Aortic Stiffness in Untreated Adult Patients With Human Immunodeficiency Virus Infection

Giuseppe Schillaci, Giuseppe V.L. De Socio, Giacomo Pucci, Massimo R. Mannarino, Johny Helou, Matteo Pirro, Elmo Mannarino

Abstract—HIV infection is associated with chronic immune activation, subclinical inflammation, and an atherogenic metabolic profile. It remains controversial whether HIV infection is a risk factor for accelerated arteriosclerosis independent from the effects of antiretroviral drugs. We investigated whether aortic stiffness, an early marker of arteriosclerosis, is increased in HIV patients who were not under antiretroviral treatment. In 39 untreated HIV-infected patients and 78 individually matched age-, sex-, and blood pressure–matched HIV-uninfected control subjects, we determined aortic pulse wave velocity (PWV), a direct noninvasive measure of aortic stiffness, by tonometric method. Subjects with overt cardiovascular disease or major cardiovascular risk factors were excluded from the study. Prevalence of the metabolic syndrome was higher in HIV patients (18% versus 5%; \( P = 0.025 \)). HIV patients had a higher aortic PWV \((7.5 \pm 1.4 \text{ m/s}\) versus \(6.7 \pm 1.1\text{ m/s}\); \( P = 0.001\)) than control subjects. Age, mean arterial pressure as a measure of distending pressure, and HIV infection (all \( P < 0.05\)) independently predicted aortic PWV when a consistent number of cardiovascular risk factors was simultaneously controlled for. Among HIV-infected subjects, serum \(\gamma\)-glutamyl transpeptidase concentration (\(\beta = 0.46; P = 0.003\)) and mean arterial pressure (\(\beta = 0.32; P = 0.03\)) were independent determinants of aortic PWV. In conclusion, aortic stiffness is increased in HIV-infected individuals who have never received antiretroviral therapy. PWV increases with increasing serum \(\gamma\)-glutamyl transpeptidase concentration. Our data support the hypothesis that HIV infection is a risk factor for arteriosclerosis. (Hypertension. 2008;52:308-313.)

Key Words: aortic stiffness ■ arteriosclerosis ■ cardiovascular diseases ■ HIV ■ pulse wave velocity

Atherosclerotic cardiovascular disease, a leading cause of morbidity and mortality in the general population, is an increasing concern also for patients with HIV infection. A high level of cardiovascular morbidity had been reported for HIV-infected patients before the availability of highly active antiretroviral therapy,1,2 and this issue has become even more relevant since treatment extended their life span.3,4 The mortality rate in HIV-infected patients receiving combination antiretroviral therapy has decreased over time, in fact most, young persons with HIV infection can expect to survive for >35 years in the late highly active antiretroviral therapy era. However, mortality is still higher in HIV-infected subjects than in the general population of a similar age.5 The association of increased cardiovascular risk in persons with HIV infection and exposure to antiretroviral therapy is not completely understood. Two of the main sources of cardiovascular disease in this population are believed to be vascular inflammation and dyslipidemia.6–9 Several studies have demonstrated that patients using combination antiretroviral therapy, particularly containing a protease inhibitor, develop atherogenic changes in their lipoprotein profile10 and early signs of arteriosclerosis.11–13

It remains controversial whether HIV infection is a risk factor for accelerated arteriosclerosis per se. Because a major limitation of studies investigating cardiovascular outcomes in the young to middle-aged population of patients with HIV infection is the low absolute event rate, there is a need for studies of surrogate cardiovascular end points. Large-artery stiffness is a simple and reproducible marker of subclinical arteriosclerotic disease and has been identified as a strong predictor of cardiovascular mortality in different clinical settings.14,15 Increased arterial stiffness has been observed recently in HIV-infected patients receiving antiretroviral therapy.13,16,17 To the best of our knowledge, no study to date has specifically evaluated the effects of HIV infection on aortic stiffness. In the present case-control study, we hypothesized that aortic stiffness, an early marker of arteriosclerosis, may be increased in HIV patients without prevalent cardiovascular disease or major atherosclerotic risk factors who had never received antiretroviral treatment.

Methods

Patients and Study Design

We studied adult patients with documented HIV infection who had never received antiretroviral treatment. Fifty-six consecutive patients...
were recruited at the outpatient Clinic of Infectious Diseases, University of Perugia, and examined during baseline clinical evaluation for HIV infection. A total of 39 consecutive HIV-infected adults satisfied all of the inclusion and exclusion criteria (see below). The median duration of HIV infection was 5 years (range: 1 to 15 years). The mode of infection of our study population was distributed as follows: heterosexual contact, 56%; homosexual contact, 31%; and injecting drug use, 13%. The median nadir of CD4+ T-lymphocyte count was 251 per mm$^3$ (range: 11 to 1222 per mm$^3$); the median log$_{10}$ of HIV-1 RNA was 4.71 (range: 1.59 to 6.30), and 33% of the patients had a diagnosis of AIDS (C stage of the Centers for Disease Control and Prevention classification).15

Seventy-eight HIV-negative control subjects were recruited among the staff working at the hospital and individuals examined for clinical checkup and found healthy. A case:control matching ratio of 1:2 was chosen because it has been shown to provide better information than a 1:1 ratio for small relative risks in case-control studies.16 Control subjects were individually matched with patients by age (±10 years), sex (same sex), and systolic blood pressure (±10 mm Hg). In patients as well as in control subjects, we excluded from the study subjects with arterial hypertension, serum cholesterol concentration ≥4.21 mmol/L (240 mg/dL), known diabetes or fasting glycaemia ≥7 mmol/L (126 mg/dL), serum creatinine concentration >177 μmol/L (2 mg/dL), obesity (body mass index ≥30 kg/m$^2$), clinical or laboratory evidence of valvular or coronary heart disease, previous stroke, treatment with any cardiovascular drug including nitrates, or any clinical or laboratory evidence of inflammation over a period of ≥1 month before the study began. Hypertension was defined by a blood pressure ≥140 mm Hg systolic and/or ≥90 mm Hg diastolic on 3 consecutive readings or antihypertensive drug treatment. The metabolic syndrome was defined according to the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults.20 The Framingham risk score, which has been validated in the general population as a measure of coronary heart disease risk, was calculated in HIV and control subjects.21 All of the participants gave their informed consent to participate in the study, which was approved by the institutional ethics committee.

To further examine the independent effect of HIV infection and antiretroviral treatment on arterial stiffness, we designed a second case-control study in which healthy control subjects were individually matched by age, sex, and systolic blood pressure with untreated HIV-infected patients and with HIV-infected patients who had been on ≥24 months of a stable combination antiretroviral therapy regimen, which included a protease inhibitor and 2 reverse transcriptase inhibitors. The latter subjects were drawn from a previously described series.17 For this case-control study, matching and exclusion criteria were identical in the 2 groups. By matching, age, sex distribution, and blood pressure values did not differ. HIV-infected patients had a lower body mass index, a lower total and high-density lipoprotein cholesterol concentration, and higher serum triglycerides. The median 10-year coronary heart disease risk according to the Framingham equation was similar in case and control subjects. Of note, despite the absence of major cardiovascular

### Table 1. Demographic, Clinical, and Laboratory Variables in 39 HIV Patients and in 78 Control Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-Infected Subjects (n=39)</th>
<th>Control Subjects (n=78)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>37±8</td>
<td>37±7</td>
<td>0.92</td>
</tr>
<tr>
<td>Men, %</td>
<td>67</td>
<td>67</td>
<td>1.00</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.72±0.07</td>
<td>1.70±0.09</td>
<td>0.19</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>22.7±3</td>
<td>25.3±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.91±0.07</td>
<td>0.91±0.08</td>
<td>0.37</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>49</td>
<td>42</td>
<td>0.52</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120±12</td>
<td>120±11</td>
<td>0.94</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>78±9</td>
<td>79±8</td>
<td>0.40</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>75±12</td>
<td>71±9</td>
<td>0.08</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.1±0.8</td>
<td>5.1±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>1.0±0.4</td>
<td>1.4±0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cholesterol, mmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.5 (1.0 to 2.2)</td>
<td>1.2 (0.8 to 1.6)</td>
<td>0.045</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.7±0.6</td>
<td>4.8±0.6</td>
<td>0.43</td>
</tr>
<tr>
<td>Metabolic syndrome, %</td>
<td>18</td>
<td>5</td>
<td>0.025</td>
</tr>
<tr>
<td>10-year coronary heart disease risk, %</td>
<td>3.3 (1.4 to 5.0)</td>
<td>3.2 (1.7 to 4.9)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are means±SDs, except for triglycerides and 10-year coronary heart disease risk, expressed as medians (interquartile ranges).

### Statistical Analysis

SPSS 13.0 (SPSS Inc) was used for all of the statistical analyses. Between-group differences were assessed by the use of Student t and Wilcoxon tests for continuous normally and nonnormally distributed variables, respectively. The χ$^2$ test was used for comparing categorical variables. Pearson’s or Spearman’s correlation coefficients, as appropriate, examined the degree of association between examined variables. Logarithmic transformation was used for those variables that showed a nonnormal distribution. Linear regression analysis was used to estimate the prediction of aortic PWV by including simultaneously in the model the following variables: age, sex, smoking status, body height, body mass index, total cholesterol, high-density lipoprotein cholesterol, triglycerides, mean arterial pressure, heart rate, and HIV infection status. Among HIV-infected subjects, a multivariate regression model was run that included all of the above variables, plus γ-glutamyl transpeptidase (GTT) concentration. One-way ANOVA and Tukey’s posthoc test for multiple comparisons were used in the case-control study, which compared healthy control subjects, untreated HIV-infected patients, and HIV-infected patients under stable treatment with antiretroviral drugs.

### Results

Selected clinical and biological characteristics of HIV-infected patients and control subjects are reported in Table 1. By matching, age, sex distribution, and blood pressure values were nearly identical in the 2 groups. Also waist/hip circumference ratio, smoking habits, and serum glucose concentration did not differ. HIV-infected patients had a lower body mass index, a lower total and high-density lipoprotein cholesterol concentration, and higher serum triglycerides. The median 10-year coronary heart disease risk according to the Framingham equation was similar in case and control subjects. Of note, despite the absence of major cardiovascular
risk factors, the prevalence of the metabolic syndrome was higher among HIV-infected individuals (Table 1).

Figure 1 shows that HIV-infected subjects had a significantly higher aortic PWV than uninfected control subjects (7.5±1.4 versus 6.7±1.1 m·s⁻¹; P=0.001). Aortic PWV showed a significant direct association with the 10-year Framingham risk score, both in HIV-infected patients (Spearman’s ρ=0.42; P=0.007) and among control individuals (Spearman’s ρ=0.23; P=0.04).

In a multiple regression model in which HIV status was included as a dummy explanatory variable together with age, sex, smoking habits, body height, body mass index, mean arterial pressure as a measure of distending pressure, heart rate, total and high-density lipoprotein cholesterol, and serum triglycerides, HIV infection was independently associated with aortic PWV along with age and mean arterial pressure (Table 2).

HIV-infected subjects with the metabolic syndrome (n=7) had a marginally higher age-adjusted aortic PWV than those without the syndrome (n=32; 8.2±1.5 versus 7.4±1.4 m·s⁻¹; P=0.08). Among patients with HIV infection, aortic PWV showed a direct association with log GGT (r=0.47; P=0.004; see Figure 2). In a multivariate analysis, log GGT concentration was independently associated with aortic PWV (β=0.46; P=0.003) and mean arterial pressure (β=0.32; P=0.034). Similar results were obtained when estimated 10-year coronary heart disease risk replaced mean arterial pressure in the equation. In that model, both Framingham risk score (β=0.36; P=0.026) and log GGT levels (β=0.33; P=0.042) independently predicted aortic PWV. No significant differences in age-adjusted aortic PWV were found between subjects with (n=8) or without (n=31) concomitant hepatitis C virus infection (7.7±1.0 versus 7.3±1.4 m·s⁻¹; P=0.50).

The results of the case-control study that compared healthy control subjects, untreated HIV-infected individuals, and HIV-infected patients under antiretroviral treatment are shown in Table 3. No between-group differences were found in terms of age, blood pressure, and 10-year Framingham risk score. Aortic PWV differed significantly among the 3 groups (P<0.001). In posthoc comparisons, PWV was higher among untreated subjects with HIV infection than in the healthy control subjects. Similarly, aortic PWV was higher in treated HIV patients than in untreated ones, although the last comparison only bordered statistical significance (P=0.07).

**Discussion**

In this study, HIV-infected patients who had never undergone antiretroviral treatment showed higher values of aortic stiffness than a matched group of HIV-uninfected control subjects. The association held regardless of the confounding effect of established cardiovascular risk factors. These findings are of interest because aortic stiffness is increasingly recognized as a valuable surrogate marker for cardiovascular disease and an integrated clinical marker of arterial pathology. In fact, alterations in arterial stiffness are known to precede clinical hypertension by a substantial period of time, and increased arterial stiffness has been linked to a higher rate of future cardiovascular complications and death.

Because the relative potential contribution of HIV to arteriosclerotic disease is difficult to distinguish from those of classic cardiovascular risk factors and of antiretroviral drugs, we chose to examine only HIV treatment-naïve subjects who were free from overt cardiovascular disease, hypertension, diabetes, and chronic renal disease and were not being treated with cardiovascular or hypolipidemic drugs. Moreover, patients and control subjects were accurately matched by age, sex, and blood pressure to minimize the confounding effect of these important conditions on the study findings.

Although antiretroviral therapy has been associated with an increased risk of myocardial infarction and with early

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**Table 2. Independent Determinants of Aortic PWV (in m·s⁻¹) in a Stepwise Multiple Linear Regression Analysis in the Whole Examined Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized Coefficient</th>
<th>Multiple R</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>0.30</td>
<td>0.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HIV infection, yes/no</td>
<td>0.31</td>
<td>0.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>0.20</td>
<td>0.46</td>
<td>0.021</td>
</tr>
</tbody>
</table>

Constant was 2.136. Only variables that entered the final model are reported. See text for details.
vascular structural and functional changes,7,11–13 the role of HIV infection, per se, as a risk factor for premature arteriosclerosis is more controversial. In the Strategies for Management of Antiretroviral Therapy Study, interruption of antiretroviral treatment was associated with an increased short-term risk of cardiovascular disease,27 and a high proportion of asymptomatic myocardial ischemia was found in HIV-infected adults, irrespective of their antiretroviral treatment status.28 In another study, HIV patients who had not initiated antiretroviral treatment had a higher risk of hospitalization for ischemic heart disease than a population-based control group.29

Pathogenetic research supports the direct role of HIV in accelerating arteriosclerosis. It is well established that infectious agents themselves (eg, viruses or bacteria) may play an important role in the etiology of the arteriosclerosis, thrombosis, and intimal thickening that occur after arterial injury. Endothelial activation and dysfunction have been proposed as plausible links between HIV infection and atherosclerosis. HIV infection with ongoing viral replication leads to an increased expression of adhesion molecules, including intercellular adhesion molecule-1 and E-selectin, and inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6,30 and antiretroviral therapy attenuates but does not completely normalize the endothelial activation state.6

HIV-mediated immunomodulation may also play a role. A low nadir level of CD4+ cells has been reported as a predictor of intima-media thickness progression,8 and patients with low CD4+ cell counts have been found to have a higher prevalence of premature carotid lesions than those with higher CD4+ cell counts.11 Moreover, HIV can impair the first step of reverse cholesterol transport, namely, cholesterol efflux from macrophages,31 which plays a key role in maintaining cell cholesterol homeostasis.32,33

The chronic inflammatory state associated with HIV infection could also contribute to explain the HIV-associated arterial stiffening. In recent years, multiple mechanisms of arterial inflammation have been indicated as important modulators of the initiation and progression of arteriosclerosis, and low-grade inflammation has been specifically involved in large-artery stiffness.34,35 Finally, dyslipidemia, insulin resistance, and impaired fibrinolysis, which have been described in HIV patients who had never undergone antiretroviral treatment,9 can represent additional potential mechanisms of vascular disease in HIV-infected individuals. It is worth mentioning that, in our study, despite the exclusion of subjects with major cardiovascular risk factors, HIV-infected patients had a higher prevalence of metabolic syndrome than control subjects. These data are in agreement with a recent Italian nationwide cross-sectional survey,36 which reported a higher prevalence of the metabolic syndrome in never-treated HIV-infected patients than in the general population. This could be of clinical relevance, given that the metabolic syndrome is a predictor of cardiovascular complications,37 and an important determinant of aortic stiffness in humans.22

Some other aspects of the present study deserve comment. First, serum GGT concentration was an independent determinant of aortic PWV among patients with HIV infection, and such association was not explained by coinfection with hepatitis C virus. Recent studies have demonstrated an association between serum GGT level and the development of cardiovascular disease risk factors,38,39 arterial stiffness,40 and cardiovascular disease and death.41 GGT concentration could be both a marker of insulin resistance42 and a contributor to oxidative stress pathways in several organ systems.43 Although we observed that the relation of GGT to aortic stiffness remained robust after accounting for established cardiovascular risk factors, it is conceivable that such adjustment incompletely accounts for hepatic insulin resistance and/or steatosis.

Second, despite having increased aortic stiffness, HIV-infected individuals did not differ from uninfected control subjects in terms of estimated Framingham risk score. We have found recently a limited increase in estimated cardiovascular risk in HIV-infected subjects compared with control subjects.44 These observations underline the importance of developing standardized tools for more precise coronary risk estimation tailored to HIV-infected individuals.

Third, HIV-infected patients under antiretroviral treatment tended to have a higher aortic PWV than untreated individuals. Although our study was not aimed at giving a definite answer to the question of whether HIV infection, per se, or antiretroviral treatment should account for increased cardiovascular risk among HIV-infected individuals, these findings

### Table 3. Main Characteristics of 23 HIV-Uninfected Subjects, 23 Untreated HIV-Infected Subjects, and 23 HIV-Infected Subjects Who Were Under Antiretroviral Treatment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Subjects (n=23)</th>
<th>P Value</th>
<th>HIV-Infected, Untreated (n=23)</th>
<th>P Value</th>
<th>HIV-Infected, Treated (n=23)</th>
<th>P Value (F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>39±7</td>
<td>NA</td>
<td>40±6</td>
<td>NA</td>
<td>40±6</td>
<td>0.92</td>
</tr>
<tr>
<td>Men, %</td>
<td>83</td>
<td>NA</td>
<td>83</td>
<td>NA</td>
<td>83</td>
<td>1.00</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.7±3</td>
<td>NA</td>
<td>22.8±3</td>
<td>NA</td>
<td>24.6±3</td>
<td>0.07</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>48</td>
<td>NA</td>
<td>61</td>
<td>NA</td>
<td>61</td>
<td>0.59</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>122±9</td>
<td>NA</td>
<td>120±10</td>
<td>NA</td>
<td>125±9</td>
<td>0.14</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80±7</td>
<td>NA</td>
<td>77±8</td>
<td>NA</td>
<td>77±6</td>
<td>0.30</td>
</tr>
<tr>
<td>10-year coronary heart disease risk, %</td>
<td>2.8 (1.0 to 4.2)</td>
<td>NA</td>
<td>3.1 (1.1 to 3.6)</td>
<td>NA</td>
<td>3.3 (1.6 to 4.0)</td>
<td>0.77</td>
</tr>
<tr>
<td>Aortic pulse wave velocity, m · s⁻¹</td>
<td>6.4±0.9</td>
<td>0.021</td>
<td>7.2±1.1</td>
<td>0.07</td>
<td>7.7±1.1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The subjects were individually matched by age, sex, and systolic blood pressure (see text for details). Values are means±SDs or medians (interquartile ranges). NA indicates not applicable.
suggest that HIV infection and antiretroviral therapy might each contribute to increase aortic stiffness.

Finally, one limitation of the present study is represented by its relatively small sample size. However, the remarkable difference in PWV between the groups suggests that the conclusions of this study are unlikely to be explained by chance.

Clinical Perspectives

The results of the present study provide for the first time evidence that aortic stiffness is increased in HIV treatment-naive patients free from cardiovascular disease and without major atherosclerotic risk factors. These findings suggest HIV infection as a potentially relevant contributor to arteriosclerosis and provide a conceptual background for the increased cardiovascular risk observed among HIV-infected individuals regardless of antiretroviral treatment. Close, noninvasive evaluation of preclinical atherosclerotic disease should be considered for HIV patients, especially those with additional risk factors for cardiovascular diseases, with the aim of addressing intensive lifestyle and pharmacological interventions aimed at reducing cardiovascular risk.

Disclosures

None.

References

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