Author's response to reviews

Title: Non-conversion of sputum culture among patients with smear positive pulmonary tuberculosis in Cameroon: a prospective cohort study

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Cover letter

Submission of a revised manuscript

Manuscript ID: 1216546212102442 entitled "Non-conversion of sputum culture among patients with smear positive pulmonary tuberculosis in Cameroon: a prospective cohort study"

By Eric Walter Pefura-Yone et al

Dear Editor,

We are grateful to the reviewers for their time and comments on our manuscript (referenced above). We have addressed their comments and queries and would like to submit an updated version of our manuscript for further consideration in the Journal. Changes in the main document have been inserted with the use of red color. In addition we are providing below a point-by-point response to each query from the reviewers.

We look forward to the outcome of your assessment.

Reviewer's report

Reviewer: Jann-Yuan Wang

By analyzing 86 TB patients with persistent sputum smear positivity after 2 months of anti-TB treatment in Cameroon, the authors found that 53% remained culture-positive for Mycobacterium tuberculosis. Among them, 2 were MDR-TB. Absence of hemoptysis and current smoking were independent factors for sputum culture non-conversion. The manuscript is well written, and can be further improved if considering the following comments.

Our answer: Thank you.

Major Compulsory Revisions:

1. I agree that drug resistance is one reason of treatment failure. Therefore early detection of drug resistance is important. However, what is the cause of treatment failure in the 8 patients without sputum culture conversion after 2 months of anti-TB treatment? Those patients may require other interventions rather than drug susceptibility testing.
Our answer: Treatment failure was recorded in 14 patients in this study, including 11 among patients with a positive culture at the end of the 2\textsuperscript{nd} month. For two patients, there was evidence of bacteriologic failure due to multi-resistant micro-organisms. For the remaining patients, treatment failure appeared to be clinical and with no obvious reason. All patients with treatment failure declared adequate adherence to prescribed medications. However, in the absence of direct supervision of medications intake by nurses during the continuation phase, it remains possible that poor adherence could account for some of the observed treatment failure. We have now summarised this in the discussion where it reads:

“Even in the absence of multiple drugs resistant tuberculosis, treatment failure was high in group C+ patients. These were essentially clinical failure, likely due to poor adherence; suggesting the need for an ongoing supervision of these patients throughout the entire course of their treatment.”

2. If resource is limited, every public health policy should be made based on cost-effective consideration. Before making any conclusion of suggestion, the authors should try to prove it cost-effective.

Our answer: We fully agree with the reviewer on the importance of cost-effectiveness evidence to back-up policy recommendation. Since generating such evidence is outside the scope of the current manuscript, we have added the statement below to the discussion to reflect the importance of cost-effectiveness data. It reads:

“Cost-effectiveness studies are needed to guide the choice of the appropriate strategies particularly in resources-limited settings.”

3. Predictive factor is helpful only when the outcome cannot easily be measured.

At the beginning of anti-TB treatment, predicting sputum culture non-conversion after 2 months of treatment may be important. However, in this study, the participants were all patients with persistent smear positivity. Therefore if mycobacterial culture is available, the correct response should be simply “mycobacterial culture”, rather than trying to use partially reliable clinical factors to predict the culture result.

Our answer: Thank you for raising this point. We would however like to draw the attention of the reviewer to the fact that we have investigated the predictors of culture non-conversion among patients with positive sputum smear after 2 months of treatment. In settings where
sputum culture is at best available only in major cities, and costly to patients, and where direct sputum examination is widely available, we think it is important to equip the large number healthcare practitioners with no access to sputum culture, with information that can guide the identification of those of their patients with positive sputum smear after 2 months of treatments and more likely to also have positive culture. Equip with this knowledge, they can for instance refer for further investigation via sputum culture only those of their patients who are likely to have positive culture.

Minor Essential Revisions:

1. The authors should mention in the methods section how identification of mycobacterium species were performed.

   Our answer: Fixed. It reads:

   “Sputum specimens collected from each eligible patient were decontaminated and inoculated onto Löwenstein-Jensen (LJ) medium. Cultures were incubated at 37°C and read for growth on a weekly basis and for a maximum duration of 8 weeks. The identification of the cultured strains was based on the following: culture aspects on the two media, resistance to thiophene-2-carboxylic acid hydrazide (TCH, 2 mg/l), susceptibility to para-amino-salicylic acid (PAS, 0.5 mg/l), reduction of nitrates, niacin production and catalase production at 22°C and 68°C. Drug susceptibility testing of *M. tuberculosis* complex strains isolated from cultures was performed using the indirect proportion method on LJ medium as described by Canetti et al [9]. A patient was considered to have drug-resistant *M. tuberculosis* if the number of colonies growing on a medium containing 0.2 mg/l INH, 2 mg/l EMB, 4 mg/l SM or 40 mg/l RMP exceeded 1% of the growth on a drug-free culture plate.”

2. It remains inconclusive whether TB patients without sputum culture conversion after 2 months of anti-TB treatment remain infectious, especially when the clinical isolate of *Mycobacterium tuberculosis* is all-susceptible.

   Our answer: Culture positivity confirms the viability of mycobacterium. It is therefore plausible that inhalation of these viable micro-organisms will contribute to the spread of the disease.

3. Page 8, 3rd paragraph, 1st sentence: please write down the prevalence.
Our answer: Fixed

4. Page 8, 3rd paragraph, 2nd sentence: I think “persistent culture positivity” is better than “persistent smear positivity”.

Our answer: Fixed

Reviewer's report

Reviewer: Wei-Juin Su

The authors analyzed 86 cases of sputum positive PTB without smear conversion at the end of initial phase of anti-TB treatment, and claim that the major finding of the manuscript is absence of hemoptysis and current smoking is the two main determinants of sputum non-conversion. The main basis of the conclusion is that failure rate is high among patients with positive sputum culture after intensive treatment, even in the absence of multi-drug resistant bacilli. However, high failure rate from culture-non-conversion cases among persistent S(+) cases is understandable. The main problem of this study is the authors only enrolled S(+) cases for analysis. How many are S(-)/C(+) at the end of the intensive phase? The cases with S(-)/C(+) are important covariant factors to the failure rate, thus those data need to show together. It could be more meaningful, if the failure data is analyzed also in correlation with S(-)/C(+) cases. The finding seems not new and not significant at all. Similar study was published by the same author (East Afr Med J. 2009 May;86(5):219-25). Accordingly the title of the paper may also need to change.

Our answer: Thank you for raising this important point. We did not include patients with negative sputum smear at the end of the intensive treatment phase. We are therefore unable to make the suggested comparison by the reviewer. Our focus was S(+) patients who in the study setting and many other region with high incidence of tuberculosis are those eligible further investigation via sputum culture, and also have a poorer prognosis.

The paper referred to by the reviewer is not similar to the present one in the sense that it focused on predictors of positive sputum smear after two months of treatment. No culture was conducted in the previous study. Furthermore, patients included in the two papers are from two different time-periods.
Comments to the Author

1. Out of 953 SPPTB, 97 (10.2%) are S(+) at the end of the intensive phase of treatment, and 86 were enrolled. How many are S(-)/C(+)? The authors need to clarify more on this issue.

Our answer: As said above we performed the culture only for S(+) patients as indicated in the methods section. It is not a routine in this setting to perform culture after 2 months for every patient who had a positive sputum smear at the start of the treatment.

2. Were pretreatment DST results used to modify treatment regimens? If so, in 14 failure cases how they have been modified?

Our answer: In the study setting, culture is not a routine in patients with newly diagnosed tuberculosis. Therefore drug susceptibility tests were not conducted prior to starting our patients on treatment. Culture was done only at the end of the second month and in S(+) patients only. Treatment was then adjusted according to DST. Patients with MDR TB for instance were treated according to the 21 months regimen of the World Health Organisation. Those with mono-resistance to anti-TB other than rifampicin were treated with a category II retreatment regimen. All patients with treatment failure and non-resistant to category I anti-TB were also transitioned to a category 2 regimen (2RHEZS/1RHEZ/5RHE) as per national guidelines.

3. Table 2 seems not necessary. How many cases with treatment failure are S < 2+? The authors did not clarify the outcome of those smear <2+.

Our answer: Five patients (14.3%) S<2+ at the end of the intensive treatment had a treatment failure against 9 (18.8%) S>=2+ patients (p=0.769). Furthermore, table 2 shows that bacillary load at the end of the intensive treatment was not associated with culture positivity.

4. In Table 3, the number of cases is confused. The total number of patient in analysis should be 78, not 86.

Our answer: Fixed
Editor's comment:

"In addition to addressing the comments of the reviewers, please incorporate the slightly revised 2013 WHO treatment outcome definitions, which were recently released (http://www.who.int/tb/publications/global_report/en/).

Our answer: Fixed

For table 1, it may be helpful to convert some of the continuous variables (e.g. age, BMI, CD4 count) into categorical variables to see if certain strata have an increased risk.

Our answer: We have done so for age, BMI and CD4 count without uncovering a higher risk in particular strata.

Please also confirm that the final multivariable model only contained hemoptysis and smoking and no other variables.

Our answer: Indeed the model contains only the two variables.

Comment on whether or not you think blood in sputum could be inhibiting the culture growth of TB (lab effect) rather than hemoptysis being a true predictor of culture negativity.

Our answer: Cultures were done at the end of the second month, and none of the patients had haemoptysis at that time. It would therefore be difficult to bring into the context the inhibitory role of blood on culture outcome.

Finally, please clarify the discussion paragraph 3 why extending this phase by an additional month "seems justified" when, in your study, doing so still resulted in a very high treatment failure rate (17%), particularly among the >50% who were culture positive. You present no data to show the benefit of extending intensive phase treatment and your data seem to indicate that simply extending the intensive phase by 1 month is an inadequate response."

Our answer: Thank you for this comment. We agree with the editor that there are no data in the current study to support the statement. This discussion section have been modified as:

“The current approach of the National Tuberculosis Control Program (NTCP), which consists in extending this phase by an additional month [2] needs to be clarified by further studies,
since in spite of the extension of the intensive phase, the failure rate is fairly high especially in the subgroup of patients with positive culture."