Author’s response to reviews

Title: Pleural IFN-γ release assay combined with biomarkers distinguished effectively tuberculosis from malignant pleural effusion

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Author’s response to reviews:

Dear Dr Sharma,

Thank you for sending us the reviewers’ comments on our manuscript entitled "Pleural IFN-γ release assay combined with biomarkers distinguished effectively tuberculosis from malignant pleural effusion" (INFD-D-18-01612). We appreciate the thorough and constructive suggestions made by the reviewers of our initial submission. The additional comments of the reviewers have been incorporated into the revised manuscript as described in the point-by-point response attached below. We hope that the revision we have provided adequately addresses your comments/suggestions.
Sincerely,

Guoliang Zhang

Editor Comments:

Authors should take care of the comments on methods sections (Reviewers raised) and clarify what are the new findings in the MS as compared to published work by Liao et al in 2014 (Ref 14).

Response: We thank editor and reviewers for the opportunity to revise and submit the manuscript. The key point of this study is different from Liao’s published work. Firstly, the cohort is different. In this study, patients diagnosed with pneumonia or other diseases were excluded from further analysis, we just compared the differences between TPE and MPE, and all the MPE was confirmed through positive pleural fluid cytology and/or biopsy histology. Secondly, serious biomarkers were evaluated for the discriminating diagnosis of TPE and MPE in this study, involving not only ESR, CRP in peripheral blood, but also those in plural effusion, such as ADA, CEA, WCC, monocytes %, LDH, TP and GLU. Thirdly, we assessed the diagnosis efficiency of plural IGRA combined with ADA and CEA, and found a diagnostic algorithm contributed to improvement of TBE diagnosis, which haven’t been reported by other groups. Finally, the association between pleural adhesion and IGRA and biomarkers in TPE was explored, and found that pleural IGRA couldn’t be affected by the adhesion, which hasn’t been reported by Liao’s paper.

Reviewers 1

Manuscript by Tang et al is an interesting peace of work Since Tuberculous pleural effusion is difficult to diagnose authors have made an effort to find our if IFN gamma detection could help in the diagnosis of TPE. I have following issues

1. As reported by the authors IFN detection in TPE has already been reported Ref No. 12 and 14. Similar to their report study by Liao et al (Ref No.14) have already investigated IFN gamma against ESAT-6 and peptide and have already reported its utility in TPE and it could distinguish TPE with MPE. My query is what new findings authors are trying to contribute through their study.

Response: The novelty of this study is listed as above. In summary, although the same IGRA assay was used as Liao’s paper, we pay more attention to the combination of pleural IGRA, with no influence by pleural adhesion, with other biomarkers for the discriminating diagnosis of TPE and MPE.
2. English need to be checked e.g line no.53 ( ) diseases are diagnosed multiple tests.

Response: We have gone through the manuscript and revised the text (see line 281).

Reviewer 2

Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

The manuscript "Pleural IFN-γ release assay combined with biomarkers distinguished effectively tuberculosis from malignant pleural effusion" is an interesting study where authors comparatively analysed IGRA together with ADA and CEA levels in blood and pleural fluid of TPE patients and MPE cases. The findings are well descriptive and it would be good approach for differential diagnosis of TPE from MPE.

The comments are given below:

* In material method section authors stated that tuberculous pleurisy was established by manifestation of positive M. tb culture in pleural effusion. Which culture method for M.tb culturing was used is not mentioned.

Response: The Bactec MGIT 960 culture system was used to culture Mtb in pleural effusion or sputum or other biological samples according to the manufacturer’s instructions. The corresponding text was added in the revision (see line 120-124).

* Duration of sample collection was not mentioned in methodology section.

Response: A total of hospitalized 222 patients were enrolled in this investigation at Shenzhen Third People’s Hospital from January 2010 to December 2016, see new line 108-110.

* Again in methodology section nowhere it is mentioned that samples were collected from OPD or ward of which hospital?

Response: All patients were hospitalized as indicated by line 108-109 in revision.

* ESAT-6 and ESAT-6/CFP peptide pool was procured or synthesized??

Response: Recombinant ESAT-6 was expressed in Escherichia coli and purified with Ni+ affinity chromatography. ESAT-6/CFP peptides with 20 amino acids in length were synthesized
by Hanyu Company (Shenzhen, China). We have added these information in the revision (see line 136-138).

* How ADA and CEA tests were performed, description of kit or brief description...not mentioned in methodology section.

Response: We thank your good suggestions and have supplemented a new paragraph for the details of ADA and CEA detection in the revision (see line 147-154).

* In line 273 M.tb is written incorrectly.

Response: The typo has been corrected (line 290).

* Table-3 pleural is spelled incorrectly.

Response: The typo has been corrected in new Table 3.

The article needs modification.

Response: The manuscript has been improved under the help of a naïve English speaker.

Reviewer 3

In the manuscript submitted by Tang et al. entitled “Pleural IFN-γ release assay combined with biomarkers distinguished effectively tuberculosis from malignant pleural effusion” the authors trying to establish a method to improve IFN-γ release assay (IGRA) using ELISPOT assay in peripheral blood mononuclear cells (PBMCs) and pleural fluid mononuclear cells (PFMCs). They have worked on the problem of confusion between the patients of tuberculosis plural effusion (TPE) and malignant pleural effusion (MPE). The authors have shown that use of adenosine deaminase (ADA) for tuberculosis and carcinoembryonic marker (CEA) for malignancy, during IGRA, can be helpful to distinguish between TPE and MPE. The authors have nicely planned and executed the experiments and conclusions are well supported by shown data. I have following concerns/suggestions regarding this article.

1. The material method section does not have any information regarding the experiments which were carried out using ADA and CEA, the authors should provide the details.

Response: The related information about detection of ADA and CEA has been added in the revision (line 147-154).
2. This is population-based study, the authors should also analyse their finding using statistics to provide the confidence interval (CI) values.

Response: We appreciated the suggestion and 95% confidence interval values have been provided for the sensitivity, specificity, PPV, NPV and AUC analysis on the corresponding sites of revision.

3. In table 3 authors should have made it clear which patient sample (TPE or MPE) are being evaluated in ADA and CEA groups. Also mention the number of patient samples evaluated for this particular study. Also mention what concentration of ADA and CEA was used.

Response: We have provided more details in the Table 3, including the number of patients evaluated, cut-off value, and 95% CI.

4. In table 2 the number of patient samples are not same in all the experiments particularly in TPE section, what is the reason behind it?

Response: Actually, not all patients were performed the tests referred in the manuscript, for example, when the diagnosis of TB was confirmed by bacterial evidences from sputum or pleural effusion, the patients won’t receive other examinations, which leads to disequilibrium of numbers.

5. What method was used to detect total protein?

Responses: We have added the details in the revision (line 150-154).

6. In figure 3 and figure 4 authors should mention in figure itself whether Pleural fluid was evaluated or lysate or secretion of PBMCs or PFMCs after activation with any agent were evaluated.

Response: After centrifuge of pleural effusion, the supernatant was used for the detection of ADA, CEA, and LDH, the isolated PFMCs were stimulated with Mtb-specific antigen. The notation was labeled on the figure 3 and figure 4.

7. In figure legend give more information about sample being evaluated.

Response: We have modified the legends according to the requirements (line 418-447).
8. Some typo errors are their in the manuscript the authors should correct them e.g. line 102 CEA has been mentioned as arcoembryonic antigen (CEN), it should be carcinogenic antigen (CEN).

Response: The typo has been corrected (line 59, 103).

Reviewer 4

The study by Tang et al aim to study TBE and MPE to assess the differential diagnostic efficiencies. However, the results shown and the conclusions drawn are not convincing as there are problems with the approach and a lack of detailed immunologic data that make the manuscript too preliminary, especially in the context of the current state of the field. I have following comments.

It is not convincing that the assays claimed in the manuscript can be used to distinct TPE and MPE. Authors claim that IGRA combined with recognition with ADA could provide potential distinction between these two diseases is not convincing. As one would still need cytology for the distinction of MPE and for TB confirmation of TB by Mtb presence or other means.

Response: Thanks for your suggestions! Indeed, the bacterial evidence is golden standard for TPE diagnosis, however, lee than 20% patients show to be positive Mtb culture in pleural effusion, which is insufficient for clinical requirements, so it is urgent to identify novel biomarkers or methods for the discriminating diagnosis. In this study, elevated Mtb-specific IFN-γ responses observed in the pleural effusion contributed to TPE diagnosis, plural IGRA assay doesn’t be affected by pleural adhesion, and combination of plural IGRA with CEA and ADA could differentiate TPE and MPE with higher sensitivity and specificity, indicating a promising and non-invasive diagnostic approach for patients with suspected TPE.

There is overall immunosuppression in malignancy. The authors choose to study IFN-g only. TPE is known to be th1 type inflammatory situation especially in the pleural fluid. However, there is overall immunosuppression in Malignancy.

Responses: Actually, TPE patients also show immunosuppression after non-Mtb-specific stimulation compared to healthy controls, however, the IFN-γ responses stimulated with Mtb-specific antigen ESAT6 and peptide were detected in this study. IGRA has been extensively studied for TB diagnosis during recent years, but the clinical samples mainly derive from peripheral blood. Since pleural effusion is enriched with Mtb antigen-experienced T cells compared with matched peripheral blood, we hypothesize that IGRA performed in the pleural effusion may be useful for TPE diagnosis.
The authors have not studied contribution of other cytokine(s) and immunologically relevant cell subset. There is overall age difference in the study cohort. In aged people Treg cells number is also more. The manuscript results and the conclusions drawn are confusing.

Response: Serious of studies have shown dysfunction of Th subpopulation was associated with TB development, for example, Chen X et al has reported that the frequency of Th17 cells decreased in TB patients, especially in severe TB patients, but the number of Treg increases in the blood or at the site of infection in active TB (Am J Respir Crit Care Med, 2010; Clin Immunol, 2007). Due to lack of specific cytokines for Treg cell, it is not suitable to be considered as diagnostic markers. Several cytokines have also been reported to contribute to TPE diagnosis, such as IL-27, showing the comparable diagnostic efficiency with IFN-γ and more accurate than that of ADA (Wang W, et al. Thorax, 2018 ), so it’s a good idea to assess the diagnostic performance of these biomarkers and their combinations in the future, which enables us to develop a rapid algorithm for the noninvasive TPE diagnosis.