Atherosclerosis is associated with multiple pathogenic mechanisms in HIV-infected antiretroviral-naive or treated individuals

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\textbf{Objectives:} HIV-infected patients have a greater burden of sub-clinical and clinical atherosclerotic disease compared to the general population. The primary objective of this study was to compare the relative roles of inflammation, endothelial alterations, and metabolic factors in the induction and maintenance of atherosclerosis in antiretroviral therapy (ART)-treated or ART-naive patients.

\textbf{Design:} Cross-sectional study; 79 HIV-infected patients (55 ART-treated and 24 naive individuals) were consecutively enrolled. In both groups, nearly 50% of the individuals had a high cardiovascular risk (Framingham value $>$20%).

\textbf{Methods:} Echo-Doppler \textit{(intima–media thickness (IMT))}, inflammatory, thrombophiliic, endothelial, metabolic indexes, and cholesterol efflux molecules were evaluated. Multivariate analysis adjusted for age, CD4 nadir, BMI, and Framingham’s score were used to analyze the results.

\textbf{Results:} A complex pathogenesis drives atherogenesis in HIV infection. Thus, whereas inflammation could be responsible for this process in ART-naive individuals, metabolic factors \textit{(low-density lipoprotein (LDL), apolipoprotein B (ApoB), lipoprotein A)} seem to play a more prevalent role in ART-treated patients. Notably, \textit{ABCA-1}, an ATP-binding transporter \textit{cassette protein} involved in cholesterol efflux, which is inhibited by Nef, is up-regulated in ART-treated individuals.

\textbf{Conclusion:} Atherosclerosis in HIV infection results from the interaction of multiple factors: an inflammatory and HIV-driven disease could prevail in the absence of therapy; metabolic, non-inflammatory causes may be more important in patients undergoing therapy. Approaches to atherosclerotic disease in HIV infection should consider these differences.

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\textbf{Keywords:} ABCA-1, ART, atherosclerosis, HIV, immunology

\textbf{Introduction}

Atherosclerosis is a multifactorial, chronic, and progressive alteration of arterial blood vessels characterized by the accumulation of monocytes/macrophages, smooth muscle cells, and lymphocytes in large and medium-sized arteries \cite{1,2}. The development of atherosclerotic lesions requires interactions between metabolic,
endothelial, and inflammatory components [3,4] within a complex scenario where different factors, including hypertension, smoking, diet, obesity, insulin resistance, and infections, induce endothelial cell activation [5]. Several evidences indicate that inflammation drives all phases of atherosclerosis [6,7]. Thus, inflammatory indexes, including C-reactive protein (CRP) [8], interleukin-6 (IL-6) [9], and tumor necrosis factor-α (TNF-α) [10], are consistently elevated in coronary atherosclerosis and are possible risk indexes for cardiovascular acute events.

Recent studies have demonstrated a link between infectious diseases and cardiovascular pathology; this is explained by the fact that Toll-like receptors (TLRs), which are molecules that recognize motifs expressed by pathogens, are involved in the pathogenesis of atherosclerosis (reviewed in [11]). In particular, the expression of TLR2 and TLR4 is augmented in human atherosclerosis plaques [11], and this was linked to the infiltration of inflammatory cells in the arterial wall, as well as with the progression of atherosclerotic disease [11]. Amongst the infectious agents correlated with cardiovascular disease (CVD), HIV plays an important role. Thus, HIV infection is correlated with alterations of plasma lipoprotein metabolism [12], and an increased risk of CVD and myocardial infarction (MI) is observed in HIV-infected individuals (reviewed in [13]). HIV may directly impair high-density lipoprotein (HDL) metabolism by enhancing the transfer of HDL to apolipoprotein B (ApoB), an atherogenic protein [13], and/or by reducing the expression of the ATP-binding protein (ABCA-1) transporter in macrophages [14], favoring foam cell transformation. Notably, HIV infection could also induce the development of atherosclerosis and contribute to increased CVD risk by a direct inflammatory effect. This possibility is suggested by the observation that augmented amounts of IL-6, TNF-α, adhesion molecules, lipopolysaccharide (LPS), and CRP are seen in HIV-infected patients [15,16]. Therapy of HIV disease does not seem to reduce the risk of either CVD or acute cardiovascular events, and is associated with a high incidence of vascular episodes among younger patients [17].

A possible relationship between antiretroviral therapy (ART) and CVD was reported by some but not all authors [17–20]. Cumulative exposure to protease inhibitors [17–20] and nuclear reverse transcriptase inhibitors (NRTIs) [21] was associated with CVD. This might be due to the ability of these molecules to induce metabolic impairments and mitochondrial dysfunctions [22,23]. Thus, both HIV per se and ART seem to contribute to atherogenesis during HIV infection; no data are nevertheless available comparing the relative roles of inflammatory, endothelial, and metabolic risk factors for atherosclerosis in ART-treated or in ART-naive patients. In the attempt to clarify this issue we analyzed these factors, as well as intracellular cholesterol transport, in a cross-sectional study performed in HIV-infected patients who were or were not undergoing therapy.

**Methods**

**Study population**

Seventy-nine HIV-infected male patients were longitudinally enrolled in a cross-sectional study and subdivided in two groups (ART-treated, n = 55; naive n = 24). Nearly 50% of patients in both groups were selected to have a Framingham risk score (FRS) above 20%, allowing the definition of different subpopulations as follows: high-risk ART-treated (n = 24); low-risk ART-treated (n = 31); high-risk naive (n = 12); low-risk naive (n = 12). Patients receiving statins were excluded from the study. Interviews aimed to collect the family history with a strong emphasis on CVDs, concomitant illnesses, substance abuse, smoking, diet, and alcohol consumption. Smokers were defined as individuals smoking at least 10 cigarettes/day during the previous 6 months. Biochemical and immuno-virologic parameters were collected for all patients. A complete physical examination was also performed in all individuals. Written informed consent was obtained and the study was conducted according to ‘Good Clinical Practice’ guidelines and the Declaration of Helsinki.

**Measurement of carotid intima–media thickness and plaque classification**

Measurements were performed according to the guidelines of the Mannheim Intima Media Thickness (IMT) Consensus using an ATL HDI-5000 ultrasound scanner (Philips, Monza, Italy) with a 7-MHz linear transducer. Longitudinal electrocardiography (ECG)-triggered images of the right and left common carotid arteries (CCAs) were obtained proximally at 1 and 2-cm points, with the patient in supine position, the head straight, and the neck extended. The same operator performed all the analyses and was blinded to the status (absence/presence of ART) of the patient. Measurements were performed on the posterior (far) vessel wall, and images were frozen on the isoelectric line to minimize variability during the cardiac cycle. For each patient, the mean cIMT (left + right/2) was measured to evaluate the wall thickness of the distal common carotid artery. Atherosclerotic plaques were classified according to the Modified American Heart Association Consensus Classification based on morphologic descriptions.

**Blood sample collection and peripheral blood mononuclear cell separation**

Blood was collected by venipuncture in EDTA-containing Vacutainer tubes (ethylenediaminetetraacetic acid) (Becton Dickinson, Rutherford, New Jersey, USA). Plasma was stored and peripheral blood mononuclear cells (PBMCs) were separated on lymphocyte separation
medium (Cedarlane Laboratories Limited, Hornby, Ontario, Canada).

**Stimulation of peripheral blood mononuclear cells**
Peripheral blood mononuclear cells were incubated for 18 h in the presence/absence of a pool of gag + env peptides (HIV) or cytomegalovirus protein (CMV) (Microbics Biosystems, Inc., Toronto, Ontario, Canada). 10 μg/ml of Brefeldin A (Sigma–Aldrich, St. Louis, Missouri, USA) was added to cell cultures during the last 6 h for cytokine analyses.

**Immunophenotypic analysis**
Cell subsets were evaluated using 50 μl of EDTA-treated peripheral blood incubated for 10 min at room temperature (RT) with fluorochrome-labeled mAbs [anti-CD62L, anti-CD11a, anti-HLA-DR, anti-CD4, anti-CD14 PE-Cy5 (Beckman Coulter, Fullerton, California, USA), anti-CD36 FITC, anti-CD44 PE (Serotec, Space Import srl, Milan, Italy), anti-TLR2 FITC, anti-CD49d, anti-TLR4 PE (eBioscience Inc., San Diego, California, USA)]. Erythrocytes were then lysed and cells were fixed using the Immuno-Prep EPICS Kit and Q-prep Work Station (Coulter Electronics, Miami Lakes, Florida, USA). Cytometry was performed on 200 000 events using an EPIC XL flow cytometer (Beckman–Coulter). Data were analyzed by first gating on the lymphocyte population as defined by forward and side light scatters. From this population a single-color CD4, CD8, or CD14 histogram was made, and T cells or monocytes were selected and inserted into a two-dimensional dot plot.

**Plasma tumor necrosis factor-α, monocyte chemotactrant protein-1, IL-6, soluble intercellular adhesion molecule 1, and soluble vascular cell adhesion molecule 1**
These molecules were analyzed using commercial ELISA kits (R&D Systems, Minneapolis, Minnesota, USA; Bender MedSystems, Vienna, Austria) following the manufacturer’s instructions. Plasma concentration of each protein was calculated in relation to standard curve.

**RNA extraction and reverse transcription**
RNA was extracted from PBMCs using the acid guanidium thiocyanate–phenol–chloroform method, dissolved in RNase-free water, and purified from genomic DNA with RNase-free DNase (RQ1 DNase, Promega, Madison, Wisconsin, USA). One microgram of RNA was reverse-transcribed into first-strand cDNA in a 20-μl final volume containing 1 μmol/l random hexanucleotide primers, 1 μmol/l oligo dT, and 200 U Moloney murine leukemia virus reverse transcriptase (Clontech, Palo Alto, California, USA).

**Lipoprotein signaling and cholesterol metabolism**
PCR arrays that included a set of optimized real-time PCR primers in 96-well plates were utilized (SA Biosciences Corporation, Frederick, Maryland, USA); a SYBR Green PCR mix (Finnzymes, Espoo, Finland) was used following the procedures suggested by the manufacturer. Controls for genomic DNA contamination, RNA quality, and general PCR performance were included in each array.

**Statistical analyses**
Student’s t test was performed to assess differences between means. When data were not distributed normally, Mann–Whitney U test was used. Categorical variables were analyzed using chi-square or Fisher’s exact tests as appropriate. A multivariate analysis of variance that included in the regression equation all variables was performed to verify univariate relationships with ART treatment status (BMI, CD4 nadir, and Framingham risk score >20). The general linear model was also used for non-normally distributed variables, after rank transformation. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed with the SAS/STAT, version 9.1 software (SAS Institute, Inc., Cary, North Carolina, USA).

## Results

**Study population**
Clinical and demographic characteristics of the patients are shown in Table 1; P values are from univariate analysis. Immunological and biochemical data were analyzed using FRS, BMI, and nadir CD4 as covariates in the multivariate model. All the results shown in this study derive from the multivariate analysis. A word of caution stems from the limited number of patients in each group: some differences (e.g. CVD history) might have reached statistical significance if more individuals would have been analyzed.

**Carotid intima media thickness and atherosclerotic plaques**
Echo Doppler analyses of the carotid IMT showed that IMT was consistently higher in ART-treated compared to ART-naïve HIV-infected individuals (P = 0.006) (Fig. 1a). These differences could be detected in multiple sites along the carotid axis. Additional analyses showed that carotid IMT was higher in ART-treated patients with a high cardiovascular risk score (CVS) compared to those with a low CVS and to low and high CVS-naïve patients (Fig. 1b). Fibro–calcific plaques were detected in three ART-treated and in two ART-naïve high-risk HIV-infected individuals.

**Thrombotic and metabolic markers of cardiovascular risk**
Thrombotic and metabolic parameters are reliable indexes of atherosclerotic plaques progression and were evaluated in all individuals (Table 2). Thrombotic
indexes, including fibrinogen ($P = 0.039$) and D-dimer ($P = 0.019$), were lower, whereas total cholesterol ($P = 0.015$), low-density lipoprotein (LDL)-cholesterol ($P = 0.016$), ApoB ($P = 0.01$), lipoprotein A ($P = 0.024$), and homocysteine ($P = 0.039$) were higher in ART-treated patients (Table 2). These results suggest that CVD-associated thrombotic and metabolic parameters are differently modulated by ART: therapy diminishes thrombogenesis probably by reducing viremia, whereas it increases metabolic factors linked with the atherosclerotic plaque pathogenesis.

Analysis of these parameters in patients divided according to high/low CVS showed different trends in ART-treated and ART-naive individuals. To summarize: total cholesterol, homocysteine, and ApoB levels were higher in

![Fig. 1. Mean (left + right/2) carotid intima–media thickness (IMT) measurements of the distal common carotid artery in HIV-infected antiretroviral (ART)-naive or ART-treated patients. In (b) patients are divided into four groups according to cardiovascular risk (Framingham’s score system). Boxes stretch from the 25th to the 75th percentile; lines across the boxes indicate median values; lines stretching from the boxes indicate extreme values. Outside values are displayed as separate points. Statistical significance is shown.](image-url)
Table 2. Inflammatory, thrombophilic, and metabolic parameters in ART-treated, ART-naive patients, and in ART-treated and naive patients divided into high and low risk based on Framingham’s scores greater than or less than 20.

<table>
<thead>
<tr>
<th></th>
<th>ART-treated patients (n = 55)</th>
<th>Naive patients (n = 21)</th>
<th>P values</th>
<th>High-risk ART-treated patients (n = 22)</th>
<th>Low-risk ART-treated patients (n = 23)</th>
<th>High-risk naive patients (n = 10)</th>
<th>Low-risk naive patients (n = 11)</th>
<th>P values</th>
</tr>
</thead>
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<tr>
<td>hsCRP (mg/l)</td>
<td>2.17 ± 0.37</td>
<td>5.39 ± 1.73</td>
<td>0.017</td>
<td>1.48 ± 0.27</td>
<td>2.80 ± 0.75</td>
<td>6.07 ± 2.25</td>
<td>4.77 ± 3.01</td>
<td>HR ART vs. HR naive = 0.024</td>
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<td></td>
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<td>HR ART vs. LR naive = 0.036</td>
<td>HR ART vs. LR ART = 0.025</td>
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<tr>
<td>Fibrinogen (mg/dl)</td>
<td>287.91 ± 8.42</td>
<td>323.25 ± 15.44</td>
<td>0.039</td>
<td>301.86 ± 14.33</td>
<td>273.95 ± 11.93</td>
<td>334.00 ± 23.36</td>
<td>312.50 ± 24.32</td>
<td>NS</td>
</tr>
<tr>
<td>D-dimer (ng/dl)</td>
<td>88.60 ± 21.28</td>
<td>98.33 ± 14.45</td>
<td>0.019</td>
<td>131.27 ± 47.13</td>
<td>45.87 ± 5.03</td>
<td>142.30 ± 22.89</td>
<td>58.36 ± 11.23</td>
<td>HR ART vs. LR ART = 0.025</td>
</tr>
<tr>
<td>Homocysteine (mmol/l)</td>
<td>15.66 ± 0.69</td>
<td>11.81 ± 0.54</td>
<td>0.039</td>
<td>15.29 ± 1.24</td>
<td>14.06 ± 0.98</td>
<td>12.36 ± 0.64</td>
<td>11.31 ± 0.95</td>
<td>HR ART vs. LR naive = 0.027</td>
</tr>
<tr>
<td>Glycemia (mg/dl)</td>
<td>101.62 ± 3.18</td>
<td>105.09 ± 9.77</td>
<td>NS</td>
<td>108.59 ± 6.24</td>
<td>94.96 ± 3.12</td>
<td>120.80 ± 21.25</td>
<td>90.82 ± 2.57</td>
<td>NS</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>212.09 ± 4.64</td>
<td>172.33 ± 6.54</td>
<td>0.015</td>
<td>213.23 ± 6.76</td>
<td>205.13 ± 7.93</td>
<td>172.80 ± 10.55</td>
<td>171.91 ± 9.92</td>
<td>HR ART vs. HR naive = 0.002</td>
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<td>HR ART vs. LR naive &lt; 0.001</td>
<td>HR ART vs. LR naive = 0.005</td>
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<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>45.29 ± 2.21</td>
<td>35.00 ± 2.36</td>
<td>0.0013</td>
<td>46.86 ± 4.47</td>
<td>43.78 ± 2.36</td>
<td>31.20 ± 2.18</td>
<td>38.45 ± 4.24</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dl)</td>
<td>129.64 ± 5.14</td>
<td>111.86 ± 8.57</td>
<td>0.016</td>
<td>136.59 ± 8.49</td>
<td>122.68 ± 7.73</td>
<td>125.20 ± 16.92</td>
<td>99.77 ± 7.60</td>
<td>HR ART vs. LR naive = 0.029</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>166.15 ± 9.12</td>
<td>160.86 ± 14.83</td>
<td>NS</td>
<td>180.27 ± 17.63</td>
<td>152.65 ± 10.40</td>
<td>176.90 ± 21.21</td>
<td>146.27 ± 23.53</td>
<td>HR ART vs. LR naive = 0.025</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.98 ± 0.03</td>
<td>0.82 ± 0.04</td>
<td>0.010</td>
<td>0.99 ± 0.06</td>
<td>0.92 ± 0.05</td>
<td>0.91 ± 0.05</td>
<td>0.74 ± 0.06</td>
<td>HR ART vs. LR naive &lt; 0.001</td>
</tr>
<tr>
<td>Lipoprotein A (g/l)</td>
<td>0.24 ± 0.03</td>
<td>0.14 ± 0.04</td>
<td>0.024</td>
<td>0.25 ± 0.06</td>
<td>0.22 ± 0.05</td>
<td>0.13 ± 0.04</td>
<td>0.15 ± 0.08</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.87 ± 0.02</td>
<td>0.97 ± 0.09</td>
<td>NS</td>
<td>0.81 ± 0.03</td>
<td>0.93 ± 0.04</td>
<td>1.12 ± 0.21</td>
<td>0.85 ± 0.05</td>
<td>HR ART vs. LR ART = 0.019</td>
</tr>
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</table>

Mean values, SE, and statistical significance are shown. ART, antiretroviral therapy; HDL, high-density lipoprotein; HR, high risk; hsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LR, low risk.
ART-treated patients regardless of CVS; and D-dimer was higher in patients with high CVS irrespective of treatment (Table 2). Thus, ART is associated with alterations in CVD-relevant metabolic parameters independently of CVS; CVS, on the contrary, is linked to changes in trombophilic markers.

**Inflammatory parameters and pro-inflammatory cytokines**

High-sensitivity CRP (hsCRP), IL-6, and TNF-α plasma concentrations were significantly lower in ART-treated individuals (Fig. 2a and Table 2) independently of the CVS (Fig. 2b and Table 2). Notably, the observation that concentration of hsCRP was higher in ART-naive individuals regardless of the CVS supports a crucial role of HIV per se in feeding inflammation.

Additional results showed that IFN-γ production by HIV antigens-stimulated CD4⁺ T cells was reduced ($P = 0.044$) in ART-treated patients, whereas a trend that did not reach significance was observed for TNF-α. Finally, IL-6- and IL-12 production by HIV-stimulated CD14⁺ cells was significantly reduced as well in ART-treated individuals (Fig. 2c). No differences were noticed upon stratification of patients on the basis of CVS. These data confirm that ART is efficacious in reducing the synthesis of pro-inflammatory cytokines.

**Soluble adhesion molecules**

Soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) plasma levels were significantly lower in ART-treated patients (Fig. 3a); the concentration of these molecules was higher in both high and low CVS risk, ART-naive groups compared to their ART-treated counterparts (Fig. 3b).

**Adhesion molecule on CD4⁺ T cells**

Adhesion molecules expressing CD4⁺ T cells were significantly higher in ART-treated patients (Fig. 3c) irrespective of the CVS.

**TLR4 expression on monocytes**

TLR4 are molecules expressed on a multitude of cells that initiate innate immune responses by recognizing common motifs expressed by bacterial pathogens; TLRs were

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![Figure 2](image.png)

**Fig. 2. Pro-inflammatory cytokines in HIV-infected antiretroviral (ART)-naive or ART-treated patients.** In (b) patients are divided into four groups according to cardiovascular risk score (CVS) (Framingham’s score system). (c) Shows HIV-stimulated cytokine-producing cells by PBMC of the same individuals. Boxes stretch from the 25th to the 75th percentile; lines across the boxes indicate median values; lines stretching from the boxes indicate extreme values. Outside values are displayed as separate points. Statistical significance is shown. PBMC, peripheral blood mononuclear cell.
suggested to play a crucial role in the pathogenesis of atherosclerosis. The percentage of TLR4-expressing CD14\(^+\) monocytes was significantly lower in ART-treated individuals (data not shown) \((P < 0.001)\) regardless of the CVS. This indicates a positive effect of therapy in decreasing LPS-driven TLR-dependent inflammation, as reported by other authors.

Lipoprotein signaling and cholesterol metabolism mRNA expression in peripheral blood mononuclear cells

HIV induces foam cell transformation \textit{in vitro} as a consequence of the inhibitory effect of the viral protein Nef on ABCA-1, an ATP-binding transporter cassette protein involved in cholesterol efflux and in HDL production. Data obtained in a real-time PCR array screening for the expression of 84 genes directly involved in cholesterol metabolism showed that ABCA-1 was the only gene whose expression was significantly up-regulated in ART-treated patients. Notably, the expression of 18 other genes (AKR1D1, ANGPTL3, APOB, APOL5, COLEC12, CYP11A1, CYP39A1, CYP7A1, CYP7B1, HMGCS2, IL4, LEP, LRPIB, NPC1L1, NR1I4, PRKAA2, STAB2, VLDLR) was reduced in the same patients (data not shown).

Correlations

Significant positive correlations between IL-6 and sICAM-1 and IMT were observed in ART-naive patients (IL-6: \(P = 0.043\), sICAM-1: \(P = 0.006\)), whereas a positive correlation was detected between ApoB and IMT in ART-treated individuals \((P < 0.05 in both cases)\). These results suggest a prevalent inflammatory pathogenesis of atherosclerosis in ART-naive, HIV-infected patients, whereas metabolic factors could be dominant in ART-treated individuals.

Discussion

Results herein indicate that HIV \textit{per se} and ART play crucial roles in the pathogenesis of atherosclerotic disease in HIV infection. Several factors, including...
immunosuppression, inflammation, the intrinsic ability of HIV to induce foam cell transformation, cumulative exposure to antiretroviral drugs, and mitochondrial and metabolic dysfunctions [12–16,22,23] were hypothesized to be involved in HIV disease-associated atherosclerosis. The relative role of these factors in the two distinct settings of patients with or without therapy has nevertheless not been assessed. Our results suggest that different atherogenic processes overlap in HIV infection. Thus, even if longitudinal data will be needed to support this hypothesis, whereas inflammation and active HIV replication could drive atherosclerosis in ART-naïve individuals, metabolic alterations might be prevalent in ART-treated patients.

Modifications of the thickness of the intima—media of arterial vessels are the result of the initial damage of such vessels, allowing the penetration of inflammatory cells within the arterial wall; the progressive intima—media thickening is associated with an increased likelihood of CVD, and acute cardiovascular events. Carotid IMT was significantly worse in ART-treated compared to naive patients; this difference was more evident when patients were subdivided on the basis of cardiovascular risk, as the highest values were observed in ART-treated patients. Inflammation, the suggested main culprit for intima–media alterations, is mostly driven by viral replication in HIV disease. Our data indicate that an inflammatory condition is present in HIV disease regardless of cardiovascular risk factors, suggesting a central role for HIV in driving the inflammation involved in atherosclerosis. Plasma concentrations of hsCRP, IL–6, TNF–α, D-dimer, fibrinogen, sICAM, and sVCAM, as well as indexes of endothelial activation, were higher in ART-naïve individuals, confirming earlier results [12–16, 22,23]. Therapy was associated with a reduced HIV viremia and a down-regulation of inflammation, of the prothrombotic state, and of endothelial parameters, suggesting the existence of a link between these pathways and HIV replication. It is nevertheless important to underline that it has been convincingly shown that a residual inflammatory condition persists in patients with an optimal response to therapy, and can even be observed in elite controllers [24–27].

Antiretroviral therapy-associated suppression of viral replication was associated with the reduction of all inflammatory indexes. In particular, the generation of pro-inflammatory cytokines by HIV-stimulated CD4⁺ T cells was reduced in ART-treated patients; of note, the levels of TNF–α were clearly related to the naive status, and not to the CVS. Cytokine-producing lymphocytes are present in the atherosclerotic plaques of HIV-infected individuals [28]; a down-modulation of inflammatory atherosclerotic process would also be expected as a consequence of the lower expression of TLR4 seen in ART-treated individuals, as this receptor plays an important role in the progression of disease [11].

It is important to underline that not all atherosclerosis-associated parameters were better in HIV-infected individuals undergoing ART; thus, ApoB, lipoprotein A, and LDL levels were significantly higher in treated compared to naive patients. These results indicate that metabolic factors might be important in driving the pathogenesis of atherosclerosis in HIV-infected individuals who undergo ART. Plasma HDL concentration, an index of cholesterol efflux and cholesterol reverse transport, was higher during ART as well, suggesting a possible beneficial effect of therapy on foam cell transformation. Recent results showed that the activity of ABCA-1, a key protein in the modulation of cholesterol efflux and HDL synthesis, is inhibited by the viral protein nef. Data herein show that ABCA-1 and HDL are up-regulated in ART-treated patients. ART, reducing viral replication, could diminish nef-mediated ABCA-1 inhibition; the net effect would be an improvement in cholesterol transport that, together with ART-associated reduction of inflammatory parameters, could quench atherosclerosis [29].

Results showing that alterations in metabolic parameters and homocysteine levels are more severe in ART-treated patients could allow the speculation that atherosclerotic progression seen during therapy is a metabolic rather than an inflammation-driven process. Homocysteine concentration, in particular, was significantly higher in ART-treated patients; this observation may be secondary to the calcification of endothelial tissue shown to be a part of the atherosclerotic process of HIV infection [30]. Thus, older and more vulnerable atherosclerotic plaques that undergo calcification are present in ART-treated patients. Notably, adhesion of lymphocytes expressing particular proteins including CD11a, CD44, and CD49d favors the infiltration of these cells in the plaques, leading to potentially disastrous plaque ruptures in response to an occasional inflammatory insult. Adhesion molecule-expressing CD4⁺ T lymphocytes were higher in ART-treated patients, indicating an increased risk for atherosclerosis complications in these individuals. These observations could justify the increased risk of CVD seen in ART-treated patients.

In conclusion, virus replication has a strong impact on inflammation, thrombophilic molecules, and foam cell transformation. ART suppresses viral replication and could reduce these atherogenic factors, but lipogenic alterations might worsen once therapy is initiated, resulting in the maintenance of the atherosclerotic process. Our study has some important limitations: in particular, due to the exploratory nature of the analysis, we did not apply any adjustment for multiple comparisons. A word of caution also stems from the cross-longitudinal design of the study: our initial findings suggest a hypothesis that will need to be tested on a larger, prospective cohort, as only longitudinal analyses will shed light on this issue. Data herein reinforce the importance...
of tailoring antiviral treatment in HIV disease, using antiretroviral drugs having the lowest lipogenic effect.

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Author contributions: S. Piconi, D.T. and M.C. conceived the initial concept for the study, analyzed results, and wrote the paper.

S. Parisotto, M.B. and M.S. performed immunological and endocrinological analyses.

S. Piconi, G.R., S. Passerini, P.M., E.N. and P.B. enrolled the patients and collected biological samples.

E.D.R. performed statistical analyses.

First draft of the manuscript with critical review by all authors. All authors participated in the development of the study protocol and analysis plan, reviewed the study data/analysis reports, and approved the final manuscript.

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Conflicts of interest

There are no conflicts of interest.

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