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

The authors aimed at investigating the effect of incident tuberculosis on immunological response of HIV patients on highly active antiretroviral therapy at Gondar University Hospital in North West Ethiopia. HIV and tuberculosis co-infection is a major public concern in the Sub-Saharan Africa because of synergism between HIV and tuberculosis. HIV is an important risk factor for tuberculosis and tuberculosis is the leading cause of morbidity and mortality among HIV/AIDS patients. Understanding specific factors related to immunological failure is important to plan effective interventions to reduce HIV-related morbidity and mortality, particularly in a resource-limited setting.

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.

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.

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.

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.

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

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

• 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.

• 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.

• 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.

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.

• 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.

• 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]…”

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

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
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 <100cells/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.

**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