Author’s response to reviews

Title: Interferon-γ release assay as a sensitive diagnostic tool of latent tuberculosis infection in patients with HIV: a cross-sectional study

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Article Title: Interferon-γ release assay as a sensitive diagnostic tool of latent tuberculosis infection in patients with HIV: a cross-sectional study

On behalf of my coauthors, I am pleased to resubmit our amended manuscript to BMC Infectious Diseases and would like to extend our gratitude to the Editors who have helped us improve our manuscript. We have revised our manuscript and have addressed the queries, point by point. Below we inventory each query and our response. The revised manuscript has also been uploaded as a single, final, clean version without tracked changes.

Thank you very much for the opportunity to resubmit our revised manuscript.
In this study, LTBI is defined as either TST or IGRA positive. Thus, it is a bit of a circular argument to show how IGRA or TST are associated with LTBI with this definition. High concordance / high kappa values are not surprising in this context. This deserves a bit of discussion. For instance, it is interesting that the IGRA concordance with LTBI is higher – why might that be? This also deserves more attention in the limitations paragraph (lines 345-353).

Authors' response: We appreciate these queries. Editor`s comments refers to a key point in our study that is the role of TST and IGRA for the diagnosis of LTBI. Indeed, we defined LTBI by the presence of risk factors plus any of these positive tests (TST and / or IGRA). Notwithstanding, IGRA demonstrated better agreement with the diagnosis of LTBI (Kappa: 0.769) and the agreement between TST and LTBI was poor (Kappa: 0.384). This may be explained by the fact that IGRA had better performance among those patients presenting lower levels of CD4 + T lymphocyte count. Therefore, in this population IGRA was able to detect LTBI with greater reliability. Another explanation for the better agreement between IGRA and LTBI is that recent contacts with smear-positive patients may still show negative TST results during the first 2 to 8 weeks after infection with M. tuberculosis (phenomenon of late hypersensitivity). On the other hand, it is expected that IGRA conversion occurs early after infection in the same situation. We also argue that a longitudinal study using the TST and IGRA
for the diagnosis of LTBI would better answer this question and perhaps explain the greater agreement between IGRA with diagnosis of LTBI. This argument, as suggested, was added in the paragraph of the limitations of the study. (line 348-354)

One reason why the IGRA was introduced was the bias in TST due to BCG. The authors have collected BCG status – they should look at the association between BCG positivity and TST and IGRA positivity. Does BCG explain the difference seen in the two assays?

Authors' response: This is an interesting and relevant topic to be addressed, thank you. The BCG vaccine may lead to false-positive TST results, which may interfere with the LTBI investigation. However, TST may show positive results mainly during the first two years after BCG vaccination and it is believed that among those vaccinated with BCG over 15 years ago, such interference would not be relevant (Wang, 2002). Conversely, most authors believe that IGRA is not influenced by prior BCG vaccination since the proteins included in the test (IGRA), such as ESAT-6 (Early Secretory Antigenic Target-6) and CFP-10 (Culture Filtrate Protein-10) are not present in BCG vaccine strains. However, we do not believe that the high coverage of BCG vaccination in the study population explains the disagreement among the tests, since our study population were all adults and the greatest discrepancy was observed in 21 patients (TST negative and IGRA positive).

More recent literature has been citing the prevalence of Mtb infection as one-quarter to one-third - this should be modified (line 64).

Authors’ response: We thank the reviewer for pointing out this issue. We amended the text and the reference (3) as requested. (line 63-71).

Abstract: The methods should state how LTBI was defined, not putting this definition off until the Results. I recommend moving the text from lines 47-48 up to line 43. Also, insert “level of” before “risk” on line 54.

Authors’ response: We amended the text as requested by providing the proper definition of LTBI and also inserted “level of”. (line 44-46, and 53).

Line 90 – IGRAs are not generally used for diagnosis of TB, so remove “and TB disease”.

Authors’ response: We amended the text as requested. (line 89).
This was pointed out in the original review: It is not clear why weight loss and/or fever in the index case are factors considered in risk of transmission for TB. At least that is not typical in the literature. Do the citations [4,20] include those two clinical characteristics? If not, another citation should be provided.

Authors’ response: We thank the reviewer for these thoughtful comments. Patients living with HIV showing symptoms such as cough, weight loss and fever were considered as having active TB for a long period of time with a delayed diagnosis (advanced disease) and likely to be the index case for the transmission of M. tuberculosis. According to WHO guidelines, HIV-infected adults and adolescents should be asked during all outpatient setting clinical visits for the presence of relevant symptoms of active TB such as: cough regardless of duration; fever; weight loss and night sweats (WHO, 2012). Therefore, we consider the presence of cough, fever and weight loss in the index TB.

Conde et al (reference 4), describes in detail the relevance of signs and symptoms of active TB, and De Rose et al (reference 20) highlights the importance of the duration (hours) of exposure to smear-positive patients as an independent risk for acquiring TB. Two additional references were added emphasizing the importance of weight loss and fever for increasing the diagnosis of active TB among HIV-positive patients. (reference 21 and 22).


It may be problematic that “undetermined” IGRA results were coded as positive. This could introduce a bias, and in general, “undetermined” results are coded as missing for analyses. The authors should either conduct a sensitivity analysis to see if the inclusion of “undetermined” with “positive” influences the results, or alternatively, look carefully at the quantitative IGRA values to see if this is justifiable. This may also explain why the concordance between IGRA and "LTBI" was so high. The number of "undetermined" results must be provided as well.

Authors’ response: This point is well taken. Although not having been previously described in the manuscript, we had only one patient with indeterminate IGRA result, in which a low response to mitogen was observed, but with TST positive. Inconclusive results of IGRA have been associated with HIV infection and lower levels of CD4 cell count. Interestingly, this patient had a normal CD4 + T lymphocyte count (938 cels/mm3), but we were unable to find a clear explanation for this result. We included this information to the results. (line 267-269)
Line 203 – the use of kappa statistics is mentioned for the second time in the paragraph. This seems repetitive and should be removed.

Authors’ response: Thank you for pointing out this typographical error, which we have now amended in the text. (line 201)

Walter N. Dehority (Reviewer 2):

The authors have done a thorough job responding to all queries. The manuscript is significantly improved. From a content standpoint, I feel they have revised and/or adequately addressed all concerns. A few minor grammatical/formatting issues should be addressed prior to any publication:

Authors’ response: We appreciate these comments.

Line 117: There is a 0 in front of ‘participate’

Authors’ response: Thank you for pointing out this typographical error, which we have now amended in the text. (line 115)

Line 124: ‘…with at least one positive test…’

Authors’ response: Thank you for pointing out this typographical error, which we have now amended in the text. (line 122)

Line 155: ‘…the reading was made…’ or ‘…the reading occurred…’

Authors’ response: Thank you for pointing out this grammatical error, which we have now amended in the text. (line 153)

Lines 156-157 ‘…and using ruler in millimeters of the National Health Foundation for measurement…’ is unclear. Is this meaning that mm were used for measurement by use of a ruler, per NFH recommendations?

Authors’ response: Thank you for pointing out this error. We amended the text as requested to make it clearer. (line 155-156)
Line 158 probably mean 'reactive' not 'reactor'

Authors’ response: Thank you for pointing out this typographical error, which we have now amended in the text. (line 157)

Line 268-271: Would re-write similar to 'Although no correlation was found between TST and risk, a good correlation was found between IGRA and risk…'

Authors’ response: We amended the text as requested to make it clearer. (line 267-269)

Line 271: 'Using a threshold value of 0.51 IU/mL for the IGRA yielded…'
Authors’ response: We amended the text as requested. (line 269-270)

Lines 310 to 320 and 354 to 362 are well written and add greatly to the text
Authors' response: We thank the reviewer for these thoughtful comments

Line 347: '…even with application of a scoring system…'
Authors’ response: We amended the text as requested. (line 345)

Line 370 '…following a clinical evaluation and categorization of risk…'
Authors’ response: We amended the text as requested. (line 376-377)

Line 377 '…and ease of performance.' Missing a period.
Authors’ response: We amended the text as requested. (line 383)

Lines 380-386: Probably should be re-written as it appears as a run-on sentence.
Authors’ response: We amended the text as requested. (line 386-391).