Author's response to reviews

**Title:** Efficacy and safety of thrice weekly DOTS in tuberculosis patients with and without HIV co-infection: an observational study

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**Author's response to reviews:**

Reviewer 1
We thank Reviewer 1 for the extremely useful comments. We have addressed these in the sections below.

Major:
1. Comment: the authors have used a large number of exclusion criteria which are likely to impact the results, including previous history of TB and diabetes. 38% of patients screened were excluded. Those excluded are likely to have a higher risk of treatment failure, and one may hypothesize that this is especially true for the HIV+ patients. I therefore feel that the conclusion "..outcomes in HIV co-infection were found to be similar to those reported previously with daily therapy, with no safety concerns." is premature
Response: We agree with the reviewer that excluding patients with previous history of TB and diabetes are likely to impact the result of the study. However this was done in an
attempt to prevent introduction of confounders and avoid heterogeneity in the study. In the Indian setting, patients with previous history of TB as per Indian Revised National TB Control Programme (RNTCP) guidelines are started on 'Category II' anti-tuberculosis drug regimen, which includes streptomycin administration in the initial 2 months. Moreover, 12-20% patients in this category have MDR-TB, which requires altogether a different regimen ('Category IV' treatment regimen in the RNTCP guidelines). During this study, we did not have access to newer rapid tests for MDR-TB (liquid culture, molecular methods such as LPA, GeneXpert, where turnaround time for MDR-TB diagnosis is from 2 hrs to 10 days) and Mtb culture was done on LJ medium, with usual time for susceptibilities report was >4 months. Therefore, in order to avoid any heterogeneity, only newly diagnosed TB patients with and without HIV-infection were included in the present study.

The reason for excluding diabetics is that it complicates the management of TB disease. In these patient treatment outcome is very poor (unless hyperglycemia is tightly controlled) and there are high chances of mortality. Diabetic drugs are also known to interact with first-line anti-tuberculosis drugs (mainly rifampicin) which further complicates the treatment of these patients. Sometimes, when a patient has uncontrolled hyperglycaemia, it is difficult to judge whether outcome of treatment is poor due to high sugars or TB, and interpretation of results is difficult.

2. Comment: there may be a information collection bias. Culture results at baseline and during follow-up were missing for many patients. 'treatment completed' and 'cure' are combined, but these are clearly two very different endpoints. Based on the information provided I cannot conclude that treatment outcome is similar in HIV+ and HIV-.
death is nog good endpoint to compare treatment results, as HIV+ patients are more likely to die from other causes. The ideal endpoint would be treatment failure and possible acquisition of drug resistance (which was not measured).

Response: This comment is long and thought-provoking - the response has been separated into sections:

a. Ideally baseline sputum culture results should have been available for all the patients but still they were missing in some pulmonary TB patients (PTB).

b. We agree with the reviewer that measurement of drug resistance is important to rule out drug resistance among TB cases and ideally its measurement should have been included as the secondary endpoint of this study. However, the present study lacks drug susceptibility testing (DST) because at the time of commencement of the study it was not the part of Indian Revised National Tuberculosis Control Programme guidelines and was not widely available. Moreover, in resource-limited settings its prohibitive cost makes the test difficult to afford.

Acquired drug resistance is a major problem in this area, which is undergoing indepth research currently. It is mentioned as a limitation in the manuscript [page 13, paragraph 6]. Currently, our centre is well equipped with an accredited lab for MDR-TB diagnosis (solid, liquid and molecular DST methods).

c. When studying a diverse group of patients, with many having extrapulmonary foci, it can be difficult to rely upon treatment failure, or even cure or treatment completion, as a primary endpoint. In the case of pulmonary smear and culture positive TB, 'cure' and 'treatment failure' are more reliable and relevant: both 'cure' and 'treatment completed' are the same, and the judgement of 'treatment failure' is very unambiguous. Judging cure in cases of widely disseminated TB in solid viscera, for example, can be difficult, and the World Health Organization's 2010 'Treatment of TB' guidelines would only allow 'treatment completed' as a positive outcome in this setting. Due to significantly more HIV-positive individuals having smear and culture negativity and extrapulmonary TB, a decision was made to compare using the combined 'treatment success' outcome for either 'cure' or 'treatment completed'.

3. Comment: no DST was performed to exclude drug-resistance as an important confounding. This is not trivial; drug resistance may be overrepresented among HIV+
Response: This is indeed a very pertinent point. Drug susceptibility testing (DST) is highly important in establishing baseline resistance, or subsequent acquisition of resistance. In this study, DST was not available widely, and is still not part of the Indian national guidelines for this reason. Resource constraints dictated this, and it has been discussed as a limitation in the discussion of the manuscript [page 13, paragraph 6]. However, this somewhat explains the reason some of the groups mentioned in the exclusion criteria weren't included, for example those with prior history of TB or exposure to anti-tuberculosis therapy (as they have high chances of developing drug-resistant TB) [page 4, paragraph 2]. The remaining patients were felt unlikely to have rifampicin resistance, which in India is almost exclusively used in the setting of TB therapy.

4. Comment: the reporting of the data is somewhat confusing. what doe the authors mean by “Modified intention to treat analysis was applied to the population undergoing any treatment, within the 'end of treatment' groups (n=305) and to all excluding those still under regular follow-up in the 'follow-up' groups (n=211). On treatment analysis was applied to all those receiving any ATT in the context of adverse events (n=305).”? Response: The wording of the quoted paragraph has been amended to clarify the methods of inclusion to groups for analysis: modified intention to treat was applied to end of treatment and follow-up outcomes, while adverse events underwent on treatment analysis. [page 8, paragraph 1]

5. Comment: “Follow-up included clinical assessment, monitoring for adverse events and opportunistic infections (in HIV- positive patients), routine blood investigations, sputum smear and culture if appropriate and possible, and repeat imaging as at enrolment to
monitor response to treatment.” Is rather vague and should be specified. Did the authors plan to sample sputum for culture monthly (smear is not very useful to monitor bacteriological response to treatment)? If not, why? The same for Chest X-ray, was it done / planned at specific time points (e.g. month 2 and 6).

Similarly, how was adherence monitored? Was a standardized questionnaire used?

Response: The quoted paragraph appears vague due to the heterogeneity of study participants [page 6, paragraph 2]. However, this same sub-section of the Methods contains the requested details about chest radiography/sputum bacteriology, and adherence monitoring (the former has been moved to a more natural position) [page 6, paragraphs 3 and 5]. A structured pro forma document was used - this has now been mentioned [page 6, paragraph 2]. In addition, the follow-up information has been separated from that relating to treatment: two individual sub-sections are now presented [pages 5 and 6].

Minor:

Comments:

Table 1. gender in %, viral load in log.
Table 2. please add %
Table 3. Please combine ‘smear results’ with ‘smear bacillary load’. Under load: negative, scanty, 1+, 2+, 3+

Tables 1-4 may be combined (6 tables seems out of proportion to the amount of original data)

Responses:

Table 1 has been amended.
Percentages have been added to Table 2.

Smear results have been combined with smear bacillary load [Table 3].

Tables 3 and 4 have been combined. We feel that Tables 1 and 2 should remain separate due to each being individually important.

Reviewer 2
We thank Reviewer 2 for his comments. These are addressed below.

Comment: This is a well written study and they indicate that the treatment failure was identical to that of HIV negative cases. They also infer that the rates of treatment failure are identical to that of historic daily therapy in the HIV positive group. However as stated by the authors there are several reasons for not being able to interpret this data completely. The major weakness of the study is the lack of a control arm of HIV infected cases having daily therapy and having just the comparator of HIV negative subjects as it then does not allow for a fair comparison of an appropriate 'control' arm.

Response: The lack of comparison with daily therapy is indeed a weakness. However, this was an observational study to look at outcomes in the current setting in India, where Revise National Tuberculosis Control Programme guidelines, irrespective of the HIV status (positive/negative) of TB patients, recommends thrice weekly intermittent ATT for all. This study provided the foundation for a potentially more meaningful interventional trial, comparing daily and thrice weekly intermittent ATT among HIV-TB co-infected individuals, commenced recently at our institute.

Comment: The cohort characteristics are significantly different at baseline and with disparate disease presentations and severity. In addition the drop-out rates are significantly higher in the HIV group. These factors may therefore introduce bias into the studied co-infected group. It would be important to know what the causes of drop-outs were and for instance were these due either to adverse effects or death as an early detrimetal effect of the thrice weekly approach?

Response: The study design involved consecutive assessment for recruitment, which, along with an unselected HIV-positive population with low median baseline CD4 cell count,
accounts for the wide variation in presentation among the groups [Table 1]. We accept that the characteristics given in Table 1 are accompanied by many more significant P values than non-significant, highlighting the heterogeneity of the two groups. This may well introduce bias, and may account at least in part for the higher mortality in the HIV co-infected group. However, multivariate analysis did reveal that the only significant factor among HIV-positive individuals predicting unfavourable outcome was low baseline CD4 count [page 10, paragraph 6].

Drop-out rates were higher in the HIV-positive group, but, apart from in the smear-positive pulmonary TB sub-group [Table 7], statistical comparison with the HIV-negative group did not achieve statistical significance. In particular, the percentage lost to follow up at 24 months of follow up was very similar [Table 8], and this same table demonstrates maintenance of similar proportions of treatment success even when those lost to follow up/defaulting are excluded from the analysis.

Regarding those defaulting, all four expressed a wish to stop attending the clinic and taking medicines due to a combination of stigma and difficulty attending follow up appointments. Those labelled as 'lost to follow up' specifically were unable to be contacted despite efforts by the research and clinical teams. As noted in the study profile [Figure 1], two who were lost to follow up in the treatment phase were later admitted and died from TB and advanced HIV infection.

Comment: Despite the similar relapse rates, in the completed study arm there were more treatment extensions in the co-infected arm and hence again we cannot be certain of the effect of this on their study cohort. In addition 'seriously unwell' cases are exclusion factors.
Response: We accept that treatment extensions may have impacted on the outcomes. Relapse and failure rates may have been affected. The reason extensions were allowed was that this is part of the Indian Revised National TB Control Programme guidelines, and standard practice in our Institute. The longer median duration of treatment in the HIV co-infected group may have impacted on initial treatment outcomes, i.e. treatment success, failure, death; but is unlikely to have affected relapse on follow up significantly - the extensions were of a median duration of one month, whereas Swaminathan et al (2010) found a difference in recurrence based on 9 months versus 6 months of treatment. However, we agree with the reviewer's observation that we cannot be sure of the impact.

The reason seriously unwell patients were excluded was to prevent potential confounding from HIV co-infected patients having multiple opportunistic infections, of which TB would be just one. This may have resulted in some inadvertent selection of less unwell patients, but we felt it was necessary to attempt to include people with TB as their main infection or co-infection. It must be noted that only those who were critically unwell, in intensive care settings, were excluded.

Comment: The authors also accept that lack of DST make complete interpretation of the data difficult and by definition as the cases with HIV had less mycobacterial load, it is difficult to interpret sputum and culture conversion rates as being representative of a real comparison.

Response: We accept that sputum smear conversion would be influenced by initial smear positivity grading, which was significantly higher in those without HIV infection [Table 3]. However, this should have less effect on culture conversion.

Comment: Finally the definition of treatment success and failure are in themselves difficult to
interpret as these incorporate extrapulmonary disease and despite the subset analysis of only the
smear positive pulmonary cases - the inability to directly compare the 'success' microbiologically
makes interpretation suboptimal.
Response: As stated by us in the manuscript [page 3 paragraph 1] and Reviewer 2 in his
comments, smear and/or culture negative, and extrapulmonary tuberculosis is, more difficult to
assess with regards to microbiological response. However, the way in which response to
treatment is assessed in this manuscript is as per the World Health Organization guidance on the
matter, and in our experience is reliable when there is reasonable duration of follow up
following treatment, as available in this study. We also put this forward as a merit of our study:
we have attempted to capture the real-life situation with regards to treatment of TB, so as to
understand the outcomes of thrice weekly therapy in a diverse range of presentations [page 13
paragraph 5]. This is particularly pertinent in the setting of HIV co-infection, which predisposes
to extrapulmonary and atypical presentations. In addition it, to some extent, begins to fill the
gap in the literature examining forms of TB other than smear and culture positive pulmonary
disease.
We feel that our conclusions are not unjustified. Although limitations as mentioned will reduce
the ability to judge on various points accurately, our interpretation of the results is communicated in a sufficiently uncertain manner so as to not prematurely or erroneously
conclude what cannot be done so from the findings. It may be acknowledged here that
extrapulmonary TB is a common form of TB in HIV/AIDS. This type, being a paucibacillary form of
TB, poses a real diagnostic challenge in real-world practice. Further, endpoints in this type of TB
are also difficult to define, but this does not mean that study in this area should
not be undertaken.

Reviewer 3

We appreciate Reviewer 3’s feedback and suggestions. Our responses follow.

Major revisions:

Comment 1: The abstract is strangely lacking in data. This makes it vague and will not attract readers. This should be changed.

Response: We have changed the abstract by inserting data and p values in its 'Results' section [page 2].

Comment 2: In the methods I am unclear as to how adverse events were graded. This then makes interpretation of the very high rates of nausea and vomiting and abdominal pain, alongside very low rates of hepatotoxicity and neuropathy, difficult to interpret. Please amend.

Response: This has now been clarified in the 'Follow up' sub-section of the 'Methods' section [page 6, paragraph 6]. The grading loosely followed the Common Terminology Criteria for Adverse Events (Trotti et al, Semin Radiat Oncol, 2003), but not with 5 separate grades: grades 1 and 2 were roughly equivalent to non-serious adverse events, while grade 3-5 events would be labelled as serious.

Comment 3: I am unclear in the methods as to the standard tests used. Did all have culture? Was PCR routine? This needs to be clarified. I think also at present the list of possible tests performed is distracting and could be removed.

Response: The list has been shortened, and further details regarding use of each modality have been given in the same paragraph [page 4 paragraph 4]. Only smear, culture and chest radiography were routinely carried out. PCR was used in fluid samples which were smear negative.
Comment 4: I am not clear about some exclusions. In particular diabetes and alcoholism. Is this per local guidelines? In figure 1 I think it should say why people were excluded.

Response: Diabetes mellitus was an exclusion as it was postulated to be not only a risk factor for TB, but also to influence outcomes of TB treatment negatively. The latter has since been found by several studies, e.g. Faurholt-Jepsen et al (Trop Med Int Health, 2013), Reed et al (PLoS One, 2013), Jimenez-Corona et al (Thorax, 2013). Chronic alcoholics were also excluded from the study as they are not reliable at maintaining adequate follow up. It was felt that not many subjects would be excluded in this way - as compared, for example, to excluding IV drug users and migrant workers, who make up a significant proportion of Delhi’s HIV infected population.

Figure 1, the study profile, has been updated with a list of exclusions.

Minor essential revisions:

Comment: Table 2: I am not sure I recognise miliary TB only in the lungs. Surely it is always disseminated?

Response: Table 2 has been amended, with 'lungs only' removed. We agree that this is somewhat ambiguous. The implication was that miliary shadowing was present on a chest radiograph, but there was not clinical or radiological evidence of involvement outside the lungs.

Although as stated, miliary TB is not restricted to the lungs.

Discretionary revisions:

1 Table 2: I think the numbers in the sub-groups should also be expressed as percentages

Percentages have been added to Table 2.

2 the proportion with smear positive disease is very high. Is this usual for your population?

In a paper from the same institution, Karmakar et al (Clin Dev Immunol, 2011, reference 22), 12 of
the 25 pulmonary TB patients with HIV co-infection had positive sputum smears. This is similar to the figure of 51.6% quoted in the current manuscript [Table 3]. Regarding HIV-negative patients, the 86.6% [Table 3] smear positivity is around what we would expect at a tertiary centre in a high burden country.

Conversely the culture positive proportion seems low with those smear figures. This may relate back to the questions in the methods but can you explain this please?

Table 3 and its first (*) footnote gives these details: HIV-positive, 51.6% smear positive, 46.1% culture positive; HIV-negative, 86.6% smear positive, 87.4% culture positive. Regarding the HIV-positive patients, four were smear positive but culture negative. They had not had therapy when baseline samples were taken, subsequent review of samples concurred with the initial smear grading, and they responded well to therapy.

Table 8: Is there a reason why so many HIV+ were lost to follow up?

The high attrition rate at our ART Centre has been previously published by Sharma et al (Bull World Health Organ, 2010, reference 21). Those data found 22% were lost to follow up, with 84% of these in the first year following registration. It is not clear why, but 40% had only primary school education or less, and 55% were unemployed or unskilled workers. Our experience is that patients in this demographic have a much higher rate of attrition, despite efforts to retain them.

I am very surprised at how infrequent neuropathy was if you were using stavudine. How commonly was stavudine used?

Zidovudine was given to 65% of those with HIV co-infection, the remaining 35% (53/150) received stavudine (zidovudine was the drug of choice; stavudine was reserved for those with anaemia) [page 10 paragraph 3]. Stavudine induced neuropathy is common, with two studies in sub-Saharan Africa quoting incidence of neuropathy in those on stavudine-containing ART as around
20% per person-year
(McGrath et al, J Neurovirol, 2012; van Oosterhout, PLoS One, 2012). It is unclear why this adverse event appears to be under-represented in our cohort: only 2/53 (3.8%) over mean 14.1 months. However, Indian data published appears to be more similar to our figures: 5.2% over median 48 weeks (Sivadasan et al, J Assoc Physicians India, 2009); 2% over one year (Sreenivasan and Dasegowda, J Infect Dev Ctries, 2010). This is something we intend to look at in more detail with an audit of our centre’s wider HIV data - if rates are indeed this low, it would be interesting to investigate further. Further, stavudine is being phased out and is no longer available as the first or second-line drug in ART centres in India.

6 Page 11: What is a category II regimen?
Apologies for this. Category II was previously used to refer to an ATT regimen including first line drugs and streptomycin in a longer course. This has been explained [page 5 paragraph 5].

7 Page 11: Could you give the numbers in the results for smear and culture conversion at month 2 please?
The sputum smear and culture conversion numbers and percentages have been included as requested [page 9 paragraph 5].

8 the frequency of IRIS is rather low and the timing is quite late. Do you have any explanations for this? This is important as may relate to adherence to both ATT and ART. In the study by Karmakar et al from the same ART Centre (Clin Dev Immunol, 2011, reference 22), incidence of TB-IRIS in those with HIV-TB co-infection was 4% (5/123), compared with the 9.3% (14/150) in the current study. Median time from initiation of ART to IRIS was similar: 73 in Karmakar et al’s study and 76.5 in the current study. We do not feel the incidence found in this cohort is particularly low. IRIS did occur later than expected in some cases, but none of the IRIS cases had
problems with adherence to treatment identified. IRIS may occur earlier in settings where protease inhibitors are used: these are not widely available in the Indian government-funded setting.

9 Page 16: I am unclear from your data why extending the length of treatment would be beneficial. When ATT was extended was that the intensive or continuation phases?

Extensions of therapy are advocated by the Indian Revised National TB Control Programme guidelines, and standard practice in our Institute. The intensive phase can be extended for one month only, and the continuation phase by up to three months, but the total extension allowed is of three months. This is to attempt to ensure complete sterilisation if incomplete response to treatment is judged clinically or microbiologically, though this is not necessarily beneficial in all ways, as illustrated by the study quoted by Swaminathan et al (Am J Respir Crit Care Med, 2010, reference 12). Our study did not set out to see if extension is beneficial, so that cannot be inferred from the data.

10 Page 16: Perhaps you could reference some of the more recent papers on starting ART such as Camelia and Sapit studies. The SAPIT study has already been referred to (reference 2). The CAMELIA study has now been included as reference 29 [page 13 paragraph 2].