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Full Length Research Paper

The relationship between subclinical atherosclerosis in HIV patients and cardiovascular risk factors

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HIV-infected patients are at an increased risk of cardiovascular disease. Pulse Wave Velocity (PWV) is regarded as non-invasive test for diagnosing subclinical atherosclerosis (SA). Until now, there have been very few studies which have analysed SA in HIV patients using PWV measurements. Our objectives were to analyse the prevalence of SA in a Spanish cohort of HIV patients using PWV and compare it with the data published in seronegative population; it investigates the risk factors involved in SA, and it evaluates the possible correlation between values obtained using PWV and the Framingham (FRS) and SCORE systems. We carried out a cross-sectional study of 136 consecutive HIV-positive patients. PWV was measured using the Complior© system. Values of >12 m/s were considered as diagnostic of SA. Results: 11.4% patients had SA. In the univariate analysis SA was associated with age, type 2-DM, HBP (high blood pressure), anti-HCV-positive, a higher viral low (VL) for HIV at onset and a higher percentage on the 10-year FRS. Multivariate analysis showed only a relationship between SA and the age (p = 0.001) and anti-HCV-positive (p=0.02), in patients diagnosed over a longer period (p=0.0001) and elevated homocysteine levels (p= 0.02). Conclusions: The prevalence of SA in our HIV cohort was low. This was associated with age, and anti-HCV-positive with a longer diagnosis and higher homocysteine levels. PWV did not correlate with cardiovascular risk calculated according to the FRS and SCORE system.

Key words: Subclinical atherosclerosis, pulse wave velocity, HIV, cardiovascular risk factors.

INTRODUCTION

HIV patients are at an increased risk of cardiovascular disease, which has previously been associated with several factors that include age, hypertension (HTA), a nadir CD4 count of <350 cells/mL (Ho et al., 2010), hypercholesterolemia and the duration of antiretroviral therapy (ART) (Lekakis et al., 2009).

Subclinical cardiovascular disease is an accurate reflection of the real prevalence of atherosclerosis at a potentially reversible stage, so that detecting it makes it possible to implement secondary preventive measures. Pulse wave velocity (PWV) is considered a non-invasive diagnostic test for subclinical atherosclerosis (SA) or arterial stiffness, a predictor of cardiovascular risk and a surrogate marker of vascular disease (London and Cohn, 2002). Until now, there have been very few studies using PWV to evaluate SA or arterial stiffness in HIV patients, and these included very few subjects with different and opposite results (Ho et al., 2010; Lekakis et al., 2009; Van et al., 2009; 2006; Schillaci et al., 2005; Schillaci et al., 2008).

Furthermore, there is some controversy about the impact that administering ART might have on increasing the stiffness of the arterial walls in HIV patients. One meta-analysis involving 5456 HIV-positive and 3600 HIV-negative patients did not find an association between SA and ART (Hulten et al., 2009) other authors suggest that ART has a possible protective effect on the development of early atherosclerosis (Ho et al., 2010); finally, others, on the contrary, report an increased rate of SA in HIV patients taking antiretroviral therapy administered over a longer period (Lekakis et al., 2009; Van et al., 2009;

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Charakida et al., 2009).

On the other hand, the role that coinfection with other microorganisms might play in the development of SA in HIV cohorts is claimed to be contradictory: in HIV-seropositive patients with chronic HCV infection, this was associated with SA (Freiberg et al., 2007) nevertheless, in others it was not (Tien et al., 2009).

In light of these findings, our study is unprecedented and its objective is to use PWV (Complior© device) to analyse the prevalence of early atherosclerosis in HIV patients in Spain, an area of low cardiovascular risk, and compare it with the data published on seronegative Spanish population.

Besides, it investigates the different risk factors involved in SA, such as metabolic syndrome, ART and HCV-HIV coinfection; it also evaluates the correlation between the cardiovascular disease risk estimated using the Framingham risk score (FRS) and SCORE index, and the presence of SA in HIV-infected patients.

MATERIALS AND METHODS

Study design

The study was cross-sectional and comprised 136 consecutive HIV-positive patients. They enrolled in the outpatient clinic of the Infectious Diseases Unit at the *Virgen de las Nieves* University Hospital (VNUH), Granada, Spain, and signed an informed consent agreement.

Criteria for inclusion

1. HIV patients, over 18 years of age, who had been diagnosed with the infection for at least 1 year before inclusion in the study.
2. Patients who were receiving ART and had to have followed the same antiretroviral regimen for at least 6 months at the time of inclusion, as well as with an undetectable viral load.
3. Signing of an informed consent agreement or, in cases of mental incapacity, proper authorisation by their legal guardian.

Criteria for exclusion

1. Intercurrent illness (neoplasia, active infections).
2. Pregnancy.
3. Having suffered a cardiovascular event, such as acute myocardial infarction, stroke, or peripheral arteriopathy.

Variables for analysis

Epidemiological data

Age, gender, duration of HIV (in months), HIV stage (as classified by the CDC), type of ART and time in months since its administration, other diseases, smoking habits, and family history of cardiovascular disease.

Physical examination

Weight (kg), height (cm), waist (cm), hip (cm), systolic (SBP) and diastolic (DBP) blood pressure (mmHg), body mass index (BMI) (kg/m^2) and waist/hip ratio.

Analytical data (obtained the same day that PWV was performed, and in fasting)

Lipid profile, ApoA and ApoB lipoproteins, insulin, homocysteine, urea, creatinine, creatinine clearance (MDRD) (ml/min), nadir CD4 count (cells /uL), CD4 count (cells/uL) and HIV viral load (copies /uL) on enrollment, HCV viral load and genotype, serology for syphilis, HCV and HBV; the data detailing the number of months with HCV and/or HBV coinfection and viral load at HIV diagnosis were also collected.

Pulse wave velocity (PWV) was measured using the Complior® system (Complior Colson, Createch Industrie, France)

This is a non-invasive, automatic method for estimating arterial compliance which measures the carotid-femoral PWV (Cohn et al., 1995). The software inputs are blood pressure (BP) values, heart rate (bpm), height (cm), weight (kg) and the distance (cm) between carotid and femoral pulse recording sites. It consists of two sensors or pressure transducers placed on the carotid and femoral arteries which can capture frequencies of between 0.1 and 100 Hz. Once in place, a pedal attached to a computer mouse is used to measure and record ten pulse waves. The PWV value was obtained from the mean of the measurements taken (Asmar et al., 2001). The PWV is the result of dividing the distance between the two sensors by the time it takes the wave to pass through these two points. In our study, the measurement was always performed by the same researcher. We considered early atherosclerosis as occurring at values of PWV ≥ 12 m/s (Mancia et al., 2007). The measurement of PWV was done when the patient stayed 10 min at rest and 30 min since the last cigarette smoked.

Blood pressure (BP) was measured at rest and using a manual sphygmomanometer after 5 minutes of rest or 30 min after having smoked. It was recorded twice with a 5 min break between measurements; the arithmetic mean was the definitive figure.

Definition of variables

Metabolic syndrome was defined according to the criteria of the NCEP ATP-III 2001 (NCEP, 2001), updated by the American Heart Association in 2005 (Stone et al., 2005). A diagnosis was established when three or more of the risk factors below coexisted:

1. Abdominal circumference of ≥ 94 cm for men and ≥ 80 cm for women.
2. Triglycerides (TG) ≥ 150 mg / dL or drug treatment for TG.
3. HDL-C in men <40 mg / dl, and women <50 mg / dL, or drug treatment.
4. BP ≥ 130 / ≥ 85 mmHg or drug treatment for hypertension.
5. Fasting glucose level of ≥ 100 mg / dL, or drug treatment for hyperglycaemia.

Insulin resistance was calculated using the Matthews formula of the HOMA index: insulin (IU/mL) x glucose (mmol/L)/22.5. The cut-off point for a pathological Homa index was ≥ 3.8 (3.9 for women and 3.5 for men) (Muniyappa et al. 2008).

Body mass index (BMI) was calculated according to the following formula: weight (kg)/height $(m)^2$. Cardiovascular risk was calculated using the Framingham and SCORE systems. According to the Framingham tables (National Heart, Lung and blood institute; Grundy et al., 1999), a cardiovascular risk score at 10 years of > 30% was classified as very high, 20 to 30% was quite high, 15 to

20% was moderate and<15 was low.

The SCORE system considered <4% as low risk, 4 to 5% as moderate, 5 to 8% as high and > 8% as very high. We used the tables that corresponded to the Spanish population, that is, low-risk. For young people we employed tables appropriate to their age (De Backer et al., 2003).

Cardiovascular disease (CVD): acute myocardial infarction, angina, cerebrovascular disease and peripheral vascular disease. Virological failure was considered when a patient undergoing antiretroviral therapy presented a detectable viral load of ≥40 copies/uL on at least two occasions in six months. An undetectable HIV viral load was considered as less than 40 copies /uL in plasma.

Statistical analysis

Descriptive analysis

This described the main variables collected in the study with calculations of central tendency and dispersion (mean, standard deviation) for quantitative variables, and absolute and relative frequencies for qualitative variables. The prevalence of AS was established with a confidence interval of 95%.

Univariate analysis was performed to study the relationship between risk factors and the presence of early atherosclerosis. For quantitative variables, Student's t-test was applied for independent samples and the Mann-Whitney test if not normal. For qualitative variables, the chi-square or Fisher tests were performed if the criteria of application were not met. To test whether the variables complied with the assumption of normality, we used the Kolmogorov-Smirnov test. We performed a multivariate analysis using logistic regression. Statistically significant and clinically relevant variables were introduced into the model. We used the stepwise method for variable selection, with entrance and exit tolerances of 0.05 and 0.10 respectively at each stage. For all tests, we considered significance levels of 0.05.

RESULTS

136 HIV-positive patients from the Infectious Diseases Unit of the *Virgen de las Nieves* University Hospital, Granada, Spain, were incorporated consecutively. 4 (2.94%) patients were excluded from the study for the following reasons: 2 cases of acute myocardial infarction, 1 transient ischemic attack, 1 withdrawal of informed consent. 90 men (68.2%) and 42 women (31.8%) with an average age of 42.70 ± 9.71 years had been diagnosed with HIV for 118.96 ± 78.56 months. The average PWV was 9.56 ± 2.03 m/s (3–19.81). 11.4% (15/135) of patients had a PWV ≥12 m/s. According to the Framingham tables, 10-year cardiovascular risk was 15.64 ± 11.13%. By the SCORE tables, the risk was 1.26±1.36 (0–6) and 93.18% (123/132) of the study sample had a risk of <4%. Tables 1 and 2 show the other variables of the medical and therapeutic history.

When we conducted a univariate analysis for factors associated with higher carotid-femoral arterial stiffness in our group of HIV patients, we found that patients tended to be older (p = 0.001), diabetic (p = 0.032), to have elevated BP (p = 0.02), an anti-HCV-positive (p = 0.01), a higher HIV VL at diagnosis (p = 0.02) and a higher Framingham cardiovascular risk score (p = 0.001). With regard to ART, there tended to be an association between the current use of protease inhibitor (PIs) (p = 0.07). Since the statistical study grouped the different PIs according to metabolic profile, saquinavir, atazanavir and darunavir were not associated with SA (p = 0.71), while lopinavir, fosamprenavir and tipranavir showed a tendency towards an increased PWV (p = 0.06) (Table 3). Finally, in the multivariate analysis, the only two factors associated with pathological PWV were anti-HCV-positive (OR 6.57; 95% CI 1.41–30.60; p = 0.016), irrespective of the VL or the existence or not of chronic HCV infection, and age (OR 1.15; 95% CI 1.06–1.25; p = 0.001) (Table 3).

In addition, we analysed the variables that differentiated anti-HCV-positive from anti-HCV-negative patients (Table 4). A univariate analysis showed that coinfected patients were older (p= 0.001), smokers (p= 0.01), had been diagnosed with the coinfection for a longer period (p= 0.0001), and had higher triglyceride levels (p= 0.015), insulin (p= 0.008), homocysteine (p=0.007), and higher cardiovascular risk, according to Framingham (p= 0.001), diastolic blood pressure (p= 0.039), and HOMA index (p=0.009). Finally, in the multivariate analysis, the only factor associated was homocyteine levels (OR 1.24; 95%CI 1.084-1.43; p= 0.02).

DISCUSSION

The prevalence of SA in our HIV cohort was 11.4% (9.53% in women; 7.33% in men). This figure is due to the fact that our patients are young (50th percentile=42y) and they come from a low cardiovascular risk area. The prevalence of SA in asymptomatic patients has been evaluated in various populations differing in age, study method used, site explored—for example, coronary or carotid arteries—and with varying results (Guembe et al., 2010; El-Sadr et al., 2006; Moritani et al., 2005; Ortega, 2005). In the general US population over 65 years old subclinical cardiovascular disease was reported to be 38.7% for men and 36% for women (Kuller et al., 1994). In South Korea, and using computed tomography coronary angiography, the prevalence of subclinical coronary artery disease in asymptomatic young adults of <40 years was 11% (Ha et al., 2010). A recent investigation conducted in Spain on healthy subjects found a 18.4% prevalence of subclinical atherosclerosis (Aguilar-Shea et al., 2010), which is similar to our results.

In the general population, measurement of SA by PWV is an independent predictor and a marker for high cardiovascular risk (Willum-Hansen et al., 2006). In our group of HIV patients, we found after univariate analysis that age, diabetes mellitus, higher systolic blood pressure levels and heart rate—the classic cardiovascular risk factors—were independently associated with the appearance of SA; these constitute risk factors that have been reported in the HIV-negative population, where hypertension and pre-hypertension alike are associated

Table 1. Demographic data and medical history of HIV-infected patients (N=132).

Variables	Mean ± SD	Nº (%)
Age (years)	42.7±9.71 (23–71)	
Male	43.06±9.45	
Female	41.93±10.41	
Gender		
Male		90/132 (68.2)
Female		42/132 (31.8)
Time since HIV diagnosis (in months)	118.96±78.56	
AIDS stage (A3, B3, C)		70/132 (53)
Method of HIV transmission	Heterosexual	39/132 (29.5)
	Homos/Bisexual	39/132 (29.5)
	IDU	36/132 (27.3)
	Transfusion	6/132 (4.5)
	Unknown	12/132 (9.2)
Family history of CVD		6/132 (4.5)
Type 2 diabetes mellitus		7/132 (5.3)
Treated for hypertension		43/132 (32.6)
Hyperlipidaemia		26/132 (19.7)
Smoker		86/132 (65,1)
Metabolic syndrome		35/132 (26.5)
Chronic HBV hepatitis		9/132 (6.8)
Anti-HCV-positive patients		51/132 (38.6)
Chronic HCV hepatitis	439,771±10,207,796	41/132 (31.1)
Spontaneous negative HCV VL		10/51 (19.6)
HCV viral load (UI/uL)		27/41 (65.8)
Genotype 1		
		9/41 (21.9)
Genotype 2-3		5/41 (12.1)
Genotype 4		
Treatment with pegylated interferon and ribavirin		18/51 (30)
Positive syphilis serology		14/132 (10.6)
Latent tuberculosis infection		21/132 (16)
Naïve		30/132 (22.7)
Length of time current ART administered (in months)	22.76±7.36	
Viral failure		20/132 (19)
Zidovudine, didanosine, stavudine		17/102 (16.7)
Abacavir in current ART		35/102 (34.3)
Abacavir in previous ART (6—84 months)		45/102 (44.1)
NNRTI (EFV/NVP)		59/102 (57.84)
PI		41/102 (40.22)
Nadir CD4 count (cells/ml)	233.52±225.94	

Table 1. Contd.

HIV viral load at diagnosis (copie/mL)	210,887±826,191
Cd4 (cells/mL) on enrollment	549.31±303.15
HIV viral load on enrollment (copies/mL)	19,685±106,454
Waist-hip index (cm)	0.93±0.07
BMI (Kg/m^2)	24.66±4.09

CVD, Cardiovascular disease; **ART,** antiretroviral therapy; **NNRTI**, nonnucleoside reverse transcriptase inhibitor; **PI**, protease inhbitor; **BMI,** body mass index.

Table 2. Results of PWV and laboratory tests, and risk cardiovascular factor scales (Framhingan, Score) from HIV-infected patients.

Variables	Mean ± SD	Nº (%)
Average PWV	9.56±2.03 (3–19.81).	
% patients with PWV> 12m/s		15/132 (11.4)
Total cholesterol (mg/dL)	186.46±42.63	
LDL (mg/dL)	107.75±33.19	
HDL (mg/dL)	50.34±15.98	
Triglycerides (mg/dL)	157.75±114.68	
Fasting glucose (mg/dL)	97.2±19.81	
Creatinine clearance (mL/h)	92.46±22.42	
Basal Insulin (uU/mL)	14±11.99	
Homa Index	3.73±3.67	
Homocysteine (umol/L)	12.35±4.67	
ApoA I (mg/dL)	141.58±29.54	
ApoB (mg/dL)	76.92±21.02	
ApoA/ApoB ratio	2.01±0.89	
10-year Framingham risk score	15.64±11.13%	
50th Percentile		13.43
75th Percentile		22.48
Low risk <15%		77/132 (58.3)
Moderate risk 15-20%		18/132 (13.6)
High or very high risk >20%		37/132 (28.1)
10-year SCORE risk	1.26±1.36 (0-6)	
Low risk <4%		123/132 (93.18)
Moderate risk 4-5%		6/132(4..54
High risk 5-8%		3/132 (2.22)

with increased carotid-femoral arterial stiffness (Gedikli et al., 2010). However, in our group of HIV patients, and after multivariate analysis, age remained as the only traditional risk factor associated with the development of arterial stiffness (OR 1.15; 95% CI 1.06 - 0.25; p = 0.001).

The prevalence of metabolic syndrome (MS) in our HIV cohort was 26%, a similar figure to findings reported in earlier studies of HIV patients where there was a high rate of MS, although no higher than in the HIV-negative population (Jericó et al., 2005; Mondy et al., 2007); moreover, we did not find MS to be an independent risk factor for the appearance of subclinical cardiovascular disease (p=1). Recently, the RIVANA study, conducted among the general population in Navarra, Spain, found a direct association between MS and the emergence of SCVD, measured by echocardiography and the ultrasound examination of the carotid arteries (Guembe

Table 3. Characteristics of PWV-assessed HIV-infected patients, according to the presence or absence of subclinical atherosclerosis. Results of univariate and multivariate analyses.

	PWV< 12 m/s (n=117) Mean ± SD N (%)	PWV ≥ 12 m/s (n=15) Mean ± SD N (%)	p value	OR; 95% CI	p value
Age (years)	41.51±9.17	51.93±9.09	0.001	1.15; 1.06 - 1.25	0.001
Gender					
Male	79/90 (84.2)	11/90(73.3)	0.77		
Female	38/42 (90.47)	4/42(9.53)			
Time since HIV diagnosis (months)	114.94±78.09	150.33±77.71	0.10		
AIDS stage (A3, B3, C)	63 (53.8)	7(46.6)	0.6		
Family history of CVD	4(3.4)	2(13.3)	0.14		
Diabetes mellitus	4(3.4)	3(20)	0.03	0.78; 0.29-20.75	0.40
Treated for hypertension	34(29.1)	9(60)	0.02	3.17; 0.88-14.49	0.75
Hyperlipidaemia	25 (21.4)	1(6)	0.3		
Smoker	73(62.4)	13 (86.7)	0.06		
Anti-HCV-positive patients	41 (35)	10(66.6)	0.01	6.57; 1.41-30.60	0.016
HIV-HCV co-infection	33(28.2)	8(53.3)	0.07		
Chronic HBV hepatitis	7(5.9)	2 (13.3)	0.25		
Nadir CD4 count	233.44±228.26	225.00±214.82	0.89		
HIV viral load at diagnosis (copies/mL)	144.030±418.871	685.005±2,111.918	0.02	0.59; 0.4-1	0.44
Cd4 count (cells/mL) at enrollment	530.64±298.46	693.67±310.58	0.071		
HIV viral load at enrollment (copies/mL)	21.307±112.745	9.228±26.136	0.69		
Antiretroviral therapy status:					
-ART-Naive patients	28/30(93.3)	2/30(6.7)			
-ART-Exposed patients	89/102 (87.5)	13/102(12.7)	0.52		
Non-nucleoside inhibitors (NNRTI)	53(45.2)	5(33.3)	0.35		
Protease inhibitors (PI)	33(28.2)	8(53)	0.072		
Atazanavir/ Saquinavir/ Darunavir	18(15.4)	3 (20)	0.71		
Lopinavir/Fosamprenavir/Tipranavir	16(13.7)	5(33.3)	0.06		
Abacavir in previous ART	29(24.8)	6(40)	0.22		
Abacavir in current ART	36(30.8)	9(60)	0.02	0.032; 0.19-3.92	0,86
Zidovudine, didanosine, stavudine	18(15.4)	3(20)	0.7		
Current ART administration time (months)	22.27±16.70	26.00±21.74	0.47		
Viral failure	15(12.8)	5(33.3)	0.13		
Metabolic syndrome	31(26.5)	4(26.7)	1		
Total cholesterol (mg/dL)	188.42±43.54	171.20± 32.223	0.142		
LDL (mg/dL)	109.63±33.70	93.67±25.93	0.08		
HDL (mg/dL)	50.55±16.49	48.67±11.49	0.67		
Triglycerides (mg/dL)	159±118.74	146.20±77.92	13.68		
Fasting glucose (md/dL)	95.58±16.71	109.87±34.09	0.08		
Creatinine clearance (mL/h)	93.66±23.06	84.22±16.28	0.24		
Basal insulin (uU/Ml)	13.98±12.6	14.21±6.15	0.96		
Homocysteine (umol/L)	12.06±4.46	14.89±5.93	0.09		

Table 3. Contd

ApoAI (mg/dL)	141.36±29.88	143.28±27.94	0.82		
ApoB (mg/dL)	78.02±20.99	68.55±20.22	0.25		
ApoA/ApoB ratio	1.98±0.89	2.29±0.89	0.26		
Framingham 10 years (%)	14.46±10.52%	24.84±11.84%	0.001	0.05; 0.91-1.08	0.83
SCORE 10 years	1.19±1.26	1.87±1.96	0.07		
BMI (kg/m2)	24.61±4.06	24.99±4.46	0.74		
Waist-hip index (cm)	0.93±0.07	0.93±0.074	0.61		
Average WVP (m/s)	9.11±1.51	13.144±1.99	0.0001		
SBP (mmHg)	126.28±18.48	141.80±20.21	0.012	0.4; 0.93-1.04	0.53
DBP (mmHg)	80.99±12.31	85±13.21	0.28		
HR (bpm)	80.15±13.03	88±12.21	0.029	2.74; 0.9-1.13	0.10

p. significance levels**; OR, CI,** Odds ratio, Confidential interval; **SBP**, systolic blood pressure; **DBP**, dyastolic blood pressure; **HR**, heart rate.

Table 4. Comparison between anti-HCV positive HIV patients and anti-HCV negative HIV patients. Results of multivariate analyses.

Parameter	**HIV monoinfected (n= 81)**		**HIV with Antibdy-HCV (n=51)**				
	N (%)	**Mean ± SD**	**N (%)**	**Mean ± SD**	**p value**	**OR; 95%CI**	**p value**
Age (years)		40.44±10.45		46.27±7.16	0.001	1; 0.94-1.08	0.83
Time from HIV diagnosis (months)		83.53±64.07		175.24±65.87	0.0001	1.02; 1.01-1.03	0.09
Smoker	46(56.79)		40(78.43)		0.01	0.6; 0.43-7.29	0.42
Triglycerides (mg/dL)		138.55±92.94		188.25±138.16	0.015	0.99; 0.98-1.01	0.34
Basal insulin (uU/Ml)		10.94±7.14		17.78±15.39	0.008	1.1; 0.98-1.13	0.1
Homocysteine (umol/L)		11.2±4.23		13.87±4.83	0.007	1.24; 1.084-1.43	0.02
Framingham 10 years (%)		13.17±11.29		19.56±9.75	0.001	0.026; 0.89-1.10	0.87
SBP (mmHg)		126.36±18.58		130.73±20.17	0.45		
DBP (mmHg)		79.69±12.46		84.24±11.97	0.04	0.61; 0.93-1.03	0.43
HR (bpm)		80.94±13.2		81.2±13.15	0.9		

p significance levels**; OR, CI,** Odds ratio, Confidential interval; **SBP**, systolic blood pressure; **DBP**, dyastolic blood pressure; **HR**, heart rate.

et al., 2010).

With regard to cardiovascular risk factors in association with HIV, we found no association between the nadir CD4 count (233.52 ± 225.94 (cells/uL) and increased arterial stiffness ≥12 m/s (p = 0.89), a finding that contrasts with another publication which reported a correlation between an altered PWV measurement and a certain nadir CD4 count (Ho et al., 2010). The authors included 80 HIV-positive males taking ART and with an undetectable VL; arterial stiffness was measured using carotid-femoral PWV and, after adjusting the classic cardiovascular risk factors, such as age, BP, diabetes, smoking, and covariables related to HIV, they found that a nadir CD4 count of less than 350 cells/uL was associated with an increase in PWV of 0.41 m/s. They concluded that the earlier introduction of ART to HIV-infected patients might reduce cardiovascular risk (Ho et al., 2010), which is comparatively higher than in the general population (Lekakis et al., 2009).

With reference to ART in our patient group, we found no relationship between AS and antiretroviral therapy intake (p = 0.52), or length of time using the current antiretroviral, or the existence of previous failures. We also analysed the effect that taking particular antiretrovirals might have on the development of early atherosclerosis, but found no association with the administration of PIs (p=0.07), thymidine analogue (didanosine, zidovudine, stavudine) (p=0.41), abacavir (p=0.13) or nonnucleoside reverse transcriptase inhibitors (NNRTIs) (p=0.35). The use of ART has led to a radical improvement in the life expectancy of HIV-infected patients in developed countries; this development,

however, has been accompanied by the appearance of other non-AIDS illnesses, such as cardiovascular disease. This was shown in the D:A:D cohort where there was a higher percentage of HIV patients with atherosclerosis and CVD than in the HIV-negative group, and which was associated with the use of certain PIs, such as lopinavir and indinavir, and certain nucleoside reverse transcriptase inhibitors (NRTIs), such as abacavir (Sabin et al., 2007; Friiss-Moller et al., 2003). Recently published data from the ACTG A5001/ALLRT has found no evidence to suggest that initial ART containing abacavir increases Myocardial Infarction risk (Ribaudo et al., 2011).

There is some controversy in the literature regarding the role of ART in the development of early atherosclerosis and CVD, and it is not fully clear whether HIV infection, alone or in conjunction with other factors such as antiretroviral therapy intake, is the main factor in the development of atherosclerosis in HIV-infected patients (Schillacci et al., 2009). This could be the result of using different study designs, of surveying different geographical areas and age groups, or the small number of patients in some cases. So, in one cross-sectional study that used carotid-femoral PWV to compare arterial stiffness in HIV-positive and HIV-negative children, a higher rate of subclinical atherosclerosis was found in HIV-positive children, especially those taking ART (Hulten et al., 2009).

In another Greek case-control study that compared 56 HIV-positive patients, 28 hypertensive patients and 28 healthy controls, the authors detected higher PWV measurements in HIV patients receiving ART (Lekakis et al., 2009). Another Italian cross-sectional study comprising 55 control subjects and 77 HIV-positive patients, found that HIV patients receiving ART had increased stiffness in the femoral artery, but not in the carotid artery. It should be pointed out that in that study around 20% of patients suffered virological failure and the number of patients per group was small in comparison with the number of variables analysed, thus reducing the value of their conclusions (Van et al., 2009). In contrast to these results, and in line with ours, are the findings of a meta-analysis. This analysis compared HIV-positive patients with healthy controls and found no association between the incidence of atherosclerosis and antiretroviral therapy intake (Charakida et al., 2009).

These results were reconfirmed in a clinical trial (SMART), which implemented structured treatment interruption of ART and found an increased risk of cardiovascular disease during the interruptions to treatment, or therapeutic vacation (Mondy et al., 2007).

On the other hand, when we examined the possible influence of a coinfection from other microorganisms on the appearance of early atherosclerosis, we discovered that our anti-HCV-positive cohort was independently associated ($p=0.018$) with a PWV of $\geq$12 m/s, irrespective of the HCV VL and the existence or not of chronic hepatitis C.

At present, the role of chronic HCV infection in atherosclerosis is controversial. A study of HCV-infected haemodialysis patients found that there was a significant association between HCV infection, and increased aortic wall stiffness and cardiovascular events (Oyake et al., 2008); however other studies of healthy subjects, HBV-coinfected (Moritani et al., 2005) or HIV-coinfected (Tien et al., 2009), HCV-monoinfected, and even haemodialised (Caliskan et al., 2009; Adam et al., 2008), found no relation between HCV seroprevalence, and heart attack (Arcari et al., 2006), atherosclerosis (Caliskan et al., 2009; Völzke et al., 2004) or arterial stiffness (Charakida et al., 2009; Tien et al., 2009). When developing an HCV patient typology by means of AS, we found out that they had the highest homocysteine levels. Previous research claims that in HIV patients under ART at least for six months homocysteine levels is higher (Coria-Ramirez et al., 2010). Likewise, homocysteine is regarded to be a cardiovascular risk factor (Xiao et al., 2011), which reinforces our findings.

When we assessed the Framingham risk scores (FRS), 58.3% (77/132) of our patients were classified as low risk, 28.1% (37/132) as high or very high, and 13.6% (18/132) as moderate risk. In the univariate analysis, we found an association between increased carotid-femoral arterial stiffness and the FRS, with 100% of those whose risk was > 25% having a PWV of $\geq$ 12 m/s, and contrasting with those whose risk was <13% and a PWV of <12 m/s. In the multivariate analysis, we found no association between early atherosclerosis and the Framingham results (OR: 0.05; 95% CI 0.91-1.08; $p=0.83$). With regard to cardiovascular risk measured using the tables from the SCORE study (adapted to low-risk populations, such as Spain), no significant association was found in our patients between the values obtained from the scale and subclinical atherosclerosis ($p=0.07$). A recent piece of research of Spanish HIV patients analyses the association between the development of subclinical atherosclerosis—using carotid intima-media thickness (IMCT)—and the Framingham risk score, and has not found any correlation between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients (Parra et al., 2010).

Conclusions

The prevalence of early atherosclerosis in our HIV cohort was low (11.4%) and similar to the general Spanish population. It was associated primarily with age and anti-HCV-positive in patients with higher levels of homocysteine who had been diagnosed over a longer period of time.

In our group of HIV patients, as in the general population, the classic cardiovascular risk factors (tobacco, diabetes, hypertension, hyperlipidaemia, etc.)

must be controlled; nonetheless, efforts should be increased in the case of older HIV and HIV-HCV coinfected patients (irrespective of the viral load or the existence or not of chronic HCV infection) with elevated homocysteine levels, irrespective of gender, method of HIV acquisition, immunological and virological status, ART type used, or personal or family antecedents with cardiovascular disease. There was no correlation found between subclinical atherosclerosis, and the calculated Framingham risk score and SCORE index in HIV-infected patients.

Finally, our findings may have limitations regarding the design of the study becouse it is cross-sectional; therefore, it is crucial to perform other prospective analyses to help clarify the possible influence of HIV-HCV coinfection on the emergence of subclinical atherosclerosis.

REFERENCES

Adam FU, Torun D, Yigit F, Ozelsancak R, Sezer S, Ozdemir FN, Haberal M (2008). Determination of the impact of hepatitis C virus on insulin resistance and arterial stiffness in hemodialysis patients. Ren. Fail., 30: 411-415.

Aguilar-Shea AL, Gallardo-Mayo C, Garrido-Elustondo S, Calvo-Manuel E, Zamorano-Gómez JL (2010). Carotid intima-media thickness as a screening tool in cardiovascular primary prevention. Eur J Clin Invest., 10.1111/j.1365-2362.2010.02440.x.

Arcari CM, Nelson KE, Netski DM, Nieto FJ, Gaydos CA (2006). No association between hepatitis C virus seropositivity and acute myocardial infarction. Clin. Infect. Dis., 43: e53-6. Epub 2006 Aug 8.

Asmar R, Topouchian J, Pannier B (2001). Pulse wave velocity as endpoint in large-scale intervention trial. Complior Study J. Hypertens., 19: 813–818.

Caliskan Y, Oflaz H, Pusuroglu H, Boz H, Yazici H, Tamer S (2009). Hepatitis C virus infection in hemodialysis patients is not associated with insulin resistance, inflammation and atherosclerosis. Clin. Nephrol., 71: 147-57.

Charakida M, Loukogeorgakis SP, Okorie MI, Masi S, Halcox JP, Deanfield JE, Klein NJ (2009). Increased arterial stiffness in HIV-infected children: risk factors and antiretroviral therapy. Antivir. Ther.; 14: 1075–1079.

Cohn JN, Finkelstein S, Mc Veigh G, Morgan D, LeMay L, Robinson J (1995). Noninvasive pulse wave analysis for the early detection of vascular disease. Hypertension, 26: 503–508.

Coria-Ramirez E, Cisneros LN, Treviño-Perez S, Ibarra-Gonzalez I, Casillas-Rodriguez J, Majluf-Cruz A (2010).Effect of highly active antiretroviral therapy on homocysteine plasma concentrations in HIV-1-infected patients. J. Acquir. Immune Defic. Syndr., 54: 477-481.

De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J (2003). European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. Eur. Heart J., 24: 1601–1610.

El-Sadr WM, Lundgren JD, Neaton JD, Gordin F, Abrams D, Arduino Rc (2006). Strategies for Management of Antiretroviral Therapy (SMART) study Group. CD4 count-guided interruption of antiretroviral treatment. N. England J. Med., 355: 2283–2296..

Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) (2001). Expert Panel on detection, evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel III). JAMA 2001; 285: 2486–2497.

Freiberg MS, Cheng DM, Kraemer KL, Saitz R, Kuller LH, Samet JH (2007). The association between hepatitis C infection and prevalent cardiovascular disease among HIV-infected individuals. AIDS, 21: 193-197.

Friiss-Moller N, Weber R, Reiss P, Thiebaut R, Kirk O, d'Arminio A (2003). Cardiovascular disease risk factors in HIV patients' association with antiretroviral therapy. Results from DAD study. AIDS, 17: 1179–1193.

Gedikli O, Kiris A, Ozturk S, Baltaci D, Karaman K, Durmus I (2010). Effects of prehypertension on arterial stiffness and wave reflections. Clin. Exp. Hypertens., 32: 84–89.

Grundy S, Pasternak R, Greenland P, Smith S, Fuster V (1999). Assessment of cardiovascular risk by use of multiple risk factor assessment equations. A statement for health care professionals from the American Heart Association and the American College of Cardiology. Circulation, 100: 1481–1492.

Guembe MJ, Toledo E, Barba J, Martínez-Vila E, González-Diego P, Irimia P (2010). Association between metabolic syndrome or its components and symptomatic cardiovascular disease in the RIVANA-study. Atherosclerosis.; 211: 612–617.

Ha EJ, Kim Y, Cheung JY, Shim SS (2010). Coronary artery disease in asymptomatic young adults: its prevalence according to coronary artery disease risk stratification and the CT characteristics. Korean J. Radiol., 11: 425–432.

Ho JE, Deeks SG, Hecht FM, Xie Y, Schnell A, Martin JN (2010). Initiation of antiretroviral therapy at higher nadir CD4+ T-cell counts is associated with reduced arterial stiffness in HIV-infected individuals. AIDS, 24: 1897-1905.

Hulten E, Mitchell J, Scally J, Gibbs B, Villines TC (2009). HIV positivity, protease inhibitor exposure and subclinical atherosclerosis: a systematic review and meta-analysis of observational studies. Heart, 95: 1826–1835.

Jericó C, Knobel H, Montero M, Ordoñez-Llanos M, Guela A, Gimeno JL (2005). Metabolic syndrome among HIV-infected patients. Diabetes Care, 28: 144–149.

Kuller L, Borhani N, Furberg C, Gardin J, Manolio T, O Leary D, Psaty B, Robbins J (1994). Prevalence of subclinical atherosclerosis and cardiovascular disease and association with risk factors in the Cardiovascular Health Study. Am. J. Epidemiol., 139: 1164–1179.

Lekakis J, Ikonomidis I, Palios J, Tsiodras S, Karatzis E, Poulakou G (2009). Association of highly active antiretroviral therapy with increased arterial stiffness in patients infected with human immunodeficiency virus. Am. J. Hypertens., 22: 828-834.

London GM, Cohn JN (2002). Prognostic application of arterial stiffness: task forces. Am. J. Hypertens., 15: 754–758.

Mancia G, De Backer G, Dominczak A, Cifkara R, Fagard R, Germano G (2007). 2007 Guideline for the Management of arterial hypertension: the task for the Management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J. Hypertens., 25: 1105–1187.

Mondy K, Overton ET, Grubb J, Tong S, Seyfried W, Powderly W (2007). Metabolic syndrome in HIV-infected patients from an urban, midwestern US outpatient population. Clin. Infect. Dis., 44:726–734.

Moritani M, Adachi K, Arima N, Takashima T, Miyaoka Y, Niigaki M, Furuta K, Sato S, Kinoshita Y(2005). A study of arteriosclerosis in healthy subjects with HBV and HCV infection. J. Gastroenterol., 40: 1049–1053.

Moritani M, Adachi K, Arima N, Takashima T, Miyaoka Y, Niigaki M (2005). A study of arteriosclerosis in healthy subjects with HBV and HCV infection. J. Gastroenterol., 40: 1049-1053.

Muniyappa R, Lee S, Chen H, Quon MJ(2008). Current approaches for assessing insulin sensitivity and resistance *in vivo*: advantages, limitations, and appropriate usage. Am. J. Physiol. Endocrinol. Metab., 294: E15–26.

National Heart, Lung and Blood Institute (2009). Framingham Heart Study: 50 Years of Research Success. URL disponible en: http://www.nhlbi.nih.gov/about/framingham/ index.html.

Ortega CA (2005). Hacia el abordaje multifactorial del RCV. Boletín Oficial de la SAMFyC. 2005; 11:14–17. Ortega Carpio A. Hacia el abordaje multifactorial del RCV. Boletín Oficial de la SAMFyC, 11:14–17.

Oyake N, Shimada T, Murakami Y, Ishibashi Y, Satoh H,Parra S, Coll B, Aragonés G, Marsillach J, Beltrán R, Rull A (2010). Nonconcordance between subclinical atherosclerosis and the calculated Framingham risk score in HIV-infected patients: relationships with serum markers of oxidation and inflammation. HIV Med. 11: 225–231.

Ribaudo HJ, Benson CA, Zheng Y, Koletar SL, Collier AC, Lok JJ

(2011). No Risk of Myocardial Infarction Associated With Initial Antiretroviral Treatment Containing Abacavir: Short and Long-Term Results from ACTG A5001/ALLRT. Clin. Infect. Dis., 52: 929-940.
Sabin Ca, Worn SW, Weber R, Reiss P, El-Sadr W, Davis F (2007). DAD study Group. Class of antiretroviral drugs and the risk of myocardial infarction. N. England J. Med., 356: 1723–1735.
Schillacci G, Pucci G, De Socio GVL (2009). HIV infection and antiretroviral treatment: a “two-hit” model for arterial stiffness?. Am. J. Hypertens., 22: 817–818.
Schillaci G (2005). Impact of treatment with protease inhibitors on aortic stiffness in adult patients with human immunodeficiency virus infection. Arterioscler. Thromb. Vasc. Biol., 25: 2381–2385.
Schillaci G, De Socio GV, Pucci G, Mannarino MR, Helou J, Pirro M (2008). Aortic stiffness in untreated adult patients with human immunodeficiency virus infection. Hypertension., 52: 308–313.
Stone NJ, Bilek S, Rosenbaum S (2005). Recent National Cholesterol Education Program Adult Treatment Panel III update: adjustments and options. Am. J. Cardiol., 96: 53E–59E.
Tien PC, Schneider MF, Cole SR, Cohen MH, Glesby MJ, Lazar J (2009). Association of hepatitis C virus and HIV infection with subclinical atherosclerosis in the women's interagency HIV study. AIDS, 24(23): 1781-1784.
Van Vonderen MG, Smulders YM, Stehouwer CD, Danner SA, Gundy CM, Vos F (2009). Carotid intima-media thickness and arterial stiffness in HIV-infected patients: the role of HIV, antiretroviral therapy, and lipodystrophy. J. Acquir. Immune Defic. Syndr., 50: 153–161.
Van Wijk de Koning EJ, Cabezas MC, Joven J, op't Roodt J, Rabelink TJ (2006). Functional and structural markers of atherosclerosis in human immunodeficiency virus-infected patients. J. Am. Coll. Cardiol., 47: 1117–1123.
Völzke H, Schwahn C, Wolff B, Mentel R, Robinson DM, Kleine V (2004). Hepatitis B and C virus infection and the risk of atherosclerosis in a general population. Atherosclerosis, 174: 99-103.
Willum-Hansen T, Stacssen JA, Torp-Pedersen C (2006). Prognostic value of aortic pulse wave velocity as index of arterial stiffness in the general population. Circulation, 113: 664–670
Xiao Y, Zhang Y, Lv X, Su D, Li D, Xia M, Qiu J, Ling W, Ma J (2011). Relationship between lipid profiles and plasma total homocysteine, cysteine and the risk of coronary artery disease in coronary angiographic subjects. Lipids Health Dis., 10: 137.