Predictive factors of vascular intima media thickness in HIV-positive subjects

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Background: The predictive factors of intima media thickness (IMT) in the HIV-infected population are still poorly understood.

Patients and methods: We studied three groups of subjects, aged 30–50 years, to find potential predictive factors of carotid and/or femoral thickening (IMT > 1 mm in at least one area): healthy controls (G1, n = 54), HIV-infected naive (G2, n = 53) and highly active antiretroviral treatment (HAART)-treated subjects (G3, n = 133). All the subjects underwent ultrasonography of the carotid and femoral vessels to evaluate IMT.

Results: Demographic characteristics of the three groups were comparable, except for gender (G1 had a higher percentage of females) and lipid levels (higher in G3). A total of 115 subjects (47.9%) had carotid and/or femoral IMT: 26 in G1 (48.1%), 21 in G2 (39.6%) and 68 in G3 (51.1%). Independent predictive factors of carotid and/or femoral IMT were older age (OR: 2.81, 95% CI: 1.95–4.04, P < 0.01, for each additional 5 years), triglycerides ≥150 mg/dL (OR: 2.66, 95% CI: 1.27–5.57, P < 0.001), serum glucose ≥110 mg/dL (OR: 5.24, 95% CI: 1.02–27.05, P = 0.04), high homocysteinaemia (OR: 2.75, 95% CI: 1.17–6.46, P = 0.02) and high body mass index (OR: 1.10, 95% CI: 1–1.22, P = 0.05 for each additional unit); females had a lower risk (OR: 0.38, 95% CI: 0.18–0.79, P < 0.01 versus males). HAART use was not associated with IMT (OR: 0.64, 95% CI: 0.27–1.53, P = 0.32 and OR: 0.80, 95% CI: 0.30–2.13, P = 0.20 for G3 and G2 versus G1, respectively).

Conclusions: This study demonstrates that traditional risk factors for cardiovascular diseases overshadow the role of HAART in determining premature vascular lesions.

Keywords: cardiovascular risk, Doppler, HAART

Introduction

HIV-infected subjects were recognized to be at increased risk of cardiovascular diseases before the introduction of highly active antiretroviral treatment (HAART) in clinical practice, mainly secondary to opportunistic infections or vasculitis.1,2 Moreover, in the pre-HAART era, a syndrome characterized by elevated triglycerides and low cholesterol was frequently described in patients with AIDS.3-4 Abnormalities such as hyperlipidaemia, insulin resistance and fat redistribution increased after the introduction of HAART,3-7 becoming an issue of primary importance in the clinical management of the HIV-infected population.

Factors contributing to the elevated risk of cardiovascular diseases in the HIV-infected population include chronic inflammatory status secondary to HIV infection, HAART-related metabolic abnormalities and traditional risk factors for cardiovascular diseases (such as smoking and alcohol habits, blood hypertension, diabetes, age, sex and race).8 However, whether such factors may predict the occurrence of arterial damage in HIV-infected subjects is still unknown.

The correlation between intima media thickness (IMT), HIV infection and HAART is still controversial. Few data are currently available on the relative contribution of traditional risk factors for IMT in HIV subjects. Ultrasound colour-Doppler is a
well-established method for measuring IMT and, consequently, the degree of atherosclerosis. Moreover, this examination may be introduced early during the development of atherosclerosis and then frequently used to monitor the progression of atherosclerotic lesions.9,10

In our study, we evaluated the IMT of the carotid and femoral vessels to determine premature atherosclerotic lesions using an ultrasound colour-Doppler technique in HIV-negative, HIV-infected naive or HAART-receiving subjects. The aim of this study was to assess the role of traditional risk factors for IMT in the HIV-infected population and compare such factors with those observed in HIV-negative subjects.

Patients and methods

Consecutive subjects fulfilling the following criteria were enrolled: Caucasian, between 30–50 years old. Exclusion criteria were: known blood hypertension, current cardiovascular diseases, diabetes, active drug abuse, alcohol abuse (defined as alcohol consumption >30 g/day) and current AIDS-defining illnesses. Three groups of consecutive subjects were identified: healthy controls (group 1, G1), HIV-infected naive (group 2, G2) and HAART-treated subjects (group 3, G3), receiving stable HAART in the previous 12 months. An interview questionnaire was performed to establish the family history of cardiovascular diseases, concomitant illnesses (HCV and/or HBV infection), use of concomitant drugs, smoking, diet and physical activity habits. Smokers were defined as individuals smoking ≥10 cigarettes/day at least during the past year (in our cohort, all the patients who smoke, declared to smoke ≥10 cigarettes/day). Additionally, we collected data on demographic characteristics, triglycerides [normal values (nv) <150 mg/dL], total cholesterol (nv <190 mg/dL), serum glucose (nv <110 mg/dL) and homocysteine (nv <12.8 μmol/L for men and <11.5 μmol/L for women). All these blood tests were performed under fasting conditions within 1 month of ultrasonography testing. In the HIV-infected population only, data on risk factors for HIV, previous AIDS-defining illnesses, time of known HIV positivity, current immunovirological status and presence of lipoatrophy (defined as body fat abnormalities consistent with lipodystrophy, lipoaccumulation, or both, clinically evident to both the patient and physician)11 were collected.

A complete physical examination was performed, including the measurement of blood pressure (determined by using a sphygmomanometer with the subjects in a sitting position after >5 min at rest) and the calculation of body mass index (BMI).

HIV-RNA serum levels were measured using the branched chain deoxyribonucleic acid technique (Chiron Inc.: detection limit 50 copies/mL) and CD4+ cell counts using the elite flow cytometer (Coulter Corporation, Miami, FL, USA).

All subjects underwent an ultrasonography of the carotid and femoral vessels using an AU 5 ESAOTE power colour-Doppler with 7.5 MHz probes. Characteristics of the intima, pulsation index, resistance index, minimal speed, peak speed and mean speed were evaluated. An IMT >1 mm in one of the examined regions was considered to be pathological.9 All the ultrasonographies were performed by the same physician, who was unaware of the HIV status, with the same colour power Doppler. Patients were submitted to the investigation in a supine position after at least 10 min of acclimatization in a comfortable room. During the examination, the head of the patient was extra-rotated from the opposite side. Once the image had been optimized, the bifurcations of the vessels were located and the echocardiograph zoom was deployed. The common carotid, the bifurcation and at least the first 2 cm of the internal and external carotid vessels were examined in the short and long axis during the tele-diastolic phase (T wave of the electrocardiogram). Carotid IMT was measured between the bifurcation and 1 cm proximal to the bifurcation. The same procedures were then repeated for the femoral vessels.

Written informed consent was obtained from all participants, and the study was conducted in adherence with local drug regulations, guidelines on ‘Good Clinical Practice’, and the principles of the Declaration of Helsinki.

Sample size was calculated assuming a clinically relevant difference of 0.1 mm in carotid IMT between the groups of interest; we assumed an standard deviation of 0.14 mm on this measurement. To detect a difference of 0.1 mm, with 80% power, at least 50 subjects per group were required.

Univariate and multivariable logistic regression analyses were performed to find potential predictors of carotid and/or femoral thickening. Variables included in the models were: gender, age, smoking habit, BMI, higher than normal triglycerides, total cholesterol, serum glucose and homocysteine, and group (G1 as reference). Two subanalyses were then repeated including only G2 and G3 subjects, to evaluate the correlation between HAART duration and IMT >1 mm. In the first analysis, groups were stratified as naive, or exposed to 1–3, 3–5 and >5 years of HAART; in the second, we considered the total durations of non-nucleoside reverse transcriptase inhibitor (NNRTI)- and protease inhibitor (PI)-containing treatments as continuous variables.

Results

From September 2005 to September 2006, 240 Caucasian, consecutive outpatients, aged 30–50 years, were studied: 54 healthy controls (G1), 53 HIV-infected naive (G2) and 133 HAART-treated subjects (G3). Demographic characteristics of the three groups were comparable except for gender (Table 1). G3 subjects were exposed to nucleoside reverse transcriptase inhibitors (NRTIs) for a median duration of 87 months [interquartile range (IQR) 35–144]; median times of NNRTI and PI exposure were 15 (IQR 0–48) and 26.5 (IQR 0–60) months, respectively. All patients exposed to PIs received a ritonavir-boosted PI. Forty-two subjects (31.6%) were taking a first-line HAART: 17 an NNRTI-based HAART and 25 a PI-based HAART. Table 2 shows the frequency of traditional factors potentially associated with cardiovascular risk in the three groups. HAART-receiving subjects had greater lipids and homocysteine levels and were more often active smokers than those included in the other groups.

A total of 115 subjects (47.9%) had carotid and/or femoral IMT >1 mm: 26 in G1, 21 in G2 and 68 in G3 (P = NS). Among these, 36 (31.3%) had IMT >1 mm in the carotid area, 27 (23.5%) in the femoral area and 52 (45.2%) in both of the areas, with no statistically significant differences between the three groups. Median IMT at carotid and femoral regions were not statistically different among the three groups (right carotid: 0.58, 0.64 and 0.65 mm; left carotid: 0.58, 0.63 and 0.64 mm; right femoral: 0.60, 0.62 and 0.66 mm; left femoral: 0.58, 0.64 and 0.63 mm for G1, G2 and G3, respectively).

Independent predictive factors of carotid and/or femoral IMT >1 mm were older age (OR: 2.81, 95% CI: 1.95–4.04, P < 0.01 for each additional 5 years), triglycerides ≥150 mg/dL (OR: 2.66, 95% CI: 1.27–5.57, P < 0.01), serum fasting glucose ≥110 mg/dL (OR: 5.24, 95% CI: 1.02–27.05,
Intima media thickness in HIV subjects

Table 1. Demographic and clinical (whenever applicable) characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Whole population (n = 240)</th>
<th>Healthy controls (G1, n = 54)</th>
<th>HIV-infected naive (G2, n = 53)</th>
<th>HIV-infected on HAART (G3, n = 133)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males, n (%)</td>
<td>166 (69.2)</td>
<td>33 (61.1)</td>
<td>36 (67.9)</td>
<td>97 (72.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Median age, years (IQR)</td>
<td>41 (37–45)</td>
<td>42 (37–41)</td>
<td>39 (34–43)</td>
<td>41 (39–45)</td>
<td>NS</td>
</tr>
<tr>
<td>CDC stage, n (%)</td>
<td>NA</td>
<td>NA</td>
<td>A: 35 (66.0)</td>
<td>A: 41 (30.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Risk factor for HIV, n (%)</td>
<td>NA</td>
<td>NA</td>
<td>B: 9 (17.0)</td>
<td>B: 39 (29.3)</td>
<td>NA</td>
</tr>
<tr>
<td>heterosexual</td>
<td>NA</td>
<td>NA</td>
<td>C: 9 (17.0)</td>
<td>C: 53 (39.8)</td>
<td>NA</td>
</tr>
<tr>
<td>homosexual</td>
<td>NA</td>
<td>NA</td>
<td>14 (26.4)</td>
<td>56 (42.1)</td>
<td>NA</td>
</tr>
<tr>
<td>IVDU</td>
<td>NA</td>
<td>NA</td>
<td>27 (50.9)</td>
<td>34 (25.6)</td>
<td>NA</td>
</tr>
<tr>
<td>other</td>
<td>NA</td>
<td>NA</td>
<td>9 (17.0)</td>
<td>38 (28.6)</td>
<td>NA</td>
</tr>
<tr>
<td>ARV treatment duration, months (IQR)</td>
<td>NA</td>
<td>NA</td>
<td>3 (5.7)</td>
<td>5 (3.8)</td>
<td>NA</td>
</tr>
<tr>
<td>NRTI</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>87 (35–144)</td>
<td>NA</td>
</tr>
<tr>
<td>NNRTI</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>15 (0–48)</td>
<td>NA</td>
</tr>
<tr>
<td>PI</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>26.5 (0–60)</td>
<td>NA</td>
</tr>
<tr>
<td>Median CD4 cells count, cells/mm³ (IQR)</td>
<td>NA</td>
<td>NA</td>
<td>421 (378–444)</td>
<td>453 (294–709)</td>
<td>NA</td>
</tr>
<tr>
<td>Median HIV-RNA, copies/mL (IQR)</td>
<td>NA</td>
<td>NA</td>
<td>5348 (4658–20 674)</td>
<td>49 (49–274)</td>
<td>NA</td>
</tr>
</tbody>
</table>

HAART, highly active antiretroviral treatment; IQR, interquartile range; CDC, Centers for Diseases Control; IVDU, intravenous drug abusers; ARV, antiretroviral; NRTI, nucleoside reverse transcriptase inhibitors; NNRTI, non-nucleoside reverse transcriptase inhibitors; PI, protease inhibitors; NA, not applicable.

P = 0.04, high homocysteine (OR: 2.75, 95% CI: 1.17–6.46, P = 0.02) and increased BMI (OR: 1.10, 95% CI: 1–1.22, P = 0.05 for each additional unit); females showed a lower risk (OR: 0.38, 95% CI: 0.18–0.79, P < 0.01 versus males). HAART exposure and HIV infection were not statistically associated with IMT > 1 mm (OR: 0.64, 95% CI: 0.27–1.53, P = 0.32 and OR: 0.80, 95% CI: 0.30–2.13, P = 0.65 for G3 and G2 versus G1, respectively).

To evaluate the role of a prolonged HAART duration in determining IMT > 1 mm, we performed a new analysis considering only HIV-infected individuals. Older age (OR: 2.77, 95% CI: 1.82–4.22, P < 0.0001 for each additional 5 years), triglycerides ≥150 mg/dL (OR: 2.41, 95% CI: 1.05–5.56, P = 0.04) and high homocysteine (OR: 2.66, 95% CI: 1.00–7.15, P = 0.05) were confirmed to be independent predictors of IMT > 1 mm. Females (OR: 0.46, 95% CI: 0.19–1.09, P = 0.08 versus males) and serum glucose ≥110 mg/dL (OR: 4.60, 95% CI: 0.85–25.02, P = 0.07) were both marginally associated with IMT > 1 mm. Prolonged HAART duration was not associated with IMT > 1 mm (HAART duration > 5 years, RR: 1.00, 95% CI: 0.40–2.52, P = 0.99 versus naive; HAART 3–5 years, RR: 0.59, 95% CI: 0.13–2.73, P = 0.48 versus naive; HAART 1–3 years, RR: 0.37, 95% CI: 0.08–1.76, P = 0.21 versus naive).

The same factors as above were confirmed to be predictive of IMT > 1 mm also when considering the time of NNRTI- and PI-exposure as continuous variables instead of HAART duration per se.

Discussion

Various studies have hypothesized an increased risk of atherosclerosis in HIV-positive patients. To date, conflicting results from studies that have investigated the relationship between HIV infection, PI exposure, lipid abnormalities and carotid IMT have been reported. Prospective data from a large international multicohort observational study, the DAD study, showed an increased relative risk for myocardial infarction during the first 7 years of HAART. The AACTG 5078 trial did not confirm the correlation between PI exposure, HIV infection and carotid IMT; in this study, traditional risk factors for atherosclerosis such as low high-density lipoprotein cholesterol, high triglycerides, older age and high BMI were found to be predictive of greater carotid IMT. Finally, the PREVALEAT study demonstrated that carotid IMT was greater among PI-treated patients in comparison with antiretroviral-naive or NNRTI-treated patients.

To our knowledge, this is one of the first studies to have compared HIV-infected (both naive and HAART treated) and HIV-negative subjects to determine the correlation between HAART exposure, HIV infection itself and the risk of premature atherosclerosis. In a case–control study that compared HIV-infected and -uninfected patients matched for age and gender, Hsu et al. reported that HIV infection per se was an independent predictor of IMT and that carotid IMT progresses much more rapidly in HIV-positive individuals. On the contrary, Johnsen et al. found a similar IMT in HIV-infected and -uninfected women; however, the exposure to PI-containing HAART was strongly associated with an increased IMT. In a recent study, Mercie et al. demonstrated that conventional risk factors for cardiovascular diseases (older age, male gender and cigarette smoking) are major determinants of IMT progression. Differently from these studies, we also evaluated femoral IMT because of the possible risk of damage of these vessels.

Almost half of our patients had carotid and/or femoral intima media thickening. In the analysis on the whole population,
HIV-infected subjects (treated or not) did not seem at higher risk of IMT compared with HIV-negative patients. Traditional risk factors for IMT such as male gender, older age, higher BMI, hyperglycaemia and hypertriglyceridaemia were confirmed to be predictive of IMT also in HIV-positive individuals. Additionally, high homocysteine levels were statistically associated with IMT, thus confirming the possible role of this parameter as a surrogate marker of cardiovascular damage.21,22 Our study failed to show any association between HAART use and duration, HIV infection and IMT. One possible explanation is that the impact of antiretroviral treatment-related changes on metabolic parameters may take a longer time to translate into changes in vascular IMT. To further demonstrate the correlation between prolonged HAART use and increased IMT and due to the high heterogeneity of HAART regimens used in our cohort, we performed an analysis considering the total exposure to boosted PIs and NNRTIs; this analysis also confirmed that traditional risk factors may overlap with the role of HAART and HIV infection. Additionally, in our study, we did not include subjects with known diabetes and blood hypertension to avoid possible biases in the results.

Our study has some limitations, mainly related to the low number of subjects included that may result in low potency. Data from longitudinal studies with a prolonged follow-up to periodically evaluate IMT in HIV-infected patients are necessary to better determine whether HIV infection itself or HAART has a significant role in the occurrence and in the progression of subclinical atherosclerosis. In conclusion, these findings provide evidence that traditional risk factors and surrogate markers for cardiovascular disease in the general population matter for cardiovascular disease in HIV-infected patients, such as atherosclerosis. As a consequence, a periodic screening for cardiovascular risk and the correction, when possible, of these parameters should also be considered mandatory in the HIV-infected population.

**Table 2.** Factors associated with a greater cardiovascular risk

<table>
<thead>
<tr>
<th>Factor</th>
<th>Whole population (n = 240)</th>
<th>Healthy controls (G1, n = 54)</th>
<th>HIV-infected naïve (G2, n = 53)</th>
<th>HIV-infected on HAART (G3, n = 133)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median BMI (IQR)</td>
<td>22.6 (21.1–25.1)</td>
<td>22.9 (21.4–25)</td>
<td>22.7 (21.4–25.2)</td>
<td>22.4 (20.8–25.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Family history of CVD, n (%)</td>
<td>177 (73.8)</td>
<td>46 (85.2)</td>
<td>39 (73.6)</td>
<td>92 (69.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>128 (53.3)</td>
<td>26 (48.1)</td>
<td>26 (49.1)</td>
<td>76 (57.1)</td>
<td>0.04</td>
</tr>
<tr>
<td>Median triglycerides, mg/dL (IQR)</td>
<td>133 (95–218)</td>
<td>108 (82–145)</td>
<td>114 (94–182)</td>
<td>164 (104–268)</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides &gt; 150 mg/dL, n (%)</td>
<td>95 (39.6)</td>
<td>10 (18.5)</td>
<td>16 (30.2)</td>
<td>69 (51.9)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Median total cholesterol, mg/dL (IQR)</td>
<td>188 (155–223)</td>
<td>173 (145–228)</td>
<td>174 (146–209)</td>
<td>196 (168–230)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total cholesterol &gt; 190 mg/dL, n (%)</td>
<td>113 (47.1)</td>
<td>22 (40.7)</td>
<td>18 (34.0)</td>
<td>73 (54.9)</td>
<td>0.03</td>
</tr>
<tr>
<td>Median glucose, mg/dL (IQR)</td>
<td>91 (85–98)</td>
<td>87 (79–93)</td>
<td>91 (85–95)</td>
<td>93 (86–101)</td>
<td>0.05</td>
</tr>
<tr>
<td>Glucose &gt; 110 mg/dL, n (%)</td>
<td>17 (7.1)</td>
<td>1 (1.9)</td>
<td>1 (1.9)</td>
<td>15 (11.3)</td>
<td>0.03</td>
</tr>
<tr>
<td>Median homocysteine, μmol/L</td>
<td>9.8 (7.9–12)</td>
<td>9.1 (7.7–11.5)</td>
<td>8.4 (7–10.2)</td>
<td>10.5 (8.6–12.8)</td>
<td>0.04</td>
</tr>
<tr>
<td>High homocysteine, a n (%)</td>
<td>41 (17.1)</td>
<td>1 (1.9)</td>
<td>4 (7.5)</td>
<td>36 (27.1)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

IQR, interquartile range; BMI, body mass index; CVD, cardiovascular diseases.

aDefined as ≥12.8 μmol/L for men and ≥11.5 μmol/L for women.

**References**


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