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HIV Disease, Antiretroviral Therapy Safety and the Cardiovascular System. Clinical-Instrumental Assessment of Antiretroviral-Naïve *Versus* Subjects Already Treated with Antiretroviral Agents

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Abstract: *Background:* There is controversy over whether or not HIV infection and antiretroviral therapy contribute to early atherosclerosis. Ultrasonographic evaluation of carotid intima-media thickness is considered as a reliable surrogate marker of subclinical atherosclerosis and may be employed in the setting of a cardiovascular risk assessment in HIV-infected patients.

Patients and methods: A cross-sectional study evaluating classical risk factors for cardiovascular diseases, parameters of HIV infection, antiretroviral therapy and subclinical atherosclerosis in HIV-positive subjects naïve or treated with antiretroviral agents was performed. The enrolled patients underwent ultrasonography of the epi-aortic vessels using a Philips HDI 5000 power color-Doppler with 7.5-MHz probes. The 10-year risk of coronary heart disease was calculated by the Framingham equation.

Results: A total of 27 patients (19 males and 8 females; mean age 44 $\pm$ 13 years; range 32-59 years) was enrolled in the study: 11 subjects were naïve to all antiretroviral agents (group A) and 16 patients were treated with antiretroviral therapy for $\geq$36 months (group B). Mean duration of known HIV infection was significantly longer in group B than in group A, such as frequency of dyslipidemia and lipodystrophy syndrome. Prevalence of carotid plaques was significantly higher in group B than in group A (43.7% versus 0; p=0.012). In group B, patients with intermediate to high 10-year risk of coronary heart disease ($\geq$10%) showed a significantly higher intima-media thickness and prevalence of carotid lesions than those with low risk (<10%). Moreover, carotid plaques presented structural features comparable to those of classical atherosclerotic plaques observed in general population, with iso-hyperechonegic aspects and irregular surfaces.

Conclusions: Prevalence of carotid atherosclerosis is higher in HIV-infected patients previously treated with antiretrovirals than in those naïve to antiretroviral therapy and seems mostly associated with a longer duration of HIV infection, more severe lipid metabolism alterations, presence of lipodystrophy syndrome, and a more elevated 10-year risk of cardiovascular diseases.

Keywords: HIV infection, antiretroviral therapy, atherosclerosis, carotid artery, ultrasonography.

INTRODUCTION

The introduction of highly active antiretroviral therapy (HAART) in clinical practice has resulted in a dramatic reduction of morbidity and mortality associated with the human immunodeficiency virus (HIV) infection in the developed world [1]. However, long-term toxicity of antiretroviral drugs is becoming recognized and widely assessed, therefore detecting a wide range of side effects including lipodystrophy and metabolic alterations, which have frequently been associated with new combination therapies, particularly when they are based on protease inhibitors (PIs) [2].

Since new PI-containing antiretroviral regimens have led to a notable extension of life expectancy in HIV-positive patients, prolonged lipid and glucose metabolism abnormalities could significantly act on the long-term prognosis and outcome of HIV-infected persons. In the post-HAART era, long-term cardiovascular complications, including myocardial infarction, peripheral vascular diseases, and stroke have been frequently reported, and an increasing concern is mounting particularly about the increased risk of acute coronary syndromes associated with new potent antiretroviral combinations [3]. In particular, some studies have demonstrated a correlation between antiretroviral therapy and increased risk of coronary heart disease [4-7], while the association between HIV infection, PI therapy and premature atherosclerosis has inconsistently been reported in the literature during the last 15 years, i.e. since the early introduction of triple combination HAART regimens containing just HIV protease inhibitor, which occurred at the end of the year 1996 [8-10]. However, the relationship between coronary heart disease and the use of HAART in HIV-infected patients is still a matter of debate. Several studies [2-10], have investigated a possible association between antiretroviral treatment and cardiovascular disease using various statistical approaches, but they often reported inconsistent and incom-

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parable results. Classic vascular risk factors (male gender, age, family history of coronary heart diseases, smoking, arterial hypertension, diabetes, dyslipidemia) obviously contribute to an increased risk of cardiovascular complications in HIV-infected patients [11]. On the other hand, recent data has highlighted systemic inflammation as a crucial factor in the pathogenesis of carotid lesions in HIV-positive subjects [12], and Maggi *et al.* have demonstrated that the ultrasonographic structure of the carotid lesions in HIV-infected individuals substantially differ from those of classical atherosclerotic plaques and share similar features with patients affected by arteritis [13].

Because a remarkable limitation of clinical trials evaluating the incidence of cardiovascular complications in HIV-infected patients is the low event rate, surrogate markers may be helpful to predict cardiovascular risk in this population. Measurement of carotid intima-media thickness by high-resolution ultrasonography is a well-accepted, non-invasive method of evaluating subclinical atherosclerosis, and is a potent predictor of myocardial infarction and stroke [14].

The aim of our cross-sectional study is to investigate the relationship between HIV infection, antiretroviral drug history, classical cardiovascular risk factors and ultrasound evidence of carotid artery atherosclerosis. Moreover, in this study we aimed to provide a precise description of structural features characterizing carotid lesions in HIV-positive population.

PATIENTS AND METHODS

HIV-infected patients referred to our tertiary care outpatient centre between May 1st –May 31st, 2007, and who fulfilled inclusion and exclusion criteria were included into the present study.

Inclusion criteria were: age 18-60 years, proven HIV-1 infection, and receiving antiretroviral therapy for $\geq$36 months or being naïve to all antiretroviral agents. Exclusion criteria were: clinical history of coronary heart disease, cerebrovascular disease, or peripheral vascular disease; diabetes mellitus (diagnosed with fasting serum glucose levels $\geq$ 126 mg/dL or use of hypoglycaemic drugs); known alcohol abuse or drug addict; lipid-lowering medication; medication for arterial hypertension; pregnancy or lactation.

All patients hospitalized at our teaching hospital have to give their written, informed consent to use their data (anonymously) for eventual publication. The original, signed documents are part of the individual clinical charts, and they are available, upon request.

All subjects underwent laboratory examinations, including haematology testing, measurement of plasma glucose, triglycerides, total cholesterol, low-density lipoprotein (LDL) cholesterol, and high-density lipoprotein (HDL) cholesterol levels, CD4 lymphocyte count, and HIV-1 RNA viral load. Plasma HIV viral load was evaluated using the bDNA Quantiplex HIV-RNA-3 assay (Chiron Corporation, Emeryville, CA, USA), according to the manufacturer's instructions, with a lower limit of detection placed at 50 bDNA copies/mL. Physical examination included evaluation of body mass index (BMI), waist circumference, and blood pressure (measured with a single manual device located in our dedicated outpatient service for HIV-infected subjects). Traditional risk factors for coronary artery disease, and presence of lipodystrophy syndrome and/or metabolic syndrome, were carefully evaluated. The 10-year risk for myocardial infarction in all considered patients was estimated by the Framingham equation (available at the following on-line address: http://hp2010.nhlbihin.net/atpiii/calculator.asp).

Traditional risk factors for coronary heart disease were defined as follows: age (men > 45 years and women > 55 years), family history of premature coronary heart disease (men < 55 years and women < 65 years), active cigarette smoking ($\geq$ 1 cigarette smoking in the past month), hypercholesterolemia (fasting serum total cholesterol level > 200 mg/dL or fasting serum LDL cholesterol level > 130 mg/dL), decreased HDL cholesterol (fasting serum levels < 40 mg/dL), hypertriglyceridemia (fasting serum triglyceride levels > 150 mg/dL), arterial hypertension (blood pressure $\geq$ 140/90 mmHg), and obesity (BMI $\geq$ 30 Kg/m^2).

A lipodystrophy syndrome was diagnosed in patients with peripheral fat loss, central fat accumulation or a mixed form (peripheral lipoatrophy and central lipohypertrophy), as assessed at physical examination. Metabolic syndrome was defined as the occurrence of 3 or more of the following abnormalities: abdominal obesity (waist circumference > 102 cm for men and > 88 cm for women), hypertriglyceridemia (fasting serum triglyceride levels > 150 mg/dL), decreased HDL cholesterol (fasting serum levels < 40 mg/dL for men and < 50 mg/dL for women), arterial hypertension (systolic blood pressure > 130 mg/dL and/or diastolic blood pressure > 85 mmHg), and hyperglycemia (fasting glucose levels $\geq$ 100 mg/dL).

The enrolled patients were subjected to ultrasonography of the epi-aortic vessels using a Philips HDI 5000 power color-Doppler with 7.5-MHz probes (Koninklijke Philips Electronics, Eindhoven, the Netherlands). Ultrasonography was performed by a physician with 15 years of training in color-doppler carotid ultrasonography. He was blinded to the patients' treatment history and status.

The patients were placed in a supine position after at least 10 minutes of acclimatization in a comfortable room. The common carotid, including the bifurcation and at least the first 2 cm of the internal and external carotid arteries were evaluated in the short and long axis during the tele-diastolic phase. During the investigation, the head of the patient was hyper-extended and extra-rotated from the opposite side. The morphological investigation of the carotid lesions (or plaques) was performed using both ultrasonography and the ultrasound power color-Doppler to better characterize the profile of the plaque and the intima-media thickness (IMT). Particularly, the following ultrasound color-Doppler features of the carotid lesions were examined: IMT, presence of carotid plaque (defined when IMT was above 1.2 mm) echogenicity of the lesion with respect to the vessel wall (anechogenic, isoechogenic, hypoechogenic, or hyperechogenic), and features of endoluminal and parietal portions of the plaque (whether homogeneous or not). In detail, we considered isoechogenic, hypoechogenic, and iso-hypoechogenic lesions as "iso-hypoechogenic", and iso-hyperechogenic and hyperechogenic lesions as "iso-hyperechogenic".

Data are presented as mean $\pm$ standard deviation (SD) for descriptive data, while comparisons between groups were performed by Student t test or Fisher exact test (where appropriate), with significance levels placed at $p<0.05$. The study was approved by the ethics committee of the hospital and written informed consent was obtained from all the participants.

RESULTS

A total of 27 patients (19 males and 8 females; mean age 44 ± 13 years; range 32-59 years) were enrolled into the study: 11 subjects were naïve to all antiretroviral agents (group A) and 16 patients were treated with antiretroviral therapy for ≥36 months (group B). Demographic, epidemiological, clinical, laboratory and ultrasonographic characteristics of the enrolled patients are depicted in Table **1**.

Male/females ratio, mean age, frequency of classical risk factors for coronary heart disease (cigarette smoking, arterial hypertension, family history of coronary heart disease), and mean CD4 lymphocyte count were comparable in group A and B. Mean duration of known HIV infection was significantly longer in group B than in group A (9.5 and 4.7 years, respectively), such as frequency of lipodystrophy syndrome (50% and 0, respectively). Current antiretroviral therapy in

Table 1. Demographic, Epidemiological, Clinical, Laboratory, and Ultrasonographic Characteristics of our Study Population

	Group A (naïve patients)	**Group B (subjects who were previously treated with antiretroviral agents)**	***p* value**
No. of patients	11	16	n.s.
Males/females ratio	8/3	12/4	n.s.
Mean age ± SD (years)	42 ± 18	44 ± 21	n.s.
Homosexuals drug addicts	5/4/2	8/6/2	n.s.
No. of patients (%) - with current cigarette smoking - arterial hypertension - family history of coronary heart disease	 5 (45.4) 0 1 (8.3)	 7 (43.7) 2 (12.5) 1 (6.2)	 n.s. n.s. n.s.
Mean duration of HIV infection ± SD (years)	4.7 ± 2.2	9.5 ± 4.3	0.009
No. of patients (%) with AIDS diagnosis	0	3 (18.7)	n.s.
Mean CD4 lymphocyte count ± SD (cells/mm^3)	530 ± 211	635 ± 299	n.s.
No. of patients (%) with undetectable HIV viral load (HIV RNA < 50 copies/mL)	0	12 (75)	0.021
Mean HIV RNA ± SD (log_{10} copies/mL) in patients with detectable HIV viral load	3.7 ± 1.6	2.8 ± 1.2	n.s.
Mean duration of HAART ± SD (months)	0	74 ± 35	0.007
Mean concentration of total cholesterol ± SD (mg/dL)	178 ± 82	219 ± 125	0.005
Mean concentration of LDL cholesterol ± SD (mg/dL)	108 ± 59	138 ± 78	0.017
Mean concentration of HDL cholesterol ± SD (mg/dL)	46 ± 21	53 ± 29	n.s.
Mean concentration of triglycerides ± SD (mg/dL)	112 ± 61	167 ± 95	0.031
Mean concentration of glucose ± SD (mg/dL)	69 ± 25	74 ± 28	n.s.
Mean waist circumference ± SD (cm)	89 ± 41	92 ± 44	n.s.
Mean BMI ± SD (Kg/m^2)	23.9 ± 11.2	24.4 ± 10.8	n.s.
No. of patients (%) with metabolic syndrome	0	2 (12.5)	n.s.
No. of patients (%) -with lipodystrophy syndrome -lipoatrophy - mixed form	0 0 0 0	8 (50) 3 (18.7) 2 (12.5) 3 (18.7)	0.021
Mean 10-year risk of MI ± SD (%)	4.4 ± 1.9	7.3 ± 2.9	n.s.
Mean IMT ± SD (mm) - right common carotid artery - left common carotid artery - right carotid bifurcation - left carotid bifurcation - right internal carotid artery - left internal carotid artery	 0.86 ± 0.45 0.82 ± 0.47 0.95 ± 0.56 1.02 ± 0.51 0.89 ± 0.49 0.92 ± 0.53	 1.38 ± 0.64 1.59 ± 0.73 1.42 ± 0.59 1.44 ± 0.62 1.27 ± 0.55 1.33 ± 0.75	 n.s. n.s. n.s. n.s. n.s. n.s.
No. of patients (%) with carotid plaques	0	7 (43.7)	0.012

n.s., no significant; SD, standard deviation; AIDS, acquired immunodeficiency syndrome; HAART, highly active antiretroviral therapy; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; MI, myocardial infarction; IMT, intima-media thickness group B included two nucleoside reverse transcriptase inhibitors (NRTIs) in all 16 patients associated with one non-nucleoside reverse transcriptase inhibitor (NNRTIs) in 7 subjects and one protease inhibitor (PI) in the remaining 9 patients. NRTI-therapy included zidovudine in 5 subjects, tenofovir in 4, stavudine in 3, abacavir in 3, didanosine in 1, lamivudine in 11, and emtricitabine in 5. NNRTI-therapy included efavirenz in 5 patients and nevirapine in 2. PI-treatment included lopinavir-ritonavir in 4 individuals, atazanavir-ritonavir in 2, fosamprenavir-ritonavir in 2, and saquinavir-ritonavir in one patient.

With regard to lipid metabolism alterations, dyslipidemia was significantly more frequent among subjects who already received antiretroviral drugs, than among naïve ones. Particularly, mean serum concentrations of total cholesterol, LDL cholesterol and triglycerides were significantly greater in group B (219, 138, and 167 mg/dL, respectively) than in group A (178, 108, and 112 mg/dL, respectively). On the other hand, no significant differences between the two compared groups were observed with regard to mean serum HDL cholesterol and glucose levels, morphological parameters (mean waist circumference and body mass index), and prevalence of metabolic syndrome. The overall 10-year risk of myocardial infarction (estimated by the Framingham equation) was significantly higher in group B (7.3%) than in group A (4.4%).

Mean values of IMT in the right and left common carotid arteries, carotid bifurcations, and internal carotid arteries of groups A and B are reported in Table **1**. The mean IMT ± standard deviation (SD) in the common carotid arteries (calculated as the mean IMT of the right and left common carotid arteries) was 0.85 ± 0.46 in group A and 1.45 ± 0.69 in group B (p=0.065). The mean IMT ± SD in the carotid bifurcations (calculated as the mean IMT of the right and left carotid bifurcations) was 0.98 ± 0.51 in group A and 1.42 ± 0.65 in group B (p=0.082). The mean IMT ± SD in the internal carotid arteries (calculated as the mean IMT of the right and left internal carotid arteries) was 0.9 ± 0.52 in group A and 1.29 ± 0.72 in group B (p=0.059). Therefore, mean values of carotid IMT in patients previously treated with antiretroviral drugs were evidently higher than in naïve subjects, even though they did not reach the statistical significance. At the same time, prevalence of carotid plaques was greater in group B than in group A (43.7% and 0, respectively), with statistical significance (p=0.012).

Patients belonging to group B were divided into two subgroups with regard to their 10-year risk of myocardial infarction calculated by the Framingham equation: low risk (<10%) and high risk (≥10%). Mean cardiovascular risk ± SD in the "low risk" and "high risk" subgroups was 3.4 ± 1.3% and 18.9 ± 9.5%, respectively. Demographic, epidemiological, clinical, laboratory and ultrasonographic features of the two subgroups are summarized in Table **2**.

Male/females ratio, mean age, frequency of "classical" risk factors for cardiovascular disease, mean duration of known HIV infection, mean duration of antiretroviral therapy, type of current antiretroviral drugs, and immuno-virological variables were comparable in the two subgroups. On the contrary, lipid metabolism parameters were remarkably worse in "high risk" patients than in "low risk" ones: mean serum concentrations of total cholesterol, LDL cholesterol, and triglycerides were significantly higher in the "high risk" subgroup (259, 180, and 268 mg/dL, respectively) than in the "low risk" one (205, 123, and 132 mg/dL, respectively). Mean values of serum glucose levels, waist circumference, body mass index, and prevalence of metabolic syndrome did not significantly differ in the two subgroups, while prevalence of lipodystrophy was significantly higher among the "high risk" patients (100%) than among the "low risk" ones (33.3%).

Mean values of IMT in the right and left common carotid arteries, carotid bifurcations, and internal carotid arteries of both subgroups are reported in Table **2**. The mean IMT ± SD in the common carotid arteries (calculated as the mean IMT of the right and left common carotid arteries) was 0.74 ± 0.32 among "low risk" patients and 1.48 ± 0.89 among "high risk" ones (p=0.006). The mean IMT ± SD in the carotid bifurcations (calculated as the mean IMT of the right and left carotid bifurcations) was 0.91 ± 0.54 among "low risk" subjects and 1.47 ± 0.75 among "high risk" ones (p=0.004). The mean IMT ± SD in the internal carotid arteries (calculated as the mean IMT of the right and left internal carotid arteries) was 0.97 ± 0.57 among "low risk" individuals and 1.49 ± 0.63 among "high risk" ones (p=0.003). Therefore, mean values of carotid IMT were significantly higher in "high risk" than in "low risk" patients, such as the prevalence of carotid plaques (75% versus 33.3%, respectively; p=0.047).

The ultrasonographic and structural description of the carotid plaques in patients of the group B evidenced that both in "low risk" and in "high risk" subjects the lesions appeared mostly iso-hyperechogenic and irregular both in their parietal and endoluminal portions in the 100% of cases.

If patients of the group B are divided in those with lipodystrophy (8 subjects) and those without lipodystrophy (8 subjects), higher cardiovascular risk and subclinical carotid atherosclerosis were found to be associated with presence of fat redistribution syndrome. Particularly, mean 10-year risk ± SD of myocardial infarction was 10.8 ± 4.2% and 3.8 ± 1.4% in lipodystrophic and non lipodystrophic patients, respectively. The mean IMT ± SD in the common carotid arteries (calculated as the mean IMT of the right and left common carotid arteries) was 0.67 ± 0.32 among non lipodystrophic patients and 1.09 ± 0.59 among lipodystrophic ones (p=0.014). The mean IMT ± SD in the carotid bifurcation (calculated as the mean IMT of the right and left carotid bifurcation) was 0.73 ± 0.35 among non lipodystrophic patients and 1.14 ± 0.66 among lipodystrophic ones (p=0.011). The mean IMT ± SD in the internal carotid arteries (calculated as the mean IMT of the right and left internal carotid arteries) was 0.81 ± 0.48 among non lipodystrophic patients and 1.19 ± 0.72 among lipodystrophic ones (p=0.025). Presence of lipodystrophy was also associated with a longer mean duration ± SD of antiretroviral therapy than absence of lipodystrophy (95 ± 42 and 58 ± 26 months, respectively; p=0.031).

DISCUSSION

The ability of potent combination antiretroviral regimens (particularly those including PIs) to accelerate atherosclerosis and increase the risk of cardiovascular diseases has been controversial in that some studies have found an association and other studies have not found an association. Much of this

Table 2. Demographic, Epidemiological, Clinical, Laboratory, and Ultrasonographic Characteristics of Patients Included in the Group B and Divided According to the Ten-Year Risk of Myocardial Infarction: Low Risk (<10%) and High Risk (≥10%)

	Low risk subjects (<10%)	High risk subjects (≥10%)	*p* value
No. of patients %	12	4	n.s.
Males/females ratio	9/3	3/1	n.s.
Mean age ± SD (years)	41 ± 16	46 ± 22	n.s.
No. of patients (%) - with current cigarette smoking - arterial hypertension - family history of coronary heart disease	 5 (41.6) 2 (16.6) 0	 2 (50) 0 1 (25)	 n.s. n.s. n.s.
Mean duration of HIV infection ± SD (years)	9.1 ± 4.2	9.8 ± 4.7	n.s.
No. of patients (%) with AIDS diagnosis	2 (16.6)	1 (25)	n.s.
Mean CD4 lymphocyte count ± SD (cells/mm^3)	611 ± 279	649 ± 302	n.s.
No. of patients (%) with undetectable HIV viral load (HIV RNA < 50 copies/mL)	9 (75)	3 (75)	n.s.
Mean duration of HAART ± SD (months)	73 ± 35	78 ± 39	n.s.
No. of patients (%) currently treated with - NNRTIs - PIs	 5 (41.7) 7 (58.3)	 2 (50) 2 (50)	 n.s. n.s.
Mean concentration of total cholesterol ± SD (mg/dL)	205 ± 102	259 ± 135	0.006
Mean concentration of LDL cholesterol ± SD (mg/dL)	123 ± 65	180 ± 92	0.002
Mean concentration of HDL cholesterol ± SD (mg/dL)	54 ± 20	47 ± 19	n.s.
Mean concentration of triglycerides ± SD (mg/dL)	132 ± 69	268 ± 144	0.003
Mean concentration of glucose ± SD (mg/dL)	75 ± 33	71 ± 27	n.s.
Mean waist circumference ± SD (cm)	90 ± 46	94 ± 49	n.s.
Mean BMI ± SD (Kg/m^2)	23.6 ± 11.1	24.8 ± 11.4	n.s.
No. of patients (%) with metabolic syndrome	0	2 (50)	n.s.
No. of patients (%) - with lipodystrophy syndrome - lipoatrophy - lipohypertrophy - mixed form	 4 (33.3) 1 (8.3) 2 (16.6) 1 (8.3)	 4 (100) 2 (50) 0 2 (50)	0.001
Mean 10-year risk of MI ± SD (%)	3.4 ± 1.3	18.9 ± 9.5	<0.001
Mean IMT ± SD (mm) - right common carotid artery - left common carotid artery - right carotid bifurcation - left carotid bifurcation - right internal carotid artery - left internal carotid artery	 0.75 ± 0.32 0.72 ± 0.33 0.92 ± 0.56 0.89 ± 0.52 0.95 ± 0.56 0.99 ± 0.59	 1.44 ± 0.81 1.51 ± 0.86 1.48 ± 0.77 1.46 ± 0.69 1.52 ± 0.64 1.49 ± 0.61	 0.007 0.005 0.004 0.004 0.002 0.004
No. of patients (%) with carotid plaques	4 (33.3)	3 (75)	0.047
No. of carotid plaques (%) - iso-hypoechogenic - iso-hyperechogenic	0 4 (100)	0 3 (100)	n.s. n.s.
No. of carotid plaques (%) with parietal and endoluminal portions appearing - homogeneous - irregular	0 4 (100)	0 3 (100)	n.s. n.s.

n.s., no significant; SD, standard deviation; AIDS, acquired immunodeficiency syndrome; HAART, highly active antiretroviral therapy; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; LDL, low-density lipoprotein; HDL, high-density lipoprotein; BMI, body mass index; MI, myocardial infarction; IMT, intima-media thickness

controversy stems from the fact that HIV-infected patients often have multiple concomitant risk factors for coronary heart disease (including cigarette smoking, arterial hypertension, alcohol abuse, active drug addiction, sedentary life, hyperlipidemia, insulin resistance and hyperglycemia), so that to distinguish the real pathogenetic role of HIV infection and antiretroviral drugs is very difficult [15, 16].

In a large retrospective study using the Veterans' Affairs Database (which included 36,766 patients followed up for an average of 40 months, between years 1993 and 2001), Bozzette *et al.* [17] showed that PI therapy was not associated with an increased risk of coronary heart disease. In contrast, Mary-Krause *et al.* [4] showed that exposure to PIs was associated with a higher risk of cardiovascular diasease, and the myocardial infarction rates increased in relation to duration of PI therapy (10.8 events per 10,000 person-years in men with <18 months PI use; 33.8 events per 10,000 person-years in those with >30 months PI use).

Moreover, recent prospective studies involving large cohorts of HIV-infected patients have documented an increased incidence of myocardial infarction and cerebrovascular diseases in association with a prolonged exposure to combination antiretroviral therapies. Even if the absolute risk of cardiovascular events remains low, it should be balanced against the remarkable benefits from HAART in terms of improvement in immune function and related morbidity and mortality.

The Data Collection on Adverse Events of Anti-HIV Drugs (DAD) Study [5,18] is a prospective, observational study of 11 previously established cohorts comprising 23,468 HIV-infected patients followed in 21 countries in Europe, United States and Australia. During this study, a total of 126 episodes of myocardial infarction were diagnosed, leading to a crude incidence rate of 3.5 per 1,000 patient-years. The authors showed that the incidence of myocardial infarction increased significantly with increasing exposure to combination antiretroviral therapy, and the adjusted risk rate per year of exposure ranged from 0.32 for no HAART use to 2.93 for ≥6 years of HAART use. This suggested that during the first four to six years of combination antiretroviral treatment there was approximately a 26% increase in the relative risk of suffering from a myocardial infarction, but the absolute risk of coronary events was low and must be balanced against the remarkable benefits from antiretroviral therapy.

Contradictory reports have been published concluding that antiretroviral agents, and particularly PIs, do or do not promote premature atherosclerosis in HIV-infected patients. PI-based HAART frequently induce remarkable alterations in lipid and glucose metabolism (including hypercholesterolemia, hypertriglyceridemia, insulin resistance, hyperglycemia, and visceral fat accumulation), that are established risk factors for premature atherosclerotic disease. However, these metabolic factors do not fully account for the premature atherosclerotic lesions observed in these patients, suggesting that other mechanisms or mediators might be involved.

To investigate possible correlation between HIV disease and subclinical atherosclerosis, ultrasonographic evaluation of epi-aortic vessels has recently been employed by several authors. A study performed by de Saint-Martin *et al.* [9] assessed 154 HIV-infected subjects and showed an association between treatment with PIs and increased IMT as measured by ultrasonography. Johnsen *et al.* [19] examined the impact of HIV infection and PIs on 183 women and demonstrated that both HIV infection and PI-based treatment increased metabolic abnormalities. These authors pointed out that global metabolic changes rather than a direct effect of antiretroviral agents were responsible for the increased cardiovascular risk in this population.

Lorenz *et al.* [20] conducted a case-control study involving 292 HIV-positive subjects and 1168 HIV-negative controls, assessing vascular risk factors and carotid IMT in both populations. In this study, HIV infection and HAART were found to be independent risk factors for early carotid atherosclerosis, and the observed IMT elevation suggested that vascular risk was 4-14% greater and the "vascular age" was 4-5 years higher in HIV-positive individuals than in HIV-negative controls.

Maggi *et al.* [8] evidenced a relationship between use of PIs and premature atherosclerotic lesions in HIV-positive patients, and observed that the structure of carotid lesions in subjects receiving HAART may be different than that of classical atherosclerotic plaques described in HIV-negative persons. A study including 61 HIV-infected patients and 47 HIV-uninfected controls showed a significantly higher proportion of iso-hypoechogenic lesions in HIV-positive subjects compared with HIV-negative atherosclerotic patients. Moreover, carotid lesions associated with HIV infection were mostly homogeneous both in their parietal and endoluminal portions, with a smooth or slightly irregular surface. Therefore, in this study epi-aortic lesions observed in HIV-positive patients had a structure substantially different from that of the plaques in atherosclerotic subjects, although they showed similar features with patients affected by arteritis [13].

In contrast to the above-mentioned studies, other authors have failed to demonstrate a direct effect of antiretroviral agents on the arterial wall disease. Currier *et al.* [11] evaluated 45 patients and did not find an association between HIV infection or PI use with increased carotid IMT. These authors observed that PIs may increase the risk of cardiovascular diseases indirectly by promoting changes in lipid metabolism or body fat composition.

In a cross-sectional analysis of 242 men and 85 women with HIV infection who underwent carotid ultrasonography and coronary computed tomography, Mangili *et al.* [10] found more abnormal surrogate markers than expected at a relative young age. However, increased carotid IMT and coronary artery calcium scores were not associated with use of HAART and PIs, but the positive associations were primarily with traditional and novel cardiovascular risk factors (such as age, waist circumference, systolic blood pressure, apolipoprotein B level, and C-reactive protein level).

Lebech *et al.* [21] evaluated risk factors for premature atherosclerosis in 25 HIV-positive and 14 HIV-negative non-smoking patients with high or low serum cholesterol concentrations. In non-smoking HIV-infected subjects receiving HAART no signs of early atherosclerosis were found, not even in patients with hypercholesterolemia. Increased carotid IMT correlated only with reduced HDL cholesterol levels, but not with increased LDL cholesterol levels or PI therapy.

The activation of the endothelium induced by either HIV infection itself or by a leucocyte-mediated inflammatory cascade triggered by the same virus leads to the increased expression of endothelial cellular adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), vascular adhesion molecule 1 (VCAM-1), E-selectin, P-selectin, thrombomodulin, tissue plasminogen activator (tPA), and plasminogen activator inhibitor 1 (PAI-1). A significant association between increasing serum concentrations of adhesion molecules and risk of future myocardial infarction has been shown in apparently healthy men and women, and these molecules are now considered as soluble biomarkers of endothelial inflammation and early atherosclerosis [22, 23].

Increased serum levels of ICAM-1, VCAM-1, E-selectin, and thrombomodulin were demonstrated in patients with advanced HIV infection and opportunistic diseases, and a correlation between ICAM-1 concentrations and the progression of disease as well as the reduction of CD4 lymphocyte count was also reported. If circulating adhesion molecules indicate vascular endothelium injury, it seems clear that endothelium injury is associated with the progression and severity of HIV disease. Moreover, the available evidence demonstrates that certain PIs could induce endothelial dysfunction, including a decrease of endothelium-dependent vasorelaxation, inhibition of the nitric oxide synthase system, increase of oxidative stress, and activation of mitogen-activated protein kinases [23-25]. Moreover, the course of atherosclerosis in patients with HIV infection seems also influenced by polymorphisms in the SDF1 and CX3C1 genes by metabolic variables and by the CD4 lymphocyte count [26, 27].

In our study, although limited by the absence of a control group, the prevalence of carotid plaques were significantly higher in HIV-infected patients receiving HAART than in those naïve to antiretroviral agents, and atherosclerosis was significantly associated with longer mean duration of known HIV infection, greater prevalence of hyperlipidemia (particularly higher mean concentrations of total cholesterol, LDL cholesterol and triglycerides), and higher frequency of lipodystrophy syndrome. Mean 10-year risk of coronary heart disease and mean values of carotid IMT were more elevated in patients who already received antiretroviral therapy than in naïve subjects, but without a statistical significance.

With regard to patients already treated with antiretroviral drugs in the past, mean values of carotid IMT and prevalence of carotid plaques were significantly higher among subjects with high ten-year risk ($\geq$10%) of coronary events than among those with low risk (<10%), so that carotid IMT evaluated by ultrasonography seems a reliable surrogate marker predictive of cardiovascular risk. In detail, “high risk” patients differed from “low risk” patients because of worse lipid metabolism alterations and a greater prevalence of lipodystrophy syndrome.

Contrary to previous results obtained by Maggi *et al.* [13], in our study carotid lesions observed in patients already treated with antietrovirals were comparable to classical atherosclerotic plaques described in general population, with iso-hyperechogenic structure and irregular endoluminal and parietal surfaces in all reported cases.

To conclude, the association between HIV disease and premature atherosclerosis is debated still today, but owing to the notable extension of life expectancy in HIV-positive subjects, cardiovascular complications are expected to become significantly more frequent, and require a routine and appropriate monitoring and management of the broad spectrum of risk factors supporting cardiovascular complications. Further, large studies including a multi-dimensional assessment are certainly requested in order to better clarify the relationship between HIV infection and atherosclerosis.

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