Author's response to reviews

Title: Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN-g for the evaluation of pleural tuberculosis: a prospective cohort study.

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The Chief Editor
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Re: Comparison of same day diagnostic tools including Gene Xpert and unstimulated IFN-γ for the evaluation of pleural tuberculosis: a prospective cohort study.

Thank you for reviewing our paper and sending back useful comments. This has helped to clarify areas of uncertainty and has improved the presentation of our manuscript. We have provided a point-by-point rebuttal to the editors and the reviewer’s comments. Where we have amended the manuscript, we have outlined this in red text.

Best wishes,

Keertan Dheda

Editors’ Comments:

1. The costs / benefits ratio of the different techniques should be analysed, considering not only ADA, IFN-γ and Xpert MTB/RIF assay, but also some classical approaches like pleural biopsy, pleural fluid analysis and thoracoscopy.
Response 1: This is answered in detail to the reviewers comment below. However, in a nutshell what the reviewer is proposing is way beyond the scope of this paper and would constitute a paper on its own. We have, however, addressed the comment satisfactorily and appropriately.

2. The interest of the Xpert MTB/RIF assay in providing information about rifampicin susceptibility should be better discussed regarding prognosis of pleural TB
Response 2: We have addressed the issue of rifampicin susceptibility in the response below.
3. The possible effect of BCG vaccination on levels of IFN gamma should be discussed.
Response 3: This is addressed with the reviewer comments below.

4. The authors should clearly indicate the limit of their study due to the small sample size and that further studies encompassing a larger number of patients are needed to validate the conclusions.
Reviewer 4: We have addressed the concern about sample size and made some cautionary comments about this issue. Details are provided in the response to reviewer’s comments below.

**Reviewer 1: Gaetano Caramori**

**General comments**
The topic is clinically relevant. The diagnosis of pleural TB is challenging due to the paucibacillarity nature of the biological samples as discussed by the authors in the introduction. Because of this, to find methods that facilitate the diagnosis of this clinical manifestation of TB is important. The data are interesting and well presented.

**Major Compulsory Revisions**

1. It would be interesting to analyze the costs / benefits ratio of the different techniques. Some methods may be more effective but also more expensive particularly in the less affluent countries where pleural TB is also more common. This aspect could be analysed considering not only ADA, IFN# and Xpert MTB/RIF assay, but also the role of classical approaches like pleural biopsy, pleural fluid analysis and thoracoscopy.
Response 1: The author raises an important issue about cost-effectiveness of investigations versus affordability. There are two parts to address this reviewer’s comment:
   i) Firstly, what the reviewer proposes is not possible as there is no standardised test for unstimulated interferon gamma and therefore a comparative cost analysis cannot be performed. Even if a commercially available research kit were to be used, they vary considerably in price and the various different kits are not clinically validated. Furthermore, these kits are only made in 96 well plate format and therefore it would be impossible to determine a ‘per patient’ cost as this would be determined by the volume of patients passing through a hospital or institution (in other words, the test cost per patient varies dramatically depending on the number of tests requested in that institution per day). For this reason, a comparative cost analysis is impossible.
   ii) Even if the concerns in (i) could be addressed, what this reviewer is proposing is beyond the scope of the current study and would constitute a separate paper. We have considerable experience in performing cost analyses (see Pooran & Dheda, PLoS ONE, 2013 and Pooran & Dheda, BMC Pulmonary Medicine, 2010) and collecting and comparing all the different cost components, and to present the models used, and do all the relevant sensitivity analyses is a huge task that would add considerably to the length of this paper and would clearly constitute a separate publication.
Nevertheless, this reviewer makes an important point about highlighting the importance of cost in resource poor settings and in TB endemic countries. Thus, conceptually we agree this is an important comment. We have now highlighted this in the discussion and have added the following text: “Affordability and cost effectiveness remains an important consideration in resource poor TB endemic countries. A comprehensive cost effectiveness analysis was beyond the scope of this paper, and we were unable to perform a simple cost analysis given the lack of a clinically validated commercially available unstimulated interferon gamma assay. Although the GeneXpert MTB/ RIF assay is now being rolled out in many TB endemic countries [28], as we have demonstrated, sensitivity is largely sub-optimal [6, 29]. Although ADA is widely available, specificity may also be also sub-optimal, as we and others have previously demonstrated. Nevertheless, it remains a widely available relatively low cost test. Diagnosing drug-resistant pleural TB also merits cost consideration. However, the GeneXpert MTB/ RIF assay has a poor sensitivity in this context and thus whatever diagnostic modality is used (unstimulated interferon gamma, ADA, or GeneXpert MTB/ RIF) pleural tissue or fluid culture is still required for susceptibility testing. There is an urgent need to make available a commercially and clinically validated, relatively rapid, single patient use assay for the measurement of unstimulated interferon gamma levels in pleural fluid and other forms of EPTB.”

2. The author says that Xpert MTB/RIF assay is also useful to provide information about rifampicin susceptibility: in your opinion is this an important issue that could make Xpert/MTB/RIF assay a test with a significant burden in the prognosis of pleural TB?
Response 2: We have already addressed this issue in the response above. In summary, although the GeneXpert MTB/ RIF assay is able to provide a rifampicin result, a sensitivity of approximately only 25% renders this test of little use in this context. Thus, whatever method it used (ADA, interferon gamma, GeneXpert, etc.) pleural fluid and tissue will still need to be sent off for culture in order to determine susceptibility to rifampicin. We have also addressed this issue in the paragraph that we have now put into the discussion as outlined in comment (1). Thus, susceptibility determination is important but given the low mycobacterial burden in pleural fluid, existing techniques such as PCR are not able to address this challenge.

**Discretionary Revisions**

Page 13, line 11: why the word “Although” has A in capital letter? Do the previous phrase need a dot?
Response: Thank you for highlighting this, we have made sure that the word is preceded by a full stop in the previous sentence.

**Reviewer 2: Surendra Sharma**

The study seems to be interesting. The idea and the concept are nice and further studies can be designed to validate it in a large number of samples. However, there are some points which need to be taken into consideration.

**Minor Essential Revisions:**
1. IFN Gamma higher levels in TB Patients might be due to the reason that out of 40 TB positive patients 20 were vaccinated by BCG.

Response 1: Thanks for this comment. Prior BCG vaccination cannot be a reason to explain this finding as the proportion of BCG vaccinated individuals was equal in both the TB and the non-TB group (see table 1 – 50 and 52%, respectively). Furthermore, the interferon gamma in the pleural fluid compartment differs markedly in those with TB from those with non-TB. Furthermore, we are measuring interferon gamma secretion from effector T-cells rather than from long-lived memory T-cells which would be generated by BCG vaccination. In any event, such cells would not be expected to be found in the pleural space but rather in the skin and central lymphoid organs.

2. The author should be discussing the IFN gamma results with the BCG vaccinated patients also.

Response 2: We have already responded to this comment in the response above.

3. Sample size seems inadequate do decide any technique to be inefficient or efficient. Sample size could have been larger as South Africa is a high TB burden country.

Response 3: Although South Africa is a high TB burden country, sample sizes are determined by statistical estimations and also by resource constrains. The confidence intervals of the interferon gamma sensitivity and specificity vary by between approximately 3 and 10%. Clearly for specificity estimation the study is well powered. The sensitivity estimates are reasonable, but we agree they could be better with a larger sample size (5% on either side of the median rather than ~10% as it is now). We have alluded to this in the limitation section of the discussion saying that a larger sample size would have improved our sensitivity estimates. We have now inserted the following text under the limitations section “The confidence intervals of the sensitivity estimates for interferon gamma and the GeneXpert MTB/ RIF assay are not ideal and therefore our findings should be confirmed using larger sample numbers and from different parts of the world.”

Although at first glance the sample sizes may appear small, one needs to bear in mind that pleural biopsies were done in all the patients, in addition GeneXpert assays and unstimulated interferon gamma and other tests were run in all the patients. These tests are not routinely performed in a TB endemic country and were expensive. Therefore, it is always challenging to perform studies with large sample sizes in TB endemic countries. One should perhaps qualify this by saying this that to-date the largest comprehensive study of evaluation of GeneXpert in the context of TB pleural effusion, and in the context of diagnostic studies, is of respectable size. Nevertheless, we have alluded to the sample size limitation.