Author's response to reviews

Title: Co-infection by human immunodeficiency virus type 1 (HIV-1) subtype c and human t cell leukemia virus type 1 (HTLV-1): does immune activation lead to a faster progression to AIDS?

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Author's response to reviews: see over
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Dear Editor

Please, find the revised version of the manuscript “Co-infection by Human Immunodeficiency Virus type 1 (HIV-1) Subtype C and Human T Cell Leukemia Virus Type 1 (HTLV-1): Does Immune Activation Lead to a Faster Progression to AIDS?” with the modifications highlighted in yellow. We are very pleased about the revision done by the reviewers; this will sure make the paper much clearer and better. As per your and the reviewer’s suggestions, we have modified the manuscript in order to accommodate the recommendations indicated by the reviewer’s comments. In this new version, we took into account the reviewers’ comments, as detailed in the point-by-point response seen below. We thus that our manuscript can now be accepted for publication in BMC Infectious Diseases. Hereby I declare that all authors have seen and approved the content.

Sincerely yours,

Eduardo Samo Gudo Jr.
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Point-by-point response to reviewers

Reviewer: Javier Carbone

Major Compulsory Revisions.

1 - Information regarding sample size calculation for comparison of means should be provided.

Answer: In the statistical analysis section we added the following information regarding sample size calculation "Taking into consideration that the major goal of our study was to compare activation marker between co-infected versus HIV mono-infected patients, two sample comparison mean with a ratio 1:2 (case:control) was used to determine the required sample size in these groups. Due to lack of information regarding comparison of activation markers between these groups, we calculated a sample size enough to detect a least a difference of 10% in the mean of these cells frequencies with a standard deviation of 14 at a significance level of 5%.”.

2 - Information regarding clinical issues that could affect lymphocyte activation is not included.

Answer: We added in the manuscript a table (table 2) with the relevant clinical presentation of co-infected and HIV mono-infected groups, as seen below
Table 2. Clinical presentation of study groups

<table>
<thead>
<tr>
<th>Clinical presentation</th>
<th>HIV/HTLV-1, n(%) (n=29)</th>
<th>HIV, n(%) (n=59)</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assymptomatic</td>
<td>7 (24.1)</td>
<td>14 (23.7)</td>
<td></td>
</tr>
<tr>
<td>Papular pruritic eruptions</td>
<td>3 (10.3)</td>
<td>14 (23.7)</td>
<td></td>
</tr>
<tr>
<td>Dermatitis</td>
<td>2 (6.9)</td>
<td>2 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Seborrhoeic dermatitis</td>
<td>2 (6.9)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Folliculitis</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Herpes zoster &lt; 5 years</td>
<td>3 (10.3)</td>
<td>4 (6.8)</td>
<td>0.570</td>
</tr>
<tr>
<td>Tinea capitis</td>
<td>5 (17.2)</td>
<td>8 (13.6)</td>
<td></td>
</tr>
<tr>
<td>Candidias</td>
<td>3 (10.3)</td>
<td>4 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Tuberculosis &lt; 1 year</td>
<td>1 (3.5)</td>
<td>4 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Weight loss &gt; 10%</td>
<td>3 (10.3)</td>
<td>7 (11.9)</td>
<td></td>
</tr>
<tr>
<td>Chronic diarrhoea</td>
<td>0 (0.0)</td>
<td>1 (1.7)</td>
<td></td>
</tr>
</tbody>
</table>

* Fisher chi square test

In the **Study design and subject** section we added the following information “Co-infected and mono-infected were comparable regarding clinical presentation of opportunistic diseases (table 2).”

In addition, in the **discussion** section we added “Cases and controls were matched by age and clinical stage (WHO) so that to be comparable in terms of clinical presentation (see table 2). Clinical staging system is performed on the basis of patient’s clinical presentation. This information is important when interpreting the differences in the activation markers between these groups”

**3 - The authors should analyse if there are significant differences in the distinct lymphocyte subsets, between distinct clinical stages in coinfected and non coinfected patients to access if there is association between activation and clinical progression.**

**Answer:** Samples size constraints is a limitation to this stratification. Therefore, although we were not able to perform statistical calculation, a pattern of changes in the activation markers through different HIV clinical stages was
assessed. A graphic with this information was generated and included in the revised version of the manuscript (see below)

In addition, in the **results** section, we added an item describing these results (*Activation markers on T cells correlate with HIV clinical stage*)
Figure 4. Changes in T cell subsets in the distinct HIV clinical stages HIV mono-infected and HIV+HTLV co-infected patients.

Reviewer: C J Castro-Sansores

Major Compulsory Revisions

1 - Why subtype C for the HIV-1 was considered for all the patients, if they were not sequenced all (just 14 mono-infected and 14 co-infected).
   Answer: The total number of samples analyzed was increased in both groups. Now this information is provided for 20 co-infected and 24 mono-infected samples. Due to the dominance of subtype C in both groups, the amount of samples sequenced were calculated so that the number of samples sequenced in each group is statistically representative of the group. In addition, we added the following statement in the discussion session “Of note, all samples sequenced in both groups were founded to be HIV subtype C, ruling out any linkage between HIV subtype in mono and co-infected groups, and immunological/clinical behavior”.

2 - There are nothing of the figure 5-A and 5B.
   Answer: We included a new figure so that now that it is correct

Minor Essential Revisions

3 - In relation to the serology for the HTLV, there were no seropositive patients for the HTLV-2?
**Answer:** In the present study we applied a Western blot that discriminate HTLV-1 from HTLV-2 (*HTLV BLOT 2.4, Genelabs® Diagnostics, Switzerland*). No sample was found to be HTLV-2.

In the **HTLV Serology** section we added the following information “*All HTLV positive samples in our study population were typed as HTLV-1 by Western blot.*”.

In the **discussion** section we added the following statement: “*We found no evidence of HTLV-2 in our study population. This is in keeping with two recent studies conducted among blood donors in Maputo city and suggests that only HTLV-1 (but not HTLV-2) circulates in Mozambique*. 

**Discretionary Revisions**

4 - *In relation to the stool evaluation, the results of the parasite finding must be placed until the end of the paragraph.*

**Answer:** we re-placed the sentence at the end of the corresponding item.

Reviewer: Carlos Brites

**Minor questions:**

1 - *How the patients were selected? Were they selected from a larger cohort, or consecutively included in the study?*

**Answer:** We included the following information in the study design and subjects section “between March and June 2006, 724 HIV 1/2 infected patients were consecutively invited to participate and 704 (97.4%) accepted to be part of this study. They were all screened for HTLV-1 infection and 32 patients (4.5%, 32/704) were founded to be co-infected by HTLV-1 and HIV. Three patients with a positive HTLV-1 antibody test did not return to collect their result and were excluded of the study. Co-infected were matched at a ratio 1:2 with HIV mono-infected by age, sex and HIV clinical stage system as defined by WHO. Co-infected and HIV mono-infected were matched without prior knowledge of CD4+T cell counts results*.”
2 - Why controls were not matched like coinfected patients? In a blood bank there are many health donors and it would be easy to do;

Answer: In the study design and subjects we included the following information “Healthy controls were not matched by age and sex as we did with co-infected and HIV-mono-infected because they were recruited on a consecutive basis from the routine blood donors at the blood bank of Maputo Central Hospital. In addition, most of blood donors are males and younger as demonstrated by two previous studies conducted at the same Blood Bank”.

3 - What would be the mechanisms of immune activation caused by HTLV? In discussion section, it would be very important to approach this point.

Answer: In the discussion we included the following statement “HTLV-1 is a strong activator of immune system. Immune activation and exaggerated immune response has been demonstrated to be the main pathogenetic mechanism involved in the HTLV-1 associated inflammatory syndromes. The immunodominant Tax protein encoded by HTLV transactivates and modulates a large number of genes playing a key role in triggering several pathways leading to cell activation. Available data demonstrate that a large proportion of asymptomatic carries progress with high levels of immune activation.

4 - The authors said stool examination would rule out other potential causes of immune activation, but there is no information on clinical characteristics of the patients. Were they presenting any opportunistic infection during the study period? It could be a potential cause of immune modulation.

Answer: Please see the answer to the question 2 by Javier Carbone