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Correlation of Cough Frequency with Treatment Efficacy in Pulmonary Tuberculosis Patients in Lima, Peru

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Correlation of Cough Frequency with Treatment Efficacy in Pulmonary Tuberculosis patients in Lima, Peru

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Introduction

Since the vast majority of people suffering from tuberculosis (TB) come from the poorer and most vulnerable segments of society, global TB control targets cannot be met unless this group of people is reached with essential health services. Early diagnosis and effective treatment are important aspects that help reduce the adverse social and financial consequences of the disease for TB patients and their families. Consequently, all influential policy documents on TB control always highlight the importance of developing strategies that ensure global access to essential health services for all TB patients.

Despite the successes achieved by the adoption of the Directly Observed Therapy, short course (DOTS) strategy in countries such as Peru, the recent emergence of multidrug resistant (MDR) TB and extremely drug resistant (XDR) TB has slowed down the progress toward the ultimate goal of TB control and elimination. Originally named after one of the components of the strategy, DOTS comprises four other critical components including: government commitment, case detection by sputum smear microscopy, uninterrupted drug supply and standardized reporting and recording. Given this new understanding, a fast, simple and cost-effective tool to assess anti-TB treatment efficacy becomes a paramount aspect of this goal.

The thesis is organized in the following sections. The background section provides an overview of tuberculosis and the problem of multidrug resistant tuberculosis around the world. These sections are followed by a review of the current state of TB in Peru and MDR TB as the new threat facing the Peruvian national TB program. Following the background, the author presents an original prospective cohort study on the correlation of cough frequency and treatment efficacy of pulmonary TB patients in Lima, Peru. The main objective of this study was to test

the hypothesis that cough frequency will decrease in patients receiving effective treatment for susceptible TB strains. This was done through a survey administered to pulmonary TB patients during their treatment. The purpose of undertaking this study was to explore the use of cough frequency as a clinical marker for treatment efficacy. Understanding this correlation promises to deliver an inexpensive and reliable method of determining treatment efficacy, especially in the era of MDR TB and XDR TB.

Tuberculosis

Current State of Tuberculosis

The Mycobacterium tuberculosis complex includes five species: *M. tuberculosis, M. bovis, M. canetti, M. africanum, and M. microti*. Most human disease is caused by *M. tuberculosis*. These bacilli, transmitted on airborne droplets, can cause a lung disease that if left untreated will kill about 50% of patients.[1] About 9 million people around the world developed tuberculosis (TB) for the first time in 2010, and nearly 2 million people died with or from the disease. Globally, TB is currently responsible for more years of healthy life lost (2.5 percent of all disability-adjusted life years, or DALYs) than any other infectious disease, bar AIDS and malaria.[2] Only AIDS is responsible for more deaths. Ninety five percent of cases and deaths occur in developing countries and TB accounts for approximately seven percent of all deaths in developing countries.[3]

The total cost of the global TB epidemic can be difficult to grasp. Often the local health services and families are responsible for the direct monetary costs associated with the diagnosis and treatment of the disease. In addition, the loss of income and decreased production when

those afflicted by the disease—often the most productive members of society— are too sick to work or die prematurely are indirect costs borne by the local communities.

Finally, one should not forget that like many other diseases, tuberculosis also carries with it enormous psychological and social costs that cannot be properly quantified in DALYs or dollars but are nonetheless very real to those who suffer from the disease.

Further exacerbating the global TB problem is the continuing emergence of multidrug-resistant (MDR) TB. MDR-TB is resistant to treatment with isoniazid and rifampin—the two most potent first-line TB drugs. Diagnosis and treatment of MDR-TB in endemic areas is a relatively new public health challenge. Successful response requires isolation of infectious patients, rapid procurement of and treatment with second-line medications, and consistent directly observed therapy (DOT). On a global scale, many rural communities do not have adequate resources or the expertise needed to address this daunting public health problem. Higher morbidity and mortality rates occur with MDR-TB than with drug susceptible TB.[4]

MDR-TB is a growing challenge to all regions of the world. TB control programs that are not achieving basic standards can create MDR-TB cases because of inadequate DOT. These same programs will likely have a difficult time managing the costs of treating MDR-TB cases. Small TB programs may struggle to manage outbreaks in a timely manner. Avoidable delays in diagnosis can result in the surprisingly rapid expansion of infectious cases and contacts with latent MDR-TB disease, which can cause the potential for MDR-TB to be firmly entrenched in the community for many years.

In the United States, from the 1950s until the mid 1980s, there was approximately a 5% annual decline in the case rate of TB. From 1985 to the early 1990s, however, the incidence of the disease increased by 20%.[4] This increase primarily affected young, urban, racial and ethnic

minority populations. Tuberculosis was more prevalent among the homeless, illicit drug users and inmates of correctional facilities.[5] Another important trend during this time period centered on an increase in the number of immigrants to the United States from countries with high prevalence of TB. In two decades, the percentage of TB cases in the United States attributed to foreign-born persons had increased from 22% in 1985 to 58% in 2007.[1]

After 1992, the increasing incidence of TB in the United States stabilized and then began to decline once again. By 2010, the case reports of TB had declined by over 50% reaching 11,181 cases (or 3.6 cases per 100,000).[6] Despite the advances in the control and elimination of this disease, TB rates among Hispanics, blacks, and Asians were seven, eight, and 25 times greater, respectively, than among whites. The TB case rate in 2010 for foreign-born persons was 11 times greater than the rate for US-born persons. Unfortunately, the target of TB elimination was not reached by 2010, a goal established in 1989. Moreover, the decrease in overall case rates has slowed in recent years from 7.8% during the 1990s to 3.8% over the past few years.[6]

Microbiology of Tuberculosis

Unlike other bacteria, mycobacteria are acid and alcohol fast. This means that if stained by an aniline dye, such as carbolfuchsin, their thick, lipid-rich wall resists decolorization when they come into contact with acid and alcohol. This unique property of mycobacteria has led to the medical community to refer to them as "acid-fast bacilli" (AFB) and highlights the importance of mycobacteria's wall to their survival.[7] On the other hand, this property also allows for the detection of AFB in sputum specimens. This is often done by the simple Ziehl-Neelsen (ZN) staining technique, widely used throughout the world.[7] This is an inexpensive tool that can be carried out rapidly.

Mycobacteria are slow growers. Each generation time is often measured in hours rather than minutes. Consequently, the normal methods of obtaining cultures from clinical specimens are difficult because of overgrowth by other bacteria. Fortunately, the thick cell wall of mycobacteria also enables them to resist alkalis and detergents, which are used to reduce contamination during culture.[7]

Transmission and Risk of Infection

People infected with *M. tuberculosis* carry live tubercle bacilli, usually in their lungs. However, the bacilli may be present in small numbers and dormant or latent. In this case, there may be no apparent disease and the person is termed to have a latent TB infection (LTBI) Disease occurs when the bacteria overcome the body's immune defenses, and become numerous enough to cause damage to tissues.[8] Patients with pulmonary TB are the most important source of infection. Infection generally occurs by inhaling droplet nuclei. These nuclei contain tubercle bacilli in infectious particles usually less than 5 micrometers in size.[8] These are spread into the air by coughing, sneezing, talking, spitting, or singing, and they can remain suspended in the air for long periods of time, especially in dark, unventilated environments.[8] For example, a single cough can produce 3,000 infectious droplet nuclei. Droplet nuclei are so small that they pass undetected through most defenses and penetrate into the terminal alveoli of the lungs, where multiplication and infection begins.[8]

The probability of transmission of the disease is determined by several different factors: 1) the infectiousness of the source case (that is, how many tubercle bacilli are being coughed into the air); 2) The immune status of the host and the susceptibility to contacts; 3) The duration of exposure and closeness of contact; 4) The environment in which the exposure takes place, especially small, poorly ventilated places.[7] Patients with positive sputum smear for acid-fast

bacilli or with cavities on chest radiograph (CXR) are much more infectious than those with negative sputum smears or without cavities on CXR.[7] Following infection, the tubercle bacilli are ingested by alveolar macrophages in the lungs. It is here that they multiply slowly with bacterial cell division occurring every 25 to 32 hours. This facilitates their spread to the local lymph nodes, and then to the rest of the body.[8] Approximately two to eight weeks after this primary infection, most infected humans develop an immune response to the tubercle bacilli called delayed-type hypersensitivity. Unless there is a defect in the cell-mediated immune response, it stops further multiplication of the bacteria, and the only evidence of infection is a positive response to an immunological test, most commonly the tuberculin skin test.[9]

If disease develops, infiltrates and lesions usually appear within the lung tissue.[8] The immune response of the patient results in a pathological lesion, which is characteristically localized, often with extensive tissue destruction and cavitation.[10] These cavitating lesions often contain many actively dividing bacilli.[10] Sputum from patients with these lesions is usually smear-positive.[10]

The proportion of any population infected depends on the rate and duration of exposure, which can vary from one group of people to another. There are, however, some common patterns. Infection rates are always observed to increase monotonically with age.[11] This is partially explained by the ability of this infection to remain dormant for many years. Although the infection rates in boys and girls are usually indistinguishable, adult men appear to suffer from higher infection rates than adult women. This discrepancy is most likely not related to the intrinsic susceptibility of men to the infection. In general, men probably suffer more from TB than women because they are more exposed to infection.[12,13]

The size of the infecting dose of tubercle bacilli and the immune status of the host determine the risk of progression from infection to disease. The estimated lifetime risk of disease for a newly infected person is 10%, with approximately half of that risk occurring in the first two years after infection.[14] This development is most common in children under five years of age and adults with advanced immunosuppression.[14] Infants and young children up to the age of five years who are infected with *M. tuberculosis* appear to be at relatively high risk, particularly of severe forms (mainly miliary TB and TB meningitis), because of their immature immune systems. The risk is also high during adolescence and remains stable during adulthood. The elderly, especially over the age of 65, also suffer from higher risk.[15]

Other factors that enhance the risk of developing TB following infection include under-nutrition, toxins (tobacco, alcohol, corticosteroids, immunosuppressive drugs), and other diseases (diabetes mellitus, silicosis, leukemia, measles, and whooping cough in children). However, the most important risk factor remains HIV co-infection.[16]

Clinical Manifestations

Clinical diagnoses of tuberculosis distinguish between pulmonary and extrapulmonary disease, the former being of much greater importance epidemiologically.

Pulmonary Tuberculosis

Patients with pulmonary TB present with a chronic productive cough, fever, and weight loss.[8] Cough occurs in a variety of circumstances, notably in acute upper and lower respiratory infections. However, these acute infections often resolve within three weeks. Thus, a patient with a cough longer than three weeks, especially after a course of antibiotics, should be investigated for pulmonary TB.[17]

The diagnosis of pulmonary TB in most hospitals in countries with limited resources is based on sputum smear microscopy and chest radiography. Most countries have a reference laboratory where *M. tuberculosis* can be cultured from clinical specimens, such as sputum. Because *M. tuberculosis* is a slow-growing organism taking two to three months to become visible on culture medium, cultures are not usually helpful in making an individual diagnosis. Mycobacterial cultures are commonly used for monitoring drug-sensitivity patterns in patients with recurrent TB and for monitoring the community prevalence of drug-resistant TB.[18] Taking sputum specimens (three per suspect) for smear microscopy of AFB is a cheap and simple way to screen for pulmonary TB.[19] If sputum smear examination is negative, patients suspected of having pulmonary TB should have a chest radiography.[19] Chest radiography suggestive of disease usually show upper lobe disease, bilateral disease, and cavitations. These findings are more common in HIV-negative patients than HIV-positive patients. However, no pattern is absolutely diagnostic of TB.[10]

Extrapulmonary Tuberculosis

Extrapulmonary TB accounts for 20% of all cases of TB in HIV-seronegative patients. This percentage increases to approximately 50% in HIV co-infection.[20] Among HIV-seronegative patients extrapulmonary TB is most common in females and young patients.[20] The common signs or forms of extrapulmonary TB include pleural effusion, lymphadenopathy, pericardial effusion, miliary disease, and meningitis. Patients usually present with constitutional symptoms and local features related to the site of disease. If patients cough for longer than three weeks, sputum smear examination and chest radiography are often carried out, because of the likely possibility of coexisting pulmonary disease. Definitive diagnosis of extrapulmonary TB depends on having diagnostic tools, such as radiographs, ultrasound scans, procedures to obtain

and analyze fluid samples, and procedures for tissue biopsies and histological analysis.[20] This degree of diagnostic sophistication is often unavailable in many areas of the world. For example, in one study in Tanzania only 18 percent of patients diagnosed with extrapulmonary TB had laboratory confirmation of the diagnosis.[21]

Childhood Tuberculosis

Approximately 11% of all cases of TB occur in children (classified as patients under the age of 15). Children are most commonly infected with *M. tuberculosis* as a result of transmission from an adult in their close environment (usually the household) with smear-positive disease. Most children remain asymptomatic, and a positive tuberculin test may be the only evidence of infection. For those who progress to disease, pulmonary TB is the most common manifestation in both children with and without HIV-coinfection. Extrapulmonary disease, however, is more frequent in those who are HIV positive and meningitis appears to be the most common manifestation in very young children (age less than 3). Although more common in children than adults, the patterns of extrapulmonary TB in children and the diagnostic problems encountered are similar to those described for adults.[22]

The diagnosis of childhood pulmonary TB has always been difficult because of the lack of sputum production and the paucity or absence of tubercle bacilli in respiratory secretions, since they typically remain confined to perihilar nodes that do not rupture into the bronchus.[23] A presumptive diagnosis, therefore, usually relies on a combination of clinical features, history of contact with a sputum-positive case, CXR and tuberculin skin test.[17] Unfortunately, CXR findings and clinical features are often nonspecific. *M. tuberculosis* recovery from gastric aspiration, induced sputum, and nasopharyngeal aspiration show promise as alternative

diagnostic techniques but are not practical under routine clinical conditions in limited resource settings.[23]

Multi-Drug Resistant Tuberculosis

Introduction

Multidrug-resistant (MDR) tuberculosis (TB) is defined as TB caused by *Mycobacterium tuberculosis* strains resistant to at least the two "first-line" anti-TB medications, isoniazid and rifampin. MDR TB is a growing global health threat. The World Health Organization (WHO) estimates that about 500,000 cases of MDR TB occur annually, or 5.4% of the 9.3 million cases of TB that occur worldwide.[24,25] The treatment required for this form of the disease consists of combination therapy with four to six drugs for a period of time averaging two years.[18] These "second line" agents are significantly more toxic, less potent and more costly. Consequently, MDR TB has lower cure rates, higher mortality and a much higher cost for a single treatment course.[18,26,27] Even in settings with optimal public health program resources, including directly observed therapy and intensive case management, overall cure rates are lower, ranging from 65 to 75%.[28,29]

Given the complexities of the disease, MDR TB requires a high level of laboratory capacity for accurate and effective diagnosis as well as fairly intensive management. Unfortunately, there has been very little progress in the area of diagnostic and treatment services needed to handle the current, estimated annual case burden. As of late 2010, the Stop TB Partnership Working Group for MDR TB reports that since 2000 approximately 100,000 MDR TB patients have been treated or are currently undergoing treatment in appropriate program conditions approved by the group's Green Light Committee quality assurance mechanism.[30]

This represents less than 10% of all the MDR TB cases that have occurred around the globe during the same time period. Further evidence of the need to improve diagnostic and treatment capacity for MDR TB around the world is the very high mortality rate for the disease. Currently, ~ 150,000 or 30% of the annual MDR TB incident cases in the world die of the disease. Even if patients are able to enter good treatment programs, mortality ranges from 8 to 21%.[27,28,29]

Risk Factors for MDR TB

Understanding the risk factors for MDR TB can help clinicians and public health officials identify potential cases early. Early detection might facilitate rapid diagnosis, initiation of appropriate treatment sooner and use of enhanced infection control measures to reduce the transmission of MDR TB strains.

Human Immunodeficiency Virus (HIV) infection is among the strongest risk factors for progression from TB infection to active TB disease. The risk for progression is over 20 times higher for those patients co-infected with TB and HIV compared with those without HIV infection.[31] In a prospective study of 169 patients by Vernon et al, HIV positive patients under treatment for TB had a greater risk of developing rifabutin resistance during the course of TB treatment.[32] Indeed, a direct association between HIV infection and TB drug resistance has been suggested by a number of reports.[18,33]

In addition to HIV infection, there are several risk factors that increase the likelihood that a given patient who presents with TB has MDR TB. These factors include a history of previous treatment for TB, especially if this treatment course failed, and having contact with a known case of MDR TB.[18]

Diagnosis

In settings where resources and health infrastructure are limited, TB is diagnosed based on the results of sputum smear staining and the identification of acid fast bacilli (AFB) using microscopy. Culture and susceptibility tests are not routinely performed.[34] According to the World Health Organization's (WHO) TB treatment guidelines for national programs, a newly diagnosed TB patient with positive AFB sputum smears should be started on standard treatment with a first-line, four-drug combination regimen (isoniazid, rifampin, pyrazinamide and ethambutol). This regimen should only be changed if the patient remains sputum smear-positive for AFB after 3 months of treatment. At this point evaluation by sputum culture and drug susceptibility testing is recommended.[34] Laboratory evaluation of sputum with culture and subsequent drug susceptibility testing is based on use of solid media. Although inexpensive, the results from this approach confirming MDR TB can take two months or longer. This approach to the diagnosis and management of TB around the world is largely the result of limitations of resources and capacity. Unfortunately, it also leads to extensive delays in the diagnosis of MDR TB among patients. Undetected MDR TB cases can lead to further transmission of the resistant strain and to higher mortality, especially in those patients with HIV infection.[33]

Over the past few years, however, there has been an increase in the availability of more practical and less expensive rapid diagnostic technology. For example, working in a high MDR TB, middle-income Russian community, Balavanova et al. used the Mycobacterium Growth Indicator Tube (MGIT) system to significantly reduce the time of diagnosis from months to weeks.[35] However, this liquid-based system required substantial resources, including various supplies and equipment maintenance, making its generalizability to other areas of the world less than ideal.

Alternatively, fairly rapid and very inexpensive culture-based methodologies, such as the microscopic observation drug susceptibility (MODS) assay and the Griess methods are also being effectively implemented in resource-constrained settings. Moore et al. implemented the MODS method in an operational setting in Peru.[36] This liquid culture method takes approximately 7 days to diagnose TB and detects bacterial resistance to all antibiotics at the same time. The sensitivity for detection of TB with this method is 97.8% while the agreement between MODS and the reference standard for susceptibility is 100% for rifampin and 97% for isoniazid. Similarly, Asencios et al. implemented a rapid drug susceptibility test (DST) in Lima, Peru at a national reference laboratory based on the direct Greiss method.[37] This is a calorimetric method that uses a nitrate reductase reaction to indicate growth of *Mycobacterium tuberculosis* on a modified Lowenstein-Jensen medium 1 to 3 weeks before colonies become visible. Its implementation in Peru yielded a sensitivity and specificity respectively of 99.1% and 100% for isoniazid and 93.5% and 100% for rifampin. The average time from sputum collection to DST result was 31 days, approximately one third of the time required for conventional DST.

Newer and more rapid molecular-based technologies applied directly to sputum samples to detect rifampin resistance have been developed over the past decade. For example, in 2000, Traore et al. utilized a line probe assay (LiPA) to detect genetic mutations conferring rifampin resistance without requiring culture. Given that rifampin mono-resistance is highly predictive of multidrug resistance, the authors concluded that this method would allow for diagnosis of MDR TB within 1 week and often within a few days.[38] More recently, the specimen processing required by these technologies is being more simplified to allow for easier uptake and incorporation into general laboratory use. All of these tests look for mutations in the core region

of the rpoB gene to detect rifampin resistance since numerous studies have shown that this region encodes at least 95% of all rifampin-resistant tuberculosis.[39] Helb et al., for example, described in 2010 an automated molecular test for the presence of *Mycobacterium tuberculosis* (MTB) and resistance to rifampin (RIF). The Gene Xpert System's MTB/RIF assay showed high sensitivity and produced results in less than two hours with minimal hands-on time.[40] Similarly, Boehme et al. has replicated these results in 1730 patients from four different countries and settings including Peru, Azerbaijan, South Africa and India.[41] In this study, the MTB/RIF assay had a 97.6% sensitivity and 98.1% specificity in identifying resistance to rifampin. Although promising, these rapid susceptibility tests still use sophisticated technology, which is costly to manufacture and maintain. For example, the need for an annual calibration was identified as a potential challenge for implementation at peripheral laboratories, especially in rural areas of these countries.

Management Strategies

The WHO guidelines serve as a highly useful resource in developing treatment regimens for patients with available drug susceptibility and in implementing an empiric treatment for those patients that are still waiting for drug susceptibility information.[18] These guidelines are the basis of treatment for many MDR TB treatment programs globally, including in Peru.

The guidelines recommend that all newly diagnosed TB patients should have their HIV status evaluated.[18] HIV prevalence among TB patients has been estimated to be as high as 80 to 90% in some areas of sub-Saharan Africa.[42] In this area of the world, however, HIV testing of TB patients varies widely. Currently, Kenya and Malawi test over 80% of TB patients, but estimates are lower in Uganda (60%), Zambia (60%) and South Africa (40%).[42] Tuberculosis is

often the first clinical indication that a person has underlying HIV infection, and TB services can be an extremely important entry point to HIV prevention, care and treatment. In addition, early detection of HIV status of TB patients can make a difference in overall outcome. Initiation of anti-retroviral therapy (ART) for persons with HIV during TB treatment has been shown to reduce mortality by approximately 50%.[42] However, early initiation of ART (within a few weeks of starting TB treatment) means a large number of tablets to ingest, which may discourage treatment adherence. In addition, combination of ART and TB treatment may lead to an increase of adverse effects, drug–drug interactions and immune reconstitution inflammatory syndrome (IRIS).[42]

After HIV testing, quick identification of drug resistance among TB patients should be undertaken, especially in areas where HIV is highly prevalent and drug resistance among the community's TB patient is known to be common or increasing.[18] The process should start by assessing risk factors for MDR TB as previously mentioned, and the use of rapid anti-TB drug susceptibility testing, if available, when TB is first diagnosed. Even if the patient has no risk factors for MDR TB at the start of treatment, one should still consider the possibility of resistance if a patient's clinical course is not progressing favorably within a reasonable time frame.[43]

In general, an individualized approach to treatment, whereby a given patient's drug regimen is tailored to the susceptibility of the cultured strains, is recommended.[18] More often than not, however, results from susceptibility testing for first and second-line anti-TB medications are not available at the beginning of treatment. Consequently, an empiric approach to therapy using a broader range of medications and then tailoring the regimen once results are available is followed, especially in resource limited settings.[28]

Previous TB treatment is a strong determinant of drug resistance, and previously treated patients comprise a significant proportion (13%) of the global TB notifications in 2007.[35] Of all the forms of drug resistance, it is most critical to detect multidrug resistance because it makes regimens with first-line drugs much less effective and resistance can be further amplified. At the global level, 15% of previously treated patients have MDR, which is five times higher than the global average of 3% in new patients.[35]

While awaiting the results of conventional drug susceptibility testing, WHO recommends administering an empiric re-treatment regimen with first-line drugs for TB patients returning after defaulting or relapsing from their first treatment course if country-specific data show low or medium levels of MDR in these patients. The empiric treatment consists of two months of Isoniazid, rifampin, pyrazinamide, ethambutol and streptomycin, followed by isoniazid, rifampin, pyrazinamide and ethambutol for 1 month. The regimen then ends with five months of Isoniazid, rifampin and ethambutol.[35]

If MDR TB is confirmed or suspected, treatment based on DST results or a standard MDR treatment regimen should be instituted as soon as possible in order to achieve earlier sputum conversion to having no growth of *Mycobacterium tuberculosis*. The study by Holtz et al. provides an in-depth and well conducted analysis of factors associated with favorable treatment outcomes among a cohort of MDR TB patients treated in Latvia.[44] This analysis actually demonstrates the strong correlation between sputum culture conversion, including when it occurs, and final treatment outcomes. Reducing the time to sputum culture conversion is an important infection control measure because patients with MDR TB and positive sputum cultures are infectious and may transmit the disease to other persons, family members, and health care

providers. This is especially true in settings with limited resources where infection control capacity is less adequate.

Treatment regimens recommended by the WHO for patients who have failed first-line treatment or belonging to groups with high likelihood of MDR TB should consist of at least four drugs with either certain, or almost certain, effectiveness. In these cases, effectiveness is determined by each country's drug susceptibility patterns at first.[35] As the results to susceptibility testing become available, the regimen can be individualized to increase treatment effectiveness. Table 1, adapted from the WHO guidelines for managing MDR TB, categorizes all the different anti-TB medications in five groups in order to facilitate the approach to the development of a treatment regimen.[18] Each group is comprised of at least four medications and are grouped based on their relative efficacy. As the guidelines indicate, optimal treatment is based on the use of any remaining first line agents (group 1) to which a given patient's *Mycobaterium tuberculosis* isolate remains susceptible, followed by the inclusion of an injectable agent from group 2 and a fluoroquinolone from group 3. Agents with bacteriostatic activity (group 4) and those medications with only in vitro or anecdotal reports of activity (group 5) should be added as needed.[18]

Due to high number of medications needed to treat MDR TB, patients are often exposed to many side effects and toxicities, limiting the actual availability of effective treatment options. Thus, identifying and managing these side effects and toxicities as much as possible is often a critical aspect of the management of MDR TB in order to reduce the incidence of abandonment of therapy and increase cure rates.[18, 45]

The Impact of Treatment on TB Transmission

There are few epidemiologic studies that examine the risk to contacts of patients treated for variable lengths of time before discharge. Of these studies, the only randomized controlled trial was limited by the high endemic rates of TB in South India in the late 1950s, minimizing the difference in exposures between the groups. These original studies from Madras, India, supporting ambulatory care, had shown that compared to treatment in a nosocomial setting, treatment at home did no increase the risk of infection or disease for persons living in the patient's home during treatment or over the following 5 years.[46,47]

However, the most direct evidence of the impact of treatment on reducing TB transmission comes from several animal experiments where large numbers of sentinel guinea pigs breathed the air exhausted from experimental TB wards. The guinea pig (*Cavea porcellus*), a rodent indigenous to the Peruvian Andes, constitutes a well-established animal model to quantify TB transmission. In an experiment conducted by Riley et al. over 60 years ago, all transmission to guinea pigs stopped when only sputum smear-positive patients started on effective therapy for drug-susceptible TB were admitted to the ward. Transmission, however, resumed when sputum smear-positive drug-resistant patients on ineffective treatment were admitted.[48] In Riley et al.'s second experiment, the authors demonstrated the rapid effect of treatment on reducing transmission. In this study, sputum smear-positive patients with drug-susceptible TB just started on treatment were only 2% as infectious as untreated sputum smear-positive patients.[49]

Recently, Escombe et al. repeated those experiments in a similar setting in Peru with HIV co-infected TB patients. This study found that 98% of guinea pig infections were attributable to just nine unsuspected or inadequately treated MDR-TB patients among the 97 pulmonary TB

patients to which the guinea pigs were exposed.[50] Only three patients with drug-susceptible TB, all of whom were not on therapy because of delays or side effects, infected a total of three guinea pigs. Globally, delays in the diagnosis of drug resistance mean that many patients may remain on ineffective therapy and continue to transmit at rates as high as 20 people per year.[8]

The Current Status of TB in Peru

Peru has suffered from tuberculosis for many years. In the 1990's, Peruvian and U.S. archaeologists discovered mummies in southern Peru more than 1,000 years old, with evidence (by PCR) of the presence of pulmonary TB.[51] They found tattoos on body sites frequently associated with tuberculous cervical lymphadenitis. Researchers also theorized about the possibility that shamans or sorcerers were exploring the use of magical rituals to stop the disease.[51] Many centuries later and despite the discovery of effective anti-TB medications, tuberculosis continues to be a serious national public health threat in Peru.

Before the DOTS program was launched in Peru in 1990, only 50% of people diagnosed with TB were able to get treatment. Of those, only half were cured.[52] During the 1980s, inflation in Peru soared uncontrollably and the government was primarily focused in negotiating an end to a guerilla war that had left over 70,000 people dead and much of the country's infrastructure, including many of its health centers, destroyed. Peru suffered from drug shortages, including anti-TB medications, a lack of any record system and overworked and demoralized public health workers. This state of affairs in the country was highlighted in 1991, when a three-year-old Peruvian boy achieved unwanted celebrity as the last case of polio in the entire Western Hemisphere.[53] He caught the virus after his local health center was destroyed by

Shining Path guerillas, preventing the administration of childhood immunization by local health workers.

During the 1990s, Peru's government recognized for the first time in history that TB control was a social, political and economic priority. This newfound appreciation for the gravity of the situation led to the first major increase of the TB budget from US$ 600,000 to US$ 5 million a year.[52] With high-level political commitment, adequate funding for drugs, and dynamic leadership, the new DOTS program in Peru had a head start. Since then, TB diagnosis and treatment are provided free of charge, drug financing is sustainable, and the program has become a model for training managerial staff from other Latin American countries.[54] Drugs, equipment and other supplies are purchased and distributed at the central level. Food packages are provided for low-income families as an incentive to comply with treatment and funding has been provided to establish patient and family support groups.[54] In sparsely populated, remote areas such as the Amazon jungle or the high Andean plateau, treatment delivery is adapted to the needs of the patient to ensure access and completion of treatment.[54]

Over the past two decades, Peru has continued to make considerable progress in the prevention and control of TB. Peru's national TB program has become one of the world's most successful ones, and it has provided some of the first evidence that widespread use of DOTS prevents new cases of TB.[54] By 1997, the entire population was covered by the DOTS program and almost 90% of TB patients were being cured. A year later, an estimated 94% of TB cases were being detected.[54] By 1999, the incidence of TB in Peru had decreased almost by half through the implementation of DOTS, preventing at least 70,000 to 90,000 deaths.[54] In 2004, the World Health Assembly, the decision-making body of the WHO, recommended that each national TB program achieve a case detection rate of 70% and a cure rate of 85% in order to

bring the worldwide TB epidemic under control.[55] By 2007, the Peruvian national TB program had surpassed both WHO control goals with a detection rate of 90% of all smear-positive cases and a cure rate of 92% of all new cases.[56] Of all the patients who were treated successfully, 80% returned as active members of the Peruvian economy and contributed 174 million *nuevos soles* (US$ 64 million) to the Gross Domestic Product.[56] Indeed, the country remains one of only a handful of high-burden countries to have met the WHO targets for TB control.[56] Over the last decade, case notifications for TB have decreased by 32.7%. Currently, the prevalence of TB in Peru stands at 38,000 cases and the incidence of the disease is 125.1 cases per 100,000 population per year. By the end of 2011, the national goal is to reduce the number of case notifications by another 50%.[56] If this trend continues, the incidence of TB in Peru could be halved every 10 years.

Part of the success of TB control in Peru is due to an increase in the number of health centers participating in the national TB program. During the 1990s, the number of health centers increased from 1,000 to 6,000 throughout the country.[52] In addition, as efforts to detect new cases intensified, the number of laboratories capable of carrying out sputum smear tests rose from about 300 to over 1000 during the same time period.[52] These efforts led to an improvement in the diagnostic capabilities of the country as evidenced by the sharp increase in the numbers of cases notified between 1990 and 1993. Since then, the number of new cases has continued to steadily declined.[52]

The continued success of the national TB program has also paralleled an increase in the funding provided by the Peruvian government. For example, from 1991 to 2005, the average yearly budget assigned to medications and laboratory was 3 million dollars. Since 2006, the budget assigned to anti-TB medications and laboratory costs has tripled to an average of US$ 10

million per year. In 2008, the total investment by the government reached a new record of approximately 105 million soles (US$ 40 million).[52] In order to facility a prompt initiation of treatment, the National Institute of Health (INS) has implemented a computerized access system, NET Lab, which allows timely access to a patient's susceptibility testing. Before 2006, patients had to wait approximately 10 months on average to start treatment. Although this delay is currently down to two months, the conditions for delivering effective treatment should continue to improve.[52] This increase in funding has resulted in the prevention of 256,000 new cases, the successful treatment of 64,000 patients and the prevention of 8,000 deaths.[52]

Despite these advances in the control of TB in Peru, the country still has one of the highest TB incidence rates in the Americas and is among the 22 countries accounting for 80% of the new TB cases occurring worldwide each year. Peru accounts for only 3% of the population of the Americas but has approximately 12% of its TB cases.[56] In 2007, the regions with the highest rates of TB were the Lima Metropolitan area (comprised of Lima city and the port of Callao), the southern coastal departments of Ica and Tacna and the Amazonian departments of Madre de Dios, Ucayali and Loreto. Of the 29,393 cases treated for newly diagnosed TB in 2007, these regions accounted for over 80%.[52]

Large cities in developed and developing countries often suffer from higher incidence rates of TB, in sectors of poverty and mayor migration. These figures are always higher than in other geographical areas of the country and can even double or triple the overall incidence. For example, Paris suffers from a TB incidence rate four times higher than the national rate while Rio de Janeiro and Buenos Aires concentrate 60% and 70%, respectively, of all the MDR TB cases in the country.[52] Lima is no exception to this global phenomenon where, as a result of the social determinants in late 1980s and early 1990's, there was a significant migration towards the

capital and other major cities in the coastal region. With over 9 million inhabitants, the Lima Metropolitan area is home to over one third of the country's population. Yet, it is responsible for 58% of all the cases in the country.[52] About 86% of all TB cases in the Lima Metropolitan area are reported in 18 of its 43 districts, which are characterized by a high degree of overcrowding and a rate of disease above the national average.

MDR TB in Peru

In 2008, MDR-TB made up 5.3% of all TB cases and 24% of all re-treatment cases in Peru. This constitutes the highest rate of MDR-TB in the Americas. In addition, more than 200 cases of XDR-TB were reported.[52] Despite these statistics, Peru is the first of the 22 countries with a high TB burden to systematically address the problem of resistance to anti-TB antibiotics. In fact, the drug resistant TB program in Lima was one of the first to show the effectiveness and feasibility of second-line treatment for MDR TB in resource-poor settings, achieving an 83% cure rate among patients who completed therapy (73% overall cure rate).[57] In order to achieve such success, however, the TB program in Lima used an integrated team composed of physicians, nurses, health promoters, and DOT volunteers, under intense training, that focused not only in the treatment of the disease but also the socioeconomic factors contributing to health disparities. A study by Mitnick et al. demonstrated a cure rate of 63% of XDR TB cases in HIV seronegative Lima patients.[58] Although these results represent a breakthrough in MDR and XDR TB care, antibiotic costs were $15,681 per patient, a price beyond the reach of most national programs.[57]

According to Peruvian government figures, from 1997 to 2007, the mortality rate of MDR TB in Peru has dropped significantly from 16.2% to 2.2%, below that of TB (2.65%).[52]

This achievement was partly due to the complete reorganization of the laboratory services dedicated to the diagnosis of TB made possible by an increase in government funding. Prior to 2005, there was only one national reference laboratory in Peru in charge of susceptibility testing for multidrug resistant TB (MDR TB). Susceptibility testing for suspected extreme drug resistant TB (XDR TB) was performed in laboratories in the United States.[52] There was no access to rapid susceptibility testing. From 2001 to 2004, approximately 13,000 cases of smear-positive TB cases and 650 MDR TB cases were not diagnosed.[52]

Over the past five years, however, the number of conventional susceptibility tests has increased by 167% to a record number of 10,275 tests. This increase in susceptibility testing is mainly due to an increase in the number of laboratories performing this test from one to six. Four of these laboratories are located in the Lima Metropolitan area. The other two are located in Lambayeque (northern Peru) and Arequipa (southern Peru). In addition, the INS is able to perform susceptibility testing for XDR TB and rapid susceptibility testing, using the Griess method, has begun in three districts of health (DISAS) in Lima. In 2005, the number of reported MDR TB cases in Peru reached its peak at 2,436. The following year, the number of cases dropped to 1,825 cases and then to 1,785 cases in 2007. This number of MDR TB cases nationwide has decreased despite an increase of the capacity for detecting multidrug resistance, as explained above.

The Lima Metropolitan area is responsible for approximately 82% of MDR TB cases and 93% of XDR TB cases in Peru.[52] In particular, thirteen districts (San Juan Lurigancho, San Martin de Porres, La Victoria, Ate, Lima Cercado, San Juan de Miraflores, Comas, El Agustino, Santa Anita, Villa Maria del Triunfo, Villa El Salvador, Independencia and Los Olivos) report a higher rate of MDR TB than the rest of the Lima Metropolitan area. Also, of the 186 confirmed

XDR TB cases in the entire country,158 (85%) of them are concentrated in the districts of La Victoria, Lima Cercado, San Martin de Porres, San Juan de Lurigancho, Ate, Santa Anita and El Agustino.[52]

Correlation of Cough Frequency with Treatment Efficacy in Pulmonary TB patients in Lima, Peru: A prospective study

The remainder of this thesis presents an original research study conducted by the author in a low-income, urban setting in Lima, Peru.

Introduction

Current World Health Organization guidelines recommend sputum-smear examination at the end of the second month of treatment in patients with recently diagnosed pulmonary TB.[34] If positive, a repeat examination is recommended at the end of the third month.[34] If the specimen obtained at the end of month 3 is still smear-positive, sputum culture and drug susceptibility testing are then recommended.[34] Treatment failure is defined as smear or culture positivity at the fifth month or later.[34] Many countries, including Peru, follow these recommendations and repeat sputum microscopy test on a monthly basis during the course of anti-TB therapy to assess the response to treatment.[60]

In 2000, Becerra et al. reported that treatment failure while on DOTS in Lima, Peru was strongly correlated with active MDR-TB. Of 173 cases of treatment failure identified, 150 (86.7%) had active, pulmonary MDR-TB.[61] Furthermore, the authors concluded that short-course chemotherapy for these patients would only serve to amplify ominous existing drug

resistance patterns. [61] During the same year, Chavez Pachas et al. performed a case control study in order to identify risk factors for treatment failure while on DOTS.[62] In this case, all 38 cases were smear-positive at 2 months of therapy, which was strongly associated with failure (OR 11.7; CI 2.4-57.5).[62] Of these patients nearly 75% had MDR-TB.[62]

Although Chavez Pachas et al. reported that positive sputum microscopy at second month of treatment is associated with subsequent treatment failure, this indicator of treatment outcome has proven insensitive at the population level. A recent systematic review and meta-analysis by Horne et al. revealed that 2 month-smear had low sensitivity (57%, CI 41-73%, seven studies) and higher, although modest, specificity (81%, CI72-87%; seven studies) when predicting treatment failure.[63] In other words, a positive sputum result during treatment did not imply that an individual will experience a poor outcome from TB. However, a negative sputum result did imply that an individual will be unlikely to experience treatment failure.[63] Thus, the widespread use of this test and its uncertain utility suggest a critical need to appraise tests and strategies suitable for resource-limited settings used to identify patients at risk for poor outcome before the three month marker recommended by the WHO.

The Case for Cough Frequency

Coughing has long been identified as an important determinant of infectiousness in TB. Cough-inducing procedures, for example, have been associated with extensive transmission of the disease. In 1982, Catazaro documented a high rate of tuberculin skin test conversion in hospital staff exposed to a smear-negative, culture-positive patient in a respiratory intensive care unit.[64] During his stay at the hospital, this patient required bronchoscopy, intubation and assisted ventilation. Of the hospital staff members who were exposed to the index case, 14 of 45 (31%) converted their tuberculin skin test.[64] Ten of 13 (77%) hospital staff members present at the time

of bronchoscopy converted, compared with 4 of 32 (12.5%) who were not present at bronchoscopy (Fischer's exact test p = 0.0006).[64]

In 1990, Malasky et al. examined the risk of conversion to a positive tuberculin skin test associated with certain medical procedures involving the lower respiratory tract.[65] The study looked at Pulmonary fellows from 14 different training programs who routinely performed procedures identified as important sources of respiratory aerosols, which have the potential for the transmission of TB. These procedures included fiberoptic bronchoscopies, endotracheal intubations and mechanical ventilation. Over a period of three years, seven of 62 (11%) Pulmonary fellows at risk converted their tuberculin skin test as opposed to one of 42 (2.4%) Infectious Disease fellows who served as controls.[65]

A case control study by Calder et al exploring the association between the production of respiratory aerosols and risk of infection took place at a Florida health clinic. Between January 1 and June 30, 1988, 30 of 76 (39.5%) staff members tested at this clinic had positive tuberculin skin test reactions.[66] The staff members whose skin test converted were more likely than those whose skin test did not convert to have been present while patients were being treated with aerosolized pentamidine (odds ratio = 15.0; 95%; CI = 1.4 - 730.0) and to have worked on the first floor of the clinic (odds ratio = 9.3; 95% CI = 1.1 - 420).[61] The clinic building was poorly ventilated, and aerosolized pentamidine treatments were given in a room from which the air tended to flow into the hallway.[66]

In 1969, Loudon et al. conducted a study in Dallas, TX with 63 newly diagnosed pulmonary tuberculosis patients. This was the first and only paper that looked specifically at cough frequency as an index of infectivity and to measure changes in cough frequency in TB patients during treatment with anti-TB antibiotics.[67] Although cough frequency at the time of

admission did not prove to be a satisfactory index of infectivity, the decline in cough frequency during treatment with anti-TB drugs was rapid.[67] Most patients reduced their cough count to half the initial value within two weeks.[67] Thus, patient's cough frequency might be a helpful and inexpensive marker to predict TB treatment outcome.

Research Setting

Peru is a South American country with a population of approximately 29.2 million people.[68] It is a relatively poor country with a gross domestic product per capita of $8,120.[68] The life expectancy of the population ranges from 74 years in males to 77 years in females, while the adult mortality rate (per 1000 adults 15-59 years) is 110 for both sexes.[68] The country has a low prevalence of HIV of 4 (per 1000 adults 15-59 years) and, as stated before, a TB prevalence of 129 per 100,000.[68]

Approximately, 72% of the population of Peru lives in urban areas.[68] Lima, the capital city, is home to approximately 8 million Peruvians or one third of the country's population.[69] The incredible growth of Lima over the past five decades is the result of many factors that have pushed people out of their rural communities: the loss or lack of adequate farmland, natural disasters such as earthquakes and landslides, lack of employment options, and a host of personal reasons. In addition, since the outbreak of terrorist activities by the Shining Path movement in 1980 and subsequent military reactions, over a million people were displaced from towns and villages in the Ayacucho and Huancavelica highlands, most of them gravitating towards Lima.[70]

The process of urban growth in Lima has produced an urban configuration that conforms to no central plan. Without access to adequate housing of any type, and without funds or available loans, migrants set about developing their own solutions by establishing organizations

of their own. They planned a takeover of unoccupied land at the fringes of the city and, with the suddenness and effectiveness of a military operation, invaded the property. Once on the land, the migrants laid out plots with precision and raised temporary housing in a matter of hours. Called *pueblos jovenes* ("young villages"), the shantytowns quickly developed both an infrastructural and a sociopolitical permanence. By early 1990, approximately half of Lima's population lived in these conditions.[70] The disorganized expansion of Lima led to a large sector of this population without basic services, including clean water, electricity, proper sanitation and access to health care. These conditions resulted in hyperendemic clusters of TB where the annual incidence of the disease reached four times the city's average incidence.[71]

The Hospital Nacional Dos de Mayo, centrally located in Lima's historic center (Downtown district), is a 850-bed teaching hospital that is run by the Peruvian Ministry of Health (MINSA healthcare system). As with other MINSA institutions, this hospital provides services to the Peruvian poor and uninsured since its establishment in 1875. The Infectious and Tropical Diseases Service at the Hospital Nacional Dos de Mayo is the major referral center for HIV and TB patients in Lima and is the only hospital in Peru with two 4-bed, negative-pressure rooms available for TB patients.[72]

Aim

The specific aim of the study is to assess the change of pulmonary TB patients' cough frequency over time depending on TB treatment outcome. The broader aim of this study is to develop a helpful and cheap marker to predict TB treatment outcome. Ultimately, this information will enable physicians to make better decisions with regard to transmission risk of pulmonary TB patients undergoing treatment in resource-limited settings.

Hypotheses

1. In patients undergoing treatment for pulmonary tuberculosis, cough frequency diminution will occur significantly earlier in patients with susceptible TB strains versus those with resistant strains.

2. Cough frequency diminution will be an independent predictor of TB susceptibility.

Methods

Description of study population

The study population was derived from patients under the care of the Infectious and Tropical Diseases Service of the Hospital Nacional Dos de Mayo and their referral centers, on both inpatient and outpatient services. Patients with TB suspected either clinically or radiologically were recruited for the study. The study team physician, student investigator, or nurse communicated daily with the medical staff and reviewed the patient logs to identify all possible study patients who fit the inclusion criteria. All possible study patients were recruited during the study period as space and equipment permitted.

Patient identifiers including name, address and other contact information such as email or phone numbers were collected by the nurses in order to facilitate the coordination of follow-up visits. This information, however, was never available to the study team and was not used in the actual study.

Data collection

The study team interviewed those prospective subjects willing to enroll in the study. Once these patients agreed to enter the study, the study team doctor or student investigator completed a form including clinical data and demographic information. During the interview

the purpose of the study as well as its risks and benefits was explained to the patient. The study team physician or student investigator gave the informed consent form to the patient so that the patient could read it and sign it if they agreed to enter the study. If the patient (or his or her representative) chose to sign the informed consent, the study team proceeded to complete the patient's study record with information provided by the patient. Demographic information as well as risk factors or current treatment for TB was collected (known contacts, previous diagnosis of TB, INH prophylaxis, etc.). Patient's HIV status was also reviewed, and information on CD4 count and viral load, if available, was collected. Subjects underwent screening for active TB by sputum smear and MODS culture. Additionally, female subjects underwent pregnancy testing by qualitative urine β-HCG.

Inclusion criteria:

- 18 years of age or older.

- Active pulmonary tuberculosis diagnosed by positive sputum smear and/or sputum culture.

Exclusion criteria:

- Pregnant at time of subject screening.

- Unwilling or unable to provide informed consent.

- Presence of extra-pulmonary tuberculosis

The physician, student investigator and/or nurses in charge of the study explained this study design to all potential participants. Any points that were not completely understood were explained in detail. In the case of illiterate patients, the consent form was read out loud in its entirety. Consent was documented by the signature of the patient and by the signature of a

witness if present. If illiterate, consent was documented by the patient's fingerprint and by the signature of a witness. Papers used for data collection were locked in a secured locker.

The study is a prospective cohort study. Patients who fit the inclusion criteria as described above were approached and asked whether they would like to learn about the study. If patients chose to provide their informed consent, they underwent a chest x-ray (CXR), a review of the history and a physical exam. Once these exams were completed, patients were hospitalized in the Hospital Nacional Dos de Mayo for a period of between 9 and 16 days (days -1 to 14, inclusive). Day 0 is defined as the day that a new anti-TB regimen was started, whether primary treatment or retreatment in cases of MDR-TB. However, treatment was not delayed if the patient was too ill, as assessed by ward staff.

The main outcome of the study was cough frequency, recorded in the questionnaire as coughs per day on days -1 (baseline), 0 (start of treatment), 3, 7, 14, 21, 30, and 60 (each +/- 2 days). During the study the following data were collected: patient weight, temperature, self-reported cough frequency and self-reported medication compliance. At day 14, patients were discharged if appropriate for their clinical condition as determined by medical ward staff, and subsequently followed as outpatients. If the patients' health permitted, and they so desired, they were sometimes discharged as early as day 7 and subsequently followed as outpatients. When patients returned for outpatient appointments, the same information was obtained. The study terminated at day 60.

The Peruvian national guidelines for the control and prevention of TB defines treatment failure based on positive sputum microscopy results after $\geq$ 5 months of treatment or reverting to positive after two consecutive negative monthly results.[60] Since the study only followed patients

for a maximum of 60 days, treatment failure in this study was defined as the detection of resistance by sputum culture or death which was defined as those patients who died during tuberculosis therapy follow-up. For the data analysis, the patients were dichotomized by those having a good outcome and those having an adverse or poor outcome (death or treatment failure, as previously defined).

Other variables of interest included in the analysis were: age measured in years, patient sex (male or female), treatment scheme (new vs. recurrent), change of sputum during treatment assessed monthly, and HIV status.

Risks to Human Subjects

The study design was reviewed and approved by the Institutional Review boards of Asociacion Benéfica PRISMA (Lima, Peru), Hospital Nacional Dos de Mayo (Lima, Peru) and the University of Connecticut Health Center (Farmington, CT).Patients diagnosed with active TB were enrolled in the national TB control program as per the usual protocol. During the course of the study all clinical decisions and patient care were the responsibility of the medical ward staff. The study team did not interfere with ward treatment plans and provided all results of patient discussion, labs, and microbiology to the medical ward staff in a timely and appropriate manner. In addition relevant results were provided to subjects in a timely manner.

Statistical Analysis

Sample size calculations were performed using a T test, and setting a two-sided alpha to .05 and the power to 80% to compare subjects no longer coughing to subjects still coughing. Using a 20% difference in results such that, for example, 80% of those no longer coughing have

negative microbiology compared to 60% of those still coughing, a sample size of 164 is calculated. Assuming a drop-out rate of 30%, we need 234 patients to find the above difference in effect size. Therefore, the final sample size is estimated to be about 250 patients.

Data were entered first into Excel and then analyzed using STATA version 11.0 for Windows. First a brief description of demographic and clinical characteristics was tabulated. Second, cough frequency was calculated for each group according to our outcome of interest and day of follow-up. Finally, a longitudinal analysis was carried out to evaluate cough frequency over time. A marginal model was fitted using Generalized Estimating Equations to model average cough frequency trends for patients with good and poor outcomes. The crude model was determined as follows:

$$Y_{ij} = B_0 + B_1.\text{Outcome} + B_2.T_{ij} + B_3.T_{ij}.\text{Outcome}$$

Where Y_{ij} is the mean cough frequency (number of coughs per 24 hour period) in patient "i" at time "j"; B_0 is the intercept, i.e. cough frequency in number of coughs per 24 hour period among those with good outcome at baseline; B_1 is the difference in cough frequency in patients with poor outcome compared to those with good outcome at baseline; and B_2 quantifies the change in cough frequency between baseline and one selected follow up visit for participants with good outcome. The sum of B_2 and B_3 (interaction term) represents the change in cough frequency between baseline and one selected follow up for participants with poor outcome. In this model, the time variable, T_{ij}, was included as categorical because weight over time did not show linearity in the poor outcome group.

Quasi-likelihood under the independence model information criterion (QIC), an extension of the Akaike's information criterion (AIC), was applied to find the best working correlation

structure applicable for the proposed model. The Akaike's information criterion is a measure of the relative goodness of fit of a statistical model. It is grounded in the concept of information entropy, in effect offering a relative measure of the information lost when a given model is used to describe reality. It can be said to describe the tradeoff between bias and variance in model construction, or loosely speaking between accuracy and complexity of the model. Given a data set, several candidate models may be ranked according to their AIC values. From the AIC values one may also infer that, for example, the top two models are roughly in a tie and the rest are far worse. Thus, AIC provides a means for comparison among models, a tool for model selection.

Additionally, the model was adjusted for potential confounders affecting both outcome and cough frequency. Potential confounders included were age, sex, HIV status, and sputum microscopy result change during treatment. Wald test was used to report p-values, whereas robust standard errors were used to calculate 95% confidence intervals for each coefficient in the model.

Results

A total of 250 patients started anti-TB treatment during the period of study and were eligible for this study. However, 45 patients did not have TB, 6 had started treatment before the start of the study, 4 had other co-morbidities that did not allow them to participate, 3 abandoned therapy, 2 moved away before starting treatment, 2 developed altered mental status due to HIV encephalitis and 2 had extra-pulmonary TB. Therefore, only 141 (56.4%) patients were included in the analysis; 71.6% of them were males and the mean age was 30.5 years (SD: 14.1; range: 18-78).

Of the total number of patients, 13 (9.2%) had a poor outcome at the end of the study period (11 had failed and 2 died). When compared to the patient group that had a good outcome, the poor outcome group was older, with a mean age of 38.4 versus a mean age of 30.8 years ($p<0.001$). In addition, this group had an average household income of 307 *nuevos soles* ($ 110.00) per month, which is significantly lower than the good outcome group's average household income of 652 *nuevos soles* ($ 233.00) ($p < 0.04$). In addition, all 13 patients in the bad outcomes group came from only three Lima districts: Carabayllo (6), Cercado de Lima (4) and San Juan de Miraflores (3). On the other hand, 13 different districts were represented in the good outcome group. A brief description of patients' characteristics in relation to outcome status is shown in Table 2.

Table 3 shows a detailed description of cough frequency variation during treatment follow-up without accounting for intra-subject correlation. Each cough count represents the mean number of coughs per day that the patients reported during treatment. There was no significant difference between cough frequency of the outcome groups at baseline ($p = 0.12$). However, on average, during the first two weeks cough frequency did not decrease in those who developed an adverse outcome whereas it declined among those who had a good outcome.

Results of crude and adjusted marginal models are shown in Table 4. Of interest, the adjusted coefficient for adverse outcome was not significant ($p = 0.17$), indicating that the difference in cough frequency (approximately 10 coughs per 24 hr period) among patients with poor and good outcome at baseline was not statistically different. However, the interaction terms together were significant (Wald test for interaction, $p = 0.002$) indicating that changes of cough frequency over time among patients with poor outcome differed from those with good outcome (Figure 1). Based on the results of the adjusted model (Table 4), at the end of the first two weeks,

on average, the cough frequency in patients with good outcome decreased by approximately half (-62.82 cough per 24 hour period according to the adjusted model) compared to baseline; at the end of the first month, cough frequency decreased by approximately 81 coughs per 24 hour period in this group of patients. On the other hand, the cough frequency in patients with poor outcome remained constant at approximately 110 coughs per 24 hour period after the first two weeks of therapy compared to the baseline, while decreasing by approximately 15 coughs per 24 hour period after the first month of treatment.

Discussion

This study shows that, after adjusting for potential confounders, the curve of cough frequency over time among patients who developed adverse outcomes is completely different from patients classified in the good outcome group at the end of the study (Figure 1). The association continues being statistically significant after having included the monthly sputum microscopy result as confounder, pointing out that change in cough frequency over time is an independent predictor of treatment outcome.

These findings might have an important impact on public health, especially in resource-limited settings. Cough frequency assessment might be an easy, cheap, and useful way to predict anti-TB treatment outcome among pulmonary TB patients receiving therapy. Constant or increased cough frequency after the end of the first two weeks of therapy might be an important indicator that further attention is needed to help avoid deaths or failures. Remarkably, most of the divergence of cough frequency over time occurred during the first two weeks of anti-TB therapy. After that, cough frequency among poor outcome patients shows parallel trends compared to good outcome ones with, however, a slightly lower rate.

These findings suggest that we can apply strategies as soon as the end of the first two weeks to avoid deaths and failures, including MDR testing and closer monitoring. A total of 10 out of 13 patients who failed treatment in this study were diagnosed as MDR-TB cases after failing; two had TB strains resistant to isoniazid and one had a TB strain resistant to rifampin (data not shown).

The TB burden follows a strong socio-economic gradient between countries, within countries, and within communities, and the poorest have the highest risk. In India, a community survey found that the prevalence of TB was higher amongst the landless, those living below the poverty line and in *katcha* houses (houses constructed from mud, stone and wooden beams), suggesting that TB disproportionately affects those with a low standard of living index (SLI).[73] In this study, all 13 cases who failed came from districts located in the outskirts of Lima. These areas of the city have suffered from patterns of social and economic development that include rapid urbanization, inequitable economic growth and presence of large pockets of social deprivation, all of which are associated with TB. Studies assessing the burden of TB in specific vulnerable populations such as prisoners, the homeless, and certain ethnic minorities also show that there is a strong association between social deprivation and TB risk.[74,75] However, the causal pathways linking poverty and low socio-economic status to increased risk of TB are not fully understood.

Strengths of this study include the use of longitudinal, prospective data to assess cough frequency change among pulmonary TB patients commencing treatment. Another important strength is the use of longitudinal analysis with the best working correlation structure taking into account several potential confounders including changes on sputum microscopy results during follow-up, one of the best-known predictors of TB treatment outcome.

However, this study also has some limitations. Although all possible patients who fit the inclusion criteria were included in the study, only a small number of failures occurred during the period of the study. As a result of the low number of cases in the study, potential treatment outcomes were joined during analysis which might lead to misclassification. In addition, the small number of cases makes it difficult to generalize the result to other areas of the world, where the rate of MDR TB might be lower. Furthermore, the group of patients who participated in this study had a combined HIV prevalence of approximately 32%. This rate is considerably higher than the national Peruvian prevalence of approximately 1.5%.[52] As stated before, most of the patients who entered the study came from the Hospital Nacional Dos de Mayo, which acts as a referral center for the city of Lima for complicated TB cases. Consequently, the results of this study might be difficult to generalize to areas of Peru and the world where the prevalence of TB and HIV co-infection is not as high. Nevertheless, the findings agreed with the previous report by Loudon showing the association between cough frequency reduction and anti-TB treatment in Texas.[67]

Due to the small sample number, other co-morbidities which can present or include cough in their symptomatology could not be excluded. These can include chronic diseases that can affect the lungs such as cancer or emphysema. However, patients can also suffer from acute diseases such as viral upper respiratory infections, allergies or acid reflux.

Finally, the determination of cough frequency was done through the administration of a questionnaire. As with all studies based on this data-gathering method, it was subject to recall bias by the patients. Although the questionnaire asked the patients to recall the last 24 hours before every visit, it is possible that those patients who were sicker or had a higher rate of coughing remembered more accurately than those who had a lower or decreasing cough

frequency. Another important bias associated with the administration of the questionnaire is the response bias. Before participating in the study, each patient was informed about the goals of the study during the informed consent sessions. Consequently, having this information, some of the respondents might have answered questions regarding cough frequency in a way they thought might please the administrators of the questionnaire.

Conclusions and Recommendations

In summary, the findings of this study reveal that trends and change of cough frequency during anti-TB therapy can predict treatment outcome. Thus, cough frequency during the first two weeks of therapy should be used as part of the routine clinical evaluation. Patients with unchanged or increased cough frequencies after two weeks of treatment should be more closely followed as they are at risk of adverse outcomes. Follow-up monitoring might include MDR diagnosis testing, closer monitoring or determination of HIV infection status or treatment of opportunistic infections.

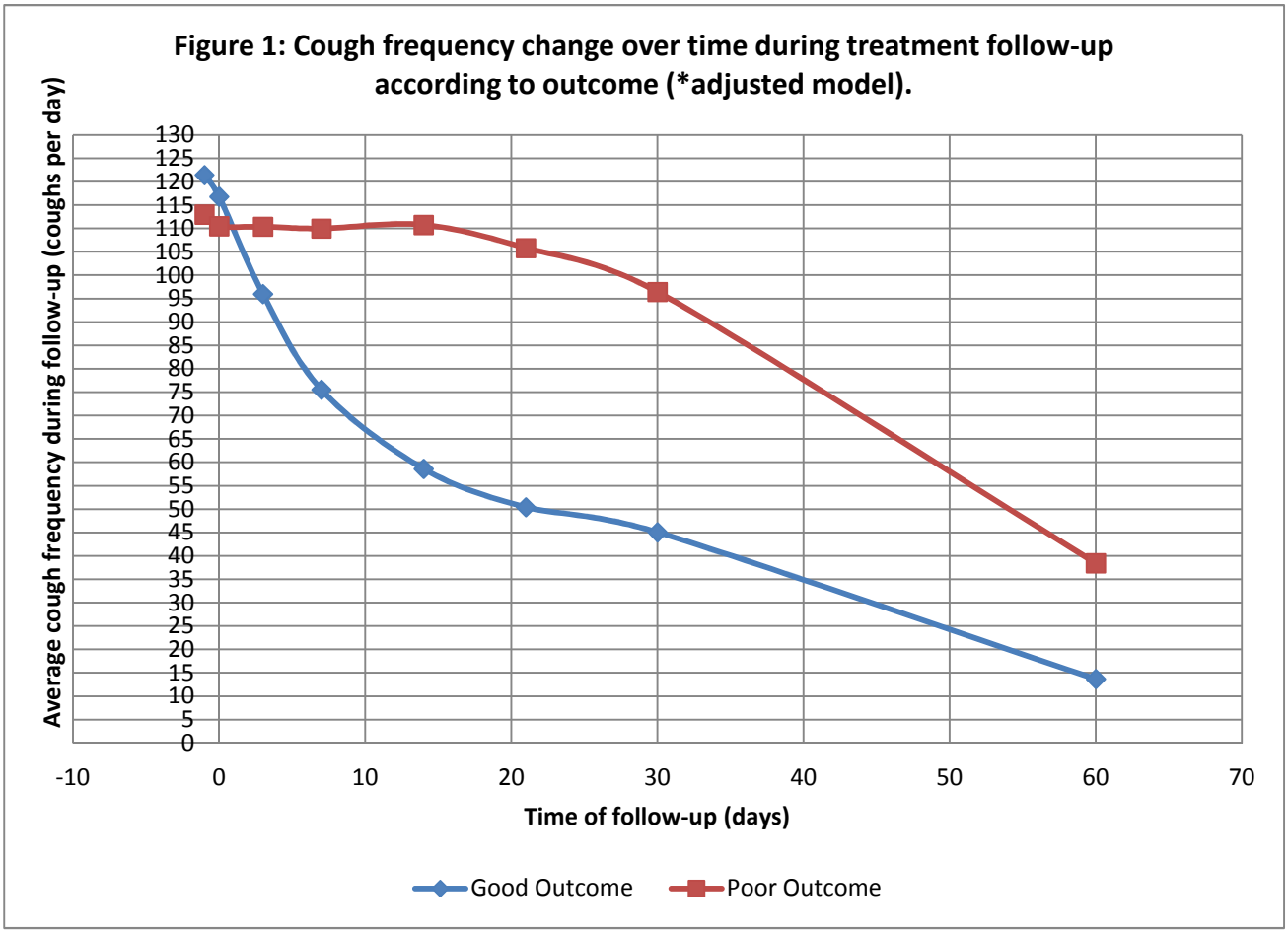
Although it would be ideal to use an ambulatory cough meter as an objective method to assess the cough frequency of pulmonary TB patients, this is currently not yet practical. Once this ideal situation arises, further studies are needed to find appropriate cough frequency cutoffs including sensitivity and specificity analysis and assess the combination of sputum microscopy results with cough frequency over time to predict TB treatment outcomes.

Tables and Figures

Table 1: Group of Drugs to Treat MDR TB*[18]		
Group 1	Available first-line agents: pyrazinamide, ethambutol	The most potent and best tolerated. They should be used if there is good laboratory evidence and clinical history to suggest that a drug from this group is effective.
Group 2	At least one injectable agent: kanamycin, amikacin, capreomycin, streptomycin	All patients should receive a Group 2 injectable agent if susceptibility is documented or suspected. Kanamycin or amikacin is generally the first choice, given the high rates of streptomycin resistance in this population.
Group 3	At least one fluoroquinolone: levofloxacin, moxifloxacin, ofloxacin	A fluoroquinolone should be added based on DST* and treatment history. When resistance to ofloxacin or XDR TB* is suspected, a higher-generation fluoroquinolone should be used.
Group 4	One or more second-line oral bacteriostatic agents: para-amino-salicylic acid cycloserine, terizadone, ethionamide or protionamide	Group 4 drugs are added until the patient is receiving at least four drugs likely to be effective. The best choice is based on treatment history, adverse-effect profile, and cost. DST* is not standardized for drugs in this group.
Group 5	Drugs of unclear role in MDR TB*: clofazimine, linezolid amoxicillin/clavulanate, thiacetazone, imipenem/cilastatin, high-dose isoniazid, clarithromycin	The efficacy of Group 5 drugs in multidrug regimens is unclear and should only be considered if there are not four drugs likely to be effective from Groups 1–4. Consultation with MDR TB* expert should be considered. If drugs are needed from Group 5, it is recommended to add at least two. DST* is not standardized for the drugs in this group.
*DST drug-sensitivity testing, MDR TB multidrug-resistant tuberculosis, XDR TB extensively drug-resistant		

Table 2: Characteristics of enrolled patients at baseline according to outcome status			
Variable	**Good Outcome (n=128)**	**Poor Outcome (n=13)**	**p-value**
Sex			
Female	36 (28.1%)	4 (30.8%)	0.82
Male	92 (71.8%)	9 (69.2%)	
Age (years)			
Mean (SD)	30.8 (13.2)	38.4 (19.4)	<0.001
Sputum result			
Negative	37 (28.9%)	3 (23.1%)	0.64
1+	49 (38.3%)	5 (38.5%)	
2+	23 (18.0%)	3 (23.1%)	
3+	19 (14.8%)	2 (15.4%)	
HIV infection			
Negative	86 (67.2%)	10 (76.9%)	0.002
Positive	42 (32.8%)	3 (23.1%)	
Monthly household income (nuevos soles)			
Mean (SD)	652 (140)	307 (73.6)	0.04
Residence by district			
District (N)	Lima Cercado (32) San Juan de Lurigancho (25) Comas (14) La Victoria (11) El Agustino (10) Ate (10) Others (26)	Carabayllo (6) Lima Cercado (4) San Juan de Miraflores (3)	

Table 3: Cough frequency change over time during follow-up according to outcome status.				
Cough frequency (coughs per hour)	**Treatment outcome**			
	Good outcome		**Poor outcome**	
	N	*Mean (SD)*	*N*	*Mean (SD)*
Day -1 (Baseline)	128	129.9 (9.2)	13	119.3 (8.7)
Day 0 (Start of Treatment)	128	125.4 (9.3)	13	118.0 (8,8)
Day 3	127	104.0 (9.2)	13	120.5 (8.5)
Day 7	120	80.3 (9.7)	12	119.3 (8.9)
Day 14	119	65.9 (9.8)	10	121.8 (9.0)
Day 21	118	60.2 (8.4)	10	115.1 (9.3)
Day 30	109	54.3 (8.9)	7	103.6 (9.2)
Day 60	64	20.7 (9.9)	3	46.0 (5.9)



*Adjusted by age, gender, HIV status and sputum microscopy

Table 4: Crude and adjusted marginal models assessing cough frequency change over time according to outcome status

	Crude model			**Adjusted model***		
	B	**95% CI**	**p-value**	**B**	**95% CI**	**p-value**
Intercept	129.90	122.7;137.1	<0.01	121.43	114.45;128.41	<0.01
Poor outcome	-10.60	-18.40;-2.81	0.145	-8.45	-15.55;-1.35	0.193
Day 0 (Start of Treatment)	-4.45	-4.67;-4.23	<0.01	-4.63	-6.41;-2.85	<0.01
Day 3	-24.72	-27.01;-22.43	<0.01	-25.45	-29.45;-21.45	<0.01
Day 7	-48.95	-50.05;-47.86	<0.01	-45.89	-49.57;-42.21	<0.01
Day 14	-63.70	-68.93;-58.47	<0.01	-62.82	-68.90;-56.74	<0.01
Day 21	-69.54	-74.73;-64.35	<0.01	-71.02	-74.78;-67.26	<0.01
Day 30	- 75.59	-79.52;-71.66	<0.01	-76.36	-81.05;-71.67	<0.01
Day 60	-109.32	-116.52;-102.12	<0.01	-107.74	-114.45;-101.03	<0.01
Poor Outcome* Day 0 (Start of Treatment)	-11.72	-15.5;-7.99	<0.01	-10.94	-14.84;-7.04	<0.01
Poor Outcome* Day 3	-10.55	-16.65;-4.45	0.01	-11.06	-16.81;-5.31	0.02
Poor Outcome* Day 7	-11.86	-15.76;-7.96	0.02	-11.45	-16.07;-6.83	0.03
Poor Outcome* Day 14	-10.54	-15.56;-5.52	0.017	-10.67	-17.73;-3.61	0.02

Poor Outcome* Day 21	-14.89	-17.87;-11.91	0.012	-15.63	-18.57;-12.69	0.018
Poor Outcome* Day 30	-23.56	-29.80;-17.32	0.02	-25.00	-33.36;-16.64	0.017
Poor Outcome* Day 60	-84.78	-91.77;-77.80	0.025	-82.99	-89.27;-76.71	0.33
*Adjusted by age, gender, HIV status and sputum microcopy						

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