Author's response to reviews

Title: The effect of Tuberculosis and antiretroviral treatment on CD4+ cell count response in HIV-positive Tuberculosis patients in Mozambique

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Author's response to reviews: see over
Dear Editor

Thank you for the useful comments made by the reviewers on our manuscript “The effect of Tuberculosis and antiretroviral treatment on CD4+ cell count response in HIV-positive Tuberculosis patients in Mozambique”.

Below we give our replies to all the comments of both reviewers and describe the revisions made to the manuscript. We addressed the additional editorial requirements as well.

We appreciated the feedback that contributed to a better manuscript. Should further (editorial) changes be necessary, please do advise us.

Yours sincerely

Miranda Brouwer
On behalf of the team of authors
Reviewer 1 comments:

Comment 1. What is the backbone NRTI used in this cohort? This factor has effect on the treatment outcome at least or more, so it should be clarified and included in the univariate and multivariate analysis.

Reply 1: This information is indeed relevant and we included it in the methods section. In addition, no difference exists in CD4+ cell count response between Nevirapine and Efavirenz containing regimens with the same NRTI backbone. A recent paper by Van Griensven et al. did find a difference in CD4+ cell count response between Nevirapine and Efavirenz containing regimens with the same NRTI backbone1. However, Hermans and colleagues argued that bias caused this difference and they did not find it in their study population2.

Action taken 1: We included the NRTI backbone information in the methods section: “The standard first line ART regimen consists of two nucleoside reverse-transcriptase inhibitors, Lamivudine and Stavudine, with either Nevirapine or Efavirenz”. Because only one patient on ART in our study received a different ART regime (with Zidovudine instead of Stavudine), we did not include the ART regimen in the univariate and multivariate analysis.

Comment 2a. How long the patients had received ART prior to TB in 32% of this cohort? Some of these might have developed TB IRIS or even TB after ARV failure for a while.

Reply 2a: We are not completely sure what the reviewer means by TB-IRIS. We understand it either as incident or unmasking TB, meaning a TB diagnosis shortly after the start of ART; or as TB-IRIS, sometimes also called paradoxical TB-IRIS.
- If the reviewer meant incident TB, the reviewer is correct that the 81 patients who started ART before TB treatment potentially have incident TB. Emerging evidence shows that CD4+ cell count response is smaller in these patients3 though earlier evidence showed a similar CD4+ cell count response in both patients with prevalent and those with incident TB compared to patients on ART without TB4. However, we cannot completely rule out a potential underestimation of the effect of TB treatment in our study.
- As for paradoxical TB-IRIS, we don’t believe it is an issue in the group that started ART prior to TB treatment in our study. According to the definition of the International Network for the Study of HIV-associated IRIS the antecedent requirement states that the TB diagnosis was made before starting ART, which is not the case in the patients that started ART before they started TB treatment (http://www.med.umn.edu/inshi/definitions/TB_IRIS/home.html accessed 4th May 2012).
- ART failure may have been a factor for the patients starting ART before they started TB treatment. This potentially influences CD4+ cell count response. We cannot ascertain this with our data. However, we believe ART failure is limited as only 9 out of 81 patients started ART more than 6 months before they started their TB treatment.

Action taken 2a: We included the potential underestimation of the effect of TB treatment as a limitation of the study: "Fifth, about one third of the patients that used ART during TB treatment started their ART before the start of TB treatment and potentially had incident TB while using ART. Emerging evidence shows that CD4+ cell count response is smaller in these patients [17]. However, earlier evidence showed a similar CD4+ cell count response in both patients with prevalent and those with incident TB compared to patients on ART without TB [18]. We cannot completely rule out a potential underestimation of the effect of TB treatment in our study. However, the majority of patients did not have incident TB while already on ART and we are confident that our results are valid."

Comment 2b: The author had better exclude the patients who initiated ART and subsequently developed TB from the analysis because the magnitude of immunologic response may be less than the patients who were naïve to ART.

Reply 2b: We do believe that it is not necessary to exclude the patients that started ART prior to TB treatment from the analysis. The majority of patients do not have incident TB and we are confident that the results are valid.

Action taken 2b: See revision of comment 2a.

Comment 3a. The last paragraph of result section should be clarified in terms of what was the time point of comparison.

Reply 3a: The time point of comparison was the whole observation period of 6 months.

Action taken 3a: We clarified this in the results section: “Over the full observation period of 6 months, using TB treatment was not statistically significant associated with the CD4+ cell count response. Patients using TB treatment had a CD4+ cell increase of 19 cells/mm3 (95% CI: -50 to 79; p=0.529) compared to patients not receiving TB treatment. ART use was statistically significantly associated with CD4+ cell response during the observation period. Patients using ART had a CD4+ cell increase of 81 cells (95% CI: 12 to 151; p=0.222) compared to patients not using ART."

Comment 3b. In addition, were the absolute CD4 or delta changes from baseline comparing?

Reply 3b: The model used the absolute CD4+ cell count values to estimate the effect of TB treatment and ART on CD4+ cell response.

Action taken 3b: We clarified this in the model description in the method section: “The model used the absolute CD4+ cell count values to estimate the effect of TB treatment and ART on CD4+ cell response.”

Comment 4. I don't think that the conclusion “CD4+ cell counts for patients not on ART at TB treatment start, remained below the cut off for initiating ART during the first three months of TB treatment; therefore some delay in getting the first CD4+ cell count would not lead to missing the opportunity to start ART.” is rational because 1. No percentile of CD4 was shown at each time point on the figure. Some patient may remain having low CD4 cell count and these patients may be abandoned to starting ART. and 2. The number of patients is too small to conclude. Most of time point had patients of less than 20 participants.

Reply 4: The reviewer is correct in stating that the number of observations at each time point is rather low. This is a real-life situation in which CD4+ cell counts are done sparsely in settings with limited access to this diagnostic tool. For the same reason we only used the general trend in the evolution in CD4+ cells by assessing the mean estimated number of CD4+ cells.
However, the use of a mixed model that incorporates both repeated measurements and missing data makes that the analysis is having its optimal power to detect changes. Every patient contributes data at each time point in the analysis, whether with a CD4+ cell estimate from the clinic, or with an estimated CD4+ cell estimate based on the modelling strategy. The amount of data is therefore larger than the number of CD4+ cell estimates reported in Figure 1, which increases the power of the analysis. Figure 1 clearly shows that within the first 12 weeks there is no evolution of CD4+ cells to speak of in the group of patients with TB treatment but without ART. We therefore feel that our conclusion is justified, although we have toned down the statement to incorporate the uncertainty around the estimates.

Action taken 4: We rephrased the conclusion: “The average CD4+ cell count for patients not on ART remained below the cut-off for initiating ART of 350 cells/mm³ during the first 12 weeks of TB treatment. Therefore, a delayed assessment of the first CD4+ cell count in itself would probably not lead to missing an opportunity to start ART based on the cut off of 350 cells/mm³.”
Reviewer 2 comments:
Comment 1a (Introduction):
The introduction should focus more on the effect of CD4 count on anti-TB treatment and ART and its relevance. There is no clear link between the literature and the formulation of the research question, the introduction mentions two studies in regard to increase in CD4 count on ATT with-out ART, more literature is required in regards to quantifying CD count on ART. If there is no such evidence the researcher should state the short fall of that evidence.
  
  Reply 1a: There was limited evidence at the time of the study and this is stated more clearly in the introduction. After our study more evidence on CD4+ cell count response in HIV-infected TB patients became available. However, the conclusion remains unclear.
  
  Action taken 1a: Revision in the introduction: “CD4+ cell response during TB treatment in HIV-infected TB patients is less clear and only a few studies addressed this question.” We included some of the recent studies in the discussion (reference 17 and 18):

Comment 1b (Introduction):
There is a clear objection but no clear aim.
  
  Reply 1b: We revised the objection and aim to be clear.
  
  Action taken 1b: Revision of the introduction: “The objective of this study is to describe the CD4+ cell count response during TB treatment and to quantify the effect of TB treatment and ART on the CD4+ cell count response. Through the CD4+ cell count response we assess whether a risk exists for missing an opportunity to start ART in the routine setting of Mozambique due to late CD4+ cell count availability in HIV-infected TB patients, and the prioritization of ART for TB-HIV co-infected patients with the lowest CD4+ cell counts.”

Comment 2 (Introduction):
Please cite: The increase in TB notifications is partly driven by the Human Immunodeficiency Virus (HIV) epidemic.
  

Comment 3 (Method):
The research design is clearly described and appropriate including the rational of the sites, although there is no clear description of the participants, i.e. the new TB patients included were they all microbiologically confirmed cases and what TB type (pulmonary vs. extra-pulmonary) was included.

  Reply 3: TB in Mozambique is diagnosed mainly by smear microscopy. Smear negative and extrapulmonary TB is diagnosed on the basis of clinical assessment and hardly ever on radiology.
  
Table 1 contains the patient characteristics. We included how many of the patients had smear positive, smear negative and extrapulmonary TB.
Action taken 3: We revised the method section: “In Mozambique, smear microscopy is the main TB diagnostic. In the participating facilities, diagnosis of sputum smear negative and extrapulmonary TB occurs mainly on clinical assessment and hardly ever on radiology.” Furthermore, we included the patient characteristics in table 1.

In comment 4 (Data collection and analysis) the reviewer mentioned a number of potential confounders:

Comment 4a: Baseline CD4 count (CD4 count before initiation of TB treatment and ART)

Reply 4a: We did not purposely collect baseline CD4+ cell counts (before initiation of both TB treatment and ART). The CD4+ cell count response is related to the baseline CD4+ cell count. However, we don’t think confounding is an issue in our study as we had a CD4+ cell count that can be considered a baseline CD4+ cell count (that is a CD4+ cell count before initiating both TB treatment and ART) for the majority of (76%) our patients. We came to 76% as follows: 81 patients had a baseline CD4+ cell count before initiation of TB treatment and ART. Another 32 had the CD4+ cell count within 5 days after the start of TB treatment while not (yet) on ART, therefore most probably reflecting the CD4+ cell count at the start of TB treatment. The CD4+ cell count results of these 32 patients can be considered as a baseline CD4+ cell count. The total 81+32=113 out of 149 makes 76%.

As the use of a mixed model that incorporates both repeated measurements and missing data, it makes that the analysis is having its optimal power to detect changes. Every patient contributes data at each time point in the analysis, whether with a CD4+ cell estimate from the TB clinic, or with an estimated CD4+ cell estimate based on the modelling strategy.

Action taken 4a: None.

Comment 4b: Microbiological results at 2 months and 5 months on TB treatment

Reply 4b: We did not collect data on microbiological results at 2 and 5 months of TB treatment and other co-morbidity/opportunistic infections. We do not believe that microbiological results at 2 and 5 month of TB treatment are potential confounders in this study. Positive microbiological results at 2 and 5 month results in a treatment outcome ‘failure’, and only a few patients (n=6, 2% of total patients included) had a treatment outcome other than cure, completion or death. Of these 6 patients, only 2 were TB treatment failures.

Action taken 4b: None.

Comment 4c: Bacillary load at initiation of TB treatment.

Reply 4c: We did collect data on bacillary load at initiation of TB treatment and these were recorded as is commonly used in TB control as 3+, 2+, 1+ or actual count. We did not perform subgroup analysis because the numbers were too small.

Action taken 4c: None.

Comment 4d: Other co-morbidity/opportunistic infections.

Reply 4d: We did not collect data on other co-morbidity/opportunistic infections. Co-morbidity is a potential confounder and information on co-morbidity

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should be included in a future study of this nature.

Action taken 4d: None.

Comment 5 (Data collection and analysis): Please stipulate the period of data collection in 2007.

Reply 5: We now included the period in the methods section.

Action taken 5: Revision in methods section “Within these clinics, we collected the information on HIV disease parameters of all new notified TB patients of 16 years and older with a positive HIV test recorded in the TB register from January to December 2007.”

Comment 6 (Results and discussion): It does not clearly state if those who were on ART at initiation of TB treatment were included or excluded in the analysis and also those with outcome of death and if these patients were on ART treatment or not. The inclusion of these individuals could have also biased the results. The graph also show that the patient on ART had a higher baseline CD4 count than those not on ART which could also be a reason for a lower and slower reconstitution. This section needs to be reviewed by a statistician or epidemiologist.

Reply 6: One of the authors, Frank van Leth, is a senior epidemiologist with many years of research experience including statistical analysis. He carefully reviewed the results and discussion sections again as suggested.

The method section explains which patients are included in the model: “With this model we used optimally all available CD4+ cell counts including all patients with at least one CD4+ cell count in the model, regardless of the number of missing values these patients have.”

Like we explained in comment 4a: The use of a mixed model that incorporates both repeated measurements and missing data makes that the analysis is having its optimal power to detect changes. Every patient contributes data at each time point in the analysis, whether with a CD4+ cell estimate from the TB clinic, or with an estimated CD4+ cell estimate based on the modelling strategy.

The differences in CD4+ cell count at TB treatment initiation are fully incorporated in the mixed model approach by including day 0 specifically in the model with absolute CD4 cell counts. This is conceptually the same as modelling at each time point the change from baseline in a model that leaves out day 0 but has every included absolute CD4+ cell count reported as a change from baseline.

Action taken 6: None.

Comment 7 (Conclusion): The conclusion relates to the objective and recommendations have been made from the results and discussion. The research fails to identify further research opportunities /issues arising from the study. Overall the writing is acceptable.

Action taken 7: We added a sentence on future research: “A prospective study will provide better insight to the question of the CD4+ cell count response during TB treatment and the effect of TB treatment and ART on this response.”
Additional editorial requirements:

Editorial requirement 1: Manuscripts should include a Competing interests section. This should be placed after the Conclusions/Abbreviations. If there are none to declare, please write 'The authors declare that they have no competing interests.'

Action taken 1: We added the sentence on competing interests: “The authors declare that they have no competing interests.”

Editorial requirement 2: Please include an Authors’ Contributions section after Competing interests. Please check the instructions for authors on the journal website for the correct format to use for Authors’ Contributions.

Action taken 2: We added the sections author contributions after the competing interests.

Editorial requirement 3: Please remove the header ‘Discussion’ in the Abstract. It should only be Background, Methods, Results and Conclusions.

Action taken 3: The header discussion is removed in the abstract.