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The Cayetano Cough Monitor: A Method for Investigating Spread of Infection in Tuberculosis

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ABSTRACT

Background: Cough is a key symptom of tuberculosis (TB) as well as the main cause of transmission. However, cough frequency (number of coughs per hour) in patients with TB has been poorly studied. The main aim of this study is to describe cough patterns before and after TB treatment and to determine baseline factors that affect cough frequency in these patients. Secondarily, we will evaluate the correlation between cough frequency and TB microbiological resolution.

Methods: This study will select participants with suspected TB from two tertiary hospitals in Lima, Peru. Participants will initially be evaluated through questionnaires, radiology, MODS broth TB-culture, auramine smear microscopy, and cough recordings. This cohort will be followed for the initial 60 days of anti-TB treatment, and throughout the study several microbiological samples as well as 24-hour cough event recordings will be collected. We will describe the variability of coughs and determine the association with baseline laboratory parameters of pulmonary TB. In addition, we will analyse the reduction in cough frequency in predicting TB cure, adjusted for potential confounders.

Discussion: This will be the first published peer-reviewed study cough frequency in TB patients since the 1960s. The data could be used to develop new technologies to predict TB outcomes during treatment. Understanding the variation of cough frequency during treatment will help clinicians and policy-makers take better decisions regarding isolation and airborne precautions in patients with TB.

Strengths and limitations of this study

- Our study has the limitation that recordings have been processed through a semi-automated algorithm. To decrease time constraints our long-time goal is to create a fully automated processing system. We anticipate that experience gained with semi-automated analysis will aid us in developing future algorithms
- A strength of this project is that its results will reflect actual cough frequency in pulmonary tuberculosis by utilising 24-hour recordings in the patients' normal-day settings (traffic, dogs barking, etc.). We expect that this will generate a novel method of evaluating cough in TB that can be used in real-world scenarios.
- The algorithm employed in this project has been validated specifically for patients with pulmonary tuberculosis, which enables us to use this algorithm in our patients.

INTRODUCTION

Tuberculosis (TB) is an infectious disease, and was responsible for 9.0 million new cases and 1.5 million deaths in 2013.[1] TB is transmitted in the air[2 ,3] and cough is the most important cause of transmission.[4] Cough in people with pulmonary TB disease arises as a result of the inflammatory response to mycobacterial pulmonary infection. A reduction in cough is assumed to result in decreased spread of infection. Despite its crucial role in TB transmission, a recent literature review[5] reported that cough frequency during TB therapy has not been studied since work carried out by Loudon in the 1960s.[6 ,7] Thus, longitudinal cough frequency studies in TB are needed.

Loudon described cough frequency in eight-hour overnight periods for nine weeks. All sounds with amplitude and frequency consistent with possible cough events were recorded and then manually reviewed.[7 ,8] His findings show a two-fold reduction in the first two weeks of treatment, from a mean of 13.6 to 4.75 coughs/hour.[7] *Mycobacterium TB* colony forming units (CFU) also reduced significantly, from 10^6 at baseline 10^3 two weeks later.[9 ,10] This evidence led to the idea that drug-susceptible TB patients become sufficiently non-infective by the second week of treatment that they no longer posed a risk to others. This and other evidence led to the often-used policy that two weeks was the necessary duration of respiratory isolation for newly diagnosed patients commenced on appropriate treatment. Current evidence[11] and guidelines affirm this position;[12 ,13] however, this two week policy has been criticised.[14 ,15] Our group has shown that drug-susceptible TB patients remain sputum culture positive for longer.[16] Most

importantly, the assumption that TB patients are no longer coughing at two weeks has never been corroborated.

The 2015 CHEST guidelines state that acoustic parameters are the best parameter to evaluate the frequency of cough.[17] In order to ensure accurate measurement, it is important to use a standardised method such as automated cough counting with a validated algorithm. Despite the recently growing literature on this topic, these methods are principally being used in the field of non-infectious chronic disease.[18-23] Whilst algorithms for cough-counting have been validated[24-28] our research protocol appears to be the first to do so specifically in patients with pulmonary TB.[29 ,30]

To address this knowledge gap, we have developed the Cayetano Cough Monitor (CayeCoM) and here describe a protocol for it to be used to study cough frequency in patients with pulmonary TB.

METHODS

Study objectives

The primary objective of this study is to describe cough frequency patterns in adults with pulmonary TB before and after treatment initiation.

The second objective of this study is to determine baseline characteristics that correlate with cough frequency, such as patient demographics, radiological findings, presence of multi drug-resistant TB (MDR-TB), and HIV status.

The third objective of this study is to test for an association between cough frequency and microbiological resolution of TB disease.

Study design

This prospective cohort study will follow adult patients with pulmonary TB through their treatment period in Lima, Peru.

Subjects with a confirmed or suspected diagnosis of active pulmonary TB will be referred to our study team. After written informed consent, we will record coughs prior to initiation of TB treatment. Subjects will provide us with early-morning sputum samples that will be tested for active pulmonary TB disease by testing at least one sputum sample using the microscopic-observation drug-susceptibility (MODS) broth culture assay[31-33] and auramine smear microscopy, which assessed the bacillary load.

Patients in whom the pulmonary TB diagnosis is confirmed by MODS will receive treatment delivered by the National TB Programme as per standard practice.[34] Figure 1 summarises the data to be collected at baseline and during the 60 days of follow-up.

Study sites

Peru has one of the highest TB incidence rates in the Americas.[35] More than one-third of the incident TB cases in the Andean region are from Peru. With respect to rates of MDR-TB and extensively drug resistant (XDR) TB, Peru ranks first in all of the Americas. However, underreporting in the region may contribute to Peru's overrepresentation, as shown in the latest Pan American Health Organization (PAHO) report.[35]

Within Peru, Lima and its metropolitan area account for most cases of MDR-TB and XDR-TB.[36] Thus, we will recruit patients from two hospitals: Hospital Nacional Dos de Mayo, located in the historic centre of Lima; and Hospital Nacional Daniel Alcides Carrión, located in Callao and which belongs to Lima's metropolitan area.

Our main site, Hospital Nacional Dos de Mayo (HNDM), is a 650-bed teaching and public national tertiary referral hospital run by the Peruvian Ministry of Health (MINSA). It provides services to the poor population from the surrounding inner city area. HNDM is the only hospital in Peru with a negative pressure ward available for TB patients. Our secondary site is another tertiary referral hospital run by MINSA, Hospital Nacional Daniel Alcides Carrión. This 462-bed teaching health facility lies in the Callao region.

Study population

The infectious disease and pulmonary physicians will refer subjects to the research team. Criteria for referral are suspicion of active pulmonary TB or a confirmed case of active pulmonary TB who has not yet started treatment. Active pulmonary TB is defined by a positive MODS culture result. Subjects will be excluded if they were less than 18 years of age, pregnant, had started a new treatment regimen for TB within the last 30 days, or are unable or unwilling to provide informed consent. If a patient changes treatment regimen, for example due to treatment failure or to an adverse drug reaction, this would

also be considered as a new regimen. Pregnancy is defined by a positive result on serum or urine beta human chorionic gonadotropin (β-hCG) assay.

Outcomes and case definitions

The primary outcomes for this study are cough frequency and microbiological data from serial sputum samples. Cough frequency is defined as the number of cough episodes, or cough epochs, within a time period. Cough epochs are defined as cough events that are within a two-second period frame.[30]

Regarding microbiological data, participants will be entered into the study if they have a positive culture result. Treatment regimens will be adjusted as needed by the treating team based on the results of the MODS drug-susceptibility testing from their sputum. Our study team will not be involved in the treatment regimen selection.

Sputum smear conversion is defined as three consecutive smear-negative results, collected at least 8 hours apart after initial smear positivity at diagnosis.[37] Culture conversion is defined as two consecutive negative culture results, taken at least 30 days apart. This last definition is the one used in the Ministry of Health (MINSA)[34] and is recommended by the World Health Organization (WHO).[38] The date of conversion will be considered as the date of the first negative sputum smear or culture contributing to conversion.

Secondary outcomes include weight, temperature, and radiological characteristics. When possible, radiological interpretation data from chest films and thoracic computed tomography (CT) scans will be obtained. Chest X-ray films (CXR) provide a high negative predictive value for the presence of active TB[39] but CT scans provide higher sensitivity for the detection of lymphadenopathy, early bronchogenic spread, and to evaluate cavitation and disease activity.[40]

Sample size

In a pilot study we estimated that the frequency of cough in TB patients before receiving treatment is approximately 327 coughs during a 24-hour period with a standard deviation of approximately 50. A sample size of 97 patients would enable us to detect a conservative decrease of the mean number of coughs in the 24-hour period of at least 45 coughs after two weeks of treatment, with a 5% Type I error probability and 80% power.

Under the hypothesis that TB patients before treatment experience a high cough frequency, we hypothesise that after two weeks of anti-TB treatment, there will be a clinical response accompanied by a significant reduction in cough frequency. Response is defined as at least a two-fold reduction in cough frequency, which was previously shown to occur within the first 2 weeks of treatment.[7] For power calculations, it is assumed that all subjects will eventually respond to treatment, according to our definition of response, and that once cough frequency has reduced in an individual it will not rise again. We assume that after the two weeks of treatment approximately 10%

of patients would maintain a high frequency of cough. Thus, a sample size of 97 patients will allow us to detect an odds ratio of at least 3.2 for the risk of patients not responding to TB treatment in two weeks of therapy, under a 95% significance and 80% power.

Study organisation

The Asociación Benéfica (A.B.) PRISMA and Universidad Peruana Cayetano Heredia in Lima, Peru will provide local administrative oversight. Overseas, oversight will be conducted by Johns Hopkins University in Baltimore, Maryland, USA.

In Lima, the Pampas office of A.B. PRISMA will provide operations and logistic support for fieldwork. An additional collaborating signal processing team will be based locally in the Universidad Nacional de Ingeniería, Lima, Peru, as well as at Tufts University, Massachusetts, USA.

Our collaborating biostatisticians are based at Tufts University, Tulane University, and Universidad Peruana Cayetano Heredia, Lima, Peru. All investigators are involved in protocol design and technical support and will remain involved in the on-going analyses.

Personnel, training and logistics

Nurses have been trained by study staff to obtain sputum samples in a best-practice fashion based on previous work,[41 ,42] and to operate and troubleshoot all recorder devices, memory cards, and battery packs. Written informed consent is required by all participants. At the time of enrolment subjects will follow the procedures outlined in Figure 1.

Subjects with active pulmonary TB will be followed throughout their TB treatment. After the identification of active pulmonary TB and based on convenience basis, subjects who consent will undergo CXR and a non-contrast thoracic CT scan.

The first day of a new TB treatment regimen is defined as “Day 0”. An initial questionnaire will be completed on that day (Supplementary 1). This questionnaire is similar to the one that was employed in a previous study.[43] Baseline cough frequency will be obtained by performing an audio recording of the patients before they obtain their microbiological results, which is usually a few days prior to treatment initiation. Hence, subjects will be recorded from at least one day prior to treatment and throughout their first two weeks of treatment. They will be subsequently recorded for 24 hours on or around days 21, 30 and 60 of treatment, although up to two days date deviation for Sundays and public holidays will be allowed.

Recordings will start at 09:00 hours and will be as continuous as possible. Occasionally incomplete recordings could be obtained due to malfunction of equipment or patient non-compliance. On the recording days clinical data will be gathered, including: weight, temperature, sputum samples for smear and MODS results. The number of days to culture positivity on the MODS assays will be recorded in order to assess the microbiological burden in the patients’ samples.

Audio recording

Design of the audio recording equipment, the CayeCoM device, builds on previous chronic cough ambulatory audio recordings.[25 ,44 ,45] The CayeCoM device is a Marantz PMD 620 professional handheld recorder, using an Audio-Technica AT899 sub-mini microphone with an AT8537 microphone power module. The microphone will be attached at the patient's lapel as shown in Figure 2. The recorder is adapted to work with an external lithium battery supply (Enix Energies 800040) to enable continuous 24-hour recordings. The audio is recorded onto a SanDisk SDHC 8 GB card, at a sample rate of 48 kHz, encoding 64 kbps in mono in MP3 format. The audio equipment is kept inside a basic pack connected to a lapel microphone. Batteries and SD cards will be exchanged daily by the study nurses. In pilot research, subjects tolerated the audio equipment well, wearing them 24 hours a day and taking them off only to bathe.

Processing of audio recordings

The recorded signals will be analysed after all patient recordings are completed. For cough analysis, software developed by our group and previously described in detail will be used.[29 ,30] This semi-automated approach has an initial automated step that removes 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.

Microbiology

The microbiological tests will be carried out in a Biosafety Containment Level 3 research laboratory situated within Universidad Peruana Cayetano Heredia in Lima, Peru. The sputum samples will be digested and decontaminated by the standard NaOH-N-acetyl cysteine method.[46] For smear microscopy, an aliquot of 100 µl is stained with Auramine O and examined with x400 magnification. Results are determined as negative, paucibacillary (1-19 acid fast bacilli [AFB] visualized in 40 fields), 1+ (20-199 AFB visualized in 40 fields), 2+ (5-50 AFB per field) and 3+ (>50 AFB per field). Culture and MODS susceptibility testing will be performed with the remaining samples, according to standard protocols.[31-33]

Radiology

Radiological information will be gathered, when possible, on a convenience basis. Priority will be given to CT scans, since they have been shown to be more sensitive. Films will be read by a local radiologist and a US board-certified radiologist blinded to the patient's demographics and outcomes. They will provide an interpretation that is standardised as per our study protocol to describe radiological findings including cavitation, consolidation, lymphadenopathy, and effusions (Supplementary 2). 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.[47-49]

Statistical methodology and analysis

All questionnaire data will be double-digitised from paper forms using Visual FoxPro 9 Service Pack 2 (Microsoft Corp. Redmond, Washington, USA) and microbiological data will be double entered using Microsoft Access 2010 (Microsoft Corp. Redmond, Washington, USA). These two data sets will be cross-compared for validity and errors. From these data, descriptive statistics will be tabulated and graphed.

Cough analysis processing results will be stored as Matlab (Mathworks, Inc, Natick MA) files containing information regarding each event and its timestamp. Algorithmically detected coughs will be annotated in the files. After manual review, isolated cough events will be grouped into cough epochs, or bursts of closely spaced individual coughs within 2 seconds, following published work on cough evaluation.[50] We have previously published a review and discussion of these various metrics (including number of individual coughs, number of cough bouts or epochs, and number of 1-sec periods containing cough).[30]

For the first study objective of describing cough frequency, cough epochs will be plotted throughout the day, and cough frequency will be summarized as the frequency of cough epochs per hour. To address the second study objective, characterising correlated of cough frequency, we will use generalised estimating equations (GEE) based Poisson or negative binomial regression with baseline microbiologic status, and trigonometric (sine/cosine) terms to model circadian periodicity, as the independent variables. In addition, a multiple logistic regression in a longitudinal generalized linear model (GLM)

framework analysis will evaluate a function of sputum bacillary load and with cough frequency that we propose as a potential predictor of TB transmissibility. In all cases we will correct for outliers, and nested models will be compared using the likelihood ratio test.

To test the association between cough frequency and microbiological resolution of TB disease associated with the third aim of this study, time-to-event survival analyses where the outcomes of interest are sputum smear conversion, and culture conversion, as defined above, and the primary predictors of interest are cough frequency at baseline, during treatment, and time to two-fold reduction in cough frequency. In addition, secondary analyses of weight, temperature, and radiological characteristics, will be conducted using generalized linear models and GEE logistic regression as appropriate.

Ethical considerations

Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA. Written informed consent will be obtained from all participants. Test results will be delivered by telephone call or at subsequent visits at which time a team physician or nurse will be able to explain the results to the study participants. TB treatment remains the responsibility of the medical staff in charge and the national TB Programme.

Discussion

We will determine cough frequency before and during anti-TB treatment using

the CayeCoM device. We will identify baseline predictors of cough frequency during TB treatment and evaluate the correlation between change in cough frequency and microbiological resolution.

The medical literature currently lacks information about cough frequency in TB. As recently noted by Turner and Bothamley,[5] cough frequency in patients undergoing TB treatment has only been studied once, almost half a century ago.[6 ,7] This previous study has the limitation of only being conducted within an 8-hour period, overnight, and thus there is no information on daytime coughing or the effect of the diurnal rhythm on cough. A similar study[51] demonstrated that the severity of cough and pathological chest x-ray findings were associated with higher levels of TB transmission. However, their study did not measure cough frequency but instead focused on a subjective characteristic: cough severity. It should be noted that to assess cough frequency one must utilise objective acoustic parameters, since self-reported cough is unreliable.[17] As reported in abstract form, the objective acoustic Leicester Cough Monitor (LCM) has been used to evaluate 24-hour cough recordings in patients with pulmonary TB before starting treatment, showing that cough frequency is reduced at night.[52] This further justifies re-evaluation of Loudon's overnight study.

Our project has several strengths and limitations. An important strength is the generation of 24-hour cough recordings, which will provide lengthy recordings, will enable evaluation of cough patterns at different times of day, and also has the benefit of being recorded during a normal day in real-world settings where we expect our device to be used in the future. Normal day recordings are filled

with 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. Similar to Loudon's proposal,[8] our algorithm will help to screen and reduce the length of the recordings to ~5% of their original length, without affecting sensitivity and improving specificity.[30] 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.

CayeCoM has been validated for 24-hour recordings,[30] whereas PulmoTrack (PulmoTrack-CC, KarmelSonix, Haifa, Israel) was validated for 25 minutes[27] and the Hull Automatic Cough Counter for 1-hour recordings.[25] Other systems have also validated their algorithms for 24-hour recordings, such as the LCM,[26 ,53] VitaloJAK,[28] and the LifeShirt System.[24] However, in contrast to our study, none of these algorithms have been validated in the setting of pulmonary TB nor within real-life settings (e.g. traffic). We expect that this project will generate a novel method of evaluating cough in TB that can be used in real-world scenarios, potentially where laboratory investigations are unavailable.

Cough frequency should provide additional information regarding the

evolution of the patients' medical condition. If a correlation with bacteriological treatment response is demonstrated, this has the possibility to contribute to patient management without relying on a laboratory in adult patients with pulmonary TB. However, we should be careful when monitoring TB patients since many might worsen their biomarkers after an initial positive response to therapy. It could assist with decisions regarding the need for on-going respiratory isolation of patients, treatment duration, and identification of patients with treatment failure who may need modification of their treatment regimens. The device also has the potential to be used remotely, as in telemedicine. This is potentially important in a country such as Peru, where the majority of doctors live in the capital, leaving most of the country without a physician in their region.

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Conflicts of interest:

All authors declare that they have no conflict of interest in relation to this work.

Ethics approval:

Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA.

Contributors:

All authors were involved in the study design and writing of the manuscript, and all reviewed the final manuscript before submission. MAB and JWL directly contributed to study design and are responsible for supervision of data gathering. AP, BHT, JWL, MZ and GL will be responsible for data management and statistical analysis for this project.

Data Sharing Statement:

No unpublished data are available. We aim to publish and disseminate our results, once the project is complete. We also expect to create and maintain an online repository for TB cough sounds as well as the statistical analysis employed.

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Figure 1 – Flow Diagram for CayeCoM Study

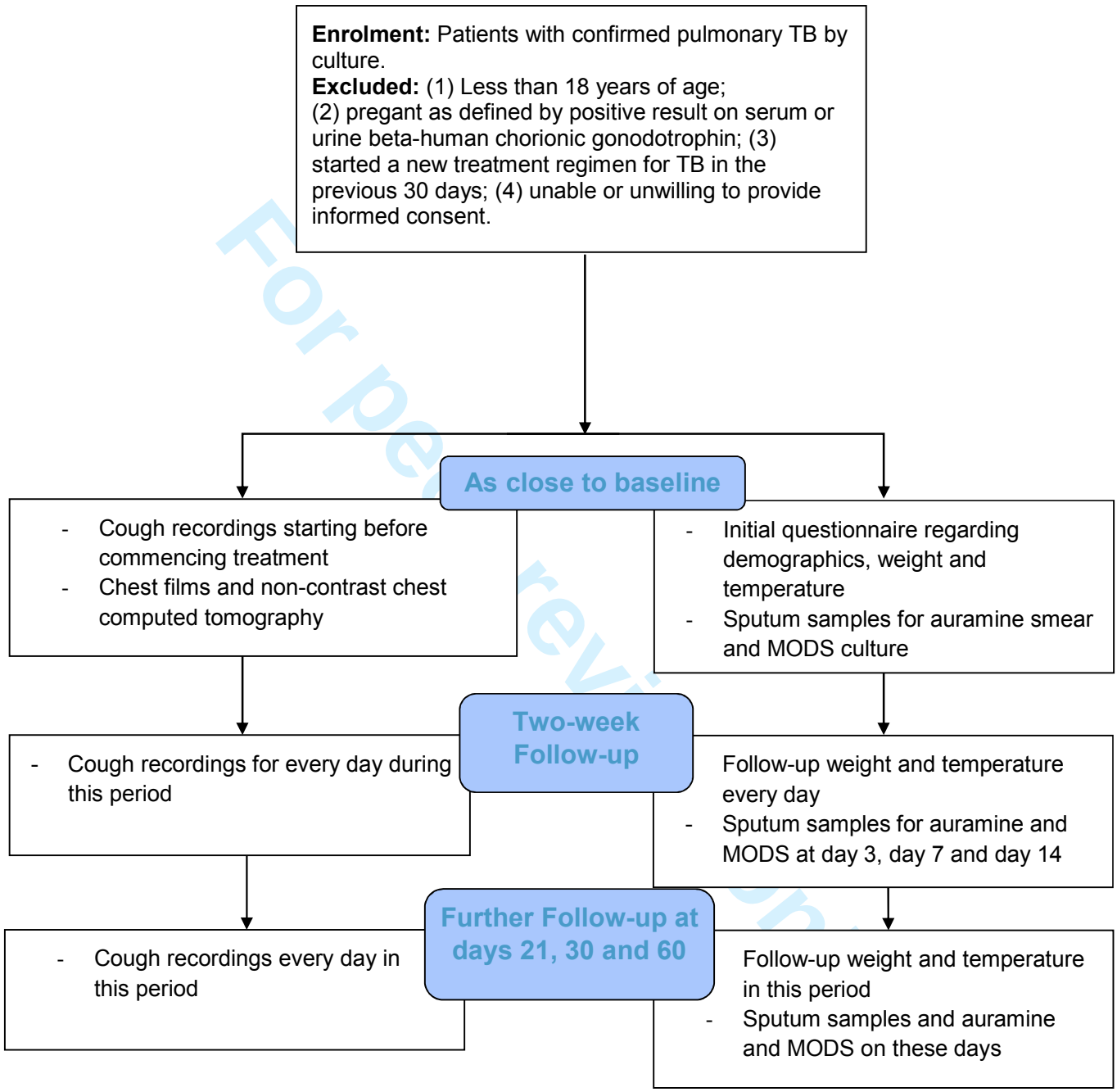

Figure 2 – Picture of the Cayetano Cough Monitor (CayeCoM)

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Entrevistador: _____

Cuestionario Inicial Para Todos Los Participantes:

Datos Demográficos:

1. Edad: _____
2. Sexo: _____

Antecedentes:

3. ¿Cuántas personas normalmente duermen en casa?: _____ Personas
4. ¿Cuántas habitaciones hay en su hogar? (sin contar baño, pasadizo, cocina, depósito, garaje): _____ habitaciones.
5. ¿Cuál es el ingreso mensual de la vivienda? S/._____
6. ¿Cuánto gasta su familia en alimentación cada semana? S/._____
7. ¿Cuántas personas en su vivienda comen de esos alimentos que compran semanalmente? _____ Personas
8. ¿Cuántas veces en el ultimo mes usted se ha acostado con bastante hambre porque no había comida en casa? _____ días

Historia de Tuberculosis:
La tuberculosis es una enfermedad que se trata con varios antibióticos a la vez, y cuyo tratamiento dura varios meses.

9. ¿Ha sido diagnosticado con TBC anteriormente?
   a. Si
   b. No → *Pase a la pregunta 15*
   c. NS
10. ¿Cuántas veces? _____
11. Si recibió tratamiento para la TBC, ¿dónde recibió la mayor parte del tratamiento?
    a. NA
    b. Mismo distrito
    c. Otro distrito
    d. Otra ciudad
    e. Otro país
12. Si recibió tratamiento para la TBC ¿por cuantos meses en total lo tomó? _____
13. Si recibió tratamiento previo, en que esquema estaba (lo mas recién): - _____
14. Si recibió tratamiento para la TBC ¿cumplió con el tratamiento previo?
    a. NA
    b. Si
    c. No→ _____
    d. NS

Factores de Riesgo

15. ¿Ha compartido un cuarto con alguien que haya tenido TBC comprobada?
    a. Si, y esta persona también tenía una tos persistente
    b. Si, pero esta persona concurrente no tenía una tos persistente
    c. No → *Pase a la pregunta 18*
    d. NS
16. ¿Dónde compartió este ambiente con alguien infectado con TBC?
    a. NA
    b. Trabajo
    c. Casa
    d. Hospital
    e. Otro:_____
17. ¿Por cuantos días compartió este ambiente con la persona con TBC comprobada? _____
18. Aparte de usted, ¿alguien mas en casa esta actualmente recibiendo medicinas para la TBC?
    a. Si → Quien: _____
    b. No
    c. NS

Creencias y Conocimiento de la Enfermedad

19. ¿Dónde escuchó de la TBC por primera vez?
    a. Familia
    b. Amigos
    c. Colegio
    d. Puesto de Salud
    e. TV
    f. Radio
    g. NS
20. ¿Puede alguien con TBC y tos infectar a sus familiares?
    a. Si
    b. No
    c. NS

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

21. ¿Qué tan contagiosa cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

22. ¿Qué tan seria cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

23. En general, ¿qué puede hacer uno por si mismo para protegerse de contraer la TBC? [Marcar el factor más importante, no sugerir repuestas].
    a. Vacunarse
    b. Comer
    c. Dormir bien, descansar
    d. Vivir una vida ordenada
    e. Mantenerse alejado de la gente con TBC
    f. Educarse
    g. Otro: _____
    h. NS

¿Cuales son los síntomas de la TBC?
[Marcar los factores, no sugerir repuestas].

24. Tos mencionada Si☐ No☐
25. Hemoptysis mencionada Si☐ No☐
26. Fiebre mencionada Si☐ No☐
27. Baja de peso mencionada Si☐ No☐
28. Fatiga, decaimiento mencionada Si☐ No☐
29. Palidez, un cierto semblante mencionada Si☐ No☐

30. ¿Qué debe hacer una persona con TBC para mejorarse? [Marcar el factor más importante, no sugerir repuestas].
    a. Tomar sus medicinas, asistir a controles
    b. Comer mas, comer mejor
    c. Descansar
    d. Tener fe
    e. Abrigarse
    f. Otro: _____
    g. NS

31. ¿Cómo puede hacer una persona con TBC para no contagiar la TBC a otros? [Marcar el factor más importante, no sugerir repuestas].
    a. Cubrirse la boca al toser
    b. Quedarase en casa, mantenerse alejado
    c. Seguir el tratamiento
    d. Separar cubiertos
    e. Otro: _____
    f. NS

32. ¿Se puede curar la TBC?
    a. Siempre
    b. Normalmente sí
    c. A veces
    d. Raramente
    e. Nunca

Direcciones: Marque una X sobre la línea la posición que escoges.
Ejemplo: ----------▯----------

33. Con que frecuencia esta tosiendo hoy, en un promedio de 24 horas?

0 1 2 3 4 5
_____
nunca poquito mucho casi siempre

Direcciones: Marque una X, en el cuadrado, todas las alternativas que corresponden.
Ejemplo: ☒

34. Que indicadores utilizó para escoger a que posición poner sus X?
    ☐ Los números: 1, 2, 3, 4, 5
    ☐ Las palabras: nunca, poquito, mucho, casi siempre
    ☐ Las figuras: 

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Direcciones: Marque con X la alternativa que corresponde.
Ejemplo: ☒

35. ¿Comparado con hace tres días, tiene más frecuencia de tos el día de hoy?
    a. ☐Tosiendo ahora menos
    b. ☐Tosiendo ahora igual
    c. ☐Tosiendo ahora más

36. El día de hoy, ¿ha tenido *solo tos, tos con flema o solo flema?*
    a. ☐Solo tos
    b. ☐Tos con flema
    c. ☐Solo flema

37. Si tiene tos, ¿a que hora en el día tiene más frecuencia: *mañana, tarde o noche*?
    a. ☐Mañana
    b. ☐Tarde
    c. ☐Noche

38. Qué tan frecuentemente tose?
    a. ☐Cada pocos segundos
    b. ☐Cada pocos minutos
    c. ☐Cada pocas horas
    d. ☐No tose

Direcciones: Marque el numero de días en total que tiene los siguientes síntomas desde que se enfermó. Marque 0 si ningún día.

39. ¿Cuantos días usted ha tenido los siguientes síntomas?
    a. Tos seca _____
    b. Tos con flema _____
    c. Tos con sangre _____
    d. Fiebre _____
    e. Falta de aire _____
    f. Perdida de peso _____
    g. Cansancio o decaimiento _____
    h. Sudor nocturno _____
    i. Falta de apetito _____

Direcciones: Responde a la pregunta en el lugar indicado.

40. Actualmente, ¿como se siente? (0 = mal, 10 = bien): _____

41. Cuando tiene tos, ¿cuantas toses tiene por hora? _____

42. Preguntas sobre VIH

		Manifiesto del paciente	Historia clínica
a.	Usted ha sido diagnosticado por VIH?	_____	_____
b.	Fecha de Diagnostico:	_____	_____
c.	Ultima Carga Viral:	_____	_____
d.	Fecha de ultima Carga Viral:	_____	_____
e.	Ultimo resultado CD4:	_____	_____
f.	Fecha de Ultimo resultado CD4:	_____	_____
g.	Tiempo tomanda TARGA (años)	_____	_____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

Radiologic Interpretation Data Form

Date of read: _____
Radiologist Name: _____

Radiologist Signature: _____

Patient code: _____
Patient gender: ☐ male ☐ female
Patient age: _____

---

1. **Type of film:**
   ☐ AP ☐ PA ☐ Lateral from R ☐ Lateral from L ☐ Thoracic CT without contrast ☐ Other: _____

2. Date of film: Day: _____ Month: _____Year: _____
3. Rotation:_____
4. Adequacy of inhalation: _____

	Site	a. Consolidation?	b. Cavitation?	c. Pneumatocele?	d. Atelectasis?
5	Right upper lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
6	- anterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
7	- apical	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
8	- posterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
9	Right middle lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
10	Right lower lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
11	- superior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
12	- basal	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
13	Left upper lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
14	- anterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
15	- apical	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
16	- posterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
17	Lingula	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
18	Left lower lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
19	- superior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
20	- basal	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no

21. Pleural effusion? ☐ yes ☐ no
left? ☐ small ☐ medium ☐ large
right? ☐ small ☐ medium ☐ large

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

22. Miliary spread? □ yes □ no
23. Pneumothorax? □ yes □ no
where and size: _____
24. Lymphadenopathy: □ yes □ no
which lymph nodes groups
□ hilar □ mediastinal
25. Pericardial effusion? □ yes □ no
left? □ small □ medium □ large
26. Bronchiectasis? □ yes □ no
where: _____
27. Fibrosis? □ yes □ no
where: _____
any retractions, deviations?_____
_____
_____
28. Mediastinal thickening? ? □ yes □ no
29. Any tree-in-bud pattern? Where?_____
_____
_____

30. Cavitation: For each cavity, please describe:
Cavity # 1:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 2:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity #3:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 4:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 5:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

More cavities? □ yes: please use another sheet to describe □ no

Other findings such as fractures, cardiac abnormalities, exudative / fibrotic densities, bronchogenic spread, mass-like lesions (calcified vs. non-calcified), please describe:

_____
_____
_____
_____
_____
_____
_____
_____

31. Normal film? □ yes □ no

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A protocol for studying cough frequency in people with pulmonary tuberculosis

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ABSTRACT

Introduction: Cough is a key symptom of tuberculosis (TB) as well as the main cause of transmission. However, a recent literature review (Turner et al. 2015, J Infect Dis) found that cough frequency (number of coughs per hour) in patients with TB has only been studied once, by Loudon in 1969 (Loudon et al. 1969, Am Rev Respire Dis). The main aim of this study is to describe cough patterns before and after TB treatment and to determine baseline factors that affect cough frequency in these patients. Secondarily, we will evaluate the correlation between cough frequency and TB microbiological resolution.

Methods: This study will select participants with culture confirmed TB from two tertiary hospitals in Lima, Peru. Based on Loudon's results, we estimated that a sample size of 107 patients was sufficient to detect clinically significant changes in cough frequency. Participants will initially be evaluated through questionnaires, radiology, MODS broth TB-culture, auramine smear microscopy, and cough recordings. This cohort will be followed for the initial 60 days of anti-TB treatment, and throughout the study several microbiological samples as well as 24-hour cough event recordings will be collected. We will describe the variability of coughs and determine the association with baseline laboratory parameters of pulmonary TB. In addition, we will analyse the reduction in cough frequency in predicting TB cure, adjusted for potential confounders.

Ethics and dissemination: Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA. We aim to publish and disseminate our findings in peer-reviewed journals. We also expect to create and maintain an online repository for TB cough sounds as well as the statistical analysis employed.

Strengths and limitations of this study

- The algorithm employed in this project has been validated specifically for patients with pulmonary tuberculosis, which enables us to use this algorithm in our patients.
- A strength of this project is that its results will reflect actual cough frequency in pulmonary tuberculosis by utilising 24-hour recordings in the patients' normal-day settings (traffic, dogs barking, etc.). We expect that this will generate a novel method of evaluating cough in TB that can be used in real-world scenarios.
- Our study has the limitation that recordings have been processed through a semi-automated algorithm. To decrease time constraints our long-time goal is to create a fully automated processing system. We anticipate that experience gained with semi-automated analysis will aid us in developing future algorithms.

INTRODUCTION

Tuberculosis (TB) is an infectious disease, and was responsible for 9.6 million new cases and 1.5 million deaths in 2014.[1] TB is transmitted in the air[2 ,3] and cough is the most important cause of transmission.[4] Cough in people with pulmonary TB disease arises as a result of the inflammatory response to mycobacterial pulmonary infection. A reduction in cough is assumed to represent adequate response to treatment, and to result in decreased risk of spread of infection. Despite its crucial role in TB transmission, a recent literature review[5] reported that cough frequency during TB therapy has not been studied since work carried out by Loudon in the 1960s.[6 ,7] Thus, longitudinal cough frequency studies in TB are needed.

Loudon described cough frequency in eight-hour overnight periods for nine weeks. All sounds with amplitude and frequency consistent with possible cough events were recorded and then manually reviewed.[8] His findings show a two-fold reduction in the first two weeks of treatment, from a mean of 13.6 to 4.75 coughs/hour.[7] *Mycobacterium TB* colony forming units (CFU) also reduced significantly, from 10^6 at baseline to 10^3 two weeks later.[9 ,10] This evidence led to the idea that drug-susceptible TB patients become sufficiently non-infective by the second week of treatment that they no longer posed a risk to others. This and other evidence led to the often-used policy that two weeks was the necessary duration of respiratory isolation for newly diagnosed patients commenced on appropriate treatment. Current evidence[11] and guidelines affirm this position;[12 ,13] however, this two week policy has been criticised.[14 ,15] Our group has shown that drug-

susceptible TB patients remain sputum culture positive for longer.[16 ,17] Most importantly, the assumption that TB patients are no longer coughing at two weeks has never been corroborated.

The 2015 CHEST guidelines state that acoustic parameters are the best parameter to evaluate the frequency of cough.[18] In order to ensure accurate measurement, it is important to use a standardised method such as automated cough counting with a validated algorithm. Despite the recently growing literature on this topic, these methods are principally being used in the field of non-infectious chronic disease.[19-24] Whilst algorithms for cough-counting have been validated[25-29] our research protocol appears to be the first to do so specifically in patients with pulmonary TB.[30 ,31]

To address this knowledge gap, we have developed the Cayetano Cough Monitor (CayeCoM) and here describe a protocol for it to be used to study cough frequency in patients with pulmonary TB.

METHODS

Study objectives

The primary objective of this study is to describe cough frequency patterns in adults with pulmonary TB before and after treatment initiation.

The second objective of this study is to determine baseline characteristics that correlate with cough frequency, such as patient demographics, radiological findings, presence of multi drug-resistant TB (MDR-TB), and HIV status.

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.

Study design

This prospective cohort study will follow adult patients with pulmonary TB through their treatment period in Lima, Peru.

Subjects with a confirmed or suspected diagnosis of active pulmonary TB will be referred to our study team. After obtaining written informed consent, we will record coughs prior to initiation of TB treatment. Subjects will provide us with early-morning sputum samples that will be tested for active pulmonary TB disease by testing at least one sputum sample using the microscopic-observation drug-susceptibility (MODS) broth culture assay[32-34] and auramine smear microscopy, to assess the bacillary load.

Patients in whom the pulmonary TB diagnosis is confirmed by MODS will receive treatment delivered by the National TB Programme as per standard practice.[35] Figure 1 summarises the data to be collected at baseline and during the 60 days of follow-up.

Study sites

Peru has one of the highest TB incidence rates in the Americas.[36] More than one-third of the incident TB cases in the Andean region are from Peru. With respect to rates of MDR-TB and extensively drug resistant (XDR) TB, Peru ranks first in all of the Americas. However, underreporting in the region

may contribute to Peru's overrepresentation, as shown in the latest Pan American Health Organisation (PAHO) report.[36]

Within Peru, Lima and its metropolitan area account for most cases of MDR-TB and XDR-TB.[37] Thus, we will recruit patients from two hospitals: Hospital Nacional Dos de Mayo, located in the historic centre of Lima; and Hospital Nacional Daniel Alcides Carrión, located in Callao and which belongs to Lima's metropolitan area.

Our main site, Hospital Nacional Dos de Mayo (HNDM), is a 650-bed teaching and public national tertiary referral hospital run by the Peruvian Ministry of Health (MINSA). It provides services to the poor population from the surrounding inner city area. HNDM is the only hospital in Peru with a negative pressure ward available for TB patients. Our secondary site is another tertiary referral hospital run by MINSA, Hospital Nacional Daniel Alcides Carrión. This 462-bed teaching health facility lies in the Callao region.

Study population

The infectious disease and pulmonary physicians will refer subjects to the research team. Criteria for referral are suspicion of active pulmonary TB or a confirmed case of active pulmonary TB who has not yet started treatment. Active pulmonary TB is defined by a positive MODS culture result. Subjects will be excluded if they were less than 18 years of age, pregnant, have started a new treatment regimen for TB within the last 7 days, or are unable or unwilling to provide informed consent. If a patient changes treatment regimen,

for example due to treatment failure or to an adverse drug reaction, this would also be considered as a new regimen. Pregnancy is defined by a positive result on serum or urine beta human chorionic gonadotropin (β-hCG) assay.

Outcomes and case definitions

The primary outcomes for this study are cough frequency and microbiological data from serial sputum samples. Cough frequency is defined as the number of cough episodes, or cough epochs, within a time period. Cough epochs are defined as cough events that are within a two-second period frame.[31]

Regarding microbiological data, participants will be entered into the study if they have a positive culture result. Treatment regimens will be adjusted as needed by the treating team based on the results of the MODS drug-susceptibility testing from their sputum. Our study team will not be involved in the treatment regimen selection.

Sputum smear conversion is defined as three consecutive smear-negative results, collected at least 8 hours apart after initial smear positivity at diagnosis.[38] Culture conversion is defined as two consecutive negative culture results, taken at least 30 days apart. This last definition is the one used in the Ministry of Health (MINSA)[35] and is recommended by the World Health Organisation (WHO).[39] The date of conversion will be considered as the date of the first negative sputum smear or culture contributing to conversion.

Secondary outcomes include weight, temperature, and radiological characteristics. When possible, radiological interpretation data from chest films and thoracic computed tomography (CT) scans will be obtained. Chest X-ray films (CXR) provide a high negative predictive value for the presence of active TB[40] but CT scans provide higher sensitivity for the detection of lymphadenopathy, early bronchogenic spread, and to evaluate cavitation and disease activity.[41]

Sample size

In a pilot study we estimated that the frequency of cough in TB patients before receiving treatment is approximately 327 coughs during a 24-hour period with a standard deviation of approximately 50. A sample size of 97 patients would enable us to detect a conservative decrease of the mean number of coughs in the 24-hour period of at least 45 coughs after two weeks of treatment, with a 5% Type I error probability and 80% power.

Under the hypothesis that TB patients before treatment experience a high cough frequency, we hypothesise that after two weeks of anti-TB treatment, there will be a clinical response accompanied by a significant reduction in cough frequency. Response is defined as at least a two-fold reduction in cough frequency, which was previously shown to occur within the first 2 weeks of treatment.[7] For power calculations, it is assumed that all subjects will eventually respond to treatment, according to our definition of response, and that once cough frequency has reduced in an individual it will not rise again. We assume that after the two weeks of treatment approximately 10%

of patients would maintain a high frequency of cough. Thus, a sample size of 97 patients will allow us to detect an odds ratio of at least 3.2 for the risk of patients not responding to TB treatment in two weeks of therapy, under a 95% significance and 80% power. 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.

Study organisation

The Asociación Benéfica (A.B.) PRISMA and Universidad Peruana Cayetano Heredia in Lima, Peru will provide local administrative oversight. Overseas, oversight will be conducted by Johns Hopkins University in Baltimore, Maryland, USA.

In Lima, the Pampas office of A.B. PRISMA will provide operations and logistic support for fieldwork. An additional collaborating signal processing team will be based locally in the Universidad Nacional de Ingeniería, Lima, Peru, as well as at Tufts University, Massachusetts, USA.

Our collaborating biostatisticians are based at Tufts University, Tulane University, and Universidad Peruana Cayetano Heredia, Lima, Peru. All investigators are involved in protocol design and technical support and will remain involved in the ongoing analyses.

Personnel, training and logistics

Nurses have been trained by study staff to obtain sputum samples in a best-practice fashion based on previous work,[42 ,43] and to operate and troubleshoot all recorder devices, memory cards, and battery packs. We will

adhere to recommended infection prevention & control practices for TB to reduce bio-risk in healthcare professionals and patients.[44] Written informed consent is required prior to research participation. At the time of enrolment, subjects will follow the procedures outlined in Figure 1.

Subjects with active pulmonary TB will be followed throughout their TB treatment. After the identification of active pulmonary TB and based on convenience basis, subjects who consent will undergo CXR and a non-contrast thoracic CT scan.

The first day of a new TB treatment regimen is defined as “Day 0”. An initial questionnaire will be completed on that day (Supplementary File 1). 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. This questionnaire is similar to the one that was employed in a previous study.[45] Baseline cough frequency will be obtained by performing an audio recording of the patients before they obtain their microbiological results, which is usually a few days prior to treatment initiation. Hence, subjects will be recorded from at least one day prior to treatment and throughout their first two weeks of treatment. They will subsequently be recorded for 24 hours on or around days 21, 30 and 60 of treatment, although up to two days date deviation for Sundays and public holidays will be allowed.

Recordings will start at 09:00 hours and will be as continuous as possible. Occasionally incomplete recordings could be obtained due to malfunction of equipment or patient non-compliance. On the recording days clinical data will be gathered, including: weight, temperature, and sputum samples for smear and MODS results. 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]

Audio recording

Design of the audio recording equipment, the CayeCoM device, builds on previous chronic cough ambulatory audio recordings.[26 ,47 ,48] The CayeCoM device is a Marantz PMD 620 professional handheld recorder, using an Audio-Technica AT899 sub-mini microphone with an AT8537 microphone power module. The microphone will be attached at the patient's lapel as shown in Figure 2. The recorder is adapted to work with an external lithium battery supply (Enix Energies 800040) to enable continuous 24-hour recordings. The audio is recorded onto a SanDisk SDHC 8 GB card, at a sample rate of 48 kHz, encoding 64 kbps in mono in MP3 format. The audio equipment is kept inside a basic pack connected to a lapel microphone. Batteries and SD cards will be exchanged daily by the study nurses. In pilot research, subjects tolerated the audio equipment well, wearing them 24 hours a day and taking them off only to bathe.

Processing of audio recordings

The recorded signals will be analysed after all patient recordings are completed. For cough analysis, software developed by our group and previously described in detail will be used.[30 ,31] Thus we provide only a brief review here and refer interested readers to our previous publications.

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,[31] 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.[49] 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.).[31]

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.[31] 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.[31] 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.

Microbiology

The microbiological tests will be carried out in a Biosafety Containment Level 3 research laboratory situated within Universidad Peruana Cayetano Heredia in Lima, Peru. The sputum samples will be digested and decontaminated by the standard NaOH-N-acetyl cysteine method.[50] For smear microscopy, an aliquot of 100 µl is stained with Auramine O and examined with x400 magnification. Results are determined as negative, paucibacillary (1-19 acid fast bacilli [AFB] visualized in 40 fields), 1+ (20-199 AFB visualized in 40 fields), 2+ (5-50 AFB per field) and 3+ (>50 AFB per field). Culture and MODS susceptibility testing will be performed with the remaining samples, according to standard protocols.[32-34]

Radiology

Radiological information will be gathered, when possible, on a convenience basis. Priority will be given to CT scans, since they have been shown to be more sensitive. Films will be read by a local radiologist and a US board-certified radiologist blinded to the patient's demographics and outcomes. They will provide an interpretation that is standardised as per our study protocol to describe radiological findings including cavitation, consolidation, lymphadenopathy, and effusions (Supplementary File 2). 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.

Statistical methodology and analysis

All questionnaire data will be double digitised from paper forms using Visual FoxPro 9 Service Pack 2 (Microsoft Corp. Redmond, Washington, USA) and microbiological data will be double entered using Microsoft Access 2010 (Microsoft Corp. Redmond, Washington, USA). These two data sets will be cross-compared for validity and errors. From these data, descriptive statistics will be tabulated and graphed.

Cough analysis processing results will be stored as Matlab (Mathworks, Inc, Natick MA) files containing information regarding each event and its timestamp. Algorithmically detected coughs will be annotated in the files. After manual review, isolated cough events will be grouped into cough epochs, or bursts of closely spaced individual coughs within 2 seconds, following published work on cough evaluation.[49] We have previously published a review and discussion of these various metrics (including number of individual coughs, number of cough bouts or epochs, and number of 1-sec periods containing cough).[31]

For the first study objective of describing cough frequency, cough epochs will be plotted throughout the day, and cough frequency will be summarised as the frequency of cough epochs per hour. Positively-skewed cough data may be log-transformed to facilitate data visualisation and analysis. To address the

second study objective, correlation of characteristics with cough frequency, we will use generalised estimating equations (GEE) based Poisson or negative binomial regression with baseline microbiologic status, and trigonometric (sine/cosine) terms to model circadian periodicity, as the independent variables. In addition, a multiple logistic regression in a longitudinal generalised linear model (GLM) framework analysis will evaluate a function of sputum bacillary load and with cough frequency that we propose as a potential predictor of TB transmissibility. In all cases we will correct for outliers, and nested models will be compared using the likelihood ratio test. 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.

To test the association between cough frequency and microbiological resolution of TB disease associated with the third aim of this study, time-to-event survival analyses where the outcomes of interest are sputum smear conversion, and culture conversion, as defined above, and the primary predictors of interest are cough frequency at baseline, during treatment, and time to two-fold reduction in cough frequency. In addition, secondary analyses of weight, temperature, and radiological characteristics, will be conducted using generalised linear models and GEE logistic regression as appropriate.

Ethical considerations

Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima,

Peru, and Johns Hopkins University, in Baltimore, USA. Written informed consent will be obtained from all participants. Test results will be delivered by telephone or at subsequent visits at which time a team physician or nurse will be able to explain the results to the study participants. TB treatment remains the responsibility of the medical staff in charge and the National TB Programme.

Discussion

We will determine cough frequency before and during anti-TB treatment using the CayeCoM device. We will identify baseline predictors of cough frequency during TB treatment and evaluate the correlation between change in cough frequency and microbiological resolution.

The medical literature currently lacks information about cough frequency in TB. As recently noted by Turner and Bothamley,[5] cough frequency in patients undergoing TB treatment has only been studied once, almost half a century ago.[6 ,7] This previous study has the limitation of only being conducted within an 8-hour period, overnight, and thus there is no information on daytime coughing or the effect of the diurnal rhythm on cough. A similar study[54] demonstrated that the severity of cough and pathological chest x-ray findings were associated with higher levels of TB transmission. However, their study did not measure cough frequency but instead focused on a subjective characteristic: cough severity. It should be noted that to assess cough frequency one must utilise objective acoustic parameters, since self-reported cough is unreliable.[18] As reported in abstract form, the objective acoustic Leicester Cough Monitor (LCM) has been used to evaluate 24-hour

cough recordings in patients with pulmonary TB before starting treatment, showing that cough frequency is reduced at night.[55] This further justifies re-evaluation of Loudon's overnight study.

Our project has several strengths and limitations. An important strength is the generation of 24-hour cough recordings, which will provide lengthy recordings, will enable evaluation of cough patterns at different times of day, and also has the benefit of being recorded during a normal day in real-world settings where we expect our device to be used in the future. 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. Similar to Loudon's proposal,[8] our algorithm will help to screen and reduce the length of the recordings to ~5% of their original length, without affecting sensitivity and improving specificity.[31] 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. 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.

CayeCoM has been validated for 24-hour recordings,[31] whereas PulmoTrack (PulmoTrack-CC, KarmelSonix, Haifa, Israel) was validated for 25 minutes[28] and the Hull Automatic Cough Counter for 1-hour recordings.[26] Other systems have also validated their algorithms for 24-hour recordings, such as the LCM,[27 ,56] VitaloJAK,[29] and the LifeShirt System.[25] However, in contrast to our study, none of these algorithms have been validated for pulmonary TB nor within real-life settings (e.g. traffic). 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.

Cough frequency should provide additional information regarding the evolution of the patients' medical condition. 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. It could assist with decisions regarding the need for on-going respiratory isolation of patients, treatment duration, and identification of patients with treatment failure who may need modification of their treatment regimens. The device also has the potential to be used remotely, as in telemedicine. This is potentially important in a country such as Peru, where the majority of doctors live in the capital, leaving most of the country without a physician in their

region. 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.

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Conflicts of interest:

All authors declare that they have no conflict of interest in relation to this work.

Ethics approval:

Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA.

Contributors:

All authors were involved in the study design and writing of the manuscript, and all reviewed the final manuscript before submission. MAB and JWL directly contributed to study design and are responsible for supervision of data gathering. AP, BHT, JWL, MZ and GL will be responsible for data management and statistical analysis for this project.

Data Sharing Statement:

We aim to publish and disseminate our results, once the project is complete. We also expect to create and maintain an online repository for TB cough sounds as well as the statistical analysis employed.

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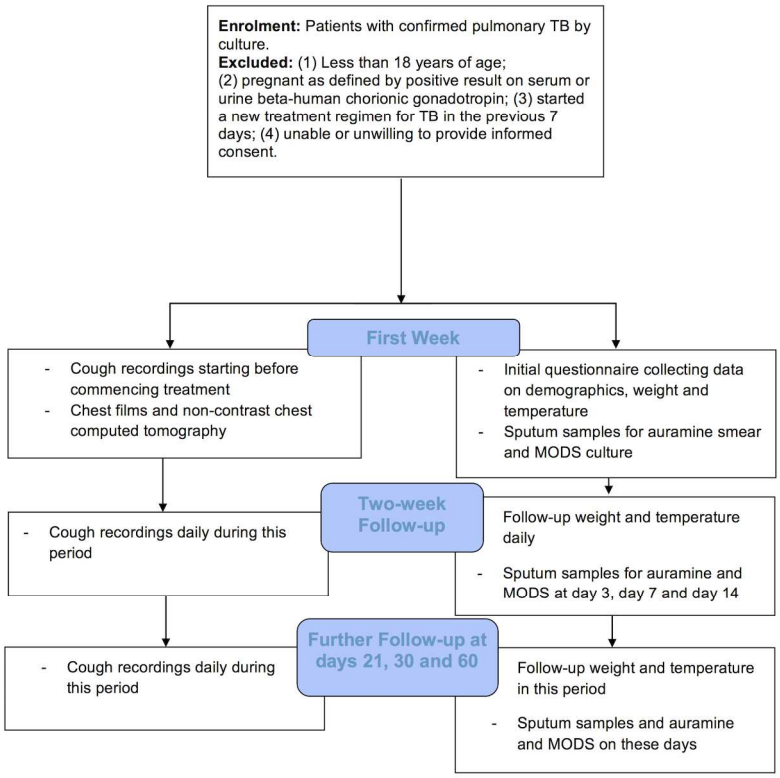

Figure 1 – Flow Diagram for CayeCoM Study
175x247mm (300 x 300 DPI)

Figure 2 – Picture of the Cayetano Cough Monitor (CayeCoM)
122x62mm (300 x 300 DPI)

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Entrevistador: _____

Cuestionario Inicial Para Todos Los Participantes:

Datos Demográficos:

1. Edad: _____
2. Sexo: _____

Antecedentes:

3. ¿Cuántas personas normalmente duermen en casa?: _____ Personas
4. ¿Cuántas habitaciones hay en su hogar? (sin contar baño, pasadizo, cocina, depósito, garaje): _____ habitaciones.
5. ¿Cuál es el ingreso mensual de la vivienda? S/._____
6. ¿Cuánto gasta su familia en alimentación cada semana? S/._____
7. ¿Cuántas personas en su vivienda comen de esos alimentos que compran semanalmente? _____ Personas
8. ¿Cuántas veces en el ultimo mes usted se ha acostado con bastante hambre porque no había comida en casa? _____ días

Historia de Tuberculosis:
La tuberculosis es una enfermedad que se trata con varios antibióticos a la vez, y cuyo tratamiento dura varios meses.

9. ¿Ha sido diagnosticado con TBC anteriormente?
   a. Si
   b. No → *Pase a la pregunta 15*
   c. NS
10. ¿Cuántas veces? _____
11. Si recibió tratamiento para la TBC, ¿dónde recibió la mayor parte del tratamiento?
    a. NA
    b. Mismo distrito
    c. Otro distrito
    d. Otra ciudad
    e. Otro país
12. Si recibió tratamiento para la TBC ¿por cuantos meses en total lo tomó? _____
13. Si recibió tratamiento previo, en que esquema estaba (lo mas recién): - _____
14. Si recibió tratamiento para la TBC ¿cumplió con el tratamiento previo?
    a. NA
    b. Si
    c. No→ _____
    d. NS

Factores de Riesgo

15. ¿Ha compartido un cuarto con alguien que haya tenido TBC comprobada?
    a. Si, y esta persona también tenía una tos persistente
    b. Si, pero esta persona concurrente no tenía una tos persistente
    c. No → *Pase a la pregunta 18*
    d. NS
16. ¿Dónde compartió este ambiente con alguien infectado con TBC?
    a. NA
    b. Trabajo
    c. Casa
    d. Hospital
    e. Otro:_____
17. ¿Por cuantos días compartió este ambiente con la persona con TBC comprobada? _____
18. Aparte de usted, ¿alguien mas en casa esta actualmente recibiendo medicinas para la TBC?
    a. Si → Quien: _____
    b. No
    c. NS

Creencias y Conocimiento de la Enfermedad

19. ¿Dónde escuchó de la TBC por primera vez?
    a. Familia
    b. Amigos
    c. Colegio
    d. Puesto de Salud
    e. TV
    f. Radio
    g. NS
20. ¿Puede alguien con TBC y tos infectar a sus familiares?
    a. Si
    b. No
    c. NS

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

21. ¿Qué tan contagiosa cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

22. ¿Qué tan seria cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

23. En general, ¿qué puede hacer uno por si mismo para protegerse de contraer la TBC? [Marcar el factor más importante, no sugerir repuestas].
    a. Vacunarse
    b. Comer
    c. Dormir bien, descansar
    d. Vivir una vida ordenada
    e. Mantenerse alejado de la gente con TBC
    f. Educarse
    g. Otro: _____
    h. NS

¿Cuales son los síntomas de la TBC?
[Marcar los factores, no sugerir repuestas].

24. Tos mencionada Si☐ No☐
25. Hemoptysis mencionada Si☐ No☐
26. Fiebre mencionada Si☐ No☐
27. Baja de peso mencionada Si☐ No☐
28. Fatiga, decaimiento mencionada Si☐ No☐
29. Palidez, un cierto semblante mencionada Si☐ No☐

30. ¿Qué debe hacer una persona con TBC para mejorarse? [Marcar el factor más importante, no sugerir repuestas].
    a. Tomar sus medicinas, asistir a controles
    b. Comer mas, comer mejor
    c. Descansar
    d. Tener fe
    e. Abrigarse
    f. Otro: _____
    g. NS

31. ¿Cómo puede hacer una persona con TBC para no contagiar la TBC a otros? [Marcar el factor más importante, no sugerir repuestas].
    a. Cubrirse la boca al toser
    b. Quedarase en casa, mantenerse alejado
    c. Seguir el tratamiento
    d. Separar cubiertos
    e. Otro: _____
    f. NS

32. ¿Se puede curar la TBC?
    a. Siempre
    b. Normalmente sí
    c. A veces
    d. Raramente
    e. Nunca

Instrucciones: Marque una X sobre la línea la posición que escoges.
Ejemplo: ----------▯----------

33. Con que frecuencia esta tosiendo hoy, en un promedio de 24 horas?

0 1 2 3 4 5

nunca poquito mucho casi siempre

Instrucciones: Marque una X, en el cuadrado, todas las alternativas que corresponden.
Ejemplo: ☒

34. Que indicadores utilizó para escoger a que posición poner sus X?
    ☐ Los números: 1, 2, 3, 4, 5
    ☐ Las palabras: nunca, poquito, mucho, casi siempre
    ☐ Las figuras: 

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Instrucciones: Marque con X la alternativa que corresponde.
Ejemplo: ☒

35. ¿Comparado con hace tres días, tiene más frecuencia de tos el día de hoy?
    a. ☐Tosiendo ahora menos
    b. ☐Tosiendo ahora igual
    c. ☐Tosiendo ahora más

36. El día de hoy, ¿ha tenido *solo tos, tos con flema o solo flema?*
    a. ☐Solo tos
    b. ☐Tos con flema
    c. ☐Solo flema

37. Si tiene tos, ¿a que hora en el día tiene más frecuencia: *mañana, tarde o noche*?
    a. ☐Mañana
    b. ☐Tarde
    c. ☐Noche

38. Qué tan frecuentemente tose?
    a. ☐Cada pocos segundos
    b. ☐Cada pocos minutos
    c. ☐Cada pocas horas
    d. ☐No tose

Instrucciones: Marque el numero de días en total que tiene los siguientes síntomas desde que se enfermó. Marque 0 si ningún día.

39. ¿Cuantos días usted ha tenido los siguientes síntomas?
    a. Tos seca _____
    b. Tos con flema _____
    c. Tos con sangre _____
    d. Fiebre _____
    e. Falta de aire _____
    f. Perdida de peso _____
    g. Cansancio o decaimiento _____
    h. Sudor nocturno _____
    i. Falta de apetito _____

Instrucciones: Responde a la pregunta en el lugar indicado.

40. Actualmente, ¿como se siente? (0 = mal, 10 = bien): _____

41. Cuando tiene tos, ¿cuantas toses tiene por hora? _____

42. Preguntas sobre VIH

	Manifiesto del paciente	Historia clínica
a. Usted ha sido diagnosticado por VIH?	_____	_____
b. Fecha de Diagnostico:	_____	_____
c. Ultima Carga Viral:	_____	_____
d. Fecha de ultima Carga Viral:	_____	_____
e. Ultimo resultado CD4:	_____	_____
f. Fecha de Ultimo resultado CD4:	_____	_____
g. Tiempo tomanda TARGA (años)	_____	_____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

Radiologic Interpretation Data Form

Date of read: _____

Radiologist Name: _____

Radiologist Signature: _____

Patient code: _____

Patient gender: □ male □ female

Patient age: _____

---

1. **Type of film:**
   □ AP □ PA □ Lateral from R □ Lateral from L □ Thoracic CT without contrast □ Other: _____

2. Date of film: Day: _____ Month: _____Year: _____
3. Rotation:_____
4. Adequacy of inhalation: _____

	Site	a. Consolidation?	b. Cavitation?	c. Pneumatocele?	d. Atelectasis?
5	Right upper lobe	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
6	- anterior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
7	- apical	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
8	- posterior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
9	Right middle lobe	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
10	Right lower lobe	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
11	- superior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
12	- basal	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
13	Left upper lobe	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
14	- anterior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
15	- apical	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
16	- posterior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
17	Lingula	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
18	Left lower lobe	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
19	- superior	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no
20	- basal	□ yes □ no	□ yes □ no	□ yes □ no	□ yes □ no

21. Pleural effusion? □ yes □ no
left? □ small □ medium □ large
right? □ small □ medium □ large

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

22. Miliary spread? □ yes □ no
23. Pneumothorax? □ yes □ no
where and size: _____
24. Lymphadenopathy: □ yes □ no
which lymph nodes groups
□ hilar □ mediastinal
25. Pericardial effusion? □ yes □ no
left? □ small □ medium □ large
26. Bronchiectasis? □ yes □ no
where: _____
27. Fibrosis? □ yes □ no
where: _____
any retractions, deviations?_____
_____
_____
28. Mediastinal thickening? ? □ yes □ no
29. Any tree-in-bud pattern? Where?_____
_____
_____

30. Cavitation: For each cavity, please describe:
Cavity # 1:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 2:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity #3:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 4:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 5:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

More cavities? □ yes: please use another sheet to describe □ no

Other findings such as fractures, cardiac abnormalities, exudative / fibrotic densities, bronchogenic spread, mass-like lesions (calcified vs. non-calcified), please describe:

_____
_____
_____
_____
_____
_____
_____
_____

31. Normal film? □ yes □ no

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A protocol for studying cough frequency in people with pulmonary tuberculosis

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ABSTRACT

Introduction: Cough is a key symptom of tuberculosis (TB) as well as the main cause of transmission. However, a recent literature review (Turner et al. 2015, J Infect Dis) found that cough frequency (number of coughs per hour) in patients with TB has only been studied once, by Loudon in 1969 (Loudon et al. 1969, Am Rev Respire Dis). The main aim of this study is to describe cough frequency patterns before and after the start of TB treatment and to determine baseline factors that affect cough frequency in these patients. Secondarily, we will evaluate the correlation between cough frequency and TB microbiological resolution.

Methods: This study will select participants with culture confirmed TB from two tertiary hospitals in Lima, Peru. Based on Loudon's results, we estimated that a sample size of 107 patients was sufficient to detect clinically significant changes in cough frequency. Participants will initially be evaluated through questionnaires, radiology, MODS broth TB-culture, auramine smear microscopy, and cough recordings. This cohort will be followed for the initial 60 days of anti-TB treatment, and throughout the study several microbiological samples as well as 24-hour recordings will be collected. We will describe the variability of cough episodes and determine its association with baseline laboratory parameters of pulmonary TB. In addition, we will analyse the reduction of cough frequency in predicting TB cure, adjusted for potential confounders.

Ethics and dissemination: Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA. We aim to publish and disseminate our findings in peer-reviewed journals. We also expect to create and maintain an online repository for TB cough sounds as well as the statistical analysis employed.

Strengths and limitations of this study

- The algorithm employed in this project has been validated specifically for patients with pulmonary tuberculosis, which enables us to use this algorithm in our patients.
- A strength of this project is that its results will reflect actual cough frequency episodes in pulmonary tuberculosis by utilising 24-hour recordings in the patients' normal-day settings (traffic, dogs barking, etc.). We expect that this will generate a novel method of evaluating cough in TB that can be used in real-world scenarios.
- Our study has the limitation that recordings have been processed through a semi-automated algorithm. To decrease time constraints our long-time goal is to create a fully automated processing system. We anticipate that experience gained with semi-automated analysis will aid us in developing future algorithms.

INTRODUCTION

Tuberculosis (TB) is an infectious disease, and was responsible for 9.6 million new cases and 1.5 million deaths in 2014.[1] TB is transmitted in the air[2 ,3] and cough is the most important cause of transmission.[4] Cough in people with pulmonary TB disease arises as a result of the inflammatory response to mycobacterial pulmonary infection. A reduction in cough is assumed to represent adequate response to treatment, and to result in decreased risk of spread of infection. Despite its crucial role in TB transmission, a recent literature review[5] reported that cough frequency during TB therapy has not been studied since work carried out by Loudon in the 1960s.[6 ,7] Thus, longitudinal cough frequency studies in TB are needed.

Loudon described cough frequency in eight-hour overnight periods for nine weeks. All sounds with amplitude and frequency consistent with possible cough events were recorded and then manually reviewed.[8] His findings show a two-fold reduction in the first two weeks of treatment, from a mean of 13.6 to 4.75 coughs/hour.[7] *Mycobacterium TB* colony forming units (CFU) also reduced significantly, from 10^6 at baseline to 10^3 two weeks later.[9 ,10] This evidence led to the idea that drug-susceptible TB patients become sufficiently non-infective by the second week of treatment that they no longer pose a risk to others. This and other evidence led to the often-used policy that two weeks was the necessary duration of respiratory isolation for newly diagnosed patients commenced on appropriate treatment. Current evidence[11] and guidelines affirm this position;[12-14] however, this two week policy has been criticised.[15 ,16] Our group has shown that drug-susceptible TB patients re-

main sputum culture positive for longer.[17 ,18] Most importantly, the assumption that TB patients are no longer coughing at two weeks has never been corroborated.

The 2015 CHEST guidelines state that acoustic parameters are the best parameter to evaluate the frequency of cough.[19] In order to ensure accurate measurement, it is important to use a standardised method such as automated cough counting with a validated algorithm. Despite the recently growing literature on this topic, these methods are principally being used in the field of non-infectious chronic disease.[20-25] Whilst algorithms for cough-counting have been validated[26-30] our research protocol appears to be the first to do so specifically in patients with pulmonary TB.[31 ,32]

To address this knowledge gap, we have developed the Cayetano Cough Monitor (CayeCoM) and here describe a protocol for it to be used to study cough frequency in patients with pulmonary TB.

METHODS

Study objectives

The primary objective of this study is to describe cough frequency patterns in adults with pulmonary TB before and after treatment initiation.

The second objective of this study is to determine baseline characteristics that correlate with cough frequency, such as patient demographics, radiological findings, presence of multi drug-resistant TB (MDR-TB), and HIV status.

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.

Study design

This prospective cohort study will follow adult patients with pulmonary TB throughout their treatment period in Lima, Peru.

Subjects with a confirmed or suspected diagnosis of active pulmonary TB will be referred to our study team. After obtaining written informed consent, we will record coughs prior to initiation of TB treatment. Subjects will provide us with early-morning sputum samples that will be tested for active pulmonary TB disease by testing at least one sputum sample using the microscopic-observation drug-susceptibility (MODS) broth culture assay[33-35] and auramine smear microscopy, to assess the bacillary load.

Patients in whom the pulmonary TB diagnosis is confirmed by MODS will receive treatment delivered by the National TB Programme as per standard practice.[36] Figure 1 summarises the data to be collected at baseline and during the 60 days of follow-up.

Study sites

Peru has one of the highest TB incidence rates in the Americas.[37] More than one-third of the incident TB cases in the Andean region are from Peru. With respect to rates of MDR-TB and extensively drug resistant (XDR) TB, Peru ranks first in all of the Americas. However, underreporting in the region

may contribute to Peru's overrepresentation, as shown in the latest Pan American Health Organisation (PAHO) report.[37]

Within Peru, Lima and its metropolitan area account for most cases of MDR-TB and XDR-TB.[38] Thus, we will recruit patients from two hospitals: Hospital Nacional Dos de Mayo, located in the historic centre of Lima; and Hospital Nacional Daniel Alcides Carrión, located in Callao and which belongs to Lima's metropolitan area.

Our main site, Hospital Nacional Dos de Mayo (HNDM), is a 650-bed teaching and public national tertiary referral hospital run by the Peruvian Ministry of Health (MINSA). It provides services to the poor population from the surrounding inner city area. HNDM is the only hospital in Peru with a negative pressure ward available for TB patients. Our secondary site is another tertiary referral hospital run by MINSA, Hospital Nacional Daniel Alcides Carrión. This 462-bed teaching health facility lies in the Callao region.

Study population

The infectious disease and pulmonary physicians will refer subjects to the research team. Criteria for referral are suspicion of active pulmonary TB or a confirmed case of active pulmonary TB who has not yet started treatment. Active pulmonary TB is defined by a positive MODS culture result. Subjects will be excluded if they were less than 18 years of age, pregnant, have started a new treatment regimen for TB within the last 7 days, or are unable or unwilling to provide informed consent. If a patient changes treatment regimen, for ex-

ample due to treatment failure or to an adverse drug reaction, this would also be considered as a new regimen. Pregnancy is defined by a positive result on serum or urine beta human chorionic gonadotropin (β-hCG) assay.

Outcomes and case definitions

The primary outcomes for this study are cough frequency and microbiological data from serial sputum samples. Cough frequency is defined as the number of cough episodes, or cough epochs, within a time period. Cough epochs are defined as cough events that are within a two-second period frame.[32]

Regarding microbiological data, participants will be entered into the study if they have a positive culture result. Treatment regimens will be adjusted as needed by the treating team based on the results of the MODS drug-susceptibility testing from their sputum. Our study team will not be involved in the treatment regimen selection.

Sputum smear conversion is defined as three consecutive smear-negative results, collected at least 8 hours apart after initial smear positivity at diagnosis.[12] Culture conversion is defined as two consecutive negative culture results, taken at least 30 days apart. This last definition is the one used in the Ministry of Health (MINSA)[36] and is recommended by the World Health Organisation (WHO).[39] The date of conversion will be considered as the date of the first negative sputum smear or culture contributing to conversion.

Secondary outcomes include weight, temperature, and radiological characteristics. When possible, radiological interpretation data from chest films and thoracic computed tomography (CT) scans will be obtained. Chest X-ray films (CXR) provide a high negative predictive value for the presence of active TB,[40] but CXR might be normal when in fact there is parenchymal disease.[41] More specifically, CT scans correctly determine pulmonary TB cases in 91% of cases whereas CXR only in 49% of cases.[41-44] In addition, CT scans provide higher sensitivity for the detection of lymphadenopathy, early bronchogenic spread, and to evaluate cavitation and disease activity.[44]

Sample size

In a pilot study we estimated that the frequency of cough in TB patients before receiving treatment is approximately 327 coughs during a 24-hour period with a standard deviation of approximately 50. A sample size of 97 patients would enable us to detect a conservative decrease of the mean number of coughs in the 24-hour period of at least 45 coughs after two weeks of treatment, with a 5% Type I error probability and 80% power.

Under the hypothesis that TB patients before treatment experience a high cough frequency, we hypothesise that after two weeks of anti-TB treatment, there will be a clinical response accompanied by a significant reduction in cough frequency. Response is defined as at least a two-fold reduction in cough frequency, which was previously shown to occur within the first 2 weeks of treatment.[7] For power calculations, it is assumed that all subjects will eventually respond to treatment, according to our definition of response,

and that once cough frequency has reduced in an individual it will not rise again. We assume that after the two weeks of treatment approximately 10% of patients would maintain a high frequency of cough. Thus, a sample size of 97 patients will allow us to detect an odds ratio of at least 3.2 for the risk of patients not responding to TB treatment in two weeks of therapy, under a 95% significance and 80% power. 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.

Study organisation

The Asociación Benéfica (A.B.) PRISMA and Universidad Peruana Cayetano Heredia in Lima, Peru will provide local administrative oversight. Overseas, oversight will be conducted by Johns Hopkins University in Baltimore, Maryland, USA.

In Lima, the Pampas office of A.B. PRISMA will provide operations and logistic support for fieldwork. An additional collaborating signal processing team will be based locally in the Universidad Nacional de Ingeniería, Lima, Peru, as well as at Tufts University, Massachusetts, USA.

Our collaborating biostatisticians are based at Tufts University, Tulane University, and Universidad Peruana Cayetano Heredia, Lima, Peru. All investigators are involved in protocol design and technical support and will remain involved in the ongoing analyses.

Personnel, training and logistics

Nurses have been trained by study staff to obtain sputum samples in a best-practice fashion based on previous work,[45 ,46] and to operate and trouble-shoot all recorder devices, memory cards, and battery packs. We will adhere to recommended infection prevention & control practices for TB to reduce bio-risk in healthcare professionals and patients.[47] Written informed consent is required prior to research participation. At the time of enrolment, subjects will follow the procedures outlined in Figure 1.

Subjects with active pulmonary TB will be followed throughout their TB treatment. After the identification of active pulmonary TB and based on convenience basis, subjects who consent will undergo CXR and a non-contrast thoracic CT scan.

The first day of a new TB treatment regimen is defined as “Day 0”. An initial questionnaire will be completed on that day (Supplementary File 1). 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. This questionnaire is similar to the one that was employed in a previous study.[48] Baseline cough frequency will be obtained by performing an audio recording of the patients before they obtain their microbiological results, which is usually a few days prior to treatment initiation. Hence, subjects will be recorded from at least one day prior to treatment and throughout their first two weeks of treatment. They will subsequently be recorded for 24 hours on or around days 21, 30 and 60 of treat-

ment, although up to two days date deviation for Sundays and public holidays will be allowed.

Recordings will start at 09:00 hours and will be as continuous as possible. Occasionally incomplete recordings could be obtained due to malfunction of equipment or patient non-compliance. On the recording days clinical data will be gathered, including: weight, temperature, and sputum samples for smear and MODS results. 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.[49]

Audio recording

Design of the audio recording equipment, the CayeCoM device, builds on previous chronic cough ambulatory audio recordings.[27 ,50 ,51] The CayeCoM device is a Marantz PMD 620 professional handheld recorder, using an Audio-Technica AT899 sub-mini microphone with an AT8537 microphone power module. The microphone will be attached at the patient's lapel as shown in Figure 2. The recorder is adapted to work with an external lithium battery supply (Enix Energies 800040) to enable continuous 24-hour recordings. The audio is recorded onto a SanDisk SDHC 8 GB card, at a sample rate of 48 kHz, encoding 64 kbps in mono in MP3 format. The audio equipment is kept inside a basic pack connected to a lapel microphone. Batteries and SD cards will be exchanged daily by the study nurses. In pilot research,

subjects tolerated the audio equipment well, wearing them 24 hours a day and taking them off only to bathe.

Processing of audio recordings

The recorded signals will be analysed after all patient recordings are completed. For cough analysis, software developed by our group and previously described in detail will be used.[31 ,32] Thus we provide only a brief review here and refer interested readers to our previous publications.

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,[32] 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.[52] 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.).[32]

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.[32] 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.[32] 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 near-

ly 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.

Microbiology

The microbiological tests will be carried out in a Biosafety Containment Level 3 research laboratory situated within Universidad Peruana Cayetano Heredia in Lima, Peru. The sputum samples will be digested and decontaminated by the standard NaOH-N-acetyl cysteine method.[53] For smear microscopy, an aliquot of 100 µl is stained with Auramine O and examined with x400 magnification. Results are determined as negative, paucibacillary (1-19 acid fast bacilli [AFB] visualized in 40 fields), 1+ (20-199 AFB visualized in 40 fields), 2+ (5-50 AFB per field) and 3+ (>50 AFB per field). Culture and MODS susceptibility testing will be performed with the remaining samples, according to standard protocols.[33-35]

Radiology

Radiological information will be gathered, when possible, on a convenience basis. Priority will be given to CT scans, since they have been shown to be more sensitive in general.[44] 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%.[54] Films will be read by a local radiologist and a US board-certified radiologist blinded to the patient's demographics and outcomes. They will provide an interpretation that is standardised as per our

study protocol to describe radiological findings including cavitation, consolidation, lymphadenopathy, and effusions (Supplementary File 2). We will explore whether these radiological findings are predictive of microbiological burden and cough frequency.

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 most importantly, to infectivity.[7 ,55-58] 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 identifies 22%.[55]

Statistical methodology and analysis

All questionnaire data will be double digitised from paper forms using Visual FoxPro 9 Service Pack 2 (Microsoft Corp. Redmond, Washington, USA) and microbiological data will be double entered using Microsoft Access 2010 (Microsoft Corp. Redmond, Washington, USA). These two data sets will be cross-compared for validity and errors. From these data, descriptive statistics will be tabulated and graphed.

Cough analysis processing results will be stored as Matlab (Mathworks, Inc, Natick MA) files containing information regarding each event and its timestamp. Algorithmically detected coughs will be annotated in the files. After manual review, isolated cough events will be grouped into cough epochs, or

bursts of closely spaced individual coughs within 2 seconds, following published work on cough evaluation.[52]

For the first study objective of describing cough frequency, cough epochs will be plotted throughout the day, and cough frequency will be summarised as the frequency of cough epochs per hour. Positively-skewed cough data may be log-transformed to facilitate data visualisation and analysis. To address the second study objective, correlation of characteristics with cough frequency, we will use generalised estimating equations (GEE) based Poisson or negative binomial regression with baseline microbiologic status, and trigonometric (sine/cosine) terms to model circadian periodicity, as the independent variables. In addition, a multiple logistic regression in a longitudinal generalised linear model (GLM) framework analysis will evaluate a function of sputum bacillary load and with cough frequency that we propose as a potential predictor of TB transmissibility. In all cases we will correct for outliers, and nested models will be compared using the likelihood ratio test. 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.

To test the association between cough frequency and microbiological resolution of TB disease associated with the third aim of this study, time-to-event survival analyses where the outcomes of interest are sputum smear conversion, and culture conversion, as defined above, and the primary predictors of interest are cough frequency at baseline, during treatment, and time to two-fold reduction in cough frequency. In addition, secondary analyses of weight,

temperature, and radiological characteristics, will be conducted using generalised linear models and GEE logistic regression as appropriate.

Ethics and dissemination

Ethical approval has been obtained from the ethics committees at A.B. PRISMA in Lima, Peru, the Universidad Peruana Cayetano Heredia in Lima, Peru, and Johns Hopkins University, in Baltimore, USA. Written informed consent will be obtained from all participants. Test results will be delivered by telephone or at subsequent visits at which time a team physician or nurse will be able to explain the results to the study participants. TB treatment remains the responsibility of the medical staff in charge and the National TB Programme. We aim to publish and disseminate our results, once the project is complete. We also expect to create and maintain an online repository for TB cough sounds as well as the statistical analysis employed.

Discussion

We will determine cough frequency before and during anti-TB treatment using the CayeCoM device. We will identify baseline predictors of cough frequency during TB treatment and evaluate the correlation between change in cough frequency and microbiological resolution.

The medical literature currently lacks information about cough frequency in TB. As recently noted by Turner and Bothamley,[5] cough frequency in patients undergoing TB treatment has only been studied once, almost half a century ago.[6 ,7] This previous study has the limitation of only being con-

ducted within an 8-hour period, overnight, and thus there is no information on daytime coughing or the effect of the diurnal rhythm on cough. A similar study[58] demonstrated that the severity of cough and pathological chest x-ray findings were associated with higher levels of TB transmission. However, their study did not measure cough frequency but instead focused on a subjective characteristic: cough severity. It should be noted that to assess cough frequency one must utilise objective acoustic parameters, since self-reported cough is unreliable.[19] As reported in abstract form, the objective acoustic Leicester Cough Monitor (LCM) has been used to evaluate 24-hour cough recordings in patients with pulmonary TB before starting treatment, showing that cough frequency is reduced at night.[59] This further justifies re-evaluation of Loudon's overnight study.

Our project has several strengths and limitations. An important strength is the generation of 24-hour cough recordings, which will provide lengthy recordings, will enable evaluation of cough patterns at different times of day, and also has the benefit of being recorded during a normal day in real-world settings where we expect our device to be used in the future. 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. Similar to Loudon's proposal,[8] our algorithm will help to

screen and reduce the length of the recordings to ~5% of their original length, without affecting sensitivity and improving specificity.[32] 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. 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.

CayeCoM has been validated for 24-hour recordings,[32] whereas PulmoTrack (PulmoTrack-CC, KarmelSonix, Haifa, Israel) was validated for 25 minutes[29] and the Hull Automatic Cough Counter for 1-hour recordings.[27] Other systems have also validated their algorithms for 24-hour recordings, such as the LCM,[28 ,60] VitaloJAK,[30] and the LifeShirt System.[26] However, in contrast to our study, none of these algorithms have been validated for pulmonary TB nor within real-life settings (e.g. traffic). 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.

Cough frequency should provide additional information regarding the evolution of the patients' medical condition. 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, we should be careful when monitoring TB

patients since some may worsen after an initial positive response to therapy. It could assist with decisions regarding the need for on-going respiratory isolation of patients, treatment duration, and identification of patients with treatment failure who may need modification of their treatment regimens. The device also has the potential to be used remotely, as in telemedicine. This is potentially important in a country such as Peru, where the majority of doctors live in the capital, leaving most of the country without a physician in their region. 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.

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Contributors:

All authors were involved in the study design and writing of the manuscript, and all reviewed the final manuscript before submission. MAB and JWL directly contributed to study design and are responsible for supervision of data gathering. AP, BHT, JWL, MZ and GL will be responsible for data management and statistical analysis for this project.

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Competing interests statement:

All authors declare that they have no competing interests in relation to this work.

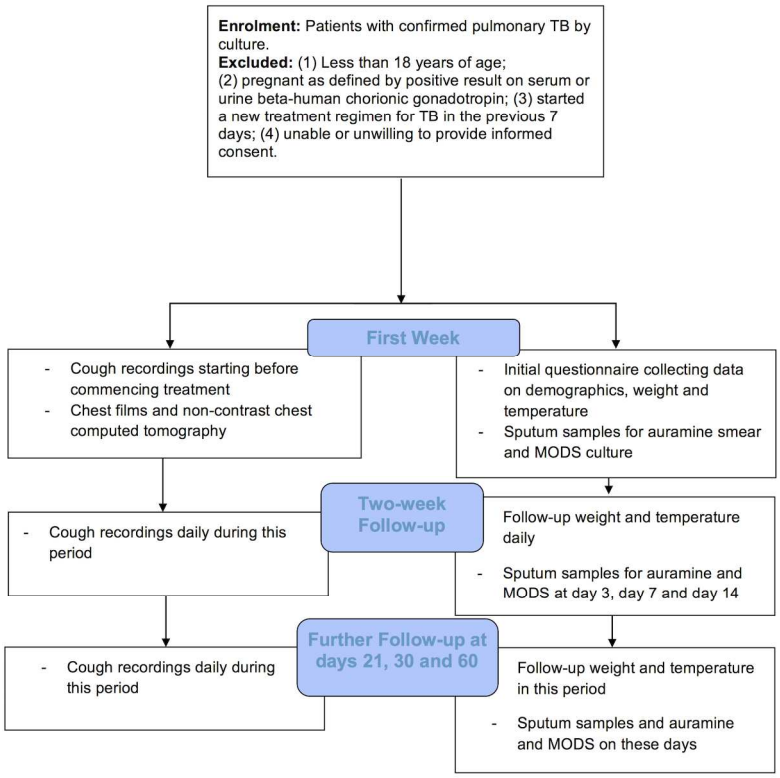

Figure 1 – Flow Diagram for CayeCoM Study
175x247mm (300 x 300 DPI)

Figure 2 – Picture of the Cayetano Cough Monitor (CayeCoM)
175x90mm (300 x 300 DPI)

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Entrevistador: _____

Cuestionario Inicial Para Todos Los Participantes:

Datos Demográficos:

1. Edad: _____
2. Sexo: _____

Antecedentes:

3. ¿Cuántas personas normalmente duermen en casa?: _____ Personas
4. ¿Cuántas habitaciones hay en su hogar? (sin contar baño, pasadizo, cocina, depósito, garaje): _____ habitaciones.
5. ¿Cuál es el ingreso mensual de la vivienda? S/._____
6. ¿Cuánto gasta su familia en alimentación cada semana? S/._____
7. ¿Cuántas personas en su vivienda comen de esos alimentos que compran semanalmente? _____ Personas
8. ¿Cuántas veces en el ultimo mes usted se ha acostado con bastante hambre porque no había comida en casa? _____ días

Historia de Tuberculosis:
La tuberculosis es una enfermedad que se trata con varios antibióticos a la vez, y cuyo tratamiento dura varios meses.

9. ¿Ha sido diagnosticado con TBC anteriormente?
   a. Si
   b. No → *Pase a la pregunta 15*
   c. NS
10. ¿Cuántas veces? _____
11. Si recibió tratamiento para la TBC, ¿dónde recibió la mayor parte del tratamiento?
    a. NA
    b. Mismo distrito
    c. Otro distrito
    d. Otra ciudad
    e. Otro país
12. Si recibió tratamiento para la TBC ¿por cuantos meses en total lo tomó? _____
13. Si recibió tratamiento previo, en que esquema estaba (lo mas recién): - _____
14. Si recibió tratamiento para la TBC ¿cumplió con el tratamiento previo?
    a. NA
    b. Si
    c. No→ _____
    d. NS

Factores de Riesgo

15. ¿Ha compartido un cuarto con alguien que haya tenido TBC comprobada?
    a. Si, y esta persona también tenía una tos persistente
    b. Si, pero esta persona concurrente no tenía una tos persistente
    c. No → *Pase a la pregunta 18*
    d. NS
16. ¿Dónde compartió este ambiente con alguien infectado con TBC?
    a. NA
    b. Trabajo
    c. Casa
    d. Hospital
    e. Otro:_____
17. ¿Por cuantos días compartió este ambiente con la persona con TBC comprobada? _____
18. Aparte de usted, ¿alguien mas en casa esta actualmente recibiendo medicinas para la TBC?
    a. Si → Quien: _____
    b. No
    c. NS

Creencias y Conocimiento de la Enfermedad

19. ¿Dónde escuchó de la TBC por primera vez?
    a. Familia
    b. Amigos
    c. Colegio
    d. Puesto de Salud
    e. TV
    f. Radio
    g. NS
20. ¿Puede alguien con TBC y tos infectar a sus familiares?
    a. Si
    b. No
    c. NS

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

21. ¿Qué tan contagiosa cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

22. ¿Qué tan seria cree que es la TBC?
    a. Nada
    b. Poquito
    c. Mucho
    d. Bastante
    e. Lo mas

23. En general, ¿qué puede hacer uno por si mismo para protegerse de contraer la TBC? [Marcar el factor más importante, no sugerir repuestas].
    a. Vacunarse
    b. Comer
    c. Dormir bien, descansar
    d. Vivir una vida ordenada
    e. Mantenerse alejado de la gente con TBC
    f. Educarse
    g. Otro: _____
    h. NS

¿Cuales son los síntomas de la TBC?
[Marcar los factores, no sugerir repuestas].

24. Tos mencionada Si☐ No☐
25. Hemoptysis mencionada Si☐ No☐
26. Fiebre mencionada Si☐ No☐
27. Baja de peso mencionada Si☐ No☐
28. Fatiga, decaimiento mencionada Si☐ No☐
29. Palidez, un cierto semblante mencionada Si☐ No☐

30. ¿Qué debe hacer una persona con TBC para mejorarse? [Marcar el factor más importante, no sugerir repuestas].
    a. Tomar sus medicinas, asistir a controles
    b. Comer mas, comer mejor
    c. Descansar
    d. Tener fe
    e. Abrigarse
    f. Otro: _____
    g. NS

31. ¿Cómo puede hacer una persona con TBC para no contagiar la TBC a otros? [Marcar el factor más importante, no sugerir repuestas].
    a. Cubrirse la boca al toser
    b. Quedarase en casa, mantenerse alejado
    c. Seguir el tratamiento
    d. Separar cubiertos
    e. Otro: _____
    f. NS

32. ¿Se puede curar la TBC?
    a. Siempre
    b. Normalmente sí
    c. A veces
    d. Raramente
    e. Nunca

Instrucciones: Marque una X sobre la línea la posición que escoges.
Ejemplo: ---------- ----------

33. Con que frecuencia esta tosiendo hoy, en un promedio de 24 horas?

0 1 2 3 4 5

nunca poquito mucho casi siempre

Instrucciones: Marque una X, en el cuadrado, todas las alternativas que corresponden.
Ejemplo: ☒

34. Que indicadores utilizó para escoger a que posición poner sus X?
    ☐ Los números: 1, 2, 3, 4, 5
    ☐ Las palabras: nunca, poquito, mucho, casi siempre
    ☐ Las figuras: 

Código del paciente: _____
Fecha de Entrevista: ___/___/____ Iniciales del Entrevistador: _____

Instrucciones: Marque con X la alternativa que corresponde.
Ejemplo: ☒

35. ¿Comparado con hace tres días, tiene más frecuencia de tos el día de hoy?
    a. ☐Tosiendo ahora menos
    b. ☐Tosiendo ahora igual
    c. ☐Tosiendo ahora más

36. El día de hoy, ¿ha tenido *solo tos, tos con flema o solo flema?*
    a. ☐Solo tos
    b. ☐Tos con flema
    c. ☐Solo flema

37. Si tiene tos, ¿a que hora en el día tiene más frecuencia: *mañana, tarde o noche*?
    a. ☐Mañana
    b. ☐Tarde
    c. ☐Noche

38. Qué tan frecuentemente tose?
    a. ☐Cada pocos segundos
    b. ☐Cada pocos minutos
    c. ☐Cada pocas horas
    d. ☐No tose

Instrucciones: Marque el numero de días en total que tiene los siguientes síntomas desde que se enfermó. Marque 0 si ningún día.

39. ¿Cuantos días usted ha tenido los siguientes síntomas?
    a. Tos seca _____
    b. Tos con flema _____
    c. Tos con sangre _____
    d. Fiebre _____
    e. Falta de aire _____
    f. Perdida de peso _____
    g. Cansancio o decaimiento _____
    h. Sudor nocturno _____
    i. Falta de apetito _____

Instrucciones: Responde a la pregunta en el lugar indicado.

40. Actualmente, ¿como se siente? (0 = mal, 10 = bien): _____

41. Cuando tiene tos, ¿cuantas toses tiene por hora? _____

42. Preguntas sobre VIH

	Manifiesto del paciente	Historia clínica
a. Usted ha sido diagnosticado por VIH?	_____	_____
b. Fecha de Diagnostico:	_____	_____
c. Ultima Carga Viral:	_____	_____
d. Fecha de ultima Carga Viral:	_____	_____
e. Ultimo resultado CD4:	_____	_____
f. Fecha de Ultimo resultado CD4:	_____	_____
g. Tiempo tomanda TARGA (años)	_____	_____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

Radiologic Interpretation Data Form

Date of read: _____

Radiologist Name: _____

Radiologist Signature: _____

Patient code: _____

Patient gender: ☐ male ☐ female

Patient age: _____

---

1. **Type of film:**
   ☐ AP ☐ PA ☐ Lateral from R ☐ Lateral from L ☐ Thoracic CT without contrast ☐ Other: _____

2. Date of film: Day: _____ Month: _____Year: _____
3. Rotation:_____
4. Adequacy of inhalation: _____

	Site	a. Consolidation?	b. Cavitation?	c. Pneumatocele?	d. Atelectasis?
5	Right upper lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
6	- anterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
7	- apical	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
8	- posterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
9	Right middle lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
10	Right lower lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
11	- superior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
12	- basal	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
13	Left upper lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
14	- anterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
15	- apical	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
16	- posterior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
17	Lingula	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
18	Left lower lobe	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
19	- superior	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
20	- basal	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no

21. Pleural effusion? ☐ yes ☐ no
left? ☐ small ☐ medium ☐ large
right? ☐ small ☐ medium ☐ large

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

22. Miliary spread? □ yes □ no
23. Pneumothorax? □ yes □ no
where and size: _____
24. Lymphadenopathy: □ yes □ no
which lymph nodes groups
□ hilar □ mediastinal
25. Pericardial effusion? □ yes □ no
left? □ small □ medium □ large
26. Bronchiectasis? □ yes □ no
where: _____
27. Fibrosis? □ yes □ no
where: _____
any retractions, deviations?_____
_____
_____
28. Mediastinal thickening? ? □ yes □ no
29. Any tree-in-bud pattern? Where?_____
_____
_____

30. Cavitation: For each cavity, please describe:
Cavity # 1:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 2:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity #3:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____

La asociación de la frecuencia de la tos con la dinámica microbiológica de la tuberculosis en pacientes con tuberculosis pulmonar activa.

CODIGO PACIENTE: _____

size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 4:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

Cavity # 5:
location: _____
size (in mm) cephalic: _____
size (in mm) caudal: _____
size (in mm) anterior-posterior: _____
presence of air/ fluid level?: □ yes □ no
Cavity wall: □ think □ thick □smooth □nodular
Cavity Wall Thickness(in mm): _____

More cavities? □ yes: please use another sheet to describe □ no

Other findings such as fractures, cardiac abnormalities, exudative / fibrotic densities, bronchogenic spread, mass-like lesions (calcified vs. non-calcified), please describe:

_____
_____
_____
_____
_____
_____
_____
_____

31. Normal film? □ yes □ no

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