Impact of Treatment With Protease Inhibitors on Aortic Stiffness in Adult Patients With Human Immunodeficiency Virus Infection

Giuseppe Schillaci, Giuseppe V.L. De Socio, Matteo Pirro, Gianluca Savarese, Massimo R. Mannarino, Franco Baldelli, Giuliano Stagni, Elmo Mannarino

Background—The role of antiretroviral therapy in acceleration of atherosclerosis in patients with human immunodeficiency virus (HIV) infection is controversial. We hypothesized that aortic stiffness, an early marker of arteriosclerosis, may be increased in HIV patients treated with protease inhibitors.

Methods and Results—In 32 HIV-infected patients treated with protease inhibitors and 32 age-, sex-, and blood pressure–matched HIV-uninfected control subjects, we obtained aortic pulse wave velocity and central aortic pressure waveform, from which aortic augmentation was calculated. HIV patients had a higher aortic pulse wave velocity (7.6 ± 1.1 versus 6.8 ± 1.2 m/s, P = 0.015) and aortic augmentation (6.8 ± 5 versus 4.6 ± 4 mm Hg, P = 0.037) than control subjects. Age and HIV infection (both P < 0.05) independently predicted aortic pulse wave velocity when a consistent number of cardiovascular risk factors was simultaneously controlled for. The cumulative duration of treatment was a predictor of aortic pulse wave velocity, each 5 years of treatment duration being independently related to a 1.35 m/s increase in pulse wave velocity.

Conclusions—Aortic stiffness is increased in HIV-positive individuals receiving antiretroviral therapy including a protease inhibitor. Pulse wave velocity increases with longer exposure to protease inhibitors. We hypothesize that arteriosclerosis is a side effect of antiretroviral treatment including a protease inhibitor. (Arterioscler Thromb Vasc Biol. 2005;25:2381-2385.)

Key Words: acquired immunodeficiency syndrome • HIV • aortic stiffness • arteriosclerosis • protease inhibitors • cardiovascular diseases

In industrialized countries, treatment with potent combination antiretroviral regimens that include protease inhibitors has resulted in a dramatic reduction in human immunodeficiency virus (HIV)-associated morbidity and mortality rates, and is one of the preferred therapeutic approaches to HIV infection. The optimism generated by such treatment has been tempered by the recognition of an increasing array of adverse metabolic effects including fat redistribution, dyslipidemia, insulin resistance, glucose intolerance, metabolic syndrome, and frank diabetes, all well-known cardiovascular risk factors in HIV-negative populations. Several studies have demonstrated that patients using antiretroviral therapy based on protease inhibitors develop atherogenic changes in their lipoprotein profile, consisting of elevations in triglyceride-rich lipoproteins and low-density lipoprotein cholesterol. There is also evidence that protease inhibitor use is associated with early signs of atherosclerosis, including endothelial dysfunction and increased intima-media thickness, but the role of protease inhibitors in this regard remains not well defined. There are reports of premature cardiovascular and cerebrovascular disease, possibly linked to both effects of the drugs and HIV infection itself. Recently, the prospective Data collection on Adverse events of anti-HIV Drugs study reported a 26% increase in the risk of myocardial infarction per year of exposure to antiretroviral therapy over the first 4 to 6 years of use, thus supporting the hypothesis that combination antiretroviral therapy may be associated with increased risk of atherosclerosis. However, these data have been questioned by the negative findings of a large retrospective study.

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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 variables other than clinical endpoints that provide a conceptual framework for the cardiovascular effects of HIV protease inhibitors. 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.11,12 To our knowledge, the impact of highly active antiretroviral treatment on arterial stiffness has never been examined in adult subjects. Because the metabolic syndrome is both a side effect of protease inhibitors13 and an important determinant of large-artery stiffness,14 the present study was established to investigate the hypothesis that treatment with protease inhibitors might induce an increase in large-artery stiffness. To this aim, a cohort of adult HIV-infected subjects free from overt cardiovascular disease was accurately matched by age, sex, and blood pressure with a group of HIV-uninfected subjects. To avoid the confounding effect of classic risk factors for atherosclerotic disease, only subjects without major cardiovascular risk factors were included in the present study.

Methods
We studied adult patients with documented HIV infection who had been on stable treatment with antiretroviral drugs including a protease inhibitor for at least 24 months. A total of 41 consecutive HIV-infected adults satisfied inclusion criteria (see below), and 32 of them agreed to participate in the study. HIV seropositivity had been documented for a median period of 10 years (range, 3 to 17 years). All patients were being treated with a combination antiretroviral therapy including a protease inhibitor (indinavir in 6 patients, nelfinavir in 21 patients, saquinavir plus ritonavir in 3 patients) for a median period of 56 months (range, 24 to 78 months). The median nadir of CD4+ T-lymphocyte count was 121/mm³ (range, 1 to 735) and 34% of the patients had a diagnosis of acquired immunodeficiency syndrome (C3 stage of the Centers for Disease Control classification15).

Thirty-two HIV-negative subjects were recruited as controls among the staff working at the hospital. Control subjects were matched with patients by age (±5 years), sex (same sex), and systolic blood pressure (±10 mm Hg). In patients as well as in controls, we excluded from the study subjects with arterial hypertension, hypercholesterolemia, known diabetes or fasting glycemia ≥7 mmol/L (126 mg/dL), serum creatinine concentration >177 µmol/L (2 mg/dL), clinical or laboratory evidence of coronary heart disease, previous stroke, or treatment with any cardiovascular drug including nitrates. 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. Hypercholesterolemia was defined by a serum cholesterol concentration ≥6.46 mmol/L (250 mg/dL) or hypolipidemic 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.16 All participants gave their informed consent to participate in the study, which was approved by the institutional ethics committee. Total cholesterol, triglycerides, and high-density lipoprotein cholesterol were determined by enzymatic-colorimetric method (Dimension Autoanalyzer; Dade Behring, Germany) after 12 hours of fasting. Subjects were examined in the morning at a room temperature of 22 ± 1°C. Blood pressure was measured by physicians in the medical center with a mercury sphygmomanometer on the left arm, after patients sat for 10 minutes or longer, as the average of 3 readings. After measuring blood pressure, arterial pulse wave velocity and waveform were obtained with an automatic device, the SphygmoCor Vx system (AtCor). Briefly, an arterial pressure waveform was recorded by applanating the radial artery with a high-fidelity hand-held tonometer at the site of maximal pulsation. Pressure waveforms obtained with this method have been validated by comparing them with those obtained by a high-fidelity intraarterial transducer.17 Central artery waveforms were derived from the radial artery waveform and pressure by using a transfer function validated previously during catheterization studies.18,19 The point at which the central aortic pressure becomes augmented by wave reflection is recognized by a computer program, and the degree of increase is expressed as the aortic augmentation, which is expressed either as a relative index or as a percentage of aortic pulse pressure (aortic augmentation index). The availability of the central aortic waveform allows the determination of systolic and diastolic duration and pressure and the calculation of the subendocardial viability ratio,20 which has been shown by Buckberg et al21,22 to be higher in both humans and experimental animals to be a measure of the propensity for myocardial ischemia on the basis of altered hemodynamic forces. Subendocardial viability ratio was calculated as (diastolic time × pressure/systolic time × pressure). Arterial pulse wave velocity was determined by the foot-to-foot method as previously described.14 Sequentially, ECG-gated pulse waveforms were obtained transecturally over the common carotid and femoral arteries in the groin (aortic pulse wave velocity) and over the carotid and radial arteries (upper limb pulse wave velocity). Pulse wave velocity was calculated as the distance between recording sites measured over the surface of the body, divided by the time interval between the feet of the pressure waves. The distance between the 2 sites was measured using a standard compass system, which avoided the measure to be influenced by thoracic and abdominal profiles. The average of 10 different cardiac cycles on each of the sites was used for the analysis. The same observer, unaware of the patient’s clinical and biochemical data, carried out all measurements.

Statistical Analysis
SPSS statistical package, release 10.0 (SPSS, Inc) was used for all statistical analyses. Between-group differences were assessed by the use of Student t and Wilcoxon tests for continuous normally and non-normally distributed variables, respectively. The χ² test was used for comparing categorical variables. Pearson correlation coefficients examined the degree of association between examined variables, and the bivariate normal ellipse (P < 0.05), ie, the contour enclosing 95% of the observations in the population, was reported. Logarithmic transformation was used for those variables which showed a non-normal distribution. Linear regression analysis was used to estimate prediction of aortic pulse wave velocity, aortic augmentation, and subendocardial viability ratio 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, systolic blood pressure, heart rate, and HIV infection status. Among HIV-infected subjects, a multivariate regression model was run which included all the above variables, plus cumulative duration of treatment.

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 body mass index, waist/hip circumference ratio, smoking habits, serum cholesterol, and serum glucose concentration did not differ. HIV-infected patients had significantly higher serum triglycerides and lower high-density lipoprotein cholesterol than control subjects and a higher prevalence of the metabolic syndrome.

As reported in Table 2, HIV-infected subjects had a significantly higher aortic pulse wave velocity, whereas upper limb pulse wave velocity did not differ. HIV-infected patients also had a higher aortic augmentation and augmentation index, and a significantly lower subendocardial viability ratio, as a result of a higher systolic (pressure × time) area and a lower diastolic area.

Among patients with HIV infection, aortic pulse wave velocity showed a direct association with the cumulative
duration of treatment ($r=0.42$, $P=0.016$; see the Figure). In a multivariate analysis, each 5-year treatment duration was independently associated to a 1.35 m/s$^2$ increase in aortic pulse wave velocity (regression coefficient 0.27, $P=0.027$), after taking into account the effects of heart rate (regression coefficient 0.05, $P=0.011$), age, and other explanatory variables.

We also investigated whether a relation exists between the severity of HIV-related immunodepression and aortic stiffness. Among HIV-infected patients, CD4+ cell count at the time of the study had no significant relation with any of the examined measures of vascular function (aortic augmentation, $r=-0.22$, $P=0.22$; aortic pulse wave velocity, $r=-0.09$, $P=0.64$; subendocardial viability ratio, $r=0.10$, $P=0.58$). However, the log-transformed nadir CD4+ cell count showed a strong inverse correlation with aortic augmentation ($r=-0.50$, $P=0.003$) and augmentation index ($r=-0.38$, $P=0.03$). Adjustment for age did not change the strength of these associations ($r=-0.47$, $P=0.008$, and $r=-0.35$, $P<0.05$, respectively).

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, systolic blood pressure, heart rate, total and high-density lipoprotein, and serum triglycerides, aortic pulse wave velocity was independently associated with HIV infection along with age (Table 3). Aortic augmentation was independently predicted by low body height, HIV infection, and systolic blood pressure, whereas a low subendocardial viability ratio was associated with HIV infection and increasing heart rate.

### Discussion
The present study demonstrates that HIV-infected patients under highly active antiretroviral treatment including a protease inhibitor have higher values of aortic stiffness than a matched group of HIV-uninfected control subjects. The association held regardless of the confounding effect of age, sex, blood pressure, and other major cardiovascular risk factors. Because aortic stiffness has been identified as an independent predictor of cardiovascular mortality, changes in arterial stiffness may, in part, mediate the association between the use of protease inhibitors and cardiovascular risk.

With the use of newer antiretroviral regimens, HIV infection has become a chronic illness in developed countries, and

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-Infected n=32</th>
<th>Controls n=32</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>41±8</td>
<td>41±8</td>
<td>0.86</td>
</tr>
<tr>
<td>Men, %</td>
<td>78</td>
<td>78</td>
<td>1.00</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73±0.08</td>
<td>1.71±0.09</td>
<td>0.34</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>25.0±4</td>
<td>25.4±3</td>
<td>0.56</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.93±0.07</td>
<td>0.90±0.08</td>
<td>0.14</td>
</tr>
<tr>
<td>Cigarette smoking, %</td>
<td>69</td>
<td>62</td>
<td>0.60</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>128±11</td>
<td>126±8</td>
<td>0.41</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>80±9</td>
<td>82±7</td>
<td>0.43</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>48±8</td>
<td>44±8</td>
<td>0.048</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>66±10</td>
<td>68±9</td>
<td>0.35</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.09±1.0</td>
<td>4.72±0.7</td>
<td>0.13</td>
</tr>
<tr>
<td>High-density lipoprotein cholesterol, mmol/L</td>
<td>1.15±0.3</td>
<td>1.41±0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.1 (1.6–3.2)</td>
<td>1.3 (0.9–2.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.81±0.8</td>
<td>4.63±0.6</td>
<td>0.30</td>
</tr>
<tr>
<td>Metabolic syndrome, %</td>
<td>28</td>
<td>6</td>
<td>0.047</td>
</tr>
</tbody>
</table>

Values are mean±SD, except for triglycerides expressed as median and interquartile range.

### Table 2. Central Hemodynamic Parameters in HIV Patients and in Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HIV-Infected n=32</th>
<th>Controls n=32</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic pulse wave velocity, m/s$^2$</td>
<td>7.6±1.1</td>
<td>6.8±1.2</td>
<td>0.015</td>
</tr>
<tr>
<td>Upper limb pulse wave velocity, m/s$^2$</td>
<td>7.6±1.3</td>
<td>7.6±1.3</td>
<td>0.88</td>
</tr>
<tr>
<td>Aortic augmentation, mm Hg</td>
<td>6.8±4</td>
<td>4.6±5</td>
<td>0.037</td>
</tr>
<tr>
<td>Aortic augmentation index</td>
<td>0.19±0.11</td>
<td>0.14±0.11</td>
<td>0.046</td>
</tr>
<tr>
<td>Diastolic pressure×time area, mm Hg×ms</td>
<td>3513±421</td>
<td>3692±532</td>
<td>0.14</td>
</tr>
<tr>
<td>Systolic pressure×time area, mm Hg×ms</td>
<td>2309±304</td>
<td>2180±332</td>
<td>0.11</td>
</tr>
<tr>
<td>Subendocardial viability ratio</td>
<td>1.54±0.2</td>
<td>1.74±0.4</td>
<td>0.017</td>
</tr>
</tbody>
</table>

Values are mean±SD.
cardiovascular disease represents an important late concern in HIV-positive individuals. Of particular interest, a linear relation has been observed between duration of treatment with antiretroviral agents and the occurrence of myocardial infarction and other cardio- and cerebrovascular events. A number of previous studies has shown an association between use of protease inhibitors and early vascular structural and functional changes in HIV-infected children and adults. In particular, endothelial dysfunction, increased carotid intima-media thickness, and carotid atherosclerotic plaques have been reported in HIV-infected adults.

Several potential mechanisms may underlie the association between treatment with protease inhibitors and large-artery stiffness. Antiretroviral therapy induces dyslipidemia, and this may contribute to accelerate atherosclerotic plaque formation. In particular, the use of protease inhibitors has been strongly associated to the development of the metabolic syndrome, which is a powerful predictor of cardiovascular morbidity and mortality, and an important determinant of aortic stiffness in humans. Indeed, in our study the prevalence of the metabolic syndrome was higher among HIV individuals treated with protease inhibitors, primarily as a result of the presence of high serum triglycerides and reduced high-density lipoprotein cholesterol concentration, of the basic components of the syndrome. On the other hand, protease inhibitors may promote atherosclerotic lesion formation independently of dyslipidemia by increasing CD36-dependent cholesterol ester accumulation in macrophages, a critical step in the initiation, progression, and rupture of atherosclerotic lesions.

Several other findings of the present study deserve to be discussed. First, a significant independent relation was found between aortic stiffness and duration of treatment. This finding, together with previous prospective data, supports the hypothesis that treatment with protease inhibitors may facilitate the progression of atherosclerotic disease in HIV-infected patients.

Second, the potential contribution of antiretroviral therapy to atherosclerotic disease is difficult to distinguish from that of classic cardiovascular risk factors. In this study, we chose to examine only subjects who were free from overt cardiovascular disease, hypertension, diabetes, chronic renal disease, or who were being treated with cardiovascular or hypolipidemic drugs. Moreover, patients and controls were accurately matched by age, sex, and blood pressure values, to minimize the confounding effect of these important conditions on the study findings.

Third, although the CD4+ T-cell count at the time of the study had no significant relation with aortic stiffness, a strong inverse correlation was found between the CD4+ T-cell count nadir and aortic augmentation. This finding supports the hypothesis that immunodepression determined by HIV infection might in itself contribute to accelerate the atherosclerotic process. Dyslipidemia, insulin resistance, inflammation, impaired fibrinolysis, and activation of vascular endothelium mediated by chronic HIV-induced low-grade inflammation may also play a role in this regard.

Peripheral applanation tonometry-based measurement of arterial pressure waveform is a sensitive, reproducible, and validated noninvasive measure of the effect of arteriosclerosis on arterial pressure as detected by the pulse waveform. Augmentation of central pressure, a consequence of earlier wave reflection which shifts the augmentation of blood pressure from diastole to systole, is a direct determinant of increased cardiac workload and diminished myocardial perfusion and has been recently implicated as a powerful risk factor for cardiovascular events independently of pulse pressure and other risk markers. Our findings support the notion that HIV protease inhibitors may have detrimental effects on central arterial pressure, cardiac workload, and myocardial perfusion that are analogous to that of aging. The observed reduction in subendocardial viability ratio, an indirect consequence of increased arterial stiffness, further reinforces these findings.

In conclusion, aortic stiffness is increased in HIV-positive individuals receiving antiretroviral therapy including a protease inhibitor. These findings provide a potential explanation for the association between treatment with protease inhibitors and cardiovascular morbidity and mortality. Although these and other data suggest that currently available protease inhibitors do elevate cardiovascular risk, this highly effective

### TABLE 3. Independent Determinants of Aortic Pulse Wave Velocity, Aortic Augmentation, and Subendocardial Viability Ratio in a Stepwise Multiple Linear Regression Analysis

<table>
<thead>
<tr>
<th>Regression Coefficient</th>
<th>Multiple R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic pulse wave velocity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>0.045</td>
<td>0.32</td>
</tr>
<tr>
<td>HIV infection, yes/no</td>
<td>0.671</td>
<td>0.43</td>
</tr>
<tr>
<td>Aortic augmentation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body height, cm</td>
<td>−0.239</td>
<td>0.30</td>
</tr>
<tr>
<td>HIV infection, yes/no</td>
<td>2.890</td>
<td>0.45</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>0.149</td>
<td>0.56</td>
</tr>
<tr>
<td>Subendocardial viability ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>−0.192</td>
<td>0.56</td>
</tr>
<tr>
<td>HIV infection, yes/no</td>
<td>−0.150</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Only variables which entered the final model are reported. See text for details.

Constant was 5.003 in Model 1, 26.406 in Model 2, and 3.011 in Model 3.
drug class will continue to be an important treatment option. For patients with preexisting cardiovascular risk factors, consideration should be given to initiating or switching to protease inhibitor-sparing antiretroviral regimens or to newer protease inhibitors which are expected to induce less metabolic complications. Until a specific therapy that can modify arterial wall properties is available, patients receiving HIV protease inhibitors, especially those with additional risk factors for cardiovascular diseases, are candidates for close noninvasive evaluation of preclinical atherosclerotic disease and intensive lifestyle and pharmacological interventions aimed at reducing cardiovascular risk.

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References

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