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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Author’s response to reviews:

Dear Catherine Stein

Editor, BMC Infectious Diseases.

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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 reviewers who have helped us improve our manuscript, by providing constructive and useful comments. We have revised our manuscript and have addressed the comments and queries provided by the reviewers below, point by point. Below we inventory each reviewer comment and our response. The revised manuscript has also been submitted with all changes tracked.

Thank you very much for the opportunity to resubmit our revised manuscript.

Kind regards,
Reviewer comments:
Reviewer #1 (Major Comments for the Author):

Harriet Mayanja-Kizza (Reviewer 1): The study looked at persons with HIV most stabilized on ART, clinically classified risk for LTBI.

Issues to consider

1. Clinical definition of risk key to study, needs better presentation

Authors’ response: We thank the reviewer for these thoughtful comments. We addressed the “clinical definition risk for LTBI” by adding more information and therefore, making it clearer.

a. Lines 120 to 130

HIGH - "Individuals at high risk for LTBI were those with a history of contact with a household member with active TB and/or contact with smear positive individuals in the past two years, associated with the following signs and symptoms observed in the index case: cough (> 3 weeks) plus at least one of the following: (a) contact with individuals who had unintentionally lost more than 10% of body weight, (b) fever (> 38 °C), and (c) night sweats."

This is a bit unclear. What was the basis for diagnosis of active Tb in the index. What is the relevance of contact with a person who had lost over 10% body weight among the smear positive
contacts. How positive was smear positive. Information like smear and degree of lung disease e.g. cavities would be more useful information here.

Authors’ response: High risk patients for LTBI was defined based upon extensive period of household contact with a smear-positive pulmonary tuberculosis person, which may have occurred during nocturnal as well as extensive diurnal periods. In addition, TB index-case patients should not have started TB treatment and presented signs and symptoms of active disease, which means cough longer than 3 weeks plus 10% loss body weight, fever or night sweats. We argue that the higher change of transmission is associated with those sharing the same house with a highly symptomatic and not treated TB index case patient.

The main diagnosis criteria for active TB in the index case was microbiology; presence of sputum with positive direct bacilloscopy (by Ziehl-Neelsen staining), culture in Löwenstein-Jensen medium or an anatomopathological examination showing caseating granulomas and acid-fast bacilli in tissue specimens. The lung tissue fragments used in the anatomopathological study were obtained by means of transbronchial biopsy. Negative microbiological results on smears or lung tissue cultures were excluded despite the presence of cavities on chest x-ray. In the manuscript, we amended the text as requested by adding a better explanation of high risk for LTBI. (p 6, line 129-134)

b. Lines 125 to 126

LOW - "Individuals at low risk were those with a history of contact with the bacillus in the past two years only outside the household, associated with one of the signs or symptoms of the index case as above."

How was contact outside household determined? Was this at work, school or other degree of contact. This group seems to be between the high and low risk.

Authors’response: This point is well taken. Individuals with low risk were those with outside household contact history not sharing the domicile, but instead they share the same physical space at work, during educational or social activities with the index case. In addition, index case must not have started TB therapy and present with only one of the following signs or symptoms of the index case: cough (> 3 weeks), fever (> 38ºC), sweating or weight loss (> 10% of body weight). The principal distinction between high and low risk patients for LTBI was presence of household contact and cough in the high-risk group.
c. Lines 127 to 130

LOW - "Other individuals at low risk were those with no history of contact with smear-positive individuals who also had a positive TST and those who had no history of treatment for LTBI in the last two years."

Again, this is unclear. How far back was a history of a positive TST? What of those with no contact and no prior TST? In this study, was there any difference between the 2 low risk groups - since they seem to have differing in their exposure to active TB contacts.

Authors' response: We appreciate these queries. Indeed, among this subgroup of low risk individuals TST turned positive in the last year. Individuals with positive TST (who were negative in the past year) and without earlier treatment for LTBI in the last two years, in the absence of contact with persons with active tuberculosis were considered low risk. This aimed to contemplate those who had turned to TST positive in the last year, but no individual fulfilled this criterion. We amended the manuscript by excluding this definition from the methods.

d. Who were the persons with "absent" risk of LTBI. Are they the ones who are double negative. if so, this is using the study results to phenotype this group.

Authors' response: We thank the reviewer for pointing out this issue. In fact, to be included in the study, HIV-infected patients must have presented high or low risk for LTBI. Double-negative tests (TST and IGRA) individuals presented any sort of risk for LTBI. Individuals who were double-negative for TST and IGRA were considered LTBI-free. (page 6, line 148-149).

2. Lines 221-222 "Briefly, among 80 patients who met the study criteria for LTBI (LTBI risk and at least one positive test, TST or IGRA), 59 (73.7%) were TST positive, while 21 (26.2%) were negative."

It appears a positive TST or IGRA were used to determine the LTBI risk phenotype. But then they were also used to study and compare with the clinically determined risk of LTBI. This needs clarification.

Authors' response: This point is again well taken. The definition of high and low risk for LTBI was solely based upon clinical criteria, although not using laboratories methods. Therefore, the results of TST and IGRA did not define risk for LTBI, but for diagnosing LTBI among HIV-positive patients presenting high and low risk for LTBI. This has been clarified in the manuscript, where we amended the Table 3 caption to make it clearer.
2a. In table 3 the term "absent" is not very clear. If this is clinical risk of LTBI, then its better to write "Risk for LTBI" rather than "LTBI" in table 3; this is better presented in table 4.

Authors' response: We thank the reviewer for these thoughtful comments. Nevertheless, Table 3 refers to TST and IGRA results (positive or negative) according to the diagnosis of patients with (80 patients) or without (10 patients) LTBI. We argue that this is an important information for the readers since the Table 4 we compared the differences in sensibility of TST and IGRA among patients presenting high and low risk for LTBI. We amended the Table 3 caption to make it clearer.

b. It may be a better idea if Table 3 and 4 could be combined. Then one can compare results among the high, low and absent LTBI risk.

Authors' response: As previously mentioned, HIV-positive patients to be included in the study must have presented any risk for LTBI. Moreover, Table 3 compared the results of TST and IGRA among patients with (80 patients) or without (10 patients) LTBI (test and the diagnosis of LTBI). Table 4 compared the differences in sensibility of TST and IGRA among patients presenting high and low risk for LTBI (test and the risk of LTBI). We amended the Table 3 caption to make it clearer.

3. Results are shown for each single test, but this does not give an indication of concordance of TST and IGRA results. One cannot tell which patients were double positive and which ones single positive. It's possible the patients positive for IGRA, may not be the same as are positive for TST.

Authors’response: We thank the reviewer for pointing out this issue. We added a new Table (6), in which we described discordant results of TST and IGRA performed in 90 HIV-positive patients under the risk for LTBI. We have amended the text and added Table 6 in the results section.

4. Table 3 and 4 need more clarification

Authors' response: We have amended Table 3 to address this comment. Table 3 captions were changed to make it clearer and we amended the head columns, by including “Diagnosis of LTBI” and replaced the terms “absent” and “present” for “yes” and “no”. Table 4 compared the differences in sensibility of TST and IGRA among patients presenting high and low risk for LTBI (test and the risk of LTBI). We amended the Table 3 caption to make it clearer.
Table 3. Description of frequencies related to the TST and IGRA result in 90 HIV infected patients at risk of LTBI.

Authors’ response: Table 3 compared the results of TST and IGRA among patients with (80 patients) or without (10 patients) LTBI (test and the diagnosis of LTBI). We amended the Table 3 caption to make it clearer.

Table 4. Description of frequencies related to the TST and IGRA result, according to the risk for LTBI in 90 HIV-infected patients studied.

Authors' response: Table 4 compared the differences in sensibility of TST and IGRA among patients presenting high and low risk for LTBI (test and the risk of LTBI).

Overall the methodology of determination and clinical classification of risk for LTBI needs better description.

Authors’ response: We thank the reviewer for these thoughtful comments. We agree that the clinical classification of risk for LTBI needed better description in the method. In the manuscript, we amended the text as requested by adding a better explanation of high risk for LTBI (p 5, line 120-146) Briefly, high risk patients for LTBI was defined based upon extensive period of household contact with a smear-positive pulmonary tuberculosis person. Low-risk patients were those with outside household contact history not sharing the domicile, but sharing the same physical space at work, during educational or social activities with the index case.

It seems both clinical and either a positive TST or IGRA were included in determining clinical risk. Also, the description of the TB contact was not very clear.

Authors’ response: The definition of high and low risk for LTBI was solely based upon clinical criteria, although not using TST and IGRA results. Therefore, the results of TST and IGRA did not define risk for LTBI and were applied to the diagnosis LTBI among HIV-positive patients. TB contact was described as those presenting contact with smear positive individuals. Household TB contact was described as an individual that shared the same house with a smear-positive pulmonary tuberculosis person occurring during nocturnal as well as extensive diurnal periods. Individuals with low risk were those with outside household contact history not sharing the domicile, but instead they share the same physical space at work, during educational or social activities with the index case. This has all been clarified in the manuscript. (p 5, line 120-135)
Walter N. Dehority (Reviewer 2):

Overall, this is an interesting study addressing an important issue in the management of patients with LTBI. The authors are to be commended on undertaking this project. A few specific comments are included below.

Introduction:

Well written, and represents a good discussion of the issues.

Authors’ response: We thank the reviewer for these thoughtful comments.

Methods:

How is active TB defined in contacts (page 5, line 120)? Did they require culture-confirmation? PCR/DNA amplification? Chest X-ray only?

Authors’ response: We appreciate this query. The definition for active TB in the index case was based upon microbiology; presence of sputum with positive direct bacilloscopy (by Ziehl-Neelsen staining), culture in Löwenstein-Jensen medium or an anatomopathological examination showing caseating granulomas and acid-fast bacilli in tissue specimens. The lung tissue fragments used in the anatomopathological study were obtained by means of transbronchial biopsy. Negative microbiological results on smears or lung tissue cultures were excluded despite the presence of cavities on chest x-ray. (p 6, line 142-148).

Page 5, line 125: What is meant by ’…contact with the bacillus…’? Is this meant to refer to smear positive individuals, those with culture positive disease, those with suspected active TB without microbiologic confirmation? I would try to clarify this point.

Authors’ response: Thank you for pointing out this typographical error, which we have now amended in the text. We amended the sentence for “those with a history of contact with smear-positive pulmonary tuberculosis person”. “those with outside household contact history not sharing the domicile, but instead they share the same physical space at work, during educational or social activities with the index case.” (p 6, line 135-137)

--Lines 127-129, page 6: It is stated that ’…those who had no contact with smear positive individuals who also had a positive TST…’ were considered low risk. But if they were in contact with a smear positive individual with a non-reactive TST, this individual may have had a reactive
IGRA test, or never have undergone IGRA testing and had floridly active TB but according to this definition, would their contacts would be considered low-risk just because the TST was not reactive? Were symptoms (or the lack thereof) part of this classification, or chest X-rays? It also states that those without treatment for LTBI in the last two years were considered low-risk. What if they had received treatment for LTBI? Did this change their classification? Some data suggests that IGRA's may exhibit sustained positivity in adults with prior TB infection. Hence, with prior LTBI (and hence possible prior LTBI treatment), a reactive IGRA may represent reactivity from an old LTBI, not from a recent exposure. I would clarify this section.

Authors’ response: We thank the reviewer for these thoughtful comments. Indeed, we agree with the referee that this low-risk classification in which persons with neither history of contact with smear-positive tuberculosis patient, nor earlier treatment for LTBI in the last two years, but with TST positivity in the last year, is controversial. We attempted to contemplate those who had turned to TST positive in the last year, but no individual fulfilled this criterion in our study. We therefore amended the manuscript by excluding this definition from the methods. (page 6. line 135 – 137)

Chest x-ray was performed for all patients included in the study, especially to rule out active tuberculosis. However, as the reviewer pointed out, radiological findings alone may indeed support the diagnosis of LTBI, but we did not use it as an isolated criterion for the diagnosis of LTBI among the HIV-positive population included in the study.

It is important to point out that those patients with earlier treatment for LTBI in the past years were excluded from the study, as it would have influenced IGRA results. We also clarified the definition of LTBI in the manuscript, by considering those with a definite risk of LTBI (high or low) with at least one positive test (TST or IGRA), in which tuberculosis disease was absent (clinical manifestations of TB and/or radiological signs suggesting TB), and with smear-negative for tuberculosis in at least two sputum samples.

What is the background data on TB in this population (Sao Paulo)? Information such as the incidence rate/prevalence may help readers determine whether the data from this study would be applicable to their patient population.

Authors’ response: We appreciate this query. Brazil is among the 20 countries with the greatest tuberculosis burden in the world, with a special importance for TB-HIV coinfection, in which tuberculosis is the main cause of death among the HIV-positive population. During 2015 in southeastern part of Brazil, especially in the city of São Paulo, 78.6% of tuberculosis patients underwent HIV testing and 9.9% of them were coinfected. We have included this information. (page 3, Lines 72-73) and reference in the manuscript.
Is Mycobacterium bovis endemic in this region? The effect of this organism on IGRA and TST testing is interesting, and if it is endemic, would probably warrant a brief discussion of how or if it would have affected your results.

Authors’ response: Mycobacterium bovis infection is not endemic in our region. However, it is worth mentioning that vaccination with BCG, universally performed at birth in Brazil, may influence the TST results, affecting its specificity with possibility of false-positive, as well as infections with other mycobacteria.

Were CXR’s done on LTBI patients? Were they asymptomatic, or was this known? How was it ensured that they likely had LTBI as opposed to active TB? This would be important and central to the study.

Authors’ response: All LTBI patients were routinely submitted to chest x-ray (page 5, line 124), and by definition, all LTBI patients were asymptomatic. Beyond routinely clinical and radiological evaluation, when indicated at least two samples of sputum were collected for BAAR identification.

There should be a definition of LTBI in the methods section. The first time this is mentioned is on page 10 in the results section, by my read (lines 213 and 221).

Authors’ response: We thank the reviewer for this query. The definition of LTBI was included in the method section (page 5-6, line 124-127), which we considered as those with a definite risk of LTBI (high or low) with at least one of the positive (TST or IGRA) tests, in which tuberculosis disease was absent (clinical manifestations of TB and/or radiological signs suggesting TB), and with smear-negative for tuberculosis in at least two sputum samples.

At least one positive test (either IGRA or TST) was required for classification of LTBI. This may represent a form of detection bias, however, as a positive result for either test was required for entry, and then the performances of each test were assessed. If, for example, IGRA positivity was one possible criteria for enrollment, and then one assessed the sensitivity and specificity of the IGRA test (upon a sample which required IGRA positivity for enrollment), it may be difficult to determine the true accuracy of this test. If one enrolled, alternatively, a group of individuals whose risk of LTBI was independent of the test being assessed (e.g. epidemiological risk factors only), this may provide a less biased estimation of the utility of the assay. Would address this issue in the text and/or results section. This is very tricky, admittedly, given the lack of a gold standard for this disease.
Authors’ response: We thank the reviewer for these thoughtful comments. Unfortunately, the definition universally accepted for diagnosing LTBI does necessarily include the performance of TST or IGRA test, which indeed may have biased our results. One attempting to assess the accuracy of a specific test for a defined disease or clinical situation, should not include the same test in the criteria for the definition. Nevertheless, our results showed a strong association between positive IGRA results and the high risk for LTBI in the HIV-positive population included in the study. Moreover, among those HIV-positive patients with lower CD4 count, IGRA accuracy was superior when compared to TST. This may represent a strong association between IGRA and epidemiological risk factors itself. In this context, a recent Brazilian Ministry of Health recommended that for those HIV-positive patients with CD4 count equal or lower than 350 cells/mm3, with a defined epidemiological high risk for LTBI and when TB disease was absent should receive therapy regardless of TST or IGRA test results. We also included update recommendations from WHO for latent TB infection in the HIV-positive population. We included these sentences and reference in the discussion section. (page 16, line 360 – 375)

Skin color is listed in Table 1. I would re-write this as ethnic or racial classifications. The categorization as 'black' or 'brown' may be offensive to some and doesn't carry any clear medical significance of which I am aware.

Authors’ response: We thank the reviewer for this query. We agree that skin color classification play an insignificant role in our study and we therefore excluded this information from Table 1.

The categorization of HIV control (viral load, CD4 counts) is important; the addition of this strengthens the manuscript.

Authors’ response: We agree with the referee. The impact of CD4 cell count on the accuracy of TST and IGRA for HIV patients with any risk for LTBI were analyzed, which showed that negative TST and positive IGRA were statistically associated with patients presenting an average CD4 cell count lower than 250 cells/mm3 (Figure 1). On the other hand, the association between viral load and the accuracy of TST and IGRA test was also assessed, but no statistical significance was identified (data not shown in the manuscript).

Discussion

Good discussion of the limitations of the study. Also a nice discussion of the limitations and advantages of IGRA and TST testing on page 14.

Authors’ response: We thank the reviewer for these thoughtful comments.
Might need a brief mention of resource-limited countries where IGRA testing may not feasible, and reliance upon the TST may still be necessary.

Authors’ response: We thank the reviewer for this query. In developing countries with high TB-HIV coinfection burden, and limited public financial resources, TST must be considered the first choice for LTBI diagnosis due to its low cost and ease to perform. We have included this sentence in the discussion section. (page 17, line 381-383)

References

Reference 8 is the 2013 'WHO Global Tuberculosis Report', accessed in 2014. However, there is a 2017 WHO 'Global Tuberculosis Report’. As this reference is 4 years out of date, would consider replacing it (and any referenced data) with more current information.

Authors’ response: Thank you for your suggestion. Add new references to numbers 3, 6, 8, 32 and change the order from the old reference 6 to 7. We replaced old reference 3, 7, and 8 with another one with more current information. We add new references new information in the text.

New Reference:


Reference 19: the 'F' in feb should be capitalized.

Authors’response; We thank the reviewer for this observation. and we changed the format to capital letter.