Author's response to reviews

**Title:** Outcome correlation of Smear-positivity but Culture-negativity during Standard Anti-tuberculosis Treatment in Taiwan

**Authors:**

- Wen-Cheng Chao (cwc081@hotmail.com)
- Yi-Wen Huang (hiwen@chhw.mohw.gov.tw)
- Ming-Chih Yu (yutbc@ms10.hient.net)
- Wen-Ta Yang (taic3057@gmail.com)
- Chou-Jui Lin (dejavu1114@gmail.com)
- Jen-Jyh Lee (e0139@tzuchi.com.tw)
- Ruay-Ming Huang (hrm@mail.hwln.mohw.gov.tw)
- Shun-Tien Chien (chientb8@mail.ccd.mohw.gov.tw)
- Jung-Yien Chien (jychien@ntu.edu.tw)

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

We are grateful for the thorough reading and insightful opinions of the reviewers. Point-to-point responses and revisions to address the opinions are as follows.

**Reviewer 1:**
MINOR ESSENTIAL REVISIONS

1. The background is clear, highlighting prior literature as well as well stated study objectives.
   
   **Reply:**
   
   We are very grateful for the thorough reading of the reviewer.

2. Definition of treatment failure needs to be included in background.
   
   **Reply:**
   
   The definition of treatment failure is now added in lines 72 to 74. According WHO guideline, treatment failure is defined as sputum smear or culture is positive at the 5th month or later during treatment (Definitions and reporting framework for tuberculosis, WHO, 2013). But, smear-positivity is not always equal to culture-positivity because of the possibility of SPCN as shown in this study. Therefore, we have incorporated this potential impact of SPCN to the assessment of TB treatment outcome in the background of this study.

3. Initially unclear at what time point SPCN was looked at, as the different treatment time points have different implications, Authors could for example specifically state out right that they looked at Months 2, 5, 9 and post treatment. This is alluded to in the literature review of 2 studies in Taiwan and Cameroon (lines 65 to 71). Line 134, generally states that SPCN was seen in 13.9% patients "during treatment", but different times have different implications. This is later better brought out in results e.g. in line 175, and discussion lines 228-230, but could be included in methodology.
   
   **Reply:**
   
   Time points of sputum follow-up during treatment are now added in lines 109 to 112. Sputum smear and culture were checked at the 2nd and 5th month after treatment for all patients as well as monthly sputum check till sputum conversion for patients with positive sputum smear according to the tuberculosis treatment guideline in Taiwan.

4. Its unclear whether by standard of care, all SPCN were given longer treatment, or
only depending on other factors eg cavitation. Was this prolonged treatment by protocol, or physician choice, or standard of care. At what SPCN time was the decision made to prolong therapy? Was there a difference in outcome between those on standard treatment compared to those with prolonged treatment, assuming these were two different groups.

**Reply:**

The decision making of prolonged treatment for SPCN is now added in lines 236 to 241.

In this study, the prolonged treatment was based on the decision of TB committee in each TB referral center. Facing sputum smear-positivity near the end of the scheduled treatment course without other evidence of treatment failure, members in TB committee tended to suggest extension of continuous treatment and follow-up of culture result instead of immediate drug modification.

In detail, the scheduled treatment course should be 9 months for patients with cavity on CXR (65%, 72/111 in SPCN (+) group) and 6 months for those without cavity (35%, 39/111 in SPCN (+) group). Cases will be discussed in TB committee if sputum smear is still positive near the end of scheduled course. Without other evidence of treatment failure, extension of continuous treatment was suggested for all these patients till the culture result of the smear-positive sample was available. It takes around two months for the availability of final negative culture results, and this explained well that SPCN (+) group had additional 49 days of Rifampicin treatment ($284 \pm 91$ vs. $235 \pm 69$ days) and additional 55 days of Isoniazide treatment ($289 \pm 90$ vs. $234 \pm 71$) compared with SPCN(-) group.

5. Table 3 - its interesting that many patients were on second line treatment. This does confuse the picture. Its initially assumed that all patients were on first line treatment (Line 81... new pulmonary TB cases......), but table 3 suggests otherwise.

**Reply:**

Indication of the use of fluoroquinolones is now added in lines 187 to 189.

This study was conducted in six TB referral centers in Taiwan, and patients suffered from adverse effects from 1st line medications were frequently referred to studying hospitals. Fluoroquinolones, including Levofloxacin and Moxifloxacin, and streptomycin were often used during drug rechallenge period. In addition, fluoroquinolones can also be used as substitutive drug for Isoniazide if patients cannot tolerate Isoniazide. If so, the necessary treatment duration will be based on the use of Rifampicin. But, if patients cannot tolerate Rifampicin, other 2nd line medications, including Prothionamide and Kanamycin, will be added. We excluded
these patients who cannot tolerate Rifampicin (53 patients in this study as shown in fig. 1) because their scheduled treatment duration is much longer than those treated with Rifampicin. Importantly, there was no difference of the use of fluoroquinolones between two groups in this study.

6. Discussion, lines 228 to 231 rather unclear to the reader, and could be confusing. May be better to revise; e.g. in line 229 " ...but only 6.8% (54/800) have culture -positive....", or " ...but only 46.6% (54/116) of these have culture -positive....".

Reply:  
Name of each denominator is now added to avoid confusion, and is now added in lines 250 to 256. The revision is shown as follows.

Our data showed that sputum SPCN mostly developed at 157±95 days after the start of treatment. Specifically, at the end of the 2nd month, 14.5% (116/800) of all patients had smear-positive result, but only 46.6% (54/116) of these smear-positive patients have culture-positive result. Similarly, at the end of the 5th month, 4.4% (35/800) of all patients had smear-positive results, but only 28.6% (10/35) of these smear-positive patients had culture-positive result.

7. Discussion lines 238-241 - of note culture is not always available, and many clinicians use smear results at month 5 to predict treatment failure. I assume this is an important issue that this study addresses, and needs to be clearly brought out, and culture requirement then cannot be emphasized as a requirement for evaluating responses to therapy.

Reply:  
A separate discussion is now added in lines 276 to 284 to address this crucial problem, and is now shown as follows.

Finally, SPCN is especially important in countries with limited laboratory capacity. In Taiwan, the laboratory capacity is adequate and all sputum samples were sent for both smear and culture at the same time. But, diagnosis of TB and assessment of treatment outcome in many countries still relies on sputum smear only. In these countries, high possibility of SPCN in patients with severe TB infection during anti-tuberculosis treatment should be taken into account to make the final judgment of treatment failure. Multi-dimensional assessment, the quality of patient supervision and chest radiography included, as we showed in previous study is crucial to avoid unnecessary modification of treatment regimen.
8. Lines 265 to 267 may need revision. Already its known and implemented in many countries that treatment is prolonged in cavitating TB, which in this study is related to SPCN. Also culture not always available. Thus the last sentence may not be a generalizable practical option in many care centers.

Reply:

The revised conclusion is now added in lines 301 to 303, and is now shown as follows.

Extension of continuation phase treatment duration in waiting culture results from smear-positive samples appears to be a practical and safe strategy with an acceptable relapse rate, although ideal regimen remains unknown at this time.

Based on our data, the suggestion of extension of continuation treatment duration should be restricted by “waiting culture results from smear-positive samples” to fit the potential application in clinical practice. There are two situations in facing positive smear near the end of scheduled treatment. The first situation is that culture had been done together with smear, and it is safe to extend the continuation treatment in waiting culture result (lines 300 to 302). If the test is smear-only, multi-dimensional assessment is crucial to judge treatment response (lines 276 to 284).

9. Figures - Figure 2 may not add a lot of information, Text could suffice. Figure 3A - correct key from SPNC to SPCN. Also wonder if this figure necessary - I do not seen to see the text referring to this figure, although the information is captured in results and discussion sections.

Reply:

The rationale of Figure 2 is now added lines 156 to 158. Figure 3 A has been corrected with SPCN, and the rationale and statement of Figure is now added in lines 171 to 173.

We used Figure 2 and Figure to demonstrate the time course and dose response effect of TB severity on the development of SPCN. We thought these two figures should be helpful for clinicians to straightforwardly apply our findings in their clinical practices.
Reviewer 2:
Reviewer's report:
This is a retrospective clinical study of significance of smear-positivity and culture-negativity (SPCN) in patients receiving antitubercular treatment. The authors attempt to evaluate relations between SPCN and demographic information, risk factors, co-morbidity, severity of infection and disease symptoms/lab findings, as well as final disease and treatment outcomes. Their aim appears to be able to predict which patients are likely to develop SPCN as well as suggest possible changes in management in patients with SPCN.

Discretionary revisions:

1. The title of the manuscript is somewhat vague – including the main finding in the title will make it more attractive. (e.g., “SPCN during TB treatment is predicted by….. but does not affect outcome.”) (discretionary revision)

   Reply:
   We are very grateful for the suggestion of this attractive title by the reviewer. The suggested title is really an excellent scientific title completely reflects our findings. But, the goal of our work is to provide evidence for clinicians in facing SPCN during standard anti-tuberculosis treatment. In such condition, outcome correlation is generally the most concerned part by clinicians. Thus, we put the outcome correlation in the start of the title, and we thought that the title with “Outcome correlation of Smear-positivity but Culture-negativity during Standard Anti-tuberculosis Treatment in Taiwan” expresses well potential clinical applications of our findings.

2. Abstract: Lines 35, 36 – Aim needs to be more specific. Line 40 – “chemotherapy data, demographics and sputum data is too vague.” Also, purpose of analysis could be included (e.g., “analyzed for association with incidence of SPCN” (Minor essential revision)

   Reply:
   Specific aims in background are now added in lines 35, 36, and purpose of each analysis is also specifically stated and is now added in lines 40 to 42. The detail explanation is now added on the reply of the next question.

3. Aim of the study: The aim of the study can be somewhat better defined. The authors mention that the aim of the study is to “investigate risk factors and clinical impact of SPNC phenomenon”. It is unclear what risk factors and what outcomes are being investigated within the aim. (Minor essential revision)
Reply:
Specific aims have been revised in lines 35, 36 as “This study aimed to investigate clinical risk factors and impacts on treatment course and relapse of sputum SPCN.”
In detail, the three specific aims in this study are shown as follows:
Aim 1: To indentify the clinical risk factors of sputum SPCN in TB patients.
Aim 2: To investigate the impact of sputum SPCN on the treatment course.
Aim 3: To determine the impact of sputum SPCN on relapse rate.

4. Introduction: The purpose of the aim is also unclear - the statement of current problem in clinical practice or gap in current knowledge is undermined. There is no mention of the purpose in the abstract. The authors do mention “great clinical impact” in line 71 and “prolonged isolation and treatment failure” in line 73; however, these issues are undermined in the introduction by the brevity of mention. What are the clinical impacts of SPCN? Reflect a little on how different situations/conclusions can affect management differently. What are the implications of SPCN? What conclusions can you draw from the currently published literature? A short review of literature will be helpful to keep the study in perspective. (Minor essential revision)
Reply:
Treatment failure judgment is a critical clinical impact of sputum SPCN, and is now added in lines 72 to 74. Other revisions of specific questions are shown as follows.

a. Line 72: What does 'final culture results' mean? Does finality refer to a conclusive result or does it refer to the last test done at a certain time point (2 months? 6 months? 2 years?) (Minor essential revision)
Reply:
“Final culture results” has been revised to “culture results of smear-positive sputum samples” to avoid confusion, and is now added in line 75, 76.

b. Line 75: List the risk factors and why you chose them. List the outcomes that you decided to study. Were these risk factors determined a priori? (Minor essential revision)
Reply:
The risk factors and outcomes to be studied have been specified, and are now added in lines 79 to 81.
The two major risk factors to be studied for sputum SPCN in this study are TB
severity and clinical predisposing factors of TB. The two important outcomes we studied are impacts of sputum SPCN on the treatment course and long term impact on the relapse rate.

5. The methods appear to be rigorous, appropriate and well described.
a. Mention that the study was retrospective (or clarify otherwise). (discretionary revision)

Reply:
“Retrospectively” is now added in the first sentence of methodology, and is now in line 83.

6. The data appears to be sound.
a. Lines 136-138: Was confounding due to relatedness of the factors studied taken into account? For example, males might be more likely to have SPCN simply because more males are smokers and alcoholics, which might be actual risk factors for SPCN. (discretionary revision)

Reply:
The statistic power of each factor may explain their roles in the development of sputum SPCN during treatment, and the explanation is now added in lines 151 to 155.

Briefly, biological risk factors of TB, including age, gender, smoking, alcohol consumption, and type 2 DM as well as TB severity, classified by cavity formation on CXR and AFS grading in sputum, were the two major factors we investigated in this study. The statistical power analysis showed that TB severity is obviously a stronger risk factor than most biological risk factors for TB, including type 2 diabetes mellitus, alcoholism, and maleness. Such results suggested that TB severity may represent the combinational biological effects of those clinical risk factors on the development of sputum SPCN. Interestingly, multivariate analysis also showed that smoking is still an independent risk factor in addition to TB severity. Such finding indicated the key role of smoking in the development of sputum SPCN. We have discussed the potential role of smoking in TB infection in the next question.

b. Lines 141-143: Was TB severity related to only SPCN positivity or was there some correlation with co-morbidity and/or co-existing risk factors such as smoking? (discretionary revision)

Reply:
Our data suggested smoking and TB severity is co-existing risk factors for
sputum SPCN during treatment.

Increasing clinical evidence showed the potential role of smoking, including passive smoking, in the incidence of TB (lines 268 to 275). A recently published report the positive correlation between childhood TB and passive smoking, and such finding indicates the potential biological effects of smoking on TB immunity instead of social effects only (Reference 21 in manuscript). In this study, we identified both smoking and TB severity is the independent risk factor for sputum SPCN, and we postulated they were two co-existing major risk factors. As we discussed for the potential sources of SPCN mycobacteria, sputum SPCN may represent host-pathogen interaction during TB infection (lines 219 to 223). Importantly, smoking was also recently found to be related to 2-month culture conversion during treatment (Reference 23 in manuscript). Smoking, thus, may has biological impacts on host-pathogen interaction in TB infection. But, underlying mechanisms of smoking in host-pathogen interaction in TB infection remains elusive and more basic studies are needed to elucidate underlying mechanisms.

c. Line 145: Please explain 'close'. It can be assumed that it was close in the time course of treatment, but this is not very clear. (discretionary revision)

Reply:

It is now revised as “sputum smear conversion curve and culture conversion curve was close to each other” to avoid confusion, and it now added in lines 159, 160.

d. Line 147: Explain 'gap'. Unless someone looks at the Figure, it is difficult to understand 'gap', which might be misunderstood to mean 'something missing'. (discretionary revision)

Reply:

We are very grateful for the suggestion, and “gap” has been revised to “discordance” for expressing the unexpected sputum SPCN phenomenon. (line 162)

e. Lines 136-139 and Lines 151-153: Multivariate regression demonstrates that maleness, or alcoholism, or DM are unrelated to SPCN. Please reflect this in stating the results in Lines 136-139. The fact that these factors tend to be more closely associated with SPCN can be explained to be likely due to concurrent association with smoking. It could be misleading to present significant p values with maleness, alcoholism and DM, in such a case. (Minor essential revision)
Reply:

The statistic powers of each factor are now added in lines 151 to 155. The explanation that TB severity and smoking were two independent risk factors for sputum SPCN during treatment has been shown in replies for question 6b and 6c.

f. Line 171: Typographical error - Instead of "that significant for..." the authors probably mean "as significant as..." (Minor essential revision)

Reply:

We are very grateful for the suggestion, and have revised to “as significant as” in line 186.

g. Line 172: What is "final positive culture"? (discretionary revision)

Reply:

“final positive culture” has been revised to “the last positive culture during treatment” to avoid confusion, and is now in line 189, 190.

h. Line 185: Explain "on the basis of". Do you mean "possibly due to..."? (discretionary revision)

Reply:

We are very grateful for the suggestion, and we thought “possibly due to” fits well with our findings. It is now revised in line 203.

7. The figures appear to be genuine.

Reply:

We are very grateful for the thorough reading of the reviewer.

8. The discussion of the findings is primarily descriptive. There appears to be adequate literature search performed and correlations sought with prior literature. The authors recognize the limitations of their study and address the reasons while suggesting further studies wherever applicable. However, the discussion can benefit from the following:

a. Discuss the importance of association of treatment duration with SPCN incidence. (discretionary revision)

Reply:

To clearly explain the association of prolonged treatment with sputum SPCN, the decision making of prolonged treatment for SPCN is now added in lines 236 to 241.

In this study, the prolonged treatment was based on the decision of TB
committee in each TB referral center. Facing sputum smear-positivity near the end of the scheduled treatment course without other evidence of treatment failure, members in TB committee tended to suggest extension of continuous treatment and follow-up of culture result instead of immediate drug modification.

Briefly, sputum SPCN leads to the prolonged treatment, and our data showing similar relapse between patients with and without SPCN suggested the prolonged treatment may be a safe and practical strategy (lines 300 to 302).

b. Line 193: Within 3 years of what? (e.g., 3 years of 'completion of treatment' or 'initiation of treatment'?)(Minor essential revision)
Reply:
It has been revised to “3 years after the completion of treatment” to avoid confusion, and is now added in line 211.

c. Lines 229 and 231: please change “46.6%” and “28.6%” to “46.6% of those” and “28.6% of those”, otherwise the sentence structure refers that these values refer to the entire pool of 800 patients. Better still, simply mentioning the culture positivity is sufficient in conveying the message in these two sentences – similar to the two sentences that follow (Lines 232 and 235). (Minor essential revision)
Reply:
Name of each denominator is now added to avoid confusion, and is now added in lines 250 to 256. The revision is shown as follows.

Our data showed that sputum SPCN mostly developed at 157±95 days after the start of treatment. Specifically, at the end of the 2nd month, 14.5% (116/800) of all patients had smear-positive result, but only 46.6% (54/116) of these smear-positive patients have culture-positive result. Similarly, at the end of the 5th month, 4.4% (35/800) of all patients had smear-positive results, but only 28.6% (10/35) of these smear-positive patients had culture-positive result.

d. Line 238-241: How did this study add to this conclusion from the previous study (reference 5)? A brief description of the incremental value provided by this study will be useful. (discretionary revision)
Reply:
The new finding of the present study compared to our previous study (reference 5) is now added in discussion part (lines 260 to 262) and conclusion (lines 301 to 303).

Briefly, we additionally showed the time course of sputum SPCN during standard treatment, and investigated the impact of sputum SPCN on treatment
course and the relapse rate after the completion of treatment for 3 years.

e. Line 257: Please expand NTM the first time it is used. (Minor essential revision)

Reply:

We are very grateful for the suggestion, and nontuberculous mycobacteria (NTM) is now added in line 292.

f. The discussion could benefit from reflecting upon how the results affect the clinical management, pointing to specific changes in management practices, with a note that the efficacy of these suggestions should be evaluated by further prospective trials. (discretionary revision)

Reply:

A separate discussion is now added in lines 276 to 284 to address the impact of sputum SPCN on judgment of treatment failure, especially in countries relies on sputum smear only. The added discussion is shown as follows.

Finally, SPCN is especially important in countries with limited laboratory capacity. In Taiwan, the laboratory capacity is adequate and all sputum samples were sent for both smear and culture at the same time. But, diagnosis of TB and assessment of treatment outcome in many countries still relies on sputum smear only. In these countries, high possibility of SPCN in patients with severe TB infection during anti-tuberculosis treatment should be taken into account to make the final judgment of treatment failure. Multi-dimensional assessment, the quality of patient supervision and chest radiography included, as we showed in previous study is crucial to avoid unnecessary modification of treatment regimen.

g. The authors' conclusions indicate that culture negativity carries less significance than smear negativity in indicating successful treatment outcome. Do the authors envision stratifying TB patients such that in a group of patients a smear only test can replace the currently practiced smear and culture test, thus saving time and cost? It may be worthwhile to discuss the pros and cons of such an approach. (discretionary revision)

Reply:

The discussion about the use smear-only test to assess treatment response has been shown in the reply of above question, and we has modified the conclusion in lines 301 to 303 to be a better general practical suggestion.

Based on our data, the suggestion of extension of continuation treatment duration should be restricted by “waiting culture result from smear-positive sample”
to fit the potential application in clinical practice.

Briefly, there are two situations in facing positive smear near the end of scheduled treatment. The first situation is that culture had been done together with smear, and it is safe to extend the continuation treatment in waiting culture result (lines 300 to 302). If the test is smear-only, multi-dimensional assessment is crucial to judge treatment response (lines 276 to 284).

9. Table 1:
a. The column of p-value may not be necessary. Since some of the factors are causally or incidentally related, it can be misleading as this column shows significance where multivariate analysis does not. Suggested removing this column from this table. (Minor essential revision)

Reply:
Table 1 showed basic characteristic factors of enrolled patients. Some readers preferred to use p-value to see key characteristics in the enrolled patient. Thus, we thus kept p-value to give readers a general spectrum of enrolled patients in two groups.

b. Is the HbA1c averaged over the period of treatment? Is it standardized as to timing of measurement, type and length of treatment of Diabetes mellitus etc? Please indicate somewhere in the manuscript (perhaps in within methods). (Minor essential revision)

Reply:
The detail information about HbA1C in the type 2 diabetes mellitus in this study is now added in line 114, 115 and line 146.
All diabetes mellitus we enrolled is type 2 diabetes mellitus and the HbA1C was measured at the start of anti-TB treatment.

c. P values cannot be negative, so p <0.000 do not exist, and are mathematically incorrect. It is okay to say <0.001 to maintain the number of decimal points. (Minor essential revision)

Reply:
We are very grateful for the correction, and it has been revised to <0.001 in Table 1.

d. Refer to Symptoms -> Cough -> under column “All”. The authors say “557 (18)”, which is perhaps a typographical error as 557 of 800 is not 18%. If otherwise, please explain. (Minor essential revision)
Reply:

We are very grateful for the correction, and it has been revised to 557 (70) in Table 1.

e. Refer to Laboratory findings -> Hemoglobin. All columns show hemoglobin measurements of 13+/−2 g/dL, however the p value of 0.095 (although not significant) appears to be very low for such equal measurements. Is this a calculation or typographical error? (Minor essential revision)

Reply:

We are very grateful for the correction of typographical error, and it has been revised to 0.995 in Table 1.

10. The manuscript language has several minor typographical and grammatical errors, which can be easily corrected by close proofreading. (Minor essential revision)

Reply:

We are very grateful for the suggestion, and close proofreading has been done.

Best wishes,

Jung-Yien Chien, MD
National Taiwan University College of Medicine, Taipei, Taiwan
Email: jychien@ntu.edu.tw

Shun-Tien Chien, MD
Chest Hospital, Ministry of Health and Welfare, Tainan, Taiwan
Email: chientb8@mail.ccd.mohw.gov.tw