PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	A protocol for studying cough frequency in people with pulmonary tuberculosis
AUTHORS	Proaño, Alvaro; Bravard, Marjory; Tracey, Brian; López, Jose; Comina, German; Zimic, Mirko; Coronel, Jorge; Lee, Gwenyth; Caviedes, Luz; Cabrera, Jose; Salas, Juan; Ticona, Eduardo; Kirwan, Daniela; Friedland, Jon; Evans, Carlton; Moore, David; Gilman, Robert

VERSION 1 - REVIEW

REVIEWER	Mark Cotton Stellenbosch University South Africa
REVIEW RETURNED	03-Nov-2015

GENERAL COMMENTS	This is an innovative and potentially important study. It is more focussed on response to treatment than diagnostic characteristics of the cough related to TB. For example, persistent non-remitting cough >2w in children is useful for PTB diagnosis (Marais Pediatrics 2006; 118: e1350). Pregnancy is excluded despite a high risk for TB in the childbearing years.These are not necessarily limitations but could be interesting to address in the present study or later on. Time to positivity for MGIT is a nice biomarker for severity of disease for liquid culture (Carroll Tuberculosis 2008; 88: 624-630) and could be useful with MODS also? I am sure that Infection Prevention & Control practise be adhered to, but this should be addressed in the protocol. Will additional subjects be recruited if some fail to complete all of the study procedures?

REVIEWER	Edward C. Jones Lopez Boston University School of Medicine and Boston Medical Center
REVIEW RETURNED	25-Nov-2015

GENERAL COMMENTS	This is an interesting clinical study performed by an experienced and complimentary group of investigators with a notable track record in tuberculosis (TB) research. As stated by the authors, there is a paucity of information regarding cough and other clinical parameters involved in transmission of Mycobacterium tuberculosis before and after treatment is initiated. As such, the proposed study would provide interesting (but mostly descriptive) data on the frequency and kinetics of cough in patients with pulmonary TB undergoing treatment. However, as written, the study has several important limitations that are likely to negatively impact its results and generalizability.

	I have organized by comments under three themes: Hypothesis: 1. Transmission of TB results from yet incompletely understood host, environmental and bacterial factors. Although central to both the disease itself and to transmission, cough is only one factor in a complex web of characteristics. By only measuring cough frequency, the proposed study reduces the stated complexity further. Even cough itself has several elements of study beyond frequency such as cough severity (strength). Therefore, the authors should restrict discussion and objectives to response to treatment rather than TB transmission. 2. Use of sputum outcomes seems outdated in a TB study focused on cough. The importance of cough in TB transmission is that cough is the generator of infectious aerosols, the infectious moiety in TB. Despite long-standing evidence that fine aerosols transmit TB, most studies continue to rely on sputum. Recent work with cough-generated aerosols in patients with TB have demonstrated the limitations of continuing to use sputum to study TB transmission. Study design: 3. As stated by the authors, plan to record 24-hr cough is both a strength and a weakness as it will be difficult to separate out ambient noise 4. The authors plan to enroll a combination of drug-susceptible and drug-resistant TB cases into the study. In most settings, drug-resistant TB cases concentrate among patients with a previous history of TB treatment (i.e retreatment TB cases); many of these patients have more severe disease than those with drug-susceptible disease. As such, I would anticipate that the kinetics of cough in drug-susceptible and drug-resistant cases will be quite variable after treatment. A Similar argument can be made for HIV (e.g. less and weaker cough in patients with advanced HIV/AIDS). Finally, many patients have persistent cough despite treatment due to TB-induced bronchiectasis, fibrosis, and residual inflammation. These considerations should be explicitly stated in the analysis plan (stratified analysis?) and sample size calculations. 5. Authors mention the cough measuring tool they will employ has been validated in TB patients but present no data. A summary of these data should be included in the protocol. 6. Authors plan to measure M. tuberculosis bacillary load using sputum acid-fast bacilli (AFB) smear microscopy. However, sputum AFB is known to be an imprecise measure of bacillary load, when compared to semi-quantitative Middlebrook cultures, liquid (MGIT 960) cultures, or quantitative cultures. 7. Inclusion criteria will accept patients with up to 30 days of antituberculous treatment despite noting that cough is likely to change within 2 weeks of treatment. Similar argument with patients placed on a new regimen due to suspected or confirmed drug resistance (i.e. partial treatment may change cough patterns). 8. Why exclude patients under 18 years of age? Children are probably group with most need of non-invasive method to monitor

	response to treatment. 9. Beyond providing more granular information on chest pathology, not clear about the risk/benefit of performing chest CT (significant radiation risk) in such a large number of patients. How will the knowledge provided by CT be applied? 10. Need to define what “as close to baseline” means (Figure 1). Study visit window likely needs to be tight given rapid cough response after treatment expected by authors. 11. The questionnaire can be improved: - Feels more as educational tool rather then TB disease severity assessment tool - Errors in Spanish (Direcciones instead of Instrucciones) - Visual cough analog scale at the end of page 2 has a 5-level ordinal scale instead of 10 (cough scale is extrapolation of standard 10-level pain scale) (see Raj AA, Birring SS (2007) Clinical assessment of chronic cough severity. Pulmonary pharmacology & therapeutics 20: 334–337). 12. Chest X-ray data capture form seems unnecessarily complicated. Has it been validated? Significance and applicability 13. The primary outcome of this relatively large and intervention-heavy study is a two-fold reduction in cough frequency. However, simple clinical observation shows that cough frequency and severity (as well as other TB symptoms) improve after treatment (assuming drug-susceptible disease and treatment adherence). As such, although interesting, a detailed description of cough kinetics after treatment seems unnecessary unless it is shown to be a surrogate marker for infectiousness and risk of transmission. The current study is not designed to assess transmission. 14. Unclear applicability in real world circumstances. Authors propose as a potential alternative (for TB diagnosis???) in settings where laboratories are not available? However, cough monitoring device appears technologically challenging and thus, too complex for most settings where TB is prevalent (and laboratory diagnosis is often lacking).

REVIEWER	Jacky Smith, Professor of Respiratory Medicine University of Manchester, United Kingdom. I am a named inventor on a patent describing a novel method for cough detection for sound. The patent is owned by the hospital in which I work and has been licensed to a medical device company, but I have not receievd any royalties to date.
REVIEW RETURNED	06-Dec-2015

GENERAL COMMENTS	This paper describes a novel and interesting protocol for a study designed to assess objective cough frequency in patients with pulmonary TB and how this responds to treatment initiation using a novel cough monitoring system. COMMENTS 1) The biggest issue with this protocol is that it is based on the

	assumption that the cough monitoring system to be used accurately quantifies cough. This is based upon a previous study where only 25hrs of data was assessed and sensitivity of only 75% achieved. I would strongly advise the authors to incorporate further validation and improvement of their algorithm into this protocol. Otherwise the accuracy of the primary endpoint is questionable and the ability to detect change may be significantly impaired. This needs to be acknowledged in the discussion of the limitations of the protocol. 2) Statistical analysis. Cough frequency data is generally positively skewed and requires log transformation for analysis. The authors could look at some of the other DBRCT pharmacological intervention studies using cough counts to guide their handling of the cough count data e.g. Khalid et al JACI 2013 and Abdulqawi et al Lancet 2015. 3) Sample size. The authors would be well advised to consider how many of their recordings may fail due to technical issues, e.g. compact flash card failure, microphone failure, issues with connects and boost the sample size accordingly.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1:

1.1 This is an innovative and potentially important study. It is more focussed on response to treatment than diagnostic characteristics of the cough related to TB. For example, persistent non-remitting cough >2w in children is useful for PTB diagnosis (Marais Pediatrics 2006; 118: e1350). Pregnancy is excluded despite a high risk for TB in the childbearing years. These are not necessarily limitations but could be interesting to address in the present study or later on.

RESPONSE. Thank you, we are aware that excluding children and pregnant women are potential limitations. This comment has been addressed with the following text in the limitations paragraph of the discussion section of the manuscript:

DISCUSSION: “This study is limited by restriction to only non-pregnant adults because this is the population for which the algorithm has been validated. However, future research is planned to include these important vulnerable populations.”

1.2 Time to positivity for MGIT is a nice biomarker for severity of disease for liquid culture (Carroll Tuberculosis 2008; 88: 624-630) and could be useful with MODS also?

RESPONSE. Thank you, we agree that time to positivity in liquid culture is an important biomarker for severity of disease and have added this measure to the manuscript. Although time to positivity in liquid culture has usually been assessed with the automated MGIT technique, the MODS technique is fundamentally similar to the MGIT test. As would be expected, we have recently validated time to culture positivity in the MODS assay as a reliable predictor of colony forming units and of severity of disease and will report these complex data in a separate publication that is currently being prepared by our research group. We have also included in our manuscript the important reference that Reviewer 1 cites. This is mentioned in the Methods section under the Personnel, training and logistics subsection:

METHODS / Personnel, training and logistics: “The number of days to culture positivity in the MODS liquid culture assay will be recorded in order to assess the microbiological burden in the patients’ samples, based on prior work done with a similar technique.[46]”

1.3 I am sure that Infection Prevention & Control practise be adhered to, but this should be addressed in the protocol.

RESPONSE. We agree and have explicitly stated this in the Methods section, under the Personnel, training and logistics subsection.

METHODS / Personnel, training and logistics: “We will adhere to recommended infection prevention & control practices for TB to reduce bio-risk in healthcare professionals and patients.[44]”

1.4 Will additional subjects be recruited if some fail to complete all of the study procedures?

RESPONSE. We thank Reviewer 1 for this helpful suggestion and have added a paragraph in the Methods section to address it. This is mentioned in the Sample Size subsection:

METHODS / Sample Size: “An additional 10% of patients will be recruited, to correct for patients who do not complete all of the study procedures. Thus, we will aim to recruit a total of 107 patients.”

Reviewer #2:
2.1 Transmission of TB results from yet incompletely understood host, environmental and bacterial factors. Although central to both the disease itself and to transmission, cough is only one factor in a complex web of characteristics. By only measuring cough frequency, the proposed study reduces the stated complexity further. Even cough itself has several elements of study beyond frequency such as cough severity (strength). Therefore, the authors should restrict discussion and objectives to response to treatment rather than TB transmission.

RESPONSE. We thank Reviewer 2 for his important comments. We agree with Reviewer 2’s point that we are not measuring transmission but rather response to treatment and this is a limitation of the study. However, infectiousness is naturally assumed to be increased / prolonged in cases of lack of response to treatment. We have sought to address this comment with the following statements:

INTRODUCTION: “A reduction in cough is assumed to represent adequate response to treatment, and to result in decreased risk of spread of infection.”

OBJECTIVES: “The third objective of this study is to test for an association between changes in cough frequency and microbiological resolution of TB disease during therapy..”

DISCUSSION: “If a correlation with bacteriological treatment response is demonstrated, then this would have the potential to contribute to patient management without relying on a laboratory in adult patients with pulmonary TB. However, it should be noted when monitoring TB patients’ response because some patients may have adverse treatment outcomes despite an initial transient positive response to therapy.”

2.2 Use of sputum outcomes seems outdated in a TB study focused on cough. The importance of cough in TB transmission is that cough is the generator of infectious aerosols, the infectious moiety in TB. Despite long-standing evidence that fine aerosols transmit TB, most studies continue to rely on sputum. Recent work with cough-generated aerosols in patients with TB have demonstrated the limitations of continuing to use sputum to study TB transmission.

RESPONSE. We thank Reviewer 2 for highlighting this. We agree that this is a limitation of the study. However, our main objective is to assess response to treatment. Regarding cough-generated aerosols, in our pilot study we tried to measure cough-generated aerosols in patients with TB; however, this was unsuccessful due to environmental (fungal) contamination. We plan to modify our methods and to re-attempt this in future TB transmission studies, and this has been addressed with the following text in the last paragraph of our Discussion:

DISCUSSION: “In future studies we intend to better assess infectiousness by additionally quantifying TB in cough-generated aerosols.”

2.3 As stated by the authors, plan to record 24-hr cough is both a strength and a weakness as it will be difficult to separate out ambient noise

RESPONSE. We agree and that is why we have been very careful in using data-quality control measures as well as manual review. This comment has been addressed with the following text in the Methods and in the Discussion sections of the manuscript:

METHODS / Processing of audio recordings: “We will employ a semi-automated approach in which cough epochs that are automatically detected will then be manually reviewed to eliminate false positives. This is necessary as our recordings will be made in very noisy environments (outside clinical settings) and false detection rates for a fully automated system remain high. For this study, a simple graphical user interface will be constructed to allow nurses to review automatically detected epochs, enabling listening to each as often as needed, and then to either accept or reject the detected cough. Thus, the review of automatically detected coughs acts to eliminate algorithmic false positive coughs.”

DISCUSSION: “Normal day recordings are confounded by background noise, which is a challenge for analysis of cough recordings, considering that traffic and environmental noise (such as dogs barking, music, and television) may generate noises similar to cough. To diminish this effect we have incorporated a time-varying estimate of the noise background as well as a data quality control. Having a semi-automated algorithm is a limitation, since it requires time and human input, but also a strength since the human ear is the gold standard for determining the characteristic sound of cough.”

2.4 The authors plan to enroll a combination of drug-susceptible and drug-resistant TB cases into the study. In most settings, drug-resistant TB cases concentrate among patients with a previous history of TB treatment (i.e retreatment TB cases); many of these patients have more severe disease than those with drug-susceptible disease. As such, I would anticipate that the kinetics of cough in drug-susceptible and drug-resistant cases will be quite variable after treatment. A Similar argument can be made for HIV (e.g. less and weaker cough in patients with advanced HIV/AIDS). Finally, many patients have persistent cough despite treatment due to TB-induced bronchiectasis, fibrosis, and residual inflammation. These considerations should be explicitly stated in the analysis plan (stratified analysis?) and sample size calculations.

RESPONSE. This is an important consideration, and will be included in the analysis, as follows:

METHODS / Statistical methodology and analysis: “We will also consider variables such as gender, HIV status, drug resistance, and previous history of TB, in our analysis, either by stratifying or by adjusting for these variables in our models.”

2.5 Authors mention the cough measuring tool they will employ has been validated in TB patients but present no data. A summary of these data should be included in the protocol.

RESPONSE. Thank you. This will be included in our protocol and cited accordingly.

METHODS / Processing of audio recordings: “Briefly, cough recordings will be analysed using a 2-step algorithm: first, event detection, followed by event classification into cough vs. non-cough. Detection of acoustic events will be based on the signal energy proportional to the voltage-squared of the signal. An acoustic event will detect if the signal energy exhibited a rapid increase above a time-

varying baseline estimate of ambient noise. The next stage of processing seeks to classify detected events. Here, the spectral features of each time frame in the acoustic event are characterised using Mel frequency cepstral coefficients and their derivatives. As described in detail elsewhere,[1] a training data set will be used to develop a classifier based on the sequential minimal optimisation (SMO) algorithm. Based on classifier outputs, each acoustic event will be marked as 'cough' or 'not-cough'.

Isolated cough events will be automatically combined into cough epochs, or bursts of closely spaced individual coughs, following previous research.[2] We will employ a definition of cough epochs, as defined in the 'Outcomes and case definitions' section above. Note that within the cough literature, a variety of metrics are available for describing cough, and there is no clear evidence as to which are most clinically meaningful. We have previously published a review and discussion of these various metrics (number of individual coughs, number of cough bouts or epochs, number of 1-sec periods containing cough, etc.).[1]

We will employ a semi-automated approach in which cough epochs that are automatically detected will then be manually reviewed to eliminate false positives. This is necessary as our recordings will be made in very noisy environments (outside clinical settings) and false detection rates for a fully automated system remain high. For this study, a simple graphical user interface will be constructed to allow nurses to review automatically detected epochs, enabling listening to each as often as needed, and then to either accept or reject the detected cough. Thus, the review of automatically detected coughs acts to eliminate algorithmic false positive coughs.

Validation: The approach described in the paragraphs above was previously validated using as gold standard a fully manual review of 60 files (15 subjects, 4 randomly selected time periods per subject) in which two nurses listened to all files in their entirety.[1] Because nurses only manually marked the start of each cough, validation was compared on the basis of the epoch definition described above. The semi-automated approach described above gave 75.5% sensitivity in detecting coughs (true positive rate of 6.8/hour) with an average false positive rate of 0.5/hour.[1] While the semi-automated approach does require time for human review, the initial automated step will remove the large majority of possible events. Thus on average, review time is reduced by nearly two orders of magnitude compared to a fully manual review in which the entire recording is reviewed. We will also maintain the privacy of subjects, as non-cough events, such as conversation, will never be reviewed by the human ear."

2.6 Authors plan to measure M. tuberculosis bacillary load using sputum acid-fast bacilli (AFB) smear microscopy. However, sputum AFB is known to be an imprecise measure of bacillary load, when compared to semi-quantitative Middlebrook cultures, liquid (MGIT 960) cultures, or quantitative cultures.

RESPONSE. Thank you, we have recently validated time to culture positivity in the MODS assay as a reliable predictor of colony forming units and will report this separately. This is mentioned in the Methods section under the Personnel, training and logistics subsection.

METHODS / Personnel, training and logistics: "The number of days to culture positivity in the MODS liquid culture assay will be recorded in order to assess the microbiological burden in the patients' samples, based on prior work done with a similar technique.[46]"

2.7 Inclusion criteria will accept patients with up to 30 days of antituberculous treatment despite noting that cough is likely to change within 2 weeks of treatment. Similar argument with patients placed on a new regimen due to suspected or confirmed drug resistance (i.e. partial treatment may change cough patterns).

RESPONSE. We agree, and have changed this to up to 7 days. This is reflected in our manuscript.

METHODS / Study Population: “had started a new treatment regimen for TB within the last 7 days”

This has also been changed in Figure 1.

2.8 8. Why exclude patients under 18 years of age? Children are probably group with most need of non-invasive method to monitor response to treatment.

RESPONSE. We thank Reviewer 2 for this comment. This has been addressed in the limitations paragraph of our Discussion.

DISCUSSION: “This study is limited by restriction to only non-pregnant adults because this is the population for which the algorithm has been validated. However, future research is planned to include these important vulnerable populations.”

2.9 Beyond providing more granular information on chest pathology, not clear about the risk/benefit of performing chest CT (significant radiation risk) in such a large number of patients. How will the knowledge provided by CT be applied?

RESPONSE. We have made the chest CT scan optional for all patients in the study, and will explain the risk/benefit ratio clearly to each patient. CT scan information will also be returned promptly to the patient’s medical team. We expect that CT information will provide us with more precise information about present bronchiectasis, atelectasis, lymph nodes that could not be observed through a simple CXR. As you mentioned earlier, patients with bronchiectasis (and other pulmonary diseases) might have different cough kinetics and we expect to address this question. We also expect that as the cavitation is greater the inflammation will be more present and cough frequency will be higher. This is now mentioned in the Methods section.

METHODS / Radiology: “Cavitations will be further described by size, location, presence or absence of an air-fluid level, and cavity wall thickness based on prior work that shows the relevance of these findings to pulmonary TB.[51-53] We will also explore whether other radiological findings are predictive of microbiological burden and cough frequency.”

2.10 Need to define what “as close to baseline” means (Figure 1). Study visit window likely needs to be tight given rapid cough response after treatment expected by authors.

RESPONSE. Thank you. We have made this clarification with the following more precise wording:

Figure 1: “As close to baseline” has been changed to “First Week”.

2.11 The questionnaire can be improved:
- Feels more as educational tool rather then TB disease severity assessment tool
- Errors in Spanish (Direcciones instead of Instrucciones)
- Visual cough analog scale at the end of page 2 has a 5-level ordinal scale instead of 10 (cough scale is extrapolation of standard 10-level pain scale) (see Raj AA, Birring SS (2007) Clinical assessment of chronic cough severity. Pulmonary pharmacology & therapeutics 20: 334–337).

RESPONSE. Our questionnaire aims to assess TB disease burden and also obtain possible risk

factors for cough frequency. Thank you for pointing out our Spanish error, we have corrected it. Regarding our visual analog scale, In our previous research in this setting, where many participants have had little schooling, visual analogue scales and scales and scales with abstract ranges (e.g. 0-10) have been bewildering and left blank by many participants. We understand that this contrasts with other research settings and thank the reviewer for this suggestion that we regret we do not feel able to follow. This is now mentioned in our manuscript:

METHOD / Personnel, training and logistics: “It should be mentioned that we used a 5-level ordinal scale instead of 10 to make it simpler for our interviewees. We have found it easier in this setting for research participants to interpret 5-levels each with defining words (never, little, much, almost always, always) rather than 10.”

2.12 Chest X-ray data capture form seems unnecessarily complicated. Has it been validated?

RESPONSE. We thank Reviewer 2 for raising this issue. The chest X-ray data capture form has not yet been validated. It will help for both CXR and CT scans. It enabled both our local radiologist and US-based radiologist to evaluate each film. Even though it is complicated it provides us with considerable radiological information that is needed when looking for explanations of cough frequency. We will use this initial questionnaire and our data obtained to make easier and more easy-friendly forms for future studies. This is expressed in the Methods section.

METHODS / Radiology: “Films will be read by a local radiologist and a US board-certified radiologist blinded to the patient’s demographics and outcomes.”

METHODS / Radiology: “We will also explore whether other radiological findings are predictive of microbiological burden and cough frequency.”

2.13 The primary outcome of this relatively large and intervention-heavy study is a two-fold reduction in cough frequency. However, simple clinical observation shows that cough frequency and severity (as well as other TB symptoms) improve after treatment (assuming drug-susceptible disease and treatment adherence). As such, although interesting, a detailed description of cough kinetics after treatment seems unnecessary unless it is shown to be a surrogate marker for infectiousness and risk of transmission. The current study is not designed to assess transmission.

RESPONSE. We agree. We do not intend to assess transmission, but rather assess response to treatment. We agree that to understand TB transmission a more complex study should be done. However, as has been mentioned earlier we have not been able to use cough-generated aerosols adequately in our setting. We are working to develop a better technique for assessing transmission, but in the meantime, feel that a detailed description of cough kinetics after treatment is a worthwhile first step. This is mentioned in our Discussion.

DISCUSSION: “We expect that this project will generate a novel method to evaluate treatment response. In future studies we intend to better assess infectiousness by additionally quantifying TB in cough-generated aerosols.”

2.14 Unclear applicability in real world circumstances. Authors propose as a potential alternative (for TB diagnosis???) in settings where laboratories are not available? However, cough monitoring device appears technologically challenging and thus, too complex for most settings where TB is prevalent (and laboratory diagnosis is often lacking).

RESPONSE. We do not intend to use cough monitoring for a potential alternative for TB diagnosis but rather to assess response to treatment. We fully understand the concern regarding the complexity of

this method and have addressed it in our Discussion.

DISCUSSION: “If a correlation with bacteriological treatment response is demonstrated, then this would have the potential to contribute to patient management without relying on a laboratory in adult patients with pulmonary TB. However, it should be noted when monitoring TB patients’ response because some patients may have adverse treatment outcomes despite an initial transient positive response to therapy.”

DISCUSSION: “Cough monitoring devices seem challenging; however we believe that this is the first step towards telemedicine in cough-TB. In Peru, many rural areas do not have facilities for laboratory diagnosis, but have at least one physician or healthcare professional. They may be trained in placing these devices. We are also working on making devices smaller, cheaper, and easier to use.”

Reviewer #3:
3.1 The biggest issue with this protocol is that it is based on the assumption that the cough monitoring system to be used accurately quantifies cough. This is based upon a previous study where only 25hrs of data was assessed and sensitivity of only 75% achieved. I would strongly advise the authors to incorporate further validation and improvement of their algorithm into this protocol. Otherwise the accuracy of the primary endpoint is questionable and the ability to detect change may be significantly impaired. This needs to be acknowledged in the discussion of the limitations of the protocol.

RESPONSE. We agree, and have included more information regarding the validation of our cough algorithm into the protocol. We have also included in the Discussion how we aim to improve our sensitivity. These improvements in response to Reviewer #3 ’s comments are shown below:

METHODS / Processing of audio recordings: “Briefly, cough recordings will be analysed using a 2-step algorithm: first, event detection, followed by event classification into cough vs. non-cough. Detection of acoustic events will be based on the signal energy proportional to the voltage-squared of the signal. An acoustic event will detect if the signal energy exhibited a rapid increase above a time-varying baseline estimate of ambient noise. The next stage of processing seeks to classify detected events. Here, the spectral features of each time frame in the acoustic event are characterised using Mel frequency cepstral coefficients and their derivatives. As described in detail elsewhere,[1] a training data set will be used to develop a classifier based on the sequential minimal optimisation (SMO) algorithm. Based on classifier outputs, each acoustic event will be marked as ‘cough’ or ‘not-cough’.

Isolated cough events will be automatically combined into cough epochs, or bursts of closely spaced individual coughs, following previous research.[2] We will employ a definition of cough epochs, as defined in the ‘Outcomes and case definitions’ section above. Note that within the cough literature, a variety of metrics are available for describing cough, and there is no clear evidence as to which are most clinically meaningful. We have previously published a review and discussion of these various metrics (number of individual coughs, number of cough bouts or epochs, number of 1-sec periods containing cough, etc.).[1]

We will employ a semi-automated approach in which cough epochs that are automatically detected will then be manually reviewed to eliminate false positives. This is necessary as our recordings will be made in very noisy environments (outside clinical settings) and false detection rates for a fully automated system remain high. For this study, a simple graphical user interface will be constructed to allow nurses to review automatically detected epochs, enabling listening to each as often as needed, and then to either accept or reject the detected cough. Thus, the review of automatically detected coughs acts to eliminate algorithmic false positive coughs.

Validation: The approach described in the paragraphs above was previously validated using as gold standard a fully manual review of 60 files (15 subjects, 4 randomly selected time periods per subject) in which two nurses listened to all files in their entirety.[1] Because nurses only manually marked the start of each cough, validation was compared on the basis of the epoch definition described above. The semi-automated approach described above gave 75.5% sensitivity in detecting coughs (true positive rate of 6.8/hour) with an average false positive rate of 0.5/hour.[1] While the semi-automated approach does require time for human review, the initial automated step will remove the large majority of possible events. Thus on average, review time is reduced by nearly two orders of magnitude compared to a fully manual review in which the entire recording is reviewed. We will also maintain the privacy of subjects, as non-cough events, such as conversation, will never be reviewed by the human ear."

DISCUSSION: "We aim to improve our sensitivity by fully automated processing remains a long-term goal for our group, and we anticipate that experience gained with semi-automated analysis will aid us in developing future algorithms. In addition we are now developing second-generation devices where the validity is improved by employing accelerometers."

3.2 Statistical analysis. Cough frequency data is generally positively skewed and requires log transformation for analysis. The authors could look at some of the other DBRCT pharmacological intervention studies using cough counts to guide their handling of the cough count data e.g. Khalid et al JACI 2013 and Abdulqawi et al Lancet 2015.

RESPONSE. Thank you, we have reviewed those articles. We agree and hope that we have adequately addressed this issue.

METHODS / Statistical methodology and analysis: "Positively-skewed cough data may be log-transformed to facilitate data visualization and analysis."

3.3 Sample size. The authors would be well advised to consider how many of their recordings may fail due to technical issues, e.g. compact flash card failure, microphone failure, issues with connects and boost the sample size accordingly.

RESPONSE. Thank you. We agree and hope that we have adequately addressed this issue in the Sample Size subsection within Methods.

METHODS / Sample Size: "An additional 10% of patients will be recruited, to correct for patients who do not complete all of the study procedures. Thus, we will aim to recruit a total of 107 patients."

VERSION 2 – REVIEW

REVIEWER	Edward C. Jones Lopez Boston Medical Center and Boston University School of medicine Boston, USA
REVIEW RETURNED	11-Jan-2016

GENERAL COMMENTS	The authors addressed most of my comments adequately. However, I still have reservations on two important issues noted in the initial review. I provide a comment under each remaining issue below. I pasted the issue raised in the initial review and the authors' response for clarity.

	2.9 Beyond providing more granular information on chest pathology, not clear about the risk/benefit of performing chest CT (significant radiation risk) in such a large number of patients. How will the knowledge provided by CT be applied? RESPONSE. We have made the chest CT scan optional for all patients in the study, and will explain the risk/benefit ratio clearly to each patient. CT scan information will also be returned promptly to the patient's medical team. We expect that CT information will provide us with more precise information about present bronchiectasis, atelectasis, lymph nodes that could not be observed through a simple CXR. As you mentioned earlier, patients with bronchiectasis (and other pulmonary diseases) might have different cough kinetics and we expect to address this question. We also expect that as the cavitation is greater the inflammation will be more present and cough frequency will be higher. This is now mentioned in the Methods section. REVIEWER COMMENT: As stated in the initial review, it is clear chest CT will provide more detailed information on chest pathology when compared to chest X-ray. I also agree the added detail provided by CT may (or may not) explain any observed differences in cough patterns. However, I am still uncomfortable with the risk/benefit ratio of such an intervention in a relatively large number of patients. The main issue is that it is not clear how this information (positive or negative correlation) will benefit either the method being studied or patients. One option would be to perform chest CT in a sub-group of patients (selected randomly). 2.13 The primary outcome of this relatively large and intervention-heavy study is a two-fold reduction in cough frequency. However, simple clinical observation shows that cough frequency and severity (as well as other TB symptoms) improve after treatment (assuming drug-susceptible disease and treatment adherence). As such, although interesting, a detailed description of cough kinetics after treatment seems unnecessary unless it is shown to be a surrogate marker for infectiousness and risk of transmission. The current study is not designed to assess transmission. RESPONSE. We agree. We do not intend to assess transmission, but rather assess response to treatment. We agree that to understand TB transmission a more complex study should be done. However, as has been mentioned earlier we have not been able to use cough-generated aerosols adequately in our setting. We are working to develop a better technique for assessing transmission, but in the meantime, feel that a detailed description of cough kinetics after treatment is a worthwhile first step. This is mentioned in our Discussion. REVIEWER COMMENT: In my opinion, reason/utility of study is still unclear if primary outcome is cough kinetics and/or response to treatment; simple clinical observation is very likely equivalent. Proposed study would make most sense if primary outcome was infectiousness, but requires different study. 2.14 Unclear applicability in real world circumstances. Authors

	propose as a potential alternative (for TB diagnosis???) in settings where laboratories are not available? However, cough monitoring device appears technologically challenging and thus, too complex for most settings where TB is prevalent (and laboratory diagnosis is often lacking). RESPONSE. We do not intend to use cough monitoring for a potential alternative for TB diagnosis but rather to assess response to treatment. We fully understand the concern regarding the complexity of this method and have addressed it in our Discussion. DISCUSSION: "If a correlation with bacteriological treatment response is demonstrated, then this would have the potential to contribute to patient management without relying on a laboratory in adult patients with pulmonary TB. However, it should be noted when monitoring TB patients' response because some patients may have adverse treatment outcomes despite an initial transient positive response to therapy." REVIEWER COMMENT: This comment is related to issue 2.13 above. Although the proposed study would provide interesting data, it is unclear to me what added clinical information it will provide beyond what is already provided by simple clinical observation. In their discussion, the authors seem to suggest that measurement of cough kinetics could inform clinical management without relying on a laboratory; it is not clear to me how this will be the case.

REVIEWER	Jacky Smith University of Manchester, United Kingdom I am a named inventor on a patent describing a method for detecting cough from acoustic recordings.
REVIEW RETURNED	27-Jan-2016

GENERAL COMMENTS	I have no further comments

VERSION 2 – AUTHOR RESPONSE

Reviewer Edward C. Jones Lopez:

2.9 Beyond providing more granular information on chest pathology, not clear about the risk/benefit of performing chest CT (significant radiation risk) in such a large number of patients. How will the knowledge provided by CT be applied?

RESPONSE. We have made the chest CT scan optional for all patients in the study, and will explain the risk/benefit ratio clearly to each patient. CT scan information will also be returned promptly to the patient's medical team. We expect that CT information will provide us with more precise information about present bronchiectasis, atelectasis, lymph nodes that could not be observed through a simple CXR. As you mentioned earlier, patients with bronchiectasis (and other pulmonary diseases) might have different cough kinetics and we expect to address this question. We also expect that as the cavitation is greater the inflammation will be more present and cough frequency will be higher. This is now mentioned in the Methods section.

REVIEWER COMMENT: As stated in the initial review, it is clear chest CT will provide more detailed information on chest pathology when compared to chest X-ray. I also agree the added detail provided by CT may (or may not) explain any observed differences in cough patterns. However, I am still uncomfortable with the risk/benefit ratio of such an intervention in a relatively large number of

patients. The main issue is that it is not clear how this information (positive or negative correlation) will benefit either the method being studied or patients. One option would be to perform chest CT in a sub-group of patients (selected randomly).

AUTHOR'S COMMENT: We agree with the Reviewer 2 that the risk/benefit ratio of the CT must be carefully weighed. As previously mentioned, we will explain the risks and benefits of the CT scan carefully to each participant. With this in consideration, we will like to highlight two sections that have been improved to mention all the benefits CT chest scans will provide when evaluating pulmonary tuberculosis. As follows:

METHODS / Outcomes and case definitions: "Chest X-ray films (CXR) provide a high negative predictive value for the presence of active TB,[1] but CXR might be normal when in fact there is parenchymal disease.[2] More specifically, CT scans correctly determine pulmonary TB cases in 91% of cases whereas CXR only in 49% of cases.[2-5] In addition, CT scans provide higher sensitivity for the detection of lymphadenopathy, early bronchogenic spread, and to evaluate cavitation and disease activity.[5]"

METHODS / Radiology:
"Priority will be given to CT scans, since they have been shown to be more sensitive in general.[5] A previous study determined that the sensitivity for the prediction of active TB through CT scans was of 96%, whereas for CXR it was merely 48%.[6]"

"Cavitations will be further described by size, location, presence or absence of an air-fluid level, and cavity wall thickness based on prior work that shows the relevance of these findings to pulmonary TB and infectivity.[7-11] It is therefore important to determine cavitations, and, as Im and collaborators have shown, CT correctly identifies cavitations in 58% of cases, whereas CXR only in 22%.[8]"

2.13 The primary outcome of this relatively large and intervention-heavy study is a two-fold reduction in cough frequency. However, simple clinical observation shows that cough frequency and severity (as well as other TB symptoms) improve after treatment (assuming drug-susceptible disease and treatment adherence). As such, although interesting, a detailed description of cough kinetics after treatment seems unnecessary unless it is shown to be a surrogate marker for infectiousness and risk of transmission. The current study is not designed to assess transmission.

RESPONSE. We agree. We do not intend to assess transmission, but rather assess response to treatment. We agree that to understand TB transmission a more complex study should be done. However, as has been mentioned earlier we have not been able to use cough-generated aerosols adequately in our setting. We are working to develop a better technique for assessing transmission, but in the meantime, feel that a detailed description of cough kinetics after treatment is a worthwhile first step. This is mentioned in our Discussion.

REVIEWER COMMENT: In my opinion, reason/utility of study is still unclear if primary outcome is cough kinetics and/or response to treatment; simple clinical observation is very likely equivalent. Proposed study would make most sense if primary outcome was infectiousness, but requires different study.

AUTHOR'S COMMENT FOR 2.13 and 2.14:
Regarding response to treatment, simple clinical observation of cough has not been rigorously validated. Problems include variability of observers, time of observation and lack of expertise. Even when interpreting chest films to determine tuberculosis, the inter-observer agreement is poor.[12 ,13] Thus, an objective tool should be used to assess response to treatment, such as time to positivity of cultures.[14] We aim to test the association between time to positivity of cultures and cough frequency. If these is found to be associated, it would provide clinicians with another important and straight-forward tool to evaluate response to treatment. By evaluating the timing of cough as well as the time to no cough, we can also help clinicians and policymakers to make the best decisions regarding airborne precautions for this deadly disease.

Regarding the transmission of tuberculosis, we have reviewed the elegant studies by Fennelly and Jones-Lopez and agree that aerosols may be a more accurate way to examine transmission than cough.[11 ,15-17] That being said, one of the risk factors for TB aerosols they mention in both their

literature review and in their data on cough, is the strength of the cough. Unfortunately they only measured the frequency of cough only in their pilot study, and for five minutes.[15] In addition they never mentioned in any of their studies that clinical observation was used for any determination but rather depend on objective measures.

While we agree that their aerosol methodology is indeed excellent it can only be done at a very high cost and without a commercial product. Multiple plates must be examined and results are only available at six weeks. The apparatus is indeed bulky and not simply used in any other circumstance than a reference centre with both the apparatus and the ability to clean it and a high powered laboratory. Not being able to obtain results before six weeks limits its use both for protection of subjects from transmission and also in terms of patient isolation or predictability. In addition, they have shown that culture-positive cough-generated aerosols were indeed associated with increased higher levels of cough frequency. Thus, we do not think that aerosol will be able to replace our highly portable and inexpensive method of cough counting.

Although the studies of the BU group have considerable merit they will not be easy to implement in a low resource site except on a highly experimental basis. A simpler approach to transmission including but not restricted to cough counting. We for example have used the time to culture rather than CFUs to speed up the process of obtaining results on culture. Thus, in summary we believe that cough counting is a valid and useful method that will yield useful results both on extent of disease as well as potentially on transmission to families in real time.

2.14 Unclear applicability in real world circumstances. Authors propose as a potential alternative (for TB diagnosis???) in settings where laboratories are not available? However, cough monitoring device appears technologically challenging and thus, too complex for most settings where TB is prevalent (and laboratory diagnosis is often lacking).

RESPONSE. We do not intend to use cough monitoring for a potential alternative for TB diagnosis but rather to assess response to treatment. We fully understand the concern regarding the complexity of this method and have addressed it in our discussion.

DISCUSSION: “If a correlation with bacteriological treatment response is demonstrated, then this would have the potential to contribute to patient management without relying on a laboratory in adult patients with pulmonary TB. However, it should be noted when monitoring TB patients’ response because some patients may have adverse treatment outcomes despite an initial transient positive response to therapy.”

REVIEWER COMMENT: This comment is related to issue 2.13 above. Although the proposed study would provide interesting data, it is unclear to me what added clinical information it will provide beyond what is already provided by simple clinical observation. In their discussion, the authors seem to suggest that measurement of cough kinetics could inform clinical management without relying on a laboratory; it is not clear to me how this will be the case.

AUTHOR’S COMMENT: Please see above, both issues have been addressed.

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Correction

Proaño A, Bravard MA, Tracey BH, *et al*. Protocol for studying cough frequency in people with pulmonary tuberculosis. *BMJ Open* 2016;6:e010365. The segmentation of the eighth author's name is incorrect. This author's last name is Lee, and middle name is O'Neill.

BMJ Open 2016;**6**:e010365corr1. doi:10.1136/bmjopen-2015-010365corr1

Correction

Proaño A, Bravard MA, Tracey BH, *et al*. Protocol for studying cough frequency in people with pulmonary tuberculosis. *BMJ Open* 2016;6:e010365. The segmentation of the tenth author's name is incorrect: his first name is Jose, middle name is Luis and last name is Cabrera, and he should be cited as Cabrera JL.

BMJ Open 2016;**6**:e010365corr2. doi:10.1136/bmjopen-2015-010365corr2