Author’s response to reviews

Title: Association of high serum vitamin D concentrations with active pulmonary TB in an HIV co-endemic setting, Harare, Zimbabwe

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01 December 2016
Your Ref: INFD-D-16-00920

Dr Alice Zwerling
BMC Infectious Diseases

Dear Dr Zwerling

Re: Association of high serum vitamin D concentrations with active pulmonary TB in an HIV co-endemic setting, Harare, Zimbabwe (INFD-D-16-00920).

We thank you for your e-mail dated 03 November 2016.

We have highlighted in yellow the edits to our revised manuscript. The edited sections are referenced by section; line and page numbers.

Reviewer reports:

Reviewer #1:

Authors’ comment:

The Reviewer’s comments were not numbered; we have added numbers to their comments for easy referencing.

(1) Thanks for allowing me to review this manuscript reporting vitamin D levels among HIV infected and non-infected and pulmonary TB infected and non-infected patients in Zimbabwe. There is equipoise on the association between serum vitamin D and risk of infection or reactivation of tuberculosis. The paper is well written in good English, and contains a thoughtful discussion section. There are design problems which limit the conclusions that can be made on the data collected, some of which are acknowledged by the authors.

Authors’ response:

We thank the Reviewer for the comments and we address the issues they raised below:

(2) The populations are not matched, with the specimens for the HIV negative cohort coming from health care workers and HIV testing volunteers, and the specimens for the HIV positive cohort coming from a previous clinical trial. There were significant differences seen between groups with regard to age and gender, so comparisons made between these different populations cannot be completely valid. This is the primary design limitation and needs to be strongly included in the discussion and conclusions.
Authors’ response:

We erred in the original submitted manuscript by referring to our study as case-control but as corrected by Reviewer 2, it is a cross-sectional study. We agree with the Reviewer’s assertion that the unmatched nature of our study design impinges on our conclusions. We acknowledged that flaw in the original manuscript; and indeed do so in the revised version; ‘The unmatched nature of our cross-sectional design probably also impedes drawing of stronger conclusions’. (Limitations section, lines 311-312, page 15) We however wish to state that for mean age, statistically significant differences were only observed between infected groups (HIV or PTB or both) versus uninfected controls with no significant differences between mono-infected and co-infected group. Whilst we do not seek to underplay the effect of age, we determined the effect of age on serum vitamin D levels in this study and indeed like others (Abu Nailah et al 2015) found no significant differences in serum vitamin D levels by age.

The effect of gender on serum vitamin D levels is widely recognized and indeed we also report gender differences in the current study. We however further compared the median vitamin D levels stratified by gender and HIV/TB status and observed statistically significant differences in females (p=0.0005) and also a statistical significant difference in males alone (p=0.04). This data is not presented in the current form of the manuscript but reinforces the fact that our findings could not be attributed solely to gender imbalance since differences were maintained even after stratifying by gender.

The recruitment of study participants for the clinical trial and HIV negative participants was in fact done during overlapping periods. This was clarified by stating ‘The RCT LAM was enrolling patients at the same period as recruitment was taking place from the city of Harare clinics, the institutional voluntary HIV testing and counseling centres in Harare’ in the revised manuscript (Methods section, Study Participants subsection, lines 118-120, pages 6-7). The sample size for the randomised controlled trial was 920 participants and from these we randomly selected the requisite number of HIV and PTB positive participants for the present study. We resorted to enrolling healthcare workers and volunteers presenting to the city clinics, voluntary counselling and testing centres because the HIV testing volunteers originated from the same source population as the TB cases that were HIV negative. The healthcare workers on the other hand, were employed at the facility admitting TB patients and were therefore exposed with some of them being possibly asymptotically infected. In our view these adequately matched the TB infected but HIV negative strata.

(3) Patient recruitment is not clearly described in the manuscript. A Figure 1 outlining recruitment success should be added (how many patients were approached for consent, how many gave consent, how many were excluded for any reason). Furthermore, timing of measurement of vitamin D may have been at different stages of HIV or TB disease (prior to or during HIV treatment, early after TB infection or reactivation, paucibacillary or multibacillary TB, low CD4 or high CD4, cavitary or non-cavitary). These variables may affect vitamin D levels at the time of measurement. These data may not be available to the authors.

Authors’ response:
A figure outlining recruitment success and process has now been incorporated into the manuscript (Figure 1) and cited in Methods section, line 105, page 6. For the recruitment from the RCT LAM, we have referenced our two publications 11, 12 (Methods section, line 120, page 7). We are also in agreement with the reviewer on the possible effect of the factors stated, on the vitamin D status but regrettably this data was not collected during the recruitment process and it is now impossible to revert to the participants now for that data.

(4) A few small additional improvements would be describing mycobacterial culture/smear/Xpert lab methods, "Mann-Whitney" instead of "Manney-Whitney", and don't reverse the order of "HIV/PTB" throughout the report.

Authors’ response:

The methodology has been improved by incorporating aspects of mycobacterial culture/smear/Xpert lab methods under a new sub section ‘Bacteriological testing’ Methods section, lines 129-145, pages 7-8. Two additional references (12,13) were inserted in that section necessitating revision of numbering of subsequent references in text. These are also highlighted in yellow. Manney-Whitney was changed to Mann-Whitney (statistical analyses section, line 174, page 9). The term ‘HIV/TB’ has been standardised throughout the manuscript.

(5) A high threshold for retaining variables in the multivariable regression was used (p<0.25), but with the few variables included, this is not inappropriate.

Authors’ response:

We agree with the reviewer, but this is biostatistically acceptable.

(6) Overall I think the conclusions are too strong for the design weaknesses, and the paper should acknowledge that it would be difficult to make a conclusion about this association with the data collected. Still, I think the paper should be revised and published.

Authors’ response: The reviewer’s comment may have been partially based on our error of describing our study as a case control. We have corrected this to a cross-sectional study. As this is an analytical cross sectional study, we believe our conclusions are valid albeit with limitations. We indeed under Limitations (Limitations section, line 311, page 15) acknowledge the innately defective nature of the study design. Further in-text the manuscript also cautions on the unavailability of other parameters that might have impacted on the findings. Included were the lack of data on sunshine exposure and the possible impact of seasonality.

Reviewer #2: General comments

The authors studied serum vitamin D levels in 551 participants from Zimbabwe, and make comparisons based on HIV status and the presence of pulmonary TB. Contrary to previous studies, vitamin D levels were higher in participants with PTB. The strengths of this study are — a fairly large sample was studied, and vitamin D levels were estimated by CLIA.
Authors’ comments:

We thank the reviewer for their comments. We address their specific comments below:

Major comments

(1) Study design: This was a cross-sectional study, not a case-control design as reported.

Authors’ response:

We thank the Reviewer for pointing out this error. Indeed our study is a cross-sectional study, an error on our part for calling it case-control. We have corrected this (Background section, line 88, page 5), Limitation, line 309, page 15).

(2) Extrapulmonary TB is common among HIV-infected persons. It is unclear whether patients with isolated extrapulmonary TB were excluded from the HIV+PTB- group.

Authors’ response:

All the TB cases were diagnosed with pulmonary TB hence our referring to PTB throughout the manuscript.

(3) If one carefully looks at the distribution of vitamin D levels (Fig 1), it appears that a handful of higher values are possibly influencing the observed difference in medians. Hence, it is prudent to rule out bias and confounding as possible explanations.

Authors’ response:

We are of the view that outliers generally tend to cause skewed distribution in the mean and the range but the median and mode will remain largely unaffected. Based on this assumption we used the median rather than the mean for cross group comparisons. We however reanalysed the data after excluding outliers and the results that were originally obtained did not vary much. We therefore retained all values as obtained in the original manuscript.

(4) One potential reason could be that HIV-negative and HIV-infected participants entered the study through different enrolment strategies. In particular, the HIV-infected patients were participants in a trial of POC urinary LAM testing strategy. Could this have introduced a systematic difference among the participants?

Authors’ response:

Indeed the participants entered the study via different enrolment strategies but it is noteworthy that although the enrolment strategies were different, enrolment was concurrent and conducted in overlapping periods. Whilst we do not dispute the possibility of systematic differences among the participants, all participants originated from the same source population.
(5) Second, were any of the participants taking multivitamin supplements before enrolment? Could this explain a few values in the higher range?

Authors’ response:

During the screening period, potential participants were excluded if they were taking multivitamins.

(6) The authors offer two potential explanations for the higher levels of vitamin D seen in PTB - low body-mass index as a result of malnutrition, and 1α-hydroxylase activity of granulomas. Were data on BMI and disease severity (such as radiological scoring) collected in this study? If available, the authors may test these suppositions.

Authors’ response:

We agree with the reviewer’s suggestion of including BMI and disease severity in the analysis, however, we did not collect data on BMI and disease severity. The data on BMI may however be referenced to findings from other studies.

Minor comments

(7) Was the vitamin D assay validated against the VDSP standards?

Authors’ response:

The Maglumi 25-OH vitamin D assay uses calibrators from SIGMA but has not yet been validated against the Vitamin D standardisation Program. However, the method is registered in the Vitamin D External Quality Assurance Scheme that has been accepted by the College of American Pathologists (CAP) as an alternative proficiency testing scheme for 25-OHD.

(8) Table 3: "Co-variates of serum vitamin D levels" - Since a logistic regression was used it would be appropriate to call this as co-variates of vitamin D status, rather than levels.

Authors’ response:

We agree with the Reviewer’s suggestion, we have changed "Co-variates of serum vitamin D levels” to "Co-variates of serum vitamin D status.

(9) The adjusted ORs may be reported in the Table 3.

Authors’ response:

The adjusted Odds ratios were deliberately left out of Table 3 as including them would clutter Table 3.
Thank you for receiving our revised manuscript and considering it for further review. We appreciate your time and look forward to your response

Thank you

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