Author's response to reviews

Title: CD4 lymphocyte dynamics in Tanzanian pulmonary tuberculosis patients with and without HIV co-infection

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Author's response to reviews: see over
Re: MS: 1237280786630599

Dear Editor

Thank you for your positive and constructive review of our manuscript: “CD4 lymphocyte dynamics in Tanzanian pulmonary tuberculosis patients with and without HIV co-infection” by Andersen AB, Range NS, Changalucha J, PrayGod G, Kidola J, Faurholt-Jepsen D, Krarup H, Grewal HMS, Friis H.

We have revised the manuscript according to your suggestions and explain our revisions in the section below.

On behalf of the group of authors

Sincerely

Åse Bengård Andersen

Point-by-point description of changes:

Ad reviewer # 1:

1) No comments required.

2) We have preserved the 2003 edition of the WHO guidelines (ref. # 10) because they were “in force” while the project was running (i.e. 2006 to 2009).

3) No comments required.
4) We have added data on the enrollment of patients and especially data on those not entering the study. We chose to present this information in the first three lines of the Results section since we felt that this would take up less space than a figure.

5) We hope that the revised version of the manuscript is less confusing and easier to read.

6) No comments required.

7) Information on the primary and secondary objectives of the study has been entered in the “Study design” section of the “Materials and methods”.

8) No comments required.

Minor suggestions for revision:

- “Absolute numbers rather than differences in abstract”. We chose to keep the differences, because we found these numbers easier to grasp “at-a-glance”.
- Ethics section: we have deleted the double information as suggested.
- More information on the study design has been added to the materials and methods section.
- There is no statistical justification why their multiple testing (e.g. table 4) has not been corrected e.g. with post hoc Bonferroni etc.
  
  We have now used one-way analyses to assess if changes in CD4 counts over time differed by HIV and PTB-status. Where this came out significant we have included a Scheffe multiple comparison post-hoc test. It is now indicated in the table, using similar letters in suprascript which groups are different. The same has been done in Table 4b. This has also been made clear in the text about statistical methods, whereas it does not affect the result section.

- Flow chart: we added the information as also mentioned earlier in three lines in the results section.
The authors state they performed linear regression. However, they use categorical data (e.g. PTB+/- and HIV +/-) as well as continuous data (CD4 count). So I suspect logistic regression was performed? Please clarify/explain.

As for the question about linear regression, we can confirm that this was what we used. Logistic regression is used when the outcome is binary, which was not the case here. We used linear regression because the dependent variable is continuous, in this case CD4 count. The independent variables can be either continuous or categorical, in this case we only used categorical variables.

Why were parameters like BMI, albumin and acute phase reactants not included in the analyses? Since these parameters also reflect “disease burden/level of progression” (like PTB and HIV status) we do not find it relevant to include. Including BMI and/or an acute phase reactants in the model lowered the CD4 level somewhat but does not change the results.

95% CI bars have been added to figures 1 and Figure 2 omitted as suggested.

Figure 2 was deleted.

We hope the revised version reads better and we acknowledge that there may be several ways to present data. However, by discussing the groups one by one as suggested (HIV negatives, HIV+ and then HIV + on ART) would make it more difficult to compare to the groups in between.

Ad reviewer #2:

We have included some of the recent papers on the timing of ART in TB patients, which were published in NEJM in 2011 (ref. # 21 and 22) and expanded the discussion accordingly. Further, data on the immune recovery in TB-AIDS patients obtained from a large retrospective, Italian HIV cohort (ref. # 7) has been introduced in the Introduction and is further discussed in the discussion section.
• **How do CD4 count change when analyses are stratified by base line CD4?**

A total of 81 (5.1%) had a CD4 count between 50-100 and 34 (2.1%) had a CD4 count below 50. There is a tendency that the HIV- with a low CD4 count experienced the highest absolute increments in CD4 numbers, but not statistically significant. No difference was observed between the HIV +.

• We have extended the discussion on the CD4 cell rise – also in HIV- TB patients, and also admitted that it is a weakness of our study that we do not have clinical data on symptoms on IRIS.

• We have tried to standardize use of abbreviations. Our terminology on PTB+ versus PTB- is consistent with what we have used in previous publications from this study site e.g. ref. 8,9 and 16. It is not so often that TB patients are culture confirmed in studies from the sub Saharan region. However, and also explained in the Materials section, the culture from some patients got contaminated. If the result from the microscopy analysis showed “Acid Fast Bacilli” the patient was considered “smear positive” and included in the analyses.

• We have expanded the description on “the-cell-leaders” a bit in the materials and methods section.

• CD4% would have been helpful, we agree, but unfortunately we do not have CD3 or CD 8 data available.

• We have expanded a bit on the nutritional intervention and also analyzed the Vitamin D status at base line and found no confounding effects. These data are planned published separately.