Author's response to reviews

Title: Serum complement C4b, fibronectin, and prolidase are associated with the pathological changes of pulmonary tuberculosis

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Author's response to reviews: see over
Dear Editor:

We have revised the manuscript in accordance with the reviewers’ comments. Here below is our description on revision according to the reviewers’ comments:

**Reviewer 1:**

Comment 1: There are some minor grammatical errors which require corrections.

Response 1: We have revised the whole manuscript to avoid any grammar error.

Comment 2: The title indicates 3 novel serum proteins when none is novel in the context of their association with TB or the processes described with TB infection, as stated by the authors in the discussion. Thus, the title should state the three proteins profiled and their association to pulmonary TB instead.

Response 2: We have changed the title to ‘Serum complement C4b, fibronectin, and prolidase are associated with the pathological changes of pulmonary tuberculosis’.

Comment 3: A few brief statements about the rationale for choosing to look at these 3 proteins should be included in the introduction.

Response 3: We have added a few brief statements as the reasons for choosing these 3 proteins. Please see page 5.

Comment 4: Why is C4b chosen instead of C3b, which would be common to all 3 complement pathways and thus capture everything, as it is also downstream of C4b
for the MBL and classical pathway? Might there not be also some background alternative pathway activation which would reflect different immune status of patients versus controls?

Response 4: C4b is a product of activated C4 in the early stage of MBL pathway, and C3 is a product of classical pathway, alternative pathway, and MBL pathway. If we use C3 as an indicator, we may not be able to recognize which pathway it is responsible for. In addition, our previous unpublished results, using iTRAQ 2D LC-MS/MS technique, showed no significant difference in serum C3 level between PTB patients and the control group. However, a significantly different C4b level was observed.

Comment 5: The authors should include explanation on power calculations performed to determine whether there were enough samples for comparison to achieve statistical significance, particularly when samples were broken into subgroups in the patient category.

Response 5: The study sample provided 88.93% power to identify significant differences between whole PTB patients and healthy controls at a statistical support level of $\alpha=0.05$ with an $d$ of 0.6 applying a two tails model calculated by Gpower3.0.5. When identifying significant differences between subgroups, the study sample provided 75.47% power. Our research has passed the power analysis. Meanwhile, the similar sample size was also used in some other published studies, ‘Scand J Clin Lab Invest. 2011; 71(6):467-72.’, ‘BMC Infect Dis. 2011; 11:71.’.
Comment 6: Reference 4 is inappropriate. The authors should cite those several studies as mentioned in the text to show defects of innate immunity to PTB.


Comment 7: "TB cavity is formed by liquid discharge..." is not referenced.


Reviewer 2:

Comment 1: The inclusion criteria appear to indicate that pleural TB alone was sufficient for inclusion. However the classification according to radiographic extent of disease appeared to indicate all subjects had pulmonary disease.

Response 1: Patients who met the study’s inclusion criteria were diagnosed as having TB, and included clinically ruling out other causes of pleural effusion, and tuberculous pleurisy. In addition, our study was focused on the pathological changes of pulmonary TB, and we chose the modified NTA classification system to indicate the pathological changes in the lung.
Comment 2: What was the reason for including the additional 72 TB patients whose blood was not sampled?

Response 2: Because of reasons such as lost of constant contact, and incomplete data of chest X-rays, 72 TB patients were not classified using the modified NTA classification system. Therefore, we included the additional 72 TB patients whose blood was not sampled for clinical data analysis.

Comment 3: Table 2 states it shows results of a one sample t test of TB patients vs. the median of the normal reference range. The medians are not given. The P values seem exaggerated, particularly since most of the TB means fall within the normal range. See, for example, triglyceride values.

Response 3: Because our clinical data showed a non-normal distribution, so we took the logarithm to make sure the transformed data show a normal distribution. The transformed median is the average value of transformed lowest level of normal reference range and transformed highest level of normal reference range. So, the median we compared to is not the median of the normal reference range. Considering this, we did not include a row of ‘medians’ in Table 2. The P values were measured using one-sample t-test by the SPSS software, version 16.0 (SPSS, Chicago, IL). Even when the TB means fall within the normal range, a significant difference can be observed.

Comment 4: Table 3 would benefit from including radiographic extent of disease.
Response 4: We have added the NTA classification into Table 3.

Comment 5: Figure 3 would benefit from having regression lines drawn. It is difficult for me to see significant relationships in many of the graphs.

Response 5: We have added the regression lines into Figure 3.

Comment 6: Table 4 probably should not include correlations of a numeric value with a binary one (yes/no). These are better analyzed by t test.

Response 6: We have revised Table 4, and deleted the binary value.

Thank you for the kind advice.

A list of changes:

1. Page 1, lines 1-3
2. Page 4, line 20
3. Page 4, line 21 to page 5, line 4
4. Page 5, lines 12-16
5. Page 5, lines 17-19
6. Page 6, line 16
7. Page 8, lines 16-20
8. Page 9, lines 1-2
9. Page 9, lines 6-7
10. Page 11, line 2

11. Page 13, lines 8-11

12. Page 23-24, changed Table 3

13. Page 25, changed Table 4

14. Changed Figure 3

Sincerely yours,

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