Author's response to reviews

Title: Impact of sputum gross appearance and volume on smear positivity of pulmonary tuberculosis: a prospective cohort study

Authors:

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

RE: MS: 7070906506912800

Title: Impact of sputum gross appearance and volume on smear positivity of pulmonary tuberculosis: a prospective cohort study

We appreciate the thoughtful comments made by reviewers. We have made as many changes as possible according to the editor's and the reviewers' recommendations, and I feel that we have been able to explain or respond to all of them. We highlighted the changes in the revised manuscript. Once again, we thank you and the reviewers for the thoughtful and helpful comments. We can see how much the reviewer's comments and recommendations have improved the quality of our manuscript.

We hope that our revision will meet with your and the reviewers’ approval and that we may anticipate publication in *BMC Infectious Disease*.

Sincerely yours,

Jae-Joon Yim, M.D.
Dear Editor,

RE: MS: 7070906506912800

Title: Impact of sputum gross appearance and volume on smear positivity of pulmonary tuberculosis: a prospective cohort study

I am the 1st author of this article, Soon Ho Yoon. It is truly honored and happy to have a discussion with two pathfinders in this topic, Ruth McNerney and Mishal Khan during the revision process. In fact, I have majored in Radiology and it has been just two years to have the responsibility for managing tuberculosis in Incheon city as a public health doctor instead of serving in the military which is an obligation of young people in Korea. When I planned this study, I have been very impressed by the achievement of those two reviewers, especially Mishal Khan. The idea of this study was also inspired by the study of Mishal Khan et al. which was published in Lancet 2007. Although I am supposed to come back to the department of Radiology, this revision process will have been recollected for me as a very special lifelong moment. I really appreciate those two reviewers and the editor.

Sincerely yours,

Soon Ho Yoon, M.D.
1. The article does not describe how the sputum specimens were sampled. Sputum is an inconsistent matrix and technicians would normally remove the mucoid/purulent fraction for examination by microscopy leaving the more watery/saliva portion that are less likely to contain the TB bacteria behind. This makes research to compare diagnostic methodologies difficult. To get equitable sampling would require homogenisation prior to division of the specimen, which may affect the performance of smear microscopy disproportionally. The authors should describe their sampling strategy and discuss its potential impact on the data obtained.

ANSWER: The national tuberculosis management guideline does not recommend homogenization of submitted sputum. Instead, a predominant portion of sputum specimen should used to assess the quality of sputum specimen if contents of sputum specimens were heterogenous. In addition, for smear, most purulent portion of specimen should be sampled with platinum wire loop. Every TB technician working for public sector attend the Quality Assurance Programs and share a common assessment and sampling strategy. We clarified it in method section at the 3rd paragraph of page 6 and 1st paragraph of page 7.

2. Clinical/radiological improvement following TB treatment is taken by the authors to indicate TB disease in microbiologically unproven cases. It is likely that TB treatment will affect cure in other bacterial infections and it might be more appropriate to classify patients as confirmed TB, possible TB and unlikely TB. The analysis should reflect this uncertainty.

ANSWER: As the reviewer recommended, we performed the additional analysis after exclusion of patients with clinically diagnosed TB. On multivariate GEE analyses, duration of respiratory symptoms (OR, 8.91, 95% CI, 2.76–28.81) and sputum volume (≥4 ml vs. <4 ml; OR, 7.99, 95% CI, 2.13–30.07) were still significantly associated with smear positivity in female patients reinforcing the importance of volume qualification. In male patients, BMI (≥23 vs. <23; OR, 5.58, 95% CI, 1.51-20.64) and gross appearance of sputum (purulent or blood-tinged sputum, rather than mucoid or saliva; OR, 6.84, 95% CI, 3.12-15.01) were significantly associated with smear positivity. We added the tables including these results as appendix and commented in method section at the 1st paragraph of page 8 and in results.
section at the 2nd paragraph of page 11. We also added the comment in discussion section at the middle of 2nd paragraph of page 12.

3. There were more male than female smokers and analysis should be undertaken to see if the impact of smoking was more significant that the effect of gender.

ANSWER: The impact of smoking was analyzed in both sexes using the generalized estimating equation (Table 3). Although the pack-year was significantly associated with smear positivity using univariate analysis in male patients, multivariate analysis including the pack-year with other variables showed no association between the pack-year and smear positivity. Similarly, there was no association between smoking and smear positivity among female patients.

4. The statement that women are less proficient than men at expectoration should be modified to include consideration of the impact of severity of disease (early vs late presentation) or other confounders such as smoking.

ANSWER: Thank you for raising a very important issue. As you indicated, there has been no firm evidence that women are less proficient at expectoration. To test this common belief, we added analysis on the association between disease severity and volume of submitted sputum. In male patients, presence of cavity (OR, 1.45, 95% CI, 0.99–2.14) and amount of smoking (≥ 10 pack-years vs. none; OR, 1.58, 95% CI, 1.02–2.46) was associated with volume of submitted sputum. On the contrary, neither presence of cavity, nor extent of disease, nor amount of smoking were associated with the increased volume of submitted sputum in females. We are convinced that the lack of association between diseases severity and volume of sputum in females reflects their inferiority at expectoration of sputum. We added the analysis in method section at the end of 1st paragraph of page 8 and in results section at the 3rd paragraph of page 11. We also added the comment in discussion section at the middle of 2nd paragraph of page 12.

5. The authors should discuss the potential impact of techniques such as sputum induction.
ANSWER: According to the reviewer’s suggestion, we added the statement on potential impact of techniques such as sputum induction in the discussion at the 1st paragraph of page 13.

Mishal Khan

1. Minor Essential Revisions
The statement that instructions are insufficient for improving sputum quality (background para 2) is questionable because studies show that sputum quality is improved by instructions. Of course not all suspects give good quality sputum after instructions, but this is often because they are not producing sputum.

ANSWER: We agree on the reviewer’s opinion. We revised the statement in background at the begin of 2nd paragraph of page 4.

2. Regarding higher positivity in women, a point to consider is that women who naturally produce a higher volume of sputum may be sicker and therefore more likely to be smear-positive. Therefore is it the larger volume or sicker patient that is correlated with sputum positivity? If a policy is made that only 4ml or larger of sputum is accepted, patients will cough up multiple times, which may or may not be the same as a patient who produces a large volume of sputum at one time.

ANSWER: As we commented in response to Dr. McNerney’ suggestion, neither presence of cavity, nor extent of disease, nor amount of smoking were associated with volume of submitted sputum in females. Furthermore, neither radiographic extent nor presence of cavity were associated with smear positivity in female patients. Based on these results, we concluded that the larger volume rather advance TB is associated with smear positivity. We added the analysis in method section at the end of 1st paragraph of page 8 and in results section at the 3rd paragraph of page 11. We also added the comment in discussion section at the middle of 2nd paragraph of page 12.
As the reviewer suggested, we admit that the smear positivity of certain volume of sputum coughed up at once might be different from same volume of sputum coughed up multiple times. Unfortunately, we do not have information on how many times the participants coughed up for each submitted specimen.

3. Major Compulsory Revision
It is not made clear what objective criteria were used to determine whether a sputum specimen was mucoid, blood stained etc. Was there a scoring system? If it is just left to the individual technician, it will be difficult to translate the findings into a policy because how well specimen quality is judged will vary from diagnostic centre to diagnostic centre.

ANSWER: As we mentioned above, the quality assessment of submitted sputum was based on a predominant portion of sputum specimen if content of sputum specimens were heterogenous. Differentiation of sputum was performed as follows. The submitted specimens having clear and watery appearance without any viscosity was designated to saliva. After the exclusion of saliva, the differentiation between mucoid and purulent sputums was based on a five-point sputum color chart (BronkoTest; Heredilab Inc., Salt Lake City, UT, USA). We added this information on method section at the 3\textsuperscript{rd} paragraph of page 6.

4. While comparisons are made between males and females in the text, as far as I can see ORs in women are not statistically compared with ORs in males.

ANSWER: As suggested, we statistically compared odds ratio of each parameter between sexes using Woolf estimator test for assessing common odds. None of the parameters which were analyzed in univariate GEE model was statistically significant between sexes.

5. Discretionary Revisions
Is there any way to condense information from tables so that 3-4 tables are presented?

ANSWER: We considered omitting some information from tables 3, 4, and 5 to make them brief. But, we think more information is better especially for an online-based journal such as BMC.
Subgroup analysis among the culture positive TB patients

Appendix 3. Results of a univariate Generalized Estimating Equation Model Predicting smear positivity according to sex in culture confirmed TB patients

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odd Ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-59 years (versus &lt; 40 years)</td>
<td>2.41 (0.88-6.58)</td>
<td>0.087</td>
</tr>
<tr>
<td>≥ 60 years (versus &lt; 40 years)</td>
<td>1.57 (0.50-4.88)</td>
<td>0.436</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥23 (versus &lt;23)</td>
<td>4.30 (1.22-15.20)</td>
<td>0.024</td>
</tr>
<tr>
<td><strong>Respiratory symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>any symptom</td>
<td>1.34 (0.44-4.07)</td>
<td>0.604</td>
</tr>
<tr>
<td>cough</td>
<td>1.83 (0.74-4.51)</td>
<td>0.192</td>
</tr>
<tr>
<td>sputum</td>
<td>2.41 (1.01-5.77)</td>
<td>0.048</td>
</tr>
<tr>
<td>hemoptysis</td>
<td>2.27 (0.28-18.58)</td>
<td>0.446</td>
</tr>
<tr>
<td>duration (&lt;3 weeks versus ≥3 weeks)</td>
<td>1.51 (0.62-3.64)</td>
<td>0.362</td>
</tr>
<tr>
<td><strong>General symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.07 (0.82-5.18)</td>
<td>0.122</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>current smoking</td>
<td>1.26 (0.53-2.99)</td>
<td>0.609</td>
</tr>
<tr>
<td>previous smoking</td>
<td>1.05 (0.43-2.56)</td>
<td>0.920</td>
</tr>
<tr>
<td>1-10 pack-years (versus 0 pack-years )</td>
<td>1.11 (0.32-3.89)</td>
<td>0.870</td>
</tr>
<tr>
<td>≥ 10 pack-years (versus 0 pack-years )</td>
<td>1.61 (0.55-4.70)</td>
<td>0.381</td>
</tr>
<tr>
<td><strong>Radiographic extent</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate or far-advanced (versus minimal)</td>
<td>1.56 (0.62-3.96)</td>
<td>0.346</td>
</tr>
<tr>
<td>cavity</td>
<td>1.94 (0.81-4.62)</td>
<td>0.136</td>
</tr>
<tr>
<td><strong>Gross appearance of sputum specimens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sputum (versus saliva )</td>
<td>5.29 (2.11-13.30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>purulent, bloody (versus saliva, mucoid)</td>
<td>6.53 (3.12-13.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Volume of sputum specimens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1ml (&lt;1ml versus ≥1ml)</td>
<td>1.48 (0.56-3.88)</td>
<td>0.430</td>
</tr>
<tr>
<td>≥2ml (&lt;2ml versus ≥2ml)</td>
<td>1.40 (0.75-2.59)</td>
<td>0.292</td>
</tr>
<tr>
<td>≥3ml (&lt;3ml versus ≥3ml)</td>
<td>1.53 (0.78-2.97)</td>
<td>0.215</td>
</tr>
<tr>
<td>≥4ml (&lt;4ml versus ≥4ml)</td>
<td>1.20 (0.47-3.07)</td>
<td>0.703</td>
</tr>
<tr>
<td>≥5ml (&lt;5ml versus ≥5ml)</td>
<td>1.26 (0.24-6.73)</td>
<td>0.784</td>
</tr>
</tbody>
</table>

Note. CI = confidence interval.
Appendix 4. Results of a multivariate Generalized Estimating Equation Model Predicting smear positivity in culture confirmed male TB patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Estimate</th>
<th>Odd Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td></td>
<td>-1.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-59 years (versus &lt; 40 years)</td>
<td></td>
<td>0.705</td>
<td>2.03 (0.66-6.19)</td>
<td>0.216</td>
</tr>
<tr>
<td>≥ 60 years (versus &lt; 40 years)</td>
<td></td>
<td>0.644</td>
<td>1.90 (0.61-5.97)</td>
<td>0.269</td>
</tr>
<tr>
<td>BMI</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥23 (versus &lt;23)</td>
<td></td>
<td>1.720</td>
<td>5.58 (1.51-20.64)</td>
<td>0.010</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sputum</td>
<td></td>
<td>0.503</td>
<td>1.65 (0.64 -4.30)</td>
<td>0.302</td>
</tr>
<tr>
<td>Gross appearance of sputum specimens</td>
<td></td>
<td>1.923</td>
<td>6.84 (3.12-15.01)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note. — Cl = confidence interval.
Appendix 5. Results of a multivariate Generalized Estimating Equation Model Predicting smear positivity in culture confirmed female TB patients

<table>
<thead>
<tr>
<th>Total</th>
<th>Estimate</th>
<th>Odd Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>-2.268</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory symptoms duration (&lt;3 weeks versus ≥3 weeks)</td>
<td>2.188</td>
<td>8.91 (2.76-28.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volume of sputum specimens ≥4ml (&lt;4ml versus ≥4ml)</td>
<td>2.078</td>
<td>7.99 (2.12-30.07)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Note. — CI = confidence interval.
Appendix 6. Results of a univariate Generalized Estimating Equation Model Predicting smear positivity in all culture confirmed patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odd Ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-59 years (versus &lt; 40 years)</td>
<td>2.23 (1.01-4.42)</td>
<td>0.048</td>
</tr>
<tr>
<td>≥ 60 years (versus &lt; 40 years)</td>
<td>0.91 (0.38-2.20)</td>
<td>0.830</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>2.97 (1.46-6.07)</td>
<td>0.003</td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥23 (versus &lt;23)</td>
<td>1.69 (0.72-3.99)</td>
<td>0.230</td>
</tr>
<tr>
<td><strong>Respiratory symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>any symptom</td>
<td>2.21 (0.99-4.96)</td>
<td>0.054</td>
</tr>
<tr>
<td>cough</td>
<td>2.33 (1.13-4.80)</td>
<td>0.022</td>
</tr>
<tr>
<td>sputum</td>
<td>2.50 (1.25-5.03)</td>
<td>0.010</td>
</tr>
<tr>
<td>hemoptysis</td>
<td>1.77 (0.52-6.05)</td>
<td>0.361</td>
</tr>
<tr>
<td>duration (&lt;3 weeks versus ≥3 weeks)</td>
<td>2.43 (1.20-4.89)</td>
<td>0.013</td>
</tr>
<tr>
<td><strong>General symptoms</strong></td>
<td>1.80 (0.85-3.80)</td>
<td>0.122</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>current smoking</td>
<td>1.41 (0.68-2.93)</td>
<td>0.351</td>
</tr>
<tr>
<td>previous smoking</td>
<td>1.10 (0.52-2.32)</td>
<td>0.812</td>
</tr>
<tr>
<td>1-10 pack-years (versus 0 pack-years)</td>
<td>1.02 (0.41-2.53)</td>
<td>0.971</td>
</tr>
<tr>
<td>≥ 10 pack-years (versus 0 pack-years)</td>
<td>2.02 (0.93-4.39)</td>
<td>0.075</td>
</tr>
<tr>
<td><strong>Radiographic extent</strong></td>
<td>2.04 (1.02-4.09)</td>
<td>0.043</td>
</tr>
<tr>
<td>2.47 (1.18-5.20)</td>
<td>0.017</td>
<td></td>
</tr>
<tr>
<td><strong>Gross appearance of sputum specimens</strong></td>
<td>3.83 (1.93-7.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sputum (versus saliva)</td>
<td>4.58 (2.60-8.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>purulent, bloody (versus saliva, mucoid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Volume of sputum specimens</strong></td>
<td>2.07 (0.96-4.46)</td>
<td>0.065</td>
</tr>
<tr>
<td>≥1ml (&lt;1ml versus ≥1ml)</td>
<td>1.67 (0.96-2.85)</td>
<td>0.062</td>
</tr>
<tr>
<td>≥2ml (&lt;2ml versus ≥2ml)</td>
<td>1.47 (0.83-2.61)</td>
<td>0.184</td>
</tr>
<tr>
<td>≥4ml (&lt;4ml versus ≥4ml)</td>
<td>1.76 (0.86-3.61)</td>
<td>0.122</td>
</tr>
<tr>
<td>≥5ml (&lt;5ml versus ≥5ml)</td>
<td>1.53 (0.50-3.05)</td>
<td>0.455</td>
</tr>
</tbody>
</table>

**Note.** — CI = confidence interval.
Appendix 7. Results of a multivariate Generalized Estimating Equation Model Predicting smear positivity in all culture confirmed patients

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Odd Ratio (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>(Intercept)</td>
<td>-2.213</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-59 years (versus &lt; 40 years)</td>
<td>0.451</td>
<td>1.57 (0.67-3.67)</td>
<td>0.298</td>
</tr>
<tr>
<td>≥ 60 years (versus &lt; 40 years)</td>
<td>0.561</td>
<td>1.75 (0.64-4.80)</td>
<td>0.275</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td>0.854</td>
<td>2.35 (1.03-5.37)</td>
<td>0.043</td>
</tr>
<tr>
<td>Respiratory symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration (&lt;3 weeks versus ≥3 weeks)</td>
<td>0.759</td>
<td>2.14 (1.00-4.56)</td>
<td>0.050</td>
</tr>
<tr>
<td>Radiographic extent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cavity</td>
<td>0.373</td>
<td>1.45 (0.62-3.39)</td>
<td>0.387</td>
</tr>
<tr>
<td>Gross appearance of sputum specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>purulent, bloody (versus saliva, mucoid)</td>
<td>1.397</td>
<td>4.04 (2.27-7.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Volume of sputum specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥4ml (&lt;4ml versus ≥4ml)</td>
<td>0.587</td>
<td>1.80 (0.76-4.26)</td>
<td>0.181</td>
</tr>
</tbody>
</table>

Note. — CI = confidence interval.