**Author's response to reviews**

**Title:** Risk factors for subclinical atherosclerosis in HIV-infected patients under and over 40 years: a case-control study

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**Version:** 2  **Date:** 19 January 2013

**Author's response to reviews:** see over
COVER LETTER

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Risk factors for subclinical atherosclerosis in HIV-infected patients under and over 40 years: a case-control study

Valéria MG Albuquerque, Josefina C Zírpoli, Demócrito B Miranda Filho, Maria de Fátima PM Albuquerque, Ulisses R Montarroyos, Ricardo AA Ximenes and Heloísa R Lacerda

January 19, 2013.

Dear Mr Vincent Lo Re

First of all, thank you for the opportunity to improve our study. To attend the reviewers’ requirements we have reanalised our data and revised the manuscript. We are now 1) sending this Cover Letter answering your demands and those of the reviewers, 2) sending a revised Manuscript, where the modifications have been inserted and 3) Uploading the Abstract (Title and Authors have not been modified).

Please do not hesitate should you require any additional information.

Kind regards.

Prof Heloísa Ramos Lacerda.

helramos@terra.com.br

Reviewer's report
Title: Risk factors for subclinical atherosclerosis in HIV-infected patients under and over 40 years: a case-control study

Version: 1 Date: 27 September 2012
Reviewer: Dana Byrne

Reviewer's report:
Major Compulsory Revisions:
1. I can't find table 1/figure 1, which I assume was the demographic differences between cases and controls. I was interested in seeing what percentage of the older adults were on what HAART regimen. The supplementary material starts at Table 2, but I'm not sure where 1 went.
ANSWER – Table 1 contains details of the two groups of patients: the group over 40 years and the group under 40 years, including patients on HAART and which regimen of treatment they were using. It has been included in the PDF of the main document.

Minor Essential Revisions:
1. Abstract line 4: change "compare de" to compare the

ANSWER – The phrase has been modified in the text.

2. Background line 7: would change the "on the one hand" phrase (take it out)-
sounds too casual

ANSWER – The phrase has been modified in the text.

3. Background Line 11: Similarly, would take out the reference to the "other hand"

ANSWER - The phrase has been modified in the text.

4. Is there a reference for Background line 20-21 "more recent data..."

ANSWER – The following reference has been inserted:


5. Page 5, line 7-8: Would put the reference at the end of the sentence and add
"and as a predictor..."

ANSWER – Thank you for the suggestion. The phrase has been modified accordingly in the text.

6. Would consider putting the table provided in the supplementary materials that would visually display the results described at the bottom of the "Characteristics of the study participants according to age group" into the paper (I assume it will be integrated and not just supplementary in the print article, but I'm not sure). Specifically I was interested in how the authors stratified by type of HAART (which is in the supplementary material).

ANSWER – This material (tables) should be inserted into the main text. We simply followed the orientation of the editors, who request that, when there is more than one table, the others should be placed in the Supplementary Material. It is our intention that they be an integral part of the article to be published.

7. Discussion Line 4 Page 12: Would change the first sentence to clarify it.
Maybe change the "latter group" to the "<40 group"

ANSWER – Thank you for the suggestion, which we have incorporated.

Discretionary Revisions:
1. Abstract Line 7: would change "in use of their first antiretroviral regimen" to something like "or individuals who remain on their first ARV regimen"
ANSWER - Thank you for the suggestion. The phrase has been modified in the text.

2. Abstract line 21: Would explain consensual union- I assume that is like same sax marriage in the US, but I am unfamiliar with the term. Is it the same?

ANSWER – “consensual union” refers to a couple that live together without being legally married. We have changed the expression to “stable partner”.

3. Page 5, line 11-13. I think the second objective could be stated a little more clearly. Reading that part of the sentence alone doesn't tell me what the second objective is (but it should)

ANSWER - Thank you for the suggestion. We have modified the text as follows:

…and secondly, to evaluate the association of the traditional risk factors for cardiovascular disease, as well as those related to HIV infection and antiretroviral therapy, with subclinical atherosclerosis in the two groups.

4. Please fix the spacing issues about halfway down page 15.

ANSWER – This has been done.

REVIEWER 2

Major Compulsory Revisions

1. The essential question is whether age interacts with atherosclerosis risk factors among HIV infected people. However, no clinically or epidemiologically valid reason is presented for the choice of 40 years as a threshold. The authors suggest that HIV prevalence among people 20-39 years in Brazil is 60%, which appears to be the rationale for the age threshold. Since they have not suggested that their study population is a representative sample of the national population, it is unclear this supports the use of the 40 years threshold. It may be more epidemiologically sound to use an internal reference e.g. above and below median age or age tertiles if the sample size permits. Alternatively, instead of splitting the sample, they could examine interaction models with age and each comorbidity. This may be statistically more efficient though possibly more involved to interpret.

ANSWER - We have chosen the cut-off point of 40 y in our study for two reasons: 1) in the exploratory analysis of our data we found that the chance of atherosclerosis was more than 10 times higher in the 40-49 y age group than in the younger group, and that this chance was even higher in people aged 50 y or over (see the table 1, below). We thought that, although we could have adjusted our analysis by age, it would be more informative to split the sample in these two groups that might have important differences between them. We thus decided to separate our population into two groups and analyze the factors related to atherosclerosis in each of them. Our findings are very interesting, as you can see. We agree that one alternative would be to test for the presence of interaction between age and each comorbidity. However this approach would not be easily understood by readers without a good epidemiological or statistical background. 2) Another point reinforced our decision, namely the 40 y cut-off divided our population down the middle (median age of the population was 40 y). This rationale has been also included in the revised version of the text.
Table 1 – Subclinical atherosclerosis in the different age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Subclinical atherosclerosis</th>
<th>OR (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence</td>
<td>Absence</td>
<td></td>
</tr>
<tr>
<td>&lt; 30 y</td>
<td>13</td>
<td>12.1</td>
<td>94</td>
</tr>
<tr>
<td>30 to 39 y</td>
<td>69</td>
<td>28.3</td>
<td>175</td>
</tr>
<tr>
<td>40 to 49 y</td>
<td>133</td>
<td>58.6</td>
<td>94</td>
</tr>
<tr>
<td>≥ 50 y</td>
<td>95</td>
<td>81.9</td>
<td>21</td>
</tr>
</tbody>
</table>

2. The outcome variable was carotid intima-media thickness (IMT). Again, the authors do not provide any justification for the choice of 0.8 mm as a threshold.

**ANSWER** - We chose a cut-off of 0.8 mm for evaluation of carotid atherosclerosis similar to Jerico et al. (See reference below). This choice was based on the fact that the measurement of the common carotid MIT in a normal population of young adults, produces values lower than 0.8 mm, with an increase from 0.01 to 0.02 mm per year (BOND, 1989; SALONEN, 1993; LIM, 2009). Additionally, individuals with carotid MIT lower than 0.8 mm have less risk of cardiovascular and cerebrovascular events (<4.6%) in six years. Given that the median age of the study population was 40 years, that seemed to be a rational choice.


3. A fascinating finding in this paper is a differential relationship between HIV-related and non-HIV related factors and cIMT by age group. The authors suggest several reasons for this observation including a) no association between antiretroviral therapy (ART) and cIMT and b) mediation of HIV-related effects by non-HIV related effects. They neglect to discuss that almost twice as many people <40yrs were not using HAART vs. >40 years, and that there was more immunocompetence and less viremia in the younger group despite longer HAART duration in the older group. An important analysis would compare cIMT values (medians, means) separately by ART use, viremia, or immune status for different age groups.

**ANSWER** – We have compared IMT separately by ART use, viremia and immune status for the different age groups, as can be seen below in table 2. We decided to insert them in the revised paper.
Table 2 - Values of the carotid intima media thickness (IMT) of the patients < 40 years and 40 years or over.

<table>
<thead>
<tr>
<th>Variables</th>
<th>&lt; 40 y group</th>
<th>≥ 40 y group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carotid MIT</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>Median ($P_{25}$; $P_{75}$)</td>
<td></td>
</tr>
<tr>
<td>HAART use</td>
<td>p = 0.069</td>
<td>p = 0.166</td>
</tr>
<tr>
<td>Yes</td>
<td>0.735 (0.691; 0.792)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>0.722 (0.677; 0.780)</td>
<td></td>
</tr>
<tr>
<td>CD4 lymphocytes (cells/mm³)</td>
<td>p = 0.373</td>
<td>p = 0.011</td>
</tr>
<tr>
<td>&lt;200</td>
<td>0.720 (0.685; 0.782)</td>
<td></td>
</tr>
<tr>
<td>200 to 350</td>
<td>0.728 (0.681; 0.792)</td>
<td></td>
</tr>
<tr>
<td>≥ 350</td>
<td>0.731 (0.693; 0.779)</td>
<td></td>
</tr>
<tr>
<td>Viral load (copies/mL)</td>
<td>p = 0.269</td>
<td>p = 0.616</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>0.745 (0.677; 0.822)</td>
<td></td>
</tr>
<tr>
<td>50 to 10,000</td>
<td>0.742 (0.705; 0.795)</td>
<td></td>
</tr>
<tr>
<td>10,000 to 100,000</td>
<td>0.723 (0.681; 0.769)</td>
<td></td>
</tr>
<tr>
<td>≥ 100,000</td>
<td>0.690 (0.682; 0.741)</td>
<td></td>
</tr>
<tr>
<td>Types HAART regimens</td>
<td>p = 0.109</td>
<td>p = 0.132</td>
</tr>
<tr>
<td>No HAART use</td>
<td>0.722 (0.677; 0.780)</td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>0.733 (0.690; 0.767)</td>
<td></td>
</tr>
<tr>
<td>NNRTI</td>
<td>0.729 (0.696; 0.784)</td>
<td></td>
</tr>
<tr>
<td>Duration of use of HAART</td>
<td>p = 0.015</td>
<td>p = 0.070</td>
</tr>
<tr>
<td>No use</td>
<td>0.722 (0.677; 0.780)</td>
<td></td>
</tr>
<tr>
<td>&lt; 5 years</td>
<td>0.730 (0.690; 0.790)</td>
<td></td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>0.757 (0.730; 0.815)</td>
<td></td>
</tr>
</tbody>
</table>
Another important sub-analysis could exclude those with traditional cardiovascular disease risk factors and to see if an HIV-related effect is unmasked.

ANSWER – We performed two new multivariate analyses: the first one (Model A) is presented in Table 3. In this model we excluded the individuals with the major traditional risk factors that were significant in the “entire group” final model such as obesity, metabolic syndrome and hypercholesterolemia, resulting in the exclusion of 333 patients: a) metabolic syndrome (166 patients); obese individuals (20 patients); hypertensives (77 individuals); patients with total cholesterol of 240 mg/dL or over (24 individuals) and smokers (46 patients). The second analysis comprised the 333 patients with at least one of the traditional risk factors for cardiovascular disease that were excluded in the previous analysis (Model B). The findings were very interesting so we decided to include them in the revised paper.

Table 3 - Multivariate model of factors related to the presence of subclinical atherosclerosis among 361 individuals without the main risk factors for cardiovascular disease (CVD) (excluding a total of 333 patients = 166 patients with metabolic syndrome, 20 obese patients, 77 hypertensive patients, 24 individuals with total cholesterol ≥ 240 mg/dL and 46 smokers) (Model A) and among 333 patients with risk factors for cardiovascular diseases (Model B).

<table>
<thead>
<tr>
<th>Variables</th>
<th>&lt; 40 years old (n=218)</th>
<th>≥ 40 years old (n=143)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR(CI)</td>
<td>p-value</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td>1.09 (1.00 – 1.19)</td>
<td>0.037</td>
</tr>
<tr>
<td>Male sex</td>
<td>3.02 (1.20 – 7.59)</td>
<td>0.018</td>
</tr>
<tr>
<td>Nonwhite race</td>
<td>3.21 (0.90 – 11.4)</td>
<td>0.071</td>
</tr>
<tr>
<td>Stable partner</td>
<td>1.62 (0.64 – 4.08)</td>
<td>0.306</td>
</tr>
<tr>
<td>TCD4 lymphocytes &lt;200</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>200 to &lt; 350 cels/mm³</td>
<td>1.07 (0.35 – 3.23)</td>
<td>0.902</td>
</tr>
<tr>
<td>≥ 350 cels/mm³</td>
<td>0.49 (0.12 – 1.98)</td>
<td>0.321</td>
</tr>
<tr>
<td>Undetectable Viral Load</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>50 to &lt; 10,000</td>
<td>0.96 (0.24 – 3.78)</td>
<td>0.954</td>
</tr>
<tr>
<td>10,000 to &lt; 100,000</td>
<td>0.47 (0.07 – 2.93)</td>
<td>0.425</td>
</tr>
<tr>
<td>≥ 100,000</td>
<td>0.33 (0.02 – 4.08)</td>
<td>0.394</td>
</tr>
<tr>
<td>HAART duration of use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART use</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>IP &lt; 5 years</td>
<td>0.51 (0.15 – 1.75)</td>
<td>0.290</td>
</tr>
<tr>
<td>Variables</td>
<td>&lt; 40 years old</td>
<td>&gt; 40 years old</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td>(n=133)</td>
<td>(n=200)</td>
</tr>
<tr>
<td>Age (continuous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.21 (1.08 – 1.36)</td>
<td>1.12 (1.04 – 1.21)</td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.59 (0.64 – 3.95)</td>
<td>2.53 (1.16 – 5.51)</td>
</tr>
<tr>
<td>Nonwhite race</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.59 (0.96 – 6.99)</td>
<td>1.28 (0.55 – 2.99)</td>
</tr>
<tr>
<td>Stable partner</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.14 (1.13 – 8.72)</td>
<td>2.04 (0.78 – 5.36)</td>
</tr>
<tr>
<td>TCD4 lymphocytes &lt;200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>200 to &lt; 350 cels/mm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.51 (0.07 – 3.38)</td>
<td>2.52 (0.85 – 7.48)</td>
</tr>
<tr>
<td>≥ 350 cels/mm³</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.82 (0.11 – 5.79)</td>
<td>10.8 (2.89 – 40.4)</td>
</tr>
<tr>
<td>Undetectable Viral Load</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>50 to &lt; 10,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.48 (0.09 – 2.40)</td>
<td>0.54 (0.15 – 1.98)</td>
</tr>
<tr>
<td>10,000 to &lt; 100,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.83 (0.11 – 6.28)</td>
<td>1.46 (0.23 – 9.11)</td>
</tr>
<tr>
<td>≥ 100,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.27 (0.00 –12.77)</td>
<td>0.91 (0.10 - 7.80)</td>
</tr>
<tr>
<td>HAART duration of use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART use</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>IP ≥ 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.75 (0.19 – 2.87)</td>
<td>1.72 (0.60 – 4.89)</td>
</tr>
<tr>
<td>IP ≥ 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.87 (0.05-15.65)</td>
<td>-</td>
</tr>
<tr>
<td>NNRTI &lt; 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.84 (0.92-8.68)</td>
<td>0.067</td>
</tr>
<tr>
<td>NNRTI ≥ 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.24 (0.02-2.53)</td>
<td>2.04 (0.63 - 6.58)</td>
</tr>
</tbody>
</table>

4. I am not familiar with this model selection procedure. Is this a variation of stepwise regression with backward and forward elimination? If so, what criteria were used to compare model fit? If not, has this method previously been described?
ANSWER – The procedure used in producing the final model of multivariate analysis was the forward one, starting with the hierarchical introduction of the variables with the highest “p” value, followed by the variables with the lowest “p” value. We decided to reformulate the analysis and maintain all the variables in the final model (as you have suggested). This was also done by the forward procedure. The results were very similar, except for the finding that > 40y group, obesity and hypertension presented an association with the presence of subclinical atherosclerosis, but nullified each other when introduced into the multivariate model. Thus hypertension was withdrawn from the final model, since obesity is related to the genesis of hypertension (see table 4 and 5, below). We have replaced the previous analysis by this new one (Table 5). As I mentioned above, we have also inserted the two other analysis of persons with metabolic risk factors for CVD and without them (Models A and B).

Table 4 - Multivariate model of the association between subclinical atherosclerosis with sociodemographic variables, habits, family history, clinical parameters, lipid profile, fasting glucose and features related to HIV by age group. (Model that contained overweight and hypertension)

<table>
<thead>
<tr>
<th>Variables</th>
<th>&lt; 40 years old</th>
<th>&gt; 40 years old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (continuous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonwhite</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI overweight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metabolic Syndrome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 to &lt; 240 mg/dL</td>
<td>0.81 (0.32 – 2.03) 0.649</td>
<td>1.19 (0.62 – 2.28) 0.596</td>
</tr>
<tr>
<td>≥ 240 mg/dL</td>
<td>3.39 (0.95 – 12.1) 0.060</td>
<td>2.88 (0.87 – 9.55) 0.083</td>
</tr>
<tr>
<td>Stable partner</td>
<td>2.54 (1.22 – 5.31) 0.013</td>
<td>2.44 (1.20 – 4.98) 0.014</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TCD4 &lt; 200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 to &lt; 350 cels/mm³</td>
<td>0.79 (0.31 – 2.03) 0.624</td>
<td>1.13 (0.56 – 2.26) 0.739</td>
</tr>
<tr>
<td>≥ 350 cels/mm³</td>
<td>0.66 (0.23 – 1.92) 0.447</td>
<td>2.90 (1.25 – 6.69) 0.013</td>
</tr>
<tr>
<td>HAART duration of use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Without HAART</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 5 years</td>
<td>1.01 (0.51 – 2.02) 0.960</td>
<td>0.87 (0.47-1.63) 0.672</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>1.75 (0.52 – 5.89) 0.368</td>
<td>2.12 (0.88-5.13) 0.095</td>
</tr>
</tbody>
</table>

Table 5 - Multivariate model of the association between subclinical atherosclerosis with sociodemographic variables, habits, family history, clinical parameters, lipid profile, fasting glucose and features related to HIV by age group.(Model that maintained only obesity and hypertension was excluded, which was the Model chosen to be inserted in the final paper)
### Table 1: Risk Factors for Increased cIMT

<table>
<thead>
<tr>
<th>Covariate</th>
<th>OR(95% CI)</th>
<th>p-value</th>
<th>OR(95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (continuous)</strong></td>
<td>1.12 (1.05 – 1.20)</td>
<td>0.001</td>
<td>1.11 (1.05 – 1.17)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Male sex</strong></td>
<td>2.77 (1.43 – 5.34)</td>
<td>0.002</td>
<td>1.62 (0.94 – 2.81)</td>
<td>0.080</td>
</tr>
<tr>
<td><strong>Nonwhite</strong></td>
<td>3.01 (1.23 – 6.53)</td>
<td>0.007</td>
<td>1.37 (0.73 – 2.57)</td>
<td>0.321</td>
</tr>
<tr>
<td><strong>BMI overweight</strong></td>
<td>1.65 (0.79 – 3.44)</td>
<td>0.344</td>
<td>1.93 (1.04 – 3.57)</td>
<td>0.036</td>
</tr>
<tr>
<td><strong>Obese</strong></td>
<td>5.13 (1.79 – 14.7)</td>
<td>0.002</td>
<td>2.53 (0.85 – 7.54)</td>
<td>0.095</td>
</tr>
<tr>
<td><strong>Metabolic Syndrome</strong></td>
<td>3.30 (1.44 – 7.58)</td>
<td>0.005</td>
<td>1.01 (0.54 – 1.88)</td>
<td>0.968</td>
</tr>
<tr>
<td><strong>Total cholesterol &lt;200</strong></td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 to &lt; 240 mg/dL</td>
<td>0.83 (0.32 – 2.03)</td>
<td>0.701</td>
<td>1.24 (0.66 – 2.32)</td>
</tr>
<tr>
<td></td>
<td>≥ 240 mg/dL</td>
<td>2.28 (0.83 – 9.40)</td>
<td>0.095</td>
<td>2.14 (0.76 – 5.98)</td>
</tr>
<tr>
<td><strong>Stable partner</strong></td>
<td>2.07 (1.03 – 4.19)</td>
<td>0.041</td>
<td>2.41 (1.24 – 4.67)</td>
<td>0.009</td>
</tr>
<tr>
<td><strong>TCD4 lymphocytes &lt;200</strong></td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 to &lt; 350 cels/mm³</td>
<td>0.86 (0.33 – 2.24)</td>
<td>0.758</td>
<td>1.18 (0.59 – 2.37)</td>
</tr>
<tr>
<td></td>
<td>≥ 350 cels/mm³</td>
<td>0.71 (0.24 – 2.09)</td>
<td>0.542</td>
<td>2.81 (1.22 – 6.47)</td>
</tr>
<tr>
<td><strong>HAART duration of use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No HAART use</td>
<td>1.0</td>
<td></td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>PI &lt; 5 years</td>
<td>0.64 (0.27 – 1.54)</td>
<td>0.323</td>
<td>0.67 (0.32 – 1.39)</td>
<td>0.287</td>
</tr>
<tr>
<td>PI ≥ 5 years</td>
<td>2.15 (0.23 – 20.1)</td>
<td>0.503</td>
<td>1.81 (0.38 – 8.59)</td>
<td>0.453</td>
</tr>
<tr>
<td>NNRTI &lt; 5 years</td>
<td>1.52 (0.74 – 3.08)</td>
<td>0.248</td>
<td>1.31 (0.66 – 2.60)</td>
<td>0.426</td>
</tr>
<tr>
<td>NNRTI ≥ 5 years</td>
<td>1.94 (0.62 – 6.07)</td>
<td>0.254</td>
<td>2.65 (1.10 – 6.37)</td>
<td>0.028</td>
</tr>
</tbody>
</table>

5. Assuming legitimacy of the model selection procedure, why not use all covariates that meet criteria for either model in both models. This enables you to discuss a similar set of covariates by the two age groups.

ANSWER – We agree with you and have included all this information in the final model.

6. The finding of increased cIMT with lower CD4 counts is unexpected. The authors cite an analysis of the Strategies for Management of Antiretroviral Therapy (SMART) study[1] and describe an increased risk of CVD with per 100 unit elevation in CD4 level. I was unable to locate this result in this study. Perhaps the authors can assist this reviewer in finding it.
REFERENCES

ANSWER – We agree with you. The article cited does not contain this information. The information cited in the text is described in two other papers that describe the findings of the SMART study, specifically related to cardiovascular disease. One study shows that in the group of the “conservative drug” (sporadic interruption of antiretrovirals), a higher CD4 level was associated with an increased risk of CVD (1.1 event for each increase of 100 cells/mm³ CI 95% 0.99-1.2, p = 0.008) (Phillips, 2008*). See below:


In the other study, a Cox regression model that included baseline dosages for IL-6, high sensitive C-reactive protein, D-dimer, CD4 lymphocytes and other HIV related characteristics as predictors for CVD in 5,098 patients of the SMART study, (of whom 252 presented a CVD event after a median follow-up period of 29 months), showed that besides those inflammatory markers, higher CD4 lymphocytes levels at baseline were associated with CVD (p <0.02) (Duprez, 2012**) (see below).

(The phrase has been modified and the wrong reference has been replaced in the revised paper).


“Results

During a median follow-up of 29 months, 252 participants experienced at least one CVD event. Numbers experiencing each type of event were: CVD death (n= 44), non-fatal MI (n=67), non-fatal stroke (n= 20), CHF (n= 30), coronary revascularization (n =63), coronary artery disease requiring drug treatment (n =51), and peripheral arterial disease (n= 51). Fifty-four participants experienced more than one CVD event. Table 1 summarizes differences in major CVD risk factors and HIV-related factors for participants with and without CVD events. P-values corresponding to univariate associations and to associations that adjust for age and gender are shown. hsCRP, IL-6 and D-dimer were associated with an increased risk of CVD in both the univariate and age and gender adjusted analyses. Kaplan-Meier curves for quartiles (quartile cut-points are given in figure legend) of each biomarker show good separation of the four
curves for IL-6 and for the upper two quartiles versus the lower two quartiles for hsCRP and Ddimer (Figure 1). In a regression model that included all three biomarkers and baseline covariates used for adjustment (see Methods), higher levels of IL-6 (p < 0.001), hsCRP (p = 0.003), and D-dimer (p = 0.002), older age (p < 0.001), male gender (p = 0.04), higher CD4+ T cell count (p = 0.02), prior AIDS (p = 0.01), smoking (p = 0.002), prior CVD (p = 0.02), diabetes (p = 0.05), antihypertensive therapy (p = 0.001), and the presence of major ECG abnormalities (p = 0.03) were associated with an increased risk of CVD…”

The authors also suggest that comorbidities among virally suppressed participants on ART in their study “may increase the chance of subclinical atherosclerosis.” However, they present no data to support this – was the burden of comorbidities higher among virally suppressed people on ART vs. non suppressed people on/off ART?

ANSWER - We have made changes in the Discussion section of the paper after obtaining new results of the analysis. This part of the Discussion has therefore been excluded.

7. The lack of association between cIMT and HDL cholesterol, LDL cholesterol and triglycerides raises the issue of confounding by lipid lowering therapy. If these data are available, the authors should account for the use of these therapies. A similar point could be made for antihypertensive therapy.

ANSWER – lipid lowering drugs, particularly statins, were used by 17 (2.4%) patients. The small number of patients on therapy is justified by the low Framingham scores of this population, a score that serves as the basis for indicating this therapy in the Brazilian guidelines. Another factor is the unavailability of the drugs for free distribution in the Brazilian HIV control program. In view of the low number of patients using this treatment, we considered that there would be no major differences in the results if they were taken into consideration. For hypertension, persons using antihypertensive medications were identified and considered as hypertensives according to international definitions.

Table 6 - Risk of cardiovascular disease using the Framingham Score

<table>
<thead>
<tr>
<th>clas_fram</th>
<th>Freq.</th>
<th>Percent</th>
<th>Cum.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>490</td>
<td>71.95</td>
<td>71.95</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>80</td>
<td>11.75</td>
<td>83.70</td>
</tr>
<tr>
<td>High risk</td>
<td>8</td>
<td>1.17</td>
<td>84.88</td>
</tr>
<tr>
<td>&lt; 30 years</td>
<td>103</td>
<td>15.12</td>
<td>100.00</td>
</tr>
<tr>
<td>Total</td>
<td>681</td>
<td>100.00</td>
<td></td>
</tr>
</tbody>
</table>

8. The time interval between cIMT and assessment of covariates is unclear. The greater the time separation, the less likely the covariates approximate biological status at the time of cIMT measurement.
ANSWER – The median time between the USG and the questionnaire was 35 days ($P_{25} = 17$ and $P_{75} = 75$ days); between the USG and CD4 and viral load (evaluated simultaneously), it was 96 days ($P_{25} = 46$ and $P_{75} = 152$ days); and between the USG and triglycerides/cholesterol (evaluated simultaneously), it was 28 days ($P_{25} = 11$ and $P_{75} = 84$ days). This information has been included in the revised manuscript.

**Minor Essential Revisions**

9. It is unclear that “consensual union” and “schooling” should be characterized as a “traditional risk factor for CHD.” The authors should provide references to support this assertion or modify this characterization (Methods, page 6).

ANSWER – We agree with you and modified this assertion.

11. Some imprecise statements made in the discussion suggest conclusions not supported by the data. “The association of male gender as a predictor of cardiovascular disease only in the younger group is in agreement with the literature…” What the data actually suggest is an association of male gender with cIMT, a marker of subclinical atherosclerosis, which is in turn a risk factor for cardiovascular disease.

ANSWER – We have modified this part of the discussion and included the following:

The association of male gender with a higher carotid IMT, which is a surrogate measure of atherosclerosis associated with cardiovascular risk factors and with cardiovascular outcomes, in the younger group only is in agreement with the literature already available on patients without HIV infection, as estrogenic activity during the premenopause slows the progression of atherosclerosis in women.

Likewise, “The …metabolic syndrome… was an independent factor … for the onset of subclinical atherosclerosis” implies some causal relation by chronology. Since assessment of the metabolic syndrome was likely done concurrently with or subsequently to assessment of cIMT, this characterization is likely incorrect.

ANSWER – We agree with you and have modified our statement as follows:

The presence of the metabolic syndrome is associated with a 2.4 to 2.8-fold greater risk of developing CVD in the general population [24] and was an independent factor associated with greater carotid IMT in younger patients in our study. This finding suggests the possibility that the metabolic syndrome is associated to the onset of subclinical atherosclerosis in this age group.

“…association between immunosuppression… and increased cardiovascular risk for both subclinical atherosclerosis and myocardial infarction” This statement conflates cardiovascular disease and cardiovascular disease risk.

ANSWER – We have changed this statement to:

….found an association between immunosuppression (CD4 counts below 200 cells/µl) and increased frequency of subclinical atherosclerosis and risk for myocardial infarction…
As well as Polak et al., we considered that “Carotid-wall intima–media thickness is a surrogate measure of atherosclerosis associated with cardiovascular risk factors and with cardiovascular outcomes”.


This occurs again during the discussion of data from the SMART study – to my knowledge, atherosclerosis risk has not been reported from SMART.

ANSWER – We have included the study that presented an extension of the SMART study, focusing on CVD:


Design

The design, methods and results of the SMART trial have been previously published [10]. Between January 2002 and January 2006, 5,472 HIV-infected patients with a CD4+ T cell count >350 cells/mm³ were randomized to intermittent ART (drug conservation, DC) or continuous ART (viral suppression, VS). For patients in the DC group, ART was not used until the CD4+ count declined to <250 cells/mm³, at which time ART was initiated (or reinitiated) until the CD4+ count increased to more than 350 cells/mm³. VS patients were to use available ART in an uninterrupted manner with the goal of maximal and continuous suppression of HIV replication. As previously reported on January 11, 2006, enrollment was stopped and participants in the DC group were advised to restart ART. All participants were followed until July 11, 2007 (study closure) [19], resulting in a minimum follow-up of 18 months for each participant and a median follow-up of 29 months.

Biomarkers and Cardiovascular Outcomes

CVD events occurring through study closure were reviewed by an Endpoint Review Committee using pre-specified criteria blinded to treatment group [20]. The CVD composite outcome used in this report includes: CVD death, non-fatal myocardial infarction (MI) (clinical and silent as measured by annual resting ECG), non-fatal stroke, congestive heart failure (CHF), coronary revascularization, coronary artery disease requiring drug treatment, and peripheral arterial disease. Cause of death was determined using the Coding of Death in HIV (CoDe) system [21]. In this report, 19 deaths of unknown causes that were unwitnessed were considered CVD on the assumption that most would be CVD-related. These unwitnessed deaths do not include violent deaths and deaths attributed to suicide, substance abuse, and accidents by the Endpoint Review Committee. In selected analyses, CVD events are grouped as non-fatal coronary heart disease (CHD) (MI, coronary revascularization, and coronary artery
disease requiring drug treatment), non-fatal atherosclerotic non-CHD (stroke and peripheral vascular disease), congestive heart failure (CHF) and CVD death. Based on strong associations of hsCRP, IL-6 and D-dimer with all-cause mortality in a nested case-control study [17] and the observation that these biomarkers were elevated in HIV-infected participants compared to those in the general population [22], these three markers were measured on stored plasma at baseline for all consenting participants by the Laboratory for Clinical Biochemistry Research at the University of Vermont. IL-6 was measured with Chemiluminescent Sandwich enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN); hsCRP with a NM™ II nephelometer, N Antiserum to Human CRP (Siemens Diagnostics, Deerfield, IL); and D-dimer levels with immunoturbidometric methods on the Sta-R analyzer, Liatest D-DI (Diagnostica Stago, Parsippany, NJ). Lipids were centrally measured on serum by Quest Diagnostics, Inc. Lipids were measured on the Olympus AU5400. LDL cholesterol was directly measured. Fasting status influences triglyceride levels but has little effect on total and HDL cholesterol levels; 52% of sample obtained at baseline were fasting. Biomarkers and lipids were analyzed blinded to CVD event status and treatment group.

Results
During a median follow-up of 29 months, 252 participants experienced at least one CVD event. Numbers experiencing each type of event were: CVD death (n = 44), non-fatal MI (n = 67), non-fatal stroke (n = 20), CHF (n = 30), coronary revascularization (n = 63), coronary artery disease requiring drug treatment (n = 51), and peripheral arterial disease (n = 51). Fifty-four participants experienced more than one CVD event. Table 1 summarizes differences in major CVD risk factors and HIV-related factors for participants with and without CVD events. P-values corresponding to univariate associations and to associations that adjust for age and gender are shown. hsCRP, IL-6 and D-dimer were associated with an increased risk of CVD in both the univariate and age and gender adjusted analyses. Kaplan-Meier curves for quartiles (quartile cut-points are given in figure legend) of each biomarker show good separation of the four curves for IL-6 and for the upper two quartiles versus the lower two quartiles for hsCRP and Ddimer(Figure 1).

In a regression model that included all three biomarkers and baseline covariates used for adjustment (see Methods), higher levels of IL-6 (p = 0.001), hsCRP (p = 0.003), and D-dimer (p = 0.002), older age (p = 0.001), male gender (p = 0.04), higher CD4+ T cell count (p = 0.02), prior AIDS (p = 0.01), smoking (p = 0.002), prior CVD (p = 0.02), diabetes (p = 0.05), antihypertensive therapy (p = 0.001), and the presence of major ECG abnormalities (p = 0.03) were associated with an increased risk of CVD...

Discretionary Revisions
12. The authors report differences in mean values of lipids. In my experience, lipid values are typically non-normally distributed. The authors may wish to indicate how they compared means if these values were not normally distributed.

ANSWER – We agree with you. The means have been replaced by the medians.

13. “No prior coronary disease” is specified as an inclusion criterion. The authors may wish to specify events (myocardial infarction, stroke, heart failure), coronary heart disease, or coronary artery disease.
ANSWER – The coronary diseases excluded were angina, heart failure and myocardial infarction. This has been stated in the revised text.

**Reviewer's report**

**Title:** Risk factors for subclinical atherosclerosis in HIV-infected patients under and over 40 years: a case-control study

**Version:** 1  **Date:** 18 October 2012  
**Reviewer:** MAhboob Alam

**Reviewer's report:**

Overall, it is a well written manuscript. However, I have one concern:

1. As your group has reported, these two patient groups are significantly different in terms of baseline cardiovascular risk profile. While, some of it may be related to HAART therapy (i.e. longer duration in older group), it is still possible that these older patients may have underlying risk of CAD due to other traditional risk factors. While multi-variate analysis is a good way to evaluate for independent associations, it still does not reduce the potential for selection bias in any case-control study. In my opinion, if possible, consider reporting analysis after you have performed PROPENSITY MATCHING for traditional cardiovascular risk factors on these patients. There is a possibility that you may have a smaller sample size after matching for tradition risk factors including diabetes mellitus, dyslipidemia, metabolic syndrome and smoking.

What is the reason behind using 40 years as a cut-off for age?

ANSWER – Because of the demand of another reviewer who had a similar concern we conducted an analysis that separated individuals with the major traditional risk factors for CVD from those without such factors, which has been included in the revised text. We consider that this is sufficient to reduce the selection bias in the two groups and that it will be more easily understood by the readers of this journal. As a result we believe that there is no need to perform PROPENSITY MATCHING.

**Editor's demand**

The authors should please provide a rational in the Methods for the cIMT cut-off choice of 0.8 mm as a threshold and justify the age group cut-offs.

1) Rationale for the cIMT cut-off choice of 0.8mm

ANSWER - We chose a cutoff of 0.8 mm for evaluation of carotid atherosclerosis similar to Jerico et al. (See reference below). This choice was based on the fact that the measurement of the common carotid MIT in a normal population of young adults, produces values lower than 0.8 mm (Aminbakhsh, 1999, Touboul, 2012), with an increase from 0.01 to 0.02 mm per year. In addition, individuals with carotid MIT lower than 0.8 mm have less risk of cardiovascular and cerebrovascular events (<4.6%) in six years. Given that the median age of the study population was 40 years, it seemed to be a rational choice.

2) Rationale for the age group cut-off.

ANSWER - We have chosen the cut-off point of 40 y in our study for two reasons: 1) in the exploratory analysis of our data we found that the chance of atherosclerosis was more than 10 times higher in the 40-49 y age group than in the younger group, and that this chance was even higher in people aged 50 y or over (see Table 1, below). We thought that, although we could have adjusted our analysis by age, it would be more informative to split the sample in these two groups that might have important differences between them. We thus decided to separate our population into two groups and analyze the factors related to atherosclerosis in each of them. Our findings are very interesting, as you can see. Another alternative would be to test for the presence of interaction between age and each comorbidity. However this approach would not be easily understood by readers without a good epidemiological or statistical background. 2) Another point reinforced our decision, namely the 40 y cut-off divided our population down the middle (median age of the population was 40 y). This rationale has been also included in the revised version of the text.

Table 7– Subclinical atherosclerosis in the different age groups

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Subclinical atherosclerosis</th>
<th>OR (CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Presence</td>
<td>Absence</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td>N</td>
</tr>
<tr>
<td>&lt; 30 y</td>
<td>13</td>
<td>12.1</td>
<td>94</td>
</tr>
<tr>
<td>30 to 39 y</td>
<td>69</td>
<td>28.3</td>
<td>175</td>
</tr>
<tr>
<td>40 to 49 y</td>
<td>133</td>
<td>58.6</td>
<td>94</td>
</tr>
<tr>
<td>≥ 50 y</td>
<td>95</td>
<td>81.9</td>
<td>21</td>
</tr>
</tbody>
</table>

3) Please have a native English speaker proofread and/or copy edit the manuscript for English usage, spelling or grammar.

ANSWER – The manuscript has been revised by a native speaker.