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

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Complete List of Authors:	Proaño, Alvaro; Universidad Peruana Cayetano Heredia, Facultad de Medicina "Alberto Hurtado" Bravard, Marjory; Massachusetts General Hospital, Department of Internal Medicine; Universidad Peruana Cayetano Heredia, Laboratory of Research and Development, Innovation For Health And Development (IFHAD) Tracey, Brian; Tufts University, Department of Electrical and Computer Engineering López, Jose; Universidad Peruana Cayetano Heredia, Facultad de Ciencias y Filosofía, Laboratorio de Bioinformática y Biología Molecular; Instituto Nacional de Salud del Niño San Borja, Sub Unidad de Atención Integral Especializada Pediatría y Sub Especialidades Comina, German; Universidad Nacional de Ingeniería, Facultad de Ciencias, Laboratorio de Ingeniería Física; Linköping University, Department of Physics, Chemistry and Biology (IFM), Optical Devices Laboratory Zimic, Mirko; Universidad Peruana Cayetano Heredia, Facultad de Ciencias y Filosofía, Laboratorio de Bioinformática y Biología Molecular; Universidad Peruana Cayetano Heredia, Facultad de Ciencias y Filosofía, Laboratorio de Investigación y Desarrollo, Laboratorio de Investigación en Enfermedades Infecciosas Coronel, Jorge; Universidad Peruana Cayetano Heredia, Facultad de Ciencias y Filosofía, Laboratorio de Investigación y Desarrollo, Laboratorio de Investigación en Enfermedades Infecciosas Lee, Gwenyth; Tulane University, Department of Global Community Health and Behavioral Sciences Caviedes, Luz; Universidad Peruana Cayetano Heredia, Facultad de Ciencias y Filosofía, Laboratorio de Investigación y Desarrollo, Laboratorio de Investigación en Enfermedades Infecciosas Cabrera, Jose; Hospital Nacional Daniel Alcides Carrión, Servicio de Neumología; Clínica Internacional, Servicio de Neumología Salas, Juan; Hospital Nacional Dos de Mayo, Servicio de Neumología Ticona, Eduardo; Hospital Nacional Dos de Mayo, Servicio de Enfermedades Infecciosas y Tropicales Kirwan, Daniela; Imperial College London, Infectious Diseases & Immunity Friedland, Jon; Imperial College London, Infectious Diseases & Immunity Evans, Carlton; Universidad Peruana Cayetano Heredia, Laboratory of Research and Development, Innovation For Health And Development (IFHAD); Imperial College London, Infectious Diseases & Immunity Moore, David; London School of Hygiene and Tropical Medicine, TB Centre Gilman, Robert; Asociación Benéfica PRISMA; Johns Hopkins University, Bloomberg School of Public Health, Department of International Health

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Corresponding author/author in charge of pre-publication contacts:

Robert H. Gilman, Department of International Health, Johns Hopkins University Bloomberg School of Public Health, Address: 615 N Wolfe St. Rm.W5515, Baltimore, MD 21205, USA, Telephone:(410)614-3959, Fax:(410)510-1284, E-mail: gilmanbob@gmail.com / rgilman@jhsph.edu

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Authors:
Alvaro **Proaño**[1], Marjory A. **Bravard**[2,3,4], Brian H. **Tracey**[5], José W. **Lopez**[6], German **Comina**[7,8], Mirko **Zimic**[6,9], Jorge **Coronel**[9], Gwenyth O'Neill **Lee**[10], Luz **Caviedes**[9†], Jose Luis **Cabrera**[11], Antonio **Salas**[12], Eduardo **Ticona**[13], Daniela E. **Kirwan**[14], Jon S. **Friedland**[14], Carlton A. **Evans**[3,14,15], David A. **Moore**[16], Robert H. **Gilman**[4,17], Tuberculosis Working Group in Peru

1 Facultad de Medicina 'Alberto Hurtado', Universidad Peruana Cayetano Heredia, Lima, Peru
2 Department of Internal Medicine, Massachusetts General Hospital, Boston, Massachusetts, United States of America
3 Innovation For Health And Development (IFHAD), Laboratory of Research and Development, Universidad Peruana Cayetano Heredia, Lima, Peru
4 Asociación Benéfica PRISMA, Lima, Perú
5 Department of Electrical and Computer Engineering, Tufts University, Medford, Massachusetts, United States of America
6 Laboratorio de Bioinformática y Biología Molecular, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú
7 Laboratorio de Ingeniería Física, Facultad de Ciencias, Universidad Nacional de Ingeniería, Lima, Perú
8 Optical Devices Laboratory - Department of Physics, Chemistry and Biology (IFM), Linköping University, Linköping 58183, Sweden
9 Laboratorio de Investigación en Enfermedades Infecciosas, Laboratorio de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú
10 Department of Global Community Health and Behavioral Sciences, Tulane University, Louisiana, New Orleans, United States of America
11 Servicio de Neumología, Hospital Nacional Alcides Carrión, Lima, Perú
12 Servicio de Neumología, Hospital Nacional Dos de Mayo, Lima, Perú
13 Servicio de Enfermedades Infecciosas y Tropicales, Hospital Nacional Dos de Mayo, Lima, Peru
14 Infectious Diseases & Immunity, Imperial College London, United Kingdom
15 Wellcome Trust Imperial College Centre for Global Health Research, London, United Kingdom
16 TB Centre, London School of Hygiene and Tropical Medicine, London, United Kingdom.
17 Program in Global Disease Epidemiology and Control, Department of International Health, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, Maryland, United States of America
† *in memoriam* Luz Caviedes who passed away in November 2012

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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.

References

1. Global Tuberculosis Report 2014. Geneva, Switzerland: World Health Organization, 2014.

2. Riley RL, Mills CC, Nyka W, et al. Aerial dissemination of pulmonary tuberculosis: a two-year study of contagion in a tuberculosis ward. Am J Epidemiol 1959;70(2):185-96.

3. Riley RL, Mills CC, O'Grady F, Sultan LU, Wittstadt F, Shivpuri DN. Infectiousness of air from a tuberculosis ward. Ultraviolet irradiation of infected air: comparative infectiousness of different patients. Am Rev Respir Dis 1962;85:511-25.

4. Loudon RG, Roberts RM. Singing and the dissemination of tuberculosis. Am Rev Respir Dis 1968;98(2):297-300.

5. Turner RD, Bothamley GH. Cough and the transmission of tuberculosis. J Infect Dis 2015;211(9):1367-72.

6. Loudon RG, Brown LC. Cough frequency in patients with respiratory disease. Am Rev Respir Dis 1967;96(6):1137-43.

7. Loudon RG, Spohn SK. Cough frequency and infectivity in patients with pulmonary tuberculosis. Am Rev Respir Dis 1969;99(1):109-11.

8. Loudon RG, Romans WE. Cough-monitoring equipment. Med Res Eng 1967;6(2):25-7.

9. Jindani A, Aber VR, Edwards EA, Mitchison DA. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am Rev Respir Dis 1980;121(6):939-49.

10. Mitchison DA. Infectivity of patients with pulmonary tuberculosis during chemotherapy. Eur Respir J 1990;3(4):385-6.

11. Datta S, Sherman JM, Bravard MA, Valencia T, Gilman RH, Evans CA. Clinical evaluation of tuberculosis viability microscopy for assessing treatment response. Clin Infect Dis 2015;60(8):1186-95.

12. Jensen PA, Lambert LA, Iademarco MF, Ridzon R, Cdc. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR Recomm Rep 2005;54(RR-17):1-141.

13. . Tuberculosis: Clinical Diagnosis and Management of Tuberculosis, and Measures for Its Prevention and Control. London, 2011.

14. Noble RC. Infectiousness of pulmonary tuberculosis after starting chemotherapy. American Journal of Infection Control 1981;9(1):6-10.

15. Sepkowitz KA. How contagious is tuberculosis? Clin Infect Dis 1996;23(5):954-62.

16. Fitzwater SP, Caviedes L, Gilman RH, et al. Prolonged infectiousness of tuberculosis patients in a directly observed therapy short-course program with standardized therapy. Clin Infect Dis 2010;51(4):371-8.

17. Boulet LP, Coeytaux RR, McCrory DC, et al. Tools for assessing outcomes in studies of chronic cough: CHEST guideline and expert panel report. Chest 2015;147(3):804-14.

18. Pavesi L, Subburaj S, Porter-Shaw K. Application and validation of a computerized cough acquisition system for objective monitoring of acute cough: a meta-analysis. Chest 2001;120(4):1121-8.

19. Smith J, Woodcock A. New developments in the objective assessment of cough. Lung 2008;186 Suppl 1:S48-54.

20. Yousaf N, Monteiro W, Parker D, Matos S, Birring S, Pavord ID. Long-term low-dose erythromycin in patients with unexplained chronic cough: a double-blind placebo controlled trial. Thorax 2010;65(12):1107-10.

21. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomised, double-blind, placebo-controlled trial. Lancet 2012;380(9853):1583-9.

22. Koehler U, Brandenburg U, Weissflog A, Sohrabi K, Gross V. [LEOSound, an innovative procedure for acoustic long-term monitoring of asthma symptoms (wheezing and coughing) in children and adults]. Pneumologie 2014;68(4):277-81.

23. Sterling M, Rhee H, Bocko M. Automated Cough Assessment on a Mobile Platform. J Med Eng 2014;2014.

24. Coyle MA, Keenan DB, Henderson LS, et al. Evaluation of an ambulatory system for the quantification of cough frequency in patients with chronic obstructive pulmonary disease. Cough 2005;1:3.

25. Barry SJ, Dane AD, Morice AH, Walmsley AD. The automatic recognition and counting of cough. Cough 2006;2:8.

26. Birring SS, Fleming T, Matos S, Raj AA, Evans DH, Pavord ID. The Leicester Cough Monitor: preliminary validation of an automated cough detection system in chronic cough. Eur Respir J 2008;31(5):1013-8.

27. Vizel E, Yigla M, Goryachev Y, et al. Validation of an ambulatory cough detection and counting application using voluntary cough under different conditions. Cough 2010;6:3.

28. Barton A, Gaydecki P, Holt K, Smith JA. Data reduction for cough studies using distribution of audio frequency content. Cough 2012;8(1):12.

29. Tracey BH, Comina G, Larson S, Bravard M, Lopez JW, Gilman RH. Cough detection algorithm for monitoring patient recovery from pulmonary tuberculosis. Conf Proc IEEE Eng Med Biol Soc 2011;2011:6017-20.

30. Larson S, Comina G, Gilman RH, Tracey BH, Bravard M, Lopez JW. Validation of an automated cough detection algorithm for tracking recovery of pulmonary tuberculosis patients. PLoS One 2012;7(10):e46229.

31. Caviedes L, Lee TS, Gilman RH, et al. Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. The Tuberculosis Working Group in Peru. J Clin Microbiol 2000;38(3):1203-8.

32. Moore DA, Mendoza D, Gilman RH, et al. Microscopic observation drug susceptibility assay, a rapid, reliable diagnostic test for multidrug-resistant tuberculosis suitable for use in resource-poor settings. J Clin Microbiol 2004;42(10):4432-7.

33. Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. N Engl J Med 2006;355(15):1539-50.

34. Norma Técnica de Salud Para la Atención Integral de las Personas Afectadas por Tuberculosis. Lima, Perú: Ministerio de Salud del Perú, 2013.

35. Tuberculosis in the Americas: Regional Report 2012. Epidemiology, Control and Financing. Washington D.C.: Pan American Health Organization, 2013.

36. Bonilla CA, Crossa A, Jave HO, et al. Management of extensively drug-resistant tuberculosis in Peru: cure is possible. PLoS One 2008;3(8):e2957.

37. American Thoracic S, Centers for Disease C, Prevention, Infectious Diseases Society of A. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med 2005;172(9):1169-227.

38. World Health O. Definitions and reporting framework for tuberculosis - 2013 revision. Geneva, Switzerland: World Health Organization, 2013.

39. Bhalla AS, Goyal A, Guleria R, Gupta AK. Chest tuberculosis: Radiological review and imaging recommendations. Indian J Radiol Imaging 2015;25(3):213-25.

40. Skoura E, Zumla A, Bomanji J. Imaging in tuberculosis. Int J Infect Dis 2015;32:87-93.

41. Alisjahbana B, van Crevel R, Danusantoso H, et al. Better patient instruction for sputum sampling can improve microscopic tuberculosis diagnosis. Int J Tuberc Lung Dis 2005;9(7):814-7.

42. Khan MS, Dar O, Sismanidis C, Shah K, Godfrey-Faussett P. Improvement of tuberculosis case detection and reduction of discrepancies between men and women by simple sputum-submission instructions: a pragmatic randomised controlled trial. Lancet 2007;369(9577):1955-60.

43. Rocha C, Montoya R, Zevallos K, et al. The Innovative Socio-economic Interventions Against Tuberculosis (ISIAT) project: an operational assessment. Int J Tuberc Lung Dis 2011;15 Suppl 2:S50-7.

44. Paul IM, Wai K, Jewell SJ, Shaffer ML, Varadan VV. Evaluation of a new self-contained, ambulatory, objective cough monitor. Cough 2006;2:7.

45. Matos S, Birring SS, Pavord ID, Evans DH. An automated system for 24-h monitoring of cough frequency: the leicester cough monitor. IEEE Trans Biomed Eng 2007;54(8):1472-9.

46. Kent PT, Kubica GP, Control CfD. Public Health Mycobacteriology: A Guide for the Level III Laboratory: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control, 1988.

47. Im JG, Itoh H, Shim YS, et al. Pulmonary tuberculosis: CT findings--early active disease and sequential change with antituberculous therapy. Radiology 1993;186(3):653-60.

48. Rodrigo T, Cayla JA, Garcia de Olalla P, et al. Characteristics of tuberculosis patients who generate secondary cases. Int J Tuberc Lung Dis 1997;1(4):352-7.

49. Van Dyck P, Vanhoenacker FM, Van den Brande P, De Schepper AM. Imaging of pulmonary tuberculosis. Eur Radiol 2003;13(8):1771-85.

50. Kelsall A, Decalmer S, Webster D, et al. How to quantify coughing: correlations with quality of life in chronic cough. Eur Respir J 2008;32(1):175-9.

51. Jones-Lopez EC, Kim S, Fregona G, et al. Importance of cough and M. tuberculosis strain type as risks for increased transmission within households. PLoS One 2014;9(7):e100984.

52. Turner R, Repossi A, Matos S, Birring S, Bothamley G. S79 Cough Prevalence And Frequency In Pulmonary Tuberculosis. Thorax 2014;69(Suppl 2):A43-A44.

53. Yousaf N, Monteiro W, Matos S, Birring SS, Pavord ID. Cough frequency in health and disease. Eur Respir J 2013;41(1):241-3.

Figure 1 – Flow Diagram for CayeCoM Study

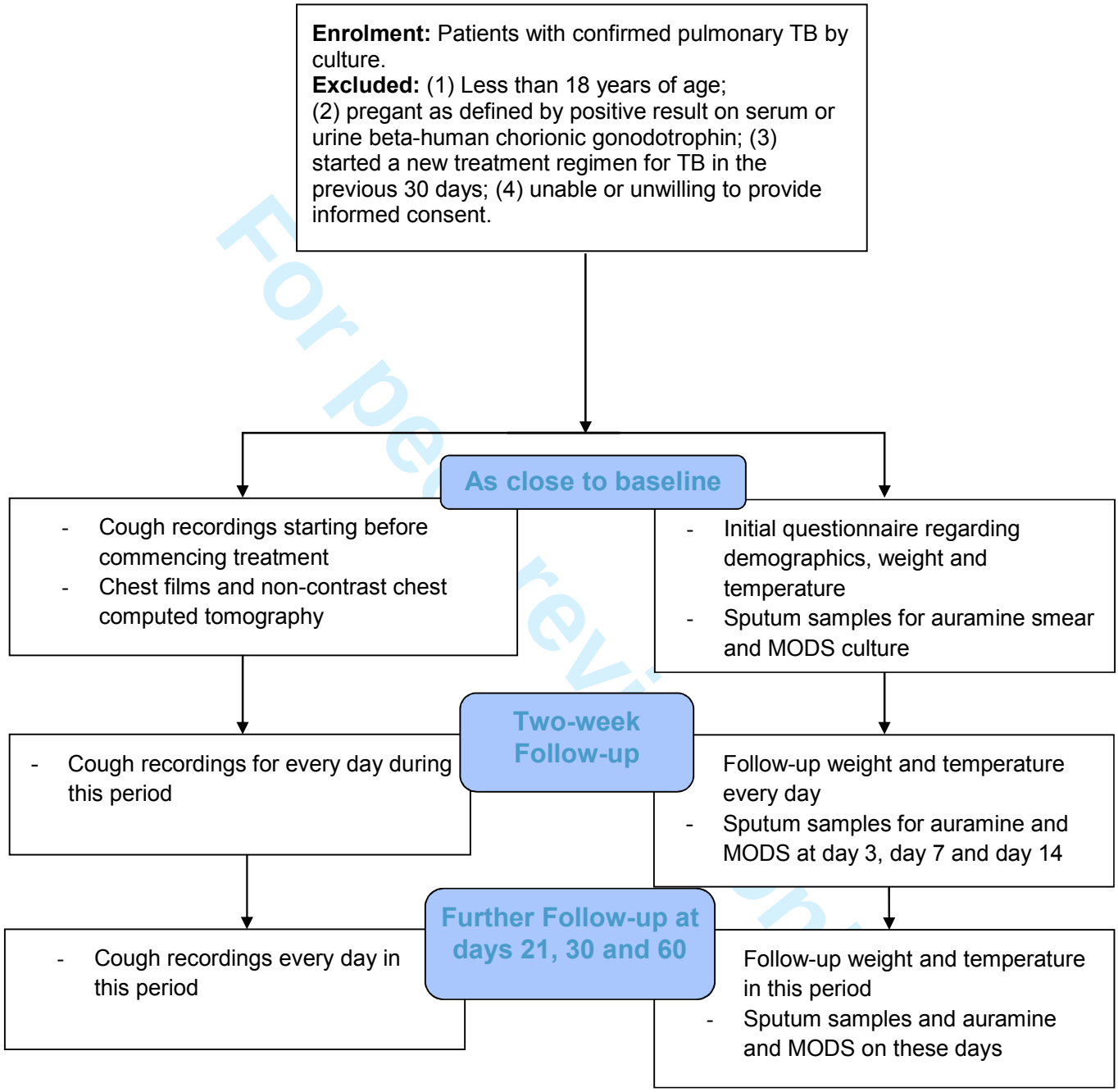

Figure 2 – Picture of the Cayetano Cough Monitor (CayeCoM)

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

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