Author’s response to reviews

Title: The Effect of Incident Tuberculosis on Immunological Response of HIV Patients taking Highly Active Antiretroviral Therapy at University of Gondar Hospital, Northwest Ethiopia: A Retrospective Follow-up Study

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Version: 3 Date: 31 May 2014

Author’s response to reviews:

Dear Editors

The authors would like to thank Reviewers for their careful review of our manuscript and providing us with their comments and suggestion to improve the quality of the manuscript. The main revised parts of the manuscript are highlighted and the following responses have been prepared to address all of the reviewers’ comments in a point–by-point fashion.

Reviewer: Dr. Brian Kigozi

This is a clinically relevant topic due to the large proportion of HIV-infected individuals presenting late for HAART treatment with concurrent opportunistic infections like tuberculosis in this population.

Major comments:

Abstract:
1. Results: A clarification should be made on the denominator used for HIV/AIDS patients used calculate percentages or proportions of patients with immunological failure and active tuberculosis (TB). In the abstract, a total of 400 HIV patients is mentioned and used as a denominator, whereas in the results section 484 HIV/AIDS patients are mentioned. There should be consistent use of the word HIV patients or HIV/AIDS serving as the denominator, not to be used interchangeably. Similarly, the word Highly Active Antiretroviral Therapy (HAART) is used interchangeably with Antiretroviral Therapy (ART) in the abstract and manuscript.

Response: to know the specific effect of TB incidence on immune recovery, we excluded patients who had active TB at ART start and in both abstract and results section analysis is restricted to 400 patients.

2. Conclusions: Conclusions derived at in the abstract differ from that of the manuscript. The conclusion should also mention male sex as a significant determinant of immunological failure. The baseline CD4 T cell count threshold
which is associated with immunological failure should be mentioned.

Response: The author completely agrees with reviewers’ comment and conclusions are modified as per your suggestion.

Manuscript

3. Introduction: authors should provide information on the number of people with TB co-infected with HIV in Ethiopia. This information may guide readers about the magnitude of the problem.

Response: thank you for bringing this point in to our attention and the authors included the information

4. Methods:

Study design and data collection: As the change in CD4+ T-cell count after HAART initiation is the primary endpoint of this study, details on how often was CD4+ T-cell counts were measured should be provided. Information on the occurrence of clinical events (i.e. occurrence of other concurrent new opportunistic infections (according to stage 4 WHO clinical staging) and hospitalizations) should also be reported during follow-up, since they are strongly related to insufficient immunological recovery and hence immunological failure.

How was the data extracted from the HAART register? Who did the data extraction? Was the data extraction standardized? How was bias minimized during data extraction? Immune Reconstitution Syndrome (IRS) is a potential confounder. How were the confounding effects of IRS minimized?

Definitions: authors should mention the duration of follow-up for the patient to be considered an immunological failure after HAART initiation.

Response: Thank you again for bringing this point to our attention. The schedule for CD4+ T-cell count measurement is mentioned. Data collection procedures are described. Definitions are also modified as per your suggestion. CD4 count that has done before the first 4 months of therapy are excluded because immune response problem before the first 4 months often represent immune reconstitution inflammatory syndromes related to pre-existing conditions. Moreover, as the study is retrospective in its nature and most of the information was missed, we couldn’t include other opportunistic infections.

5. Statistical methods: Authors should clarify whether they assessed for statistical interaction or effect modification.

Response: we have modified the multivariate analysis with the consultation of biostatistician

6. Results

• Authors should provide a study profile for patients screened from HAART register.

Response: the authors completely agree and the information is included.

As reported in Table 3, the multivariate analysis was adjusted for presence of incident TB, baseline CD4+ T-cell count, sex, education level, ART regimen,
functional status and WHO clinical stage. As variables of WHO clinical stage and incident TB were identical, it is not appropriate to include both variables in the model.

Response: Since we excluded patients who had active TB at ART start, baseline WHO clinical stage is contributed by conditions other than TB.

• Immunological failure after initiation of ART: 3rd sentence, the median time to the occurrence of immunological failure should be reported instead of the mean time. The 4th sentence reports 89 immunological failures out of these 26 were due to incident TB. Were the remaining 43 immunological failures probably due to other opportunistic infections? Information is needed for other possible causes of immunological failure. See 4 above.

Response: the median time to the occurrence of immunological failure with inter quartile range is mentioned. As we are assessing merely immunological failure but not treatment failure, development of incident TB does not necessarily herald ART failure. Therefore, all TB patients didn't get immunological failure rather we did the comparison between TB+ and TB free for immunological failure.

• The authors suggest that individuals with incident TB had immunological failure due to profound immunosuppression at HAART initiation deriving from the disease itself and increased viral replication. However, incident TB was associated with immunological failure only in the univariate analysis but not in the multivariate analysis (borderline significance), possibly indicating that the level of immunodeficiency at baseline is a more important factor to explain the immunological failure in patients with incident TB. It would be also interesting to know, how successful the treatment of incident TB was in these patients (for example providing the changes in the body mass index (BMI) over time. See Table 3; also 2nd paragraph under discussion.

Response: thank you for your salient observation and authors indicated as lower baseline CD4 is a more important factor than incident TB. BMI is also mentioned in the limitation part.

7. Discussion: This needs to concise and discuss the significant findings of the study. The results (Person years of observation, Hazard ratios and their 95% confidence intervals are appearing in the discussion, which should not be. The discussion needs to be rewritten.

Response: Thank you for bringing this point to our attention and the document is edited accordingly.

• Authors need to explain why adherence to HAART and/or anti-TB drugs was not controlled for during multivariate analysis since it is a major confounder. The problem of drug-drug-interactions, that are more likely to occur among individuals treated for incident TB on HAART, should be also discussed, since this is possibly related to lower adherence and insufficient viral suppression and hence immunological failure. HAART-Niverapine based regimens are likely to interact with Rifampicin. See discussion, third paragraph and last sentence.

Response: Assessing adherence to HAART and/or anti-TB drugs in our setting by such retrospective data was very challenging because of the patient reporting
bias. We found almost all patients have reported as they have good adherence and hence excluded from analysis. The authors also agree on the interaction of Nevirapine with Rifampicin, however, according to the national ART guideline all ART patients who developed TB were switched to efavirenz based ART drugs.

- 4th paragraph, 4th sentence: the following sentence is not clear and is not in concordance with the study findings; “……Contrary to our finding, low baseline CD4+ T- cell counts were not associated with increased risk of immunological failure [23]…”

Response: This information is replaced by the report that supports our finding.

8. Study limitations: authors should acknowledge and mention;

- Their inability to control all possible confounders because of the nature of the study design, citing some of confounders they were unable to measure and control for.
- Under estimation of individuals with immunological failure as many patients die shortly after anti-TB drugs are started while on HAART due to the profound immune suppression and adverse effects of treatment.
- Inadequate sample size.

Authors may benefit from reading the following related reference: The effect of AIDS defining conditions on immunological recovery among patients initiating antiretroviral therapy at Joint Clinical Research Centre, Uganda. AIDS Research and Therapy 2009, 6:17

Response: thank you, Authors mentioned the limitations as per your suggestion. However, regarding under estimation of individuals with immunological failure as many patients die shortly after anti-TB drugs are started, the main point of immunological failure identification was development of incident TB and hence it can’t be affected by this one. Moreover, the authors would like to thank you for providing an important reference material.

Minor revisions:

1. Words appearing for the first time like TB should be spelt in full with the abbreviation in bracket, which may be used later. See background in the abstract.

2. There are many grammatical and spelling errors which needs to be addressed.

The manuscript also requires proof reading. Examples;

- Title page: Corresponding authors should be amended to “Corresponding author.” A full stop should be removed from the title of the manuscript.
- Abstract: Methods-amend to “A retrospective cohort study was carried out on adult HIV patients who started HAART at University of Gondar Hospital between 1st September 2007 and 30th August 2008. Changes in CD4+ T- lymphocyte count and incident TB episodes occurring in the 42 months of HAART after initiation were assessed. Cumulative probabilities and median time to immunological failure were estimated using a Life table and Kaplan-Meier curves respectively.”
• Abstract: results: the word incident is missing in the first sentence. There is need to round off hazard ratios reported to 2 decimal places.

• Abstract: conclusions: the second sentence should stat as follows, “A low baseline CD4+ T cell count of <100 cells/mm3………”

• Ethical considerations should be amended to “Ethical approval.”

• Statistical methods: last sentence be amended from “…confidence intervals was to “…confidence intervals were…”

• Limitation as a subheading should be amended to “Study limitations.”

• The manuscript should follow the following chronology after the conclusion subsection; competing interests, authors’ contributions and acknowledgements.

• References: reference no.3 should provide the information source. Was the source from a website?

• Table 1, 2, 3: the title or heading should reflect the number of HIV/AIDS patients and the time period

• Consistence should be done while reporting CD4+ T- cell counts. For example in the abstract are reported as cells/ mm3 whereas in Table 3 are reported as cells/µl.

• Figures 2, 3: y-axis should report the probability of immunological failure. The word “log rank test” should appear in the Kaplan- Meir survival curves and the corresponding p-values reported. Figure 1 can be done away with.

Response: Minor revisions-Thank you for your critical review and the authors agree on the comments and the document is revised accordingly.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
No competing interests declared

Reviewer: Neil Martinson

Major Compulsory Revisions

1. Suggest a proof reading by someone whose first language is English as there are a number of errors that need correcting as well as some rephrasing to make it easier to read (eg Active TB developed in 26(6.5%) of patients within 42 months on ART; Incident TB is defined as active TB which developed after initiating ART and prevalent TB as active TB for which treatment is initiated prior to ART).

Response: Thank you for your critical review and the authors agree on the comment and the document is thoroughly revised and edited by local language experts.

2. It is a pity that the authors selected to only report those people with at least six
months of ART, as it appears most immunological failures occur early after treatment initiation. Suggest
Response: immunological failures that may occur before the first six months of therapy were not included because the routine monitoring of CD4 cell counts is done every six months, or more frequently if clinically indicated. Patients with lower baseline CD4 cell counts may have lower count for long time and to assess immunological failures it needs at least six months of follow up.

2.1. A flow diagram or text equivalent is included to show the total number of patients started on ART, the total number with >1 month of follow up, the number remaining in care at 6 months (484), 12 months, 18 months etc and the number who did not have enough data to be included in this analysis.
Response: thank you for bringing the issue in to our attention. The authors completely agree but we included the information in general term rather than by follow up time because of the different time of study censorship.

2.2. Show immunological failure rate in three or six month increments not one year.
Response: the authors completely agree and the information is included.

2.3. There are several components to the treatment failure - rank the components that contributed to diagnosis of immunological failure – possibly stratifying by incident TB.
Response: As we are assessing merely immunological failure but not treatment failure, development of incident TB does not necessarily herald ART failure. Treatment failure is normally monitored by both immunological and clinical criteria. Immunological failure may not mean treatment failure. Therefore, we didn’t rank the treatment failure.

3. End of follow up time needs to be defined. Why is there an apparent censoring time of 42 months? The retrospective review was conducted in 2013. Patients were started on HAART ~5 years earlier - there should be some who have >60 months of follow up time.
Response: The author completely agrees with reviewers comment. The period between Sept 2007 and Aug 2008 was selected to follow patients for sufficient time. However, due to the nature of the study design, the number of patients having consistent CD4 cells measurement became very small and hence we are forced to collect data of 42 months period.

4. Were there really only 484 people who started ART in the year Sept 2007-Aug 2008? What other eligibility criteria were used to include or exclude participants
Response: the authors already mentioned the information in the reviewer comment number 2.1 part

Minor Essential Revisions

5. Table 1 and Table 2 could be combined without losing data and decide whether your columns are immunological failure or incident TB.
Response: thank you for the concern. We have used Table 1 and Table 2 only to
describe the socio-demographic characteristics of the study subjects. In particular to show the TB incidence in the different clinical characteristics of study subject, we prefer to put in a separate table. Regarding the immunological failure, for each our study variable we indicated the magnitude of immunological failure in the second and third column of bivariate analysis part.

6. Similarly could the KM graphs not be combined to show interesting interactions between TB and possibly baseline CD4 count. Or just have one figure with three of four subfigures contained it?

Response: since the each KM graph is the individual statistical out comes that can’t be produced simultaneously and even results of all KM graphs can’t be merged, it is better to leave as it is.

7. Suggest the multivariable Cox analysis be redone after discussion with a statistician

7.1. Excluding those variables that are either not biologically plausible or appear to have no impact in univariable analyses.

7.2. Consider collinearity -- CD4 count is likely to be very closely correlated with WHO stage and with functional level. I suggest you only include CD4 in your multivariable model and leave out the other two.

Response: the authors agree and the analysis is modified as per your suggestion.

Discretionary Revisions

8. Can you not find a more reader-friendly term than "Immunological failure survival"?

Response: the graphs are talking about survival of patients from experiencing immunological failure and that is why we use this term. However, authors are very pleased to get suggestion of more reader-friendly term.

Level of interest: An article of limited interest

Quality of written English: Not suitable for publication unless extensively edited

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests