Atherosclerosis in the HIV and non-HIV setting: detecting and modifying cardiovascular risk
Sankatsing, R.R.

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Increased Carotid Intima-Media Thickness in HIV patients treated with Protease Inhibitors as compared to Non-Nucleoside Reverse Transcriptase Inhibitors

Raaj R Sankatsing
Ferdinand W Wit
Martin Vogel
Eric de Groot
Kees Brinkman
Juergen K Rockstroh
John JP Kastelein
Erik SG Stroes
Peter Reiss

Abstract

**Background:** Prolonged exposure to protease inhibitor (PI)-, but not non-nucleoside reverse transcriptase inhibitor (NNRTI)-containing combination antiretroviral therapy (CART) has been associated with an increased cardiovascular risk, partly explained by the different effects of these drugs on plasma lipids. Most markedly, NNRTIs have been associated with increases in high density lipoprotein cholesterol (HDL-C), which may be atheroprotective.

**Methods:** In a cross-sectional study we investigated the impact of PI- versus NNRTI-based CART in 130 HIV-1-infected patients with plasma virus suppressed to below the limit of detection, whom had been continuously exposed for at least 2 years to either one of such regimens, but not both. Carotid intima-media thickness (C-IMT) and fasting metabolic parameters were measured.

**Results:** Mean (±SD) C-IMT in patients treated with PI-based CART was 0.81 (±0.17) mm as compared to 0.71 (±0.14) mm in NNRTI treated patients (p=0.0003). HDL-C and apolipoprotein A-I (apoA-I) levels were higher in the NNRTI than in the PI group (1.39 versus 1.03 mmol/L, p<0.0001, and 1.44 versus 1.33 mmol/L, p=0.0008, respectively). Age, body mass index, duration of CART, and use of PI-based CART were positively correlated with C-IMT whereas HDL-C and apoA-I were inversely correlated with C-IMT.

**Conclusions:** Treatment of HIV-1-infected patients for two years or more with PI-based compared to NNRTI-based CART is associated with greater C-IMT, consistent with the reported higher risk of CVD in patients using PI. However, this difference seems not fully explained by a more favorable impact of NNRTI-based CART on HDL-C and apoA-I levels.
Introduction

AIDS-related mortality was profoundly reduced when combination antiretroviral therapy (CART) was introduced into clinical practice. However, CART use was also found to cause a range of metabolic complications, including insulin resistance and diabetes mellitus, as well as dyslipidemia and changes in body fat distribution or lipodystrophy.\textsuperscript{1,2} This has fueled the concern that patients using CART might be at increased risk of cardiovascular disease (CVD). The largest study that prospectively addressed this question, the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) Study, indeed revealed that CART, independent of traditional CVD risk factors, was associated with an increased risk of myocardial infarction.\textsuperscript{3} In a recent update from this study, the risk of myocardial infarction associated with CART proved to be confined to protease inhibitors.\textsuperscript{4} Conversely, no evidence was found for such an association with non-nucleoside reverse transcriptase inhibitors. This association with PI exposure could only partly be explained by the dyslipidemia seen with CART.

HIV treatment that includes protease-inhibitors (PIs), in particular when boosted by ritonavir, is often associated with increased plasma levels of triglycerides (TG) and low density lipoprotein (LDL)-cholesterol, with modest or no consequences for high density lipoprotein cholesterol (HDL-C). These effects on plasma lipids are much less pronounced when non-nucleoside reverse transcriptase inhibitors (NNRTIs) such as nevirapine or efavirenz are used.\textsuperscript{5-8} Of note, in contrast to PIs, NNRTIs have been associated with marked increases in HDL-C.\textsuperscript{5-7, 9-11} The latter is of particular interest since high HDL-C may protect against CVD due to its ability to promote reverse cholesterol transport\textsuperscript{12} and a range of additional mechanisms which include antioxidative and anti-inflammatory properties.\textsuperscript{13}

We therefore speculated that NNRTI-containing CART, in contrast to regimens that include protease inhibitors, would be associated with a slower onset of atherosclerotic vascular disease. In order to test this hypothesis, we performed a cross-sectional study in which we compared the carotid intima-media thickness (C-IMT) in HIV-1-infected patients with viral suppression below the limit of detection, who had continuously been exposed for two years or longer to either PI- or NNRTI-containing CART, but not both.
Methods

Patient Selection
All eligible patients who consecutively attended the HIV outpatient clinics of the Academic Medical Center and the Onze Lieve Vrouwe Gasthuis in Amsterdam, the Netherlands, as well as of the Universitätsklinikum in Bonn, Germany between June 2003 and February 2006, and who met the inclusion criteria for the study were approached to participate. Patients could be included if they were on either PI- or NNRTI-based CART and had been receiving such a regimen continuously for at least two years. Furthermore patients had to have documented plasma HIV-1 RNA levels to below the limit of detection (< 50 copies/mL) at the time of study entry. Patients in the PI-treated group were allowed to have switched between different PIs, but could not have been exposed to NNRTI while patients in the NNRTI-group had to have been treated with either nevirapine- (NVP) or efavirenz- (EFV) containing CART, and were not allowed to have switched between these two NNRTIs, or to have ever used PIs. The study protocol was approved by the institutional review board of the Academic Medical Center, University Hospital of Amsterdam. Patients from the Onze Lieve Vrouwe Gasthuis in Amsterdam were referred for the study to the Academic Medical Center. No ethics approval was needed for patients from Bonn according to local guidelines, given that no intervention was involved and all bloods were drawn according to routine clinical care. All subjects provided written informed consent.

Cardiovascular Risk Factor Assessment
At the study visit a detailed medical history was obtained from each patient using a standardized questionnaire including questions concerning demographic characteristics, prior and current antiretroviral treatment, family history of CVD, prior history of CVD, cigarette smoking, and use of antihypertensive, antidiabetic, or lipid lowering drugs. Patients’ height and weight, as well as blood pressure in supine position were measured in a standardized fashion. Framingham Risk Scores were calculated for each patient.

In addition, at the same visit blood was drawn following an overnight fast for assessment of total- and HDL cholesterol (HDL-c), triglycerides (using enzymatic methods, Roche Diagnostics GmbH, Mannheim, Germany), apolipoprotein A-I (apoA-I), apolipoprotein B (apoB) (using rate immunonephelometry, Dade Behring Nephelometer BNII, Marburg, Germany), high-sensitivity C-reactive protein (hs-CRP) (using an immune turbidimetric test [Roche Diagnostics GmbH, Mannheim, Germany]), aspartate aminotransferase, alanine aminotransferase (using the Pyridoxalphosphate, 37 °C UV test [Roche Diagnostics GmbH, Mannheim, Germany]), glucose (using the HK/Glucose-6-P dehydrogenase UV test [Roche Diagnostics GmbH, Mannheim, Germany]), free T4 and thyroid stimulating hormone (TSH) (using a solid phase, two-site fluorimmunometric assay [PerkinElmer Life and Analytical Sciences, Turku, Finland]). LDL cholesterol (LDL-c) was calculated using the Friedewald equation. Finally, in Amsterdam absolute and percent CD4- and CD8-positive lymphocyte subsets were determined by flow cytometry (BD
Multitest CD3FITC/CD8PE/CD45PerCP/CD4APC [BD Biosciences, San Jose, USA], and plasma HIV-RNA was measured using the Versant HIV-1 RNA 3.0 assay (bDNA)(Siemens Medical Solutions Diagnostics, Los Angeles, USA) with a lower limit of detection of 50 copies/mL.

In Bonn, HIV-RNA was measured using bDNA assay (Bayer Vital GmbH Diagnostika, Fernwald, Germany), likewise with a lower limit of detection of 50 copies/mL, while CD4- and CD8-cell counts were analyzed on a FACS calibur flow cytometer (Becton Dickinson, Heidelberg, Germany). Hepatitis B and C co-infection was not assessed.

**Measurement of Carotid Intima-Media Thickness**

The common carotid, carotid bulb, and internal carotid arterial far walls were scanned bilaterally using a standardized B-mode ultrasound imaging and image analysis protocol, the details of which have been described previously elsewhere. In summary, an Acuson 128XP ultrasound instrument (Acuson, Mountainview, CA, USA) equipped with an L7 transducer and Extended Frequency (EF) software was used. Standard views of 2 by 2 centimeters were obtained and saved as 4:1 compressed JPEG image files. For off-line image analyses in-house designed validated hardware and software was used. A subject’s carotid IMT was calculated as the average of the sum of the 3 right and 3 left carotid arterial far wall IMT measurements. Two experienced sonographers, blinded to the subject’s antiretroviral regimen, scanned all subjects both in the Netherlands and in Germany. A technician, who remained blinded to each patient’s CART history, subsequently performed the off-line image analyses.

**Statistical methods**

Baseline parameters were tabulated and compared between groups using Fisher’s exact or Wilcoxon test as appropriate. The C-IMT was compared among groups using Student’s t-tests. Univariate and multivariate linear regression was used to investigate the influence of selected covariates on the C-IMT. Covariates that were found to be statistically significantly associated with C-IMT in a univariate analysis were entered into the multivariate analyses. The final multivariate linear regression model only contained covariates that remained significantly associated with the C-IMT. Investigated covariates were: (duration of) used antiretroviral agents, (duration of) smoking, blood lipids and glucose and CRP, presence of diabetes mellitus, age, gender, blood pressure, BMI, weight, and the Framingham Risk Score. The sample size was calculated assuming a difference in IMT favouring patients using NNRTI- compared to PI-based therapy of at least 0.05 mm over 2 years with a standard deviation of 0.1. The study was originally designed to compare 40 patients using nevirapine with 80 patients using protease inhibitors, which would yield 80% power using single-sided significance testing. Before the start of the study the design was changed to also include 40 patients using efavirenz. We assumed similar findings in the NVP and EFV subgroups. The main comparison was now between the PI (n=80) and the combined NNRTI group (n=80), which had 88% power using 2-sided significance testing. Eventually we were able to recruit just 62 and 68 patients into the PI and the
combined NNRTI groups, yielding 81% power using 2-sided testing. The study was not powered to formally compare between the EFV and NVP subgroups. Statistical significance was set at a 2-sided p-value of 0.05 for all comparisons. All analyses were done using SAS software (SAS version 9.1, SAS institute, Cary, NC, USA).

Results

Patient characteristics

A total of 130 HIV-1-infected patients were enrolled in this study, of whom 62 were receiving PI-based CART and 68 NNRTI-based CART. Within the NNRTI-group, 38 patients were on NVP and 30 patients on EFV. The clinical characteristics of the patients are listed in table 1. The median age of the total cohort was 46 years, and 90% were male.

The groups of patients on PI- and NNRTI-based CART were comparable with respect to age, sex, body mass index, history of CVD, presence of current other risk factors for CVD, and concomitant use of antihypertensive, antidiabetic and lipid lowering medication. No differences were found in Framingham Risk Scores between NNRTI and PI-treated patients.

A specification of PI use is provided in table 2.

Table 1 Clinical characteristics of patients on PI- and NNRTI-based CART

<table>
<thead>
<tr>
<th></th>
<th>PI (n=62)</th>
<th>NNRTI (n=68)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45 (40-54)</td>
<td>47 (42-53)</td>
<td>0.61</td>
</tr>
<tr>
<td>Sex, female (%)</td>
<td>5 (8)</td>
<td>8 (12)</td>
<td>0.57</td>
</tr>
<tr>
<td>Body Mass Index, kg/m²</td>
<td>23 (21-25)</td>
<td>24 (22-26)</td>
<td>0.20</td>
</tr>
<tr>
<td>History of CVD, n (%)</td>
<td>6 (10)</td>
<td>4 (6)</td>
<td>0.52</td>
</tr>
<tr>
<td>Risk factors CVD, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current Smoking</td>
<td>27 (44)</td>
<td>30 (44)</td>
<td>1.0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (11)</td>
<td>3 (4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>2 (3)</td>
<td>0 (0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Family history premature CVD</td>
<td>4 (6)</td>
<td>9 (13)</td>
<td>0.25</td>
</tr>
<tr>
<td>Current Medications, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antihypertensive drugs</td>
<td>8 (13)</td>
<td>3 (4)</td>
<td>0.12</td>
</tr>
<tr>
<td>Antidiabetic drugs</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Lipid lowering drugs</td>
<td>7 (11)</td>
<td>3 (4)</td>
<td>0.19</td>
</tr>
<tr>
<td>Pack-years of smoking, y</td>
<td>21 (14-30)</td>
<td>20 (9-27)</td>
<td>0.96</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>123 (119-135)</td>
<td>121 (119-131)</td>
<td>0.37</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>78 (72-84)</td>
<td>80 (75-82)</td>
<td>0.59</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>48 (42-54)</td>
<td>44 (40-51)</td>
<td>0.11</td>
</tr>
<tr>
<td>Duration of CART use (y)</td>
<td>5.12 (3.99-8.86)</td>
<td>4.96 (3.91-6.07)</td>
<td>0.24</td>
</tr>
<tr>
<td>Framingham Risk Score</td>
<td>6 (3-12)</td>
<td>5 (2-10)</td>
<td>0.10</td>
</tr>
</tbody>
</table>

CVD denotes cardiovascular disease, SBP systolic blood pressure, DBP diastolic blood pressure, and CART combination antiretroviral therapy. Current smoking was defined as smoking within the last month.
Results of laboratory assessments (Table 3)

As per protocol all patients had a plasma HIV-1 RNA level below the limit of detection of 50 copies per milliliter. The median CD4- and CD8-positive lymphocyte counts and the CD4/CD8 ratio were not significantly different between PI- and NNRTI-treated patients. Mean HDL-C levels were significantly higher in the NNRTI-group than in the PI group (1.39 (1.16-1.66) mmol/L versus 1.03 (0.90-1.25) mmol/L, respectively; p<0.0001). Within the NNRTI-group, they were somewhat higher in the NVP subgroup compared to the EFV subgroup (1.44 (1.26-1.80) mmol/L vs. 1.37 (1.03-1.54) mmol/L, p=0.021).

Likewise apolipoprotein A-I (apoA-I) levels were significantly higher in the NNRTI group compared with the PI group (1.44 [1.30-1.59] mmol/L vs. 1.33 [1.16-1.45] mmol/L, p=0.0008), whereas triglycerides levels were lower in the NNRTI group (1.2 [0.9-1.7] mmol/L vs. 2.1 [1.4-3.4] mmol/L, p<0.0001). Glucose levels were within the normal range in both groups, but somewhat higher in the NNRTI group as compared to the PI group (5.1 [4.7-5.4] mmol/L vs. 4.6 [4.3-5.2] mmol/L, p=0.0016).

No significant differences were observed in levels of C-reactive protein, liver aminotransferases and thyroid hormones.

Carotid IMT

Mean (±SD) C-IMT was 0.81 (±0.17) mm in the PI-treated group compared to 0.71 (±0.14) mm in the NNRTI-treated group (p=0.0003) (Figure 1). In view of the fact that we had a reasonably substantial number of patients on NVP and EFV respectively, a post-hoc exploratory analysis was performed comparing each of these subgroups to the patients on PI. In this comparison both the NVP-subgroup as well as the EFV-subgroup showed thinner C-IMT as compared to the PI-group, 0.72 (±0.13) mm (NVP) vs. 0.81 (±0.17) mm (PI) (p=0.0031), and 0.70 (±0.15) mm (EFV) vs. 0.81 (±0.17) mm (PI) (p=0.0027). On univariate analyses, increasing age, systolic and diastolic blood pressure, BMI, pack years of smoking, triglycerides, apoB levels, duration of CART use, Framingham Risk Score, as well as use of any PI and use of ritonavir in particular, were associated with increased C-IMT. In contrast, increasing levels of HDL-C and apoA-I were associated with decreased C-IMT.

Of those parameters which were univariately associated with C-IMT, only age (+0.05 mm per 10 years older), BMI (+0.011 mm per 1 kg/m² increase), duration of CART use (+0.011 mm per additional year of exposure) and the use of PI (+0.10 mm for PI use) remained significantly and independently associated with C-IMT on multivariate analysis (Table 4). A trend remained for diastolic blood pressure (+0.025 mm per 10 mmHg increase) on multivariate analysis.
Figure 1  C-IMT of HIV-infected patients on PI- and NNRTI-based CART vs. PI

C-IMT (mean ± SD)

C-IMT is significantly thicker in patients using PI compared to NNRTI. Data are presented as mean ± standard deviation. * p<0.001 NNRTI vs. PI

Discussion

Our results show that exposure of HIV-1-infected adults for two years or more to PI-containing, but not NNRTI-containing CART, was significantly associated with increased C-IMT, independent of traditional cardiovascular risk factors. These findings are of interest, because increases in C-IMT are predictive of future cardiovascular events16-18. C-IMT is regarded a validated surrogate marker for cardiovascular disease19, and because of recently published results from the D:A:D Study which reported an increased risk of myocardial infarction with PI but not with NNRTI use.4 Interestingly, the association between the odds for myocardial infarction and PI exposure could only partly be explained by the dyslipidemic effects of therapy, suggesting that additional factors related to the use of PI are contributing to this risk.

Table 2  Specification of protease inhibitors used by PI treated patients

<table>
<thead>
<tr>
<th>Protease inhibitor</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APV+LPV/r</td>
<td>1 (1.61)</td>
</tr>
<tr>
<td>APV/r</td>
<td>4 (6.45)</td>
</tr>
<tr>
<td>ATZ/r</td>
<td>3 (4.84)</td>
</tr>
<tr>
<td>IDV</td>
<td>5 (8.06)</td>
</tr>
<tr>
<td>IDV/RTV</td>
<td>4 (6.45)</td>
</tr>
<tr>
<td>LPV/r</td>
<td>34 (54.84)</td>
</tr>
<tr>
<td>NFV</td>
<td>3 (4.84)</td>
</tr>
<tr>
<td>RTV (full dose)</td>
<td>4 (6.45)</td>
</tr>
<tr>
<td>SQV/RTV</td>
<td>4 (6.45)</td>
</tr>
<tr>
<td>Total</td>
<td>62 (100)</td>
</tr>
</tbody>
</table>

Footnote: APV denotes amprenavir, LPV lopinavir, ATZ atazanavir, IDV indinavir, NFV nelfinavir, RTV “full” dose ritonavir (i.e. 400-600 mg twice daily), SQV saquinavir, and r boosting dose of ritonavir (i.e. 100-200 mg twice daily)
Classical risk factors for CVD were overall balanced between the two groups with no parameter being significantly different between groups. Although in our study levels of HDL-C and apoA-I were significantly higher in the NNRTI-treated patients, and inversely related to C-IMT in the univariate analysis, this association no longer remained significant in the multivariate analysis. The latter only revealed PI use, duration of CART use, age, and BMI as significantly associated with C-IMT. However, given the strong univariate associations between PIs and IMT on the one hand and HDL and IMT on the other hand as well as the association between NNRTI and HDL, it is difficult to identify an “independent” effect of NNRTI above and beyond HDL. Of note, previous studies have shown increases in HDL-C levels both in therapy-naïve patients commencing treatment with nevirapine- or efavirenz-based CART, as well as in patients in whom nevirapine or efavirenz was substituted for a PI. In general HDL-C increases in these studies have been more pronounced with nevirapine- than efavirenz-containing CART. Although the latter was confirmed in the current study, HDL-C levels were significantly higher for patients on either NVP or EFV when compared to those on PIs. Comparison of the individual NNRTIs with PIs showed consistent results with respect to C-IMT although this analysis has to be interpreted with caution in view of more limited power. Contrary to what would be expected, we found similar levels of LDL-C in PI- and NNRTI-treated patients and slightly lower levels of glucose in PI-treated patients. While these observations may reflect inadequate matching previous studies show that, in contrast to NVP, EFV increases LDL-C levels which may have obscured a possible difference in LDL-C levels between the two groups.

It is important to realize however that, in addition to any antiretroviral treatment-associated effect not mediated by lipid changes, factors related to the HIV-1 infection itself as well as the state of immunodeficiency and/or immune activation associated with the infection may also contribute to atherogenesis and cardiovascular disease in the context of HIV-1 infection. Results from the SMART (Strategies for Management of Antiretroviral Therapy) trial have raised the awareness for this concept. In this trial the intermittent use of antiretroviral therapy guided by patients’ CD4+ lymphocyte counts (drug conservation strategy) was associated with a higher rate of fatal or nonfatal cardiovascular events compared to when antiretroviral therapy was used continuously (viral suppression strategy). These increased odds in the drug conservation arm were highest for those already entering the trial off antiretroviral therapy and to a lesser degree for those entering on therapy and discontinuing NNRTI-containing therapy. Lipid changes, including reductions in HDL-C and increased total/HDL-C ratios, were also more unfavorable in patients allocated to drug conservation. This was particularly the case in those on NNRTI at entry, which may partially explain the observed excess CVD risk in the drug conservation strategy arm. Thus, multiple factors both related and unrelated to antiretroviral therapy, likely act in concert in promoting atherogenesis in patients with HIV-1 infection. This renders it difficult to disentangle the individual contribution of any one of these factors, including the effect of particular antiretroviral drug classes. This difficulty is also illustrated by the results of
previous studies examining C-IMT in patients with HIV-1 infection. Several \textsuperscript{22-24} but not all\textsuperscript{25} previous studies which included an HIV-uninfected control group have demonstrated the presence of HIV-1 infection to be associated with increased C-IMT. For example Hsue et al. reported higher C-IMT measurements in HIV-infected subjects compared to age-matched controls.\textsuperscript{22} Similar findings were reported by these same authors in a recent cross-sectional study, in which they also showed higher cytomegalovirus (CMV)-specific interferon-\(\gamma\) CD8 T-cell responses in HIV-infected compared to uninfected subjects, which were positively associated with greater C-IMT.\textsuperscript{26} This suggests that immune activation may be one of the additional factors contributing to atherosclerosis progression in HIV-1-infected subjects. In the current study the groups were comparable with respect to levels of CD4 and CD8 positive T-lymphocytes. Neither CD4 nor CD8 counts were significantly associated with C-IMT.

In addition, the relationship between measurements of C-IMT and other surrogate markers of atherosclerosis with regard to antiretroviral therapy, particularly protease inhibitors, have been less consistent. Whereas several studies, most of which have been cross-sectional, have reported treatment with PI to be associated with greater C-IMT or more evidence of coronary plaque formation\textsuperscript{23, 24, 27}, others have not\textsuperscript{22, 28, 30}. Of note, these studies have differed substan-

\begin{table}
\centering
\caption{Results of laboratory assessments}
\begin{tabular}{llll}
\hline
\textbf{Parameter} & \textbf{Protease inhibitors} & \textbf{Non-nucleoside reverse transcriptase inhibitors} & \textbf{P} \\
\hline
Total cholesterol, mmol/L & 5.5 (4.4-6.0) & 5.4 (4.8-6.2) & 0.65 \\
HDL cholesterol, mmol/L & 1.03 (0.90-1.25) & 1.39 (1.16-1.66) & <0.0001 \\
LDL cholesterol, mmol/L & 3.09 (2.37-3.71) & 3.13 (2.57-3.86) & 0.26 \\
Triglycerides, mmol/L & 2.1 (1.4-3.4) & 1.2 (0.9-1.7) & <0.0001 \\
Apolipoprotein A, mmol/L & 1.33 (1.16-1.45) & 1.44 (1.30-1.59) & 0.0008 \\
Apolipoprotein B, mmol/L & 1.13 (0.87-1.34) & 1.04 (0.85-1.24) & 0.25 \\
AST, IU/L & 31 (26-40) & 29 (25-39) & 0.18 \\
ALT, IU/L & 32 (22-44) & 29 (22-42) & 0.68 \\
hs-CRP, mg/L & 1.7 (0.8-4.3) & 2.9 (1.0-5.6) & 0.18 \\
Glucose, mmol/L & 4.6 (4.3-5.2) & 5.1 (4.7-5.4) & 0.0016 \\
Free T4, pmol/L & 14.0 (13.0-14.8) & 15.1 (13.0-17.6) & 0.35 \\
TSH, mE/L & 1.35 (0.96-1.90) & 1.20 (0.98-1.80) & 0.48 \\
CD4+ T-cell count, cells/mm\(^3\) & 510 (350-705) & 530 (410-680) & 0.28 \\
CD4+ T-cell count, % & 25 (19-30) & 27 (19-35) & 0.095 \\
CD8+ T-cell count, cells/mm\(^3\) & 890 (625-1410) & 910 (670-1390) & 0.80 \\
CD8+ T-cell count, % & 46 (40-53) & 44 (37-54) & 0.67 \\
CD4/CD8 ratio & 0.53 (0.37-0.70) & 0.61 (0.39-0.86) & 0.35 \\
\hline
\end{tabular}
\footnote{HDL denotes high-density lipoprotein, LDL low-density lipoprotein, AST aspartate aminotransferase, ALT alanine aminotransferase, hs-CRP high sensitivity C-reactive protein, and TSH thyroid stimulating hormone.}
\end{table}
Table 4: Results of univariate and multivariate linear regression analyses with C-IMT as the dependent variable

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Univariate Estimate (mm)</th>
<th>P</th>
<th>Multivariate Estimate (mm)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of PI</td>
<td>+0.10</td>
<td>0.0003</td>
<td>+0.10</td>
<td>0.0001</td>
</tr>
<tr>
<td>Use of ritonavir (any dose)</td>
<td>+0.072</td>
<td>0.012</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ritonavir dose (per 100 mg)</td>
<td>+0.006</td>
<td>0.37</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of ART use (per year)</td>
<td>+0.018</td>
<td>0.0003</td>
<td>+0.011</td>
<td>0.017</td>
</tr>
<tr>
<td>Pack years smoking (per 10 years)</td>
<td>+0.020</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol</td>
<td>+0.023</td>
<td>0.11</td>
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<tr>
<td>HDL-cholesterol</td>
<td>-0.074</td>
<td>0.026</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>+0.021</td>
<td>0.016</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apolipoprotein A1</td>
<td>-0.11</td>
<td>0.046</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>+0.15</td>
<td>0.0025</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP</td>
<td>+0.0025</td>
<td>0.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+0.0015</td>
<td>0.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td>+0.040</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse pressure (per 10 mmHg)</td>
<td>+0.016</td>
<td>0.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SBP (per 10 mmHg)</td>
<td>+0.023</td>
<td>0.022</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (per 10 mmHg)</td>
<td>+0.047</td>
<td>0.006</td>
<td>+0.025</td>
<td>0.097</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>+0.017</td>
<td>0.0005</td>
<td>+0.011</td>
<td>0.015</td>
</tr>
<tr>
<td>Age (per 10 years)</td>
<td>+0.069</td>
<td>&lt;0.0001</td>
<td>+0.050</td>
<td>0.0003</td>
</tr>
<tr>
<td>Framingham Risk Score (per percent increase)</td>
<td>+0.008</td>
<td>&lt;0.0001</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Footnote: ART denotes antiretroviral therapy, LDL low-density lipoprotein, HDL high-density lipoprotein, hs-CRP high sensitivity C-reactive protein, SBP systolic blood pressure, DBP diastolic blood pressure, NS = not significantly associated in the multivariate analyses, therefore not included in the final multivariate model.

Initially with respect to the selection of patients and in particular with respect to inclusion criteria concerning prior exposure to antiretroviral therapy. To the best of our knowledge, few, if any, of these studies have required patients to have been exposed to just PI- or NNRTI-containing therapy without allowing them to have switched between these two antiretroviral drug classes. Furthermore, none of these earlier studies required patients to have plasma HIV-1 RNA levels suppressed to below 50 copies per milliliter as was the case in our study, in order to minimize the potentially confounding effect of ongoing HIV-1 replication on atherogenesis. Our finding that PI-containing combination therapy is associated with greater C-IMT than NNRTI-containing therapy is consistent with the results as obtained by Maggi et al.27 Using an ultrasound color Doppler technique, they reported a higher prevalence of pathological C-IMT (defined as > 1mm) and/or plaques in 105 patients (53% with viral load below the limit of detection) treated for a median of 26 months with PI-based CART (52.4%) than in 125 PI-naïve patients (59% with viral load below the limit of detection) treated for a median of 24 months with NNRTI-based CART (15.2%).
Our study has several limitations in addition to its modest sample size. First, the lack of an HIV-negative control group and an HIV-positive untreated group renders it impossible to draw any conclusions from our study as to the contribution of HIV on atherogenesis. Nevertheless, as mentioned before, most studies to date strongly suggest the virus or the immune dysfunction associated with it to indeed be a relevant contributor. Second, given the cross-sectional nature of our study design, we cannot judge to which extent the use of PI- or NNRTI-containing treatments differ with respect to their effect on atherosclerosis progression. The results from a number of studies however, do provide circumstantial evidence that the rate of atherosclerosis progression may indeed be greater for patients exposed to PI. Thirdly, we have no information regarding the presence of insulin resistance in our patients, although we did record the presence or absence of diabetes mellitus and did perform fasting glucose levels. Finally, given that the number of female patients enrolled into our study was limited, we cannot determine whether our findings equally apply to both genders.

In conclusion, our results indicate that treatment of HIV-1-infected patients for two years or more with PI-based compared to NNRTI-based CART is associated with a relative C-IMT increase in the PI group. This is in accordance with the reported higher odds for CVD in patients using PI. However, we want to emphasize that no cause-effect relationship was established between PIs and C-IMT. Also, this difference was not fully explained by a more favorable impact of NNRTI-based CART on HDL-C and apo-A1 levels. These findings stress the impact of antiretroviral therapy on atherogenesis, but also suggest that traditional CVD risk factors in the HIV-infected patient population should be identified and properly managed.
Reference List


