Author's response to reviews

Title: Additional Role of Second Washing Specimen Obtained during Single Bronchoscopy Session in Diagnosis of Pulmonary Tuberculosis

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Version: 2 Date: 1 August 2013

Author's response to reviews: see over
We appreciate your constructive comments and criticisms of our manuscript. In response to the suggestions and questions we received, we have revised our manuscript. All changes have been marked in red ink in the revised manuscript. And we’ve changed title “Additional role of second bronchial washing specimen obtained by flexible bronchoscopy in diagnosis of tuberculosis” to “Additional Role of Second Washing Specimen Obtained during Single Bronchoscopy Session in Diagnosis of Pulmonary Tuberculosis” based on co-authors’ opinions. We believe that by making these changes, there has been substantial improvement and thank you for your efforts in this regard. We also have responded to reviewers’ comments for each point below.

Response to the comments of the associate editor

C1. The authors performed bronchial washing three times; 1st one for AFB smear and culture, 2nd one for another AFB smear and culture, and 3rd for TB-PCR. In this setting, they reported the additional diagnostic role of 2nd bronchial washing fluid. What if they performed bronchial washing once, but with enough volume of saline, and requested two times of AFB smear and culture test and TB-PCR with the retrieved saline? I am not sure the results were different from those from separate bronchial washing. A prospective study comparing the results of three separate bronchial washings and those of one washing with enough volume of saline will address the issue.

R1. The associate editor raises an important issue. It is possible that the increase in the yield of diagnosis by combining first and second bronchial washing may not be due to the addition of bronchial washing but to the larger volume of saline used for washing. However, our data and current knowledge on the yield of bronchial washing cannot determine this point. As an aliquot of respiratory sample is used for examination by direct microscopy and culture, the proportion of \textit{M. tuberculosis} present in samples is important. In other words, for smear or culture to be positive, concentration of \textit{M. tuberculosis} in each bronchial washing sample is important. However, it is not certain whether a larger volume of washing sample dilute down the concentration of mycobacteria or other pathogens in bronchial washing. If the same amount of \textit{M. tuberculosis} is retrieved regardless of the volume of the saline infused, this may lead to decreased yield by reducing the concentration of certain pathogen. Since there is no available data, it is not certain whether increase in the yield from additional bronchial washing is volume dependent phenomenon or not. Therefore, as the associate editor has mentioned, the authors agree that a prospective study comparing separate bronchial washings and single bronchial washing with the same amount of total saline is required to solve this problem. This point is mentioned in the discussion section of the revised manuscript as follows (see page 12 lines 21 to 24). “Additionally, the increased yield of TB diagnosis in our study might have been related to larger cumulative volume rather than the repeated washing process. A prospective study comparing separate bronchial washings and single bronchial washing with the same amount of total saline is required to address this question.”
Response to the comments of the reviewer #1

C1. The first sentence of the introduction is not correct. According to 2011 WHO report, there were totally 1.45 million TB-related deaths in 2010, including 1.1 million deaths from TB among HIV-negative people and an additional 0.35 million deaths from HIV-associated TB.

R1. We thank the reviewer for correcting number of TB-related deaths. We have modified the number of TB-related deaths in 2010 as follows: “Pulmonary tuberculosis (TB) is a major public health challenge worldwide, with an estimated 8.8 million new cases and 1.45 million TB-related deaths in 2010.[1]” (see page 5 line 4)

C2. The authors did not clearly state the reference method of TB diagnosis in this study. What was that?

R2. All patients in our cohort were diagnosed with pulmonary tuberculosis by microbiological confirmation. (See “Study Population” under “Materials and Methods” page 6 lines 3 to 5). Microbiological confirmation of M. tuberculosis was defined as growth of M. tuberculosis or positive test result of the nucleic acid amplification for M. tuberculosis from respiratory or tissue samples. (See “Definition” under “Materials and Methods” page 7, lines 14 to 16)

C3. The location of reference #11 is not correct. It should be after the sentence started with “it is yet known whether an additional bronchial specimen…”.

R3. We thank the reviewer for pointing this out. We’ve moved the reference #11 to right place with references #9 and #10 (page 5 line 14).

C4. A flow chart showing the patient/sample flow or testing algorithm would be helpful.

R4. Thank you for your constructive comment. We have added a diagnostic algorithm in the manuscript as figure 1 (see page 6 line10).

C5. According to the manuscript, TB-PCR was requested using bronchial washing fluid from all every patient. How many patients were diagnosed as having TB by PCR?

R5. TB-PCR using the third bronchial washing sample yielded positive result in 72 (41%) patients. Among them, TB-PCR was the sole mean of diagnosis in 2 patients. We’ve inserted this result (See page 8 lines 26 to 27 and page 9 line 1).
C6. In Table 1, the number of patients showing normal chest radiography is 3. However, in the manuscript, the authors stated that clinically suspected TB case was defined as patients having symptoms with chest radiographs suggestive of pulmonary TB.

R6. In regard to the fact that chest radiograph showed no abnormality in 3 patients, subsequent chest CT scan revealed nodular lesions, which were not detected in chest radiograph. Chest CT scan was performed in those patients in an effort to further evaluate persistent symptoms such as hemoptysis. To avoid confusion, we modified definition of clinically suspected TB case as follows (see page 5 lines 27 to 28):

TB case is clinically suspected as a patient currently not receiving tuberculosis treatment with a persistent cough for >3 weeks or symptoms consistent with TB (chest pain, low-grade fever, night sweats, shortness of breath, and weight loss) with chest radiograph or computed tomograph (CT) scan suggestive of pulmonary TB.

C7. The authors did not compare a diagnostic yield of bronchoscopic sample with those of induced sputum. I think that it is better for the authors to omit the contents related to induced sputum.

R7. Thank you for your thoughtful comment. We have deleted the content regarding diagnostic yield of induced sputum from our manuscript.

C8. In discussion, the authors emphasize a role of diagnosis of drug resistant tuberculosis from 2nd washing. But, I am not sure that the result has a significant meaning because the sample size was small and there is no plausible biological explanation. The authors should provide an explanation of their observation or omit the mention from the discussion.

R8. We understand the reviewer’s concern. Our implication was that 2nd bronchial washing sample may be beneficial not only in diagnosis tuberculosis but also in obtaining M. tuberculosis strain which allows drug sensitivity test. To clarify this point, we omitted sentence “suggesting that an additional bronchial washing is also beneficial for detection of drug-resistant tuberculosis.” and inserted following sentence in the discussion (see page 12 lines 7 to 9): “It indicates that an additional bronchial washing is a meaningful process not only in increasing the diagnostic yield but also in obtaining M. tuberculosis strain which allows drug susceptibility test.”

C9. The location of quotation of the reference is not appropriate in some cases. The authors should correct the position of reference like this, considering relevance. Following references should be corrected. (Reference 1 and 2)

R9. Thank you for pointing this out. We looked through all references thoroughly and have revised the location of quotation including reference 1,2, 18 and 19.
Response to the comments of the reviewer #2

C1. There are some discrepancies between the numbers in Table 1 and 2.

R1. The discrepancy in numbers between Table 1 and Table 2 is due to that the population of two tables is different. Table 1 shows the data of all 174 patients, however, table 2 shows the data from 163 patients who had positive results of either the first or second bronchial washing or for both. To clarify the subgroup of patients we compared, we have added figure 1 explaining flow algorithm and indicated subset number of table 2 (See page 10 line 9 and Table 2 Title) and we’ve inserted following phrase (page 12 line 10) “For patients with positive results for M. tuberculosis from bronchial washing”.

C2. Despite that in Table 2 none of the clinical characteristics showed significant statistical correlation with the positivity yield, however, correlation between important clinical data and specimens’ results should be better clarified if the data in earlier reports are available, including details and duration of previous anti TB therapy in those who had, extent of radiological abnormalities versus those with no radiological or normal CXR.

R2. We thank you for your critic comments. We understand the reviewer’s concern. With regards to sputum, previous study demonstrated that patients’ clinical characteristics including symptoms or radiographic findings are correlated with microbiological positive rates in the patients confirmed by sputum culture.[1] In other words, high positive yield rate in sputum was related to severe symptoms and presence of infiltration/cavities on chest image. Given that sputum has to be expectorated by patients, the patients with severe symptoms and presence of infiltration/cavities had a greater chance to expectorate adequate sputum samples, which increases up positive yield of TB diagnosis.

However, regarding the bronchoscopic washing samples, there have been a few studies discussing the relationship between clinical- radiological characteristics of pulmonary TB and positive rates of bronchoscopy samples. According to previous study with the patients confirmed by culture through the various diagnostic samples such as spontaneous sputum, induced sputum, gastric washing, BAL or bronchial washing, the radiographic finding and clinical characteristics did not show any difference to predict positive culture results from broncoscopy.[2,3] The culture results from direct sampling through bronchoscopy don’t seem to be related to the radiographic or symptoms severity.

As we replied to your first comment, table 2 also shows the data from 163 patients who had already positive results for M. tuberculosis from bronchial washing fluids; either the first or
second bronchial washing or for both. We clarified this part in C1. We intended to identify associated factors to predict the benefits from second bronchial washing procedure and to select patients who need serial bronchial washing. And, as we described, no specific clinical or radiologic parameters are predictive of the benefits of serial bronchial washings. To clarify this point, we changed sentence “no specific clinical or radiologic parameters are predictive of the benefits of serial bronchial washings to “For patients with positive results for M. tuberculosis from bronchial washing, there are no specific clinical or radiologic parameters which can determine who needs second bronchial washing.”

Furthermore, 51 receiving tuberculosis treatment before bronchoscopy were excluded from the study (page 6 lines 5 to 6) and only 3 patients have normal chest radiography (Table 1), which is too small number to compare. Thus, we could not go through further statistical analysis for detail comparisons.

Reference


C3. Limitations of the study including its retrospective nature have to be mentioned.

R3. Limitations of our study including its retrospective nature have been mentioned in discussion section, (see page 12 lines 19 to 20).