ 515/μL‡		
Yes	0·565 (0·14)	0·78
No	0·558 (0·08)	
CD4+/CD8+ ratio < 0·67‡		
Yes	0·588 (0·14)	0·14
No	0·550 (0·09)	
Time from HIV diagnosis ≥ 12 years*		
Yes	0·584 (0·11)	0·04
No	0·538 (0·08)	
Protease inhibitors treatment		
Yes	0·581 (0·13)	0·24
No	0·552 (0·09)	

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Reference value: median.
†Reference value: percentile 75.
‡Reference value: percentile 25.

CVRF. The use of statins as primary prevention of cardiovascular events in patients at low risk is not currently recommended; however, these drugs have been shown to reduce

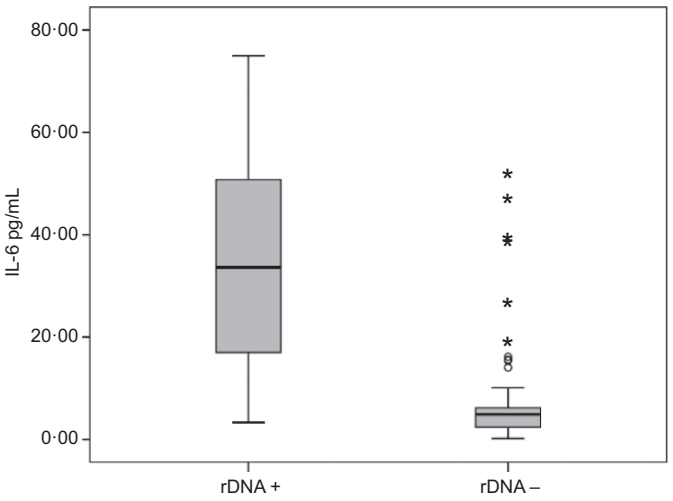

Figure 1 Relationship between IL-6 levels and microbial translocation (rDNA). IL-6: interleukin-6; rDNA+: presence of DNA-ribosomal 16S; rDNA−: absence of DNA-ribosomal 16S.

noncalcified coronary plaque volume and to slow progression of CIMT [24,25], so, in the absence of conclusive clinical data, statins use in HIV patients with subclinical atherosclerosis based on the ultrasound carotid plaque composition need to be studied.

Our second main finding is that, in patients without CVRF, atherosclerosis development depends on age and inflammation. Age is the main risk factor in most studies, so atherosclerosis should be considered as part of normal ageing, although it appears at earlier ages in HIV patients. In our results, inflammation is also strongly associated with subclinical atherosclerosis in the absence of classical CVRF, so patients with IL-6 levels > 6·6 pg/mL have a risk ninefold greater cardiovascular risk. The relationship between IL-6, sCD14 and morbimortality (including cardiovascular disease) has been assessed in animal models, cohort studies and clinical trials, although it does not apply exclusively to patients with HIV infection [26–28]. MT seems to favour clinical progression even in HIV elite controllers (patients who spontaneously control VL without ART) [29]. In our study, the association between rDNA and subclinical atherosclerosis disappeared in multivariate analysis, suggesting that rDNA could raise IL-6 levels (most patients with higher IL-6 have MT). The rationale for a high IL-6 level in absence of rDNA could be that MT is intermittent, translocation of bacterial products other than rDNA or mechanisms unrelated to MT such as viral replication in reservoirs, the role of plasma low-grade HIV viraemia on inflammation seems to be less important [30].

Our study has several limitations. We included smokers, but the percentage of current smokers was very low (12%) and they were light smokers (< 5 cigarettes/day). On the other hand, the cumulative pack-years in patients with previous smoking were relatively low (8 pack-years). Smoking was slightly more frequent in patients with subclinical atherosclerosis, but the association was not significant. Another limitation is the possible relationship between PI and subclinical atherosclerosis. Our design was not prospective, and we have not recorded the cumulative exposition to PI. Other authors have investigated this relationship with discordant results, probably because not all PI are equal (in fact, new PI are not clearly associated with cardiovascular events), and the debate about this association remains [12,31]. Finally, we did not measure other variables that could be important such as use of abacavir, history of low-level viraemias, sCD163 (strongly associated with arterial inflammation) [32] or protective cytokines such as interleukin-10, adiponectin or interleukin-27 [33].

Currently, rates of cardiovascular disease in patients with HIV infections are dropping, probably as a result of a better control of CVRF, the use of more lipid friendly ART and the starting of ART at high CD4+ levels [34]. However, by now, no treatment has been able to fully normalize the inflammatory markers, so the risk of cardiovascular disease (and other comorbidities) will likely continue increased in patients with HIV. The new role of integrase inhibitors as drugs of choice for naive patients in recent guidelines could help because they seem to have a better inflammatory profile than PI or non-nucleoside reverse transcriptase inhibitors, although data are not conclusive [35,36].

In conclusion, atherosclerosis is a major concern in middle-aged HIV patients, even if they do not have vascular risk factors. Atherosclerosis can be demonstrated in subclinical phases with a quick, simple, noninvasive test as carotid ultrasound. Given the cumulative evidence relating the findings of carotid ultrasound with cardiovascular events, guidelines should consider the inclusion of prophylaxis recommendations not solely based on scores. HIV-infected patients with a low theoretical risk could be classified in two subgroups, those with normal carotid ultrasound and those with subclinical atherosclerosis, with very different risk of cardiovascular morbidity.

As atherosclerosis in patients without vascular risk factors is related to age and inflammation, it is mandatory to insight into the mechanisms that produce inflammation (mainly MT) and the search for anti-inflammatory treatment strategies. Meanwhile, the behaviour of ART on markers of inflammation may be considered when setting priorities among the therapeutic options.

Acknowledgements

This work was supported by a grant from 'Instituto de Investigación Sanitaria y Biomédica de Alicante (ISABIAL-Fundación FISABIO)' and Gilead Sciences (Fellowship Program).

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Received 19 February 2017; accepted 26 June 2017

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