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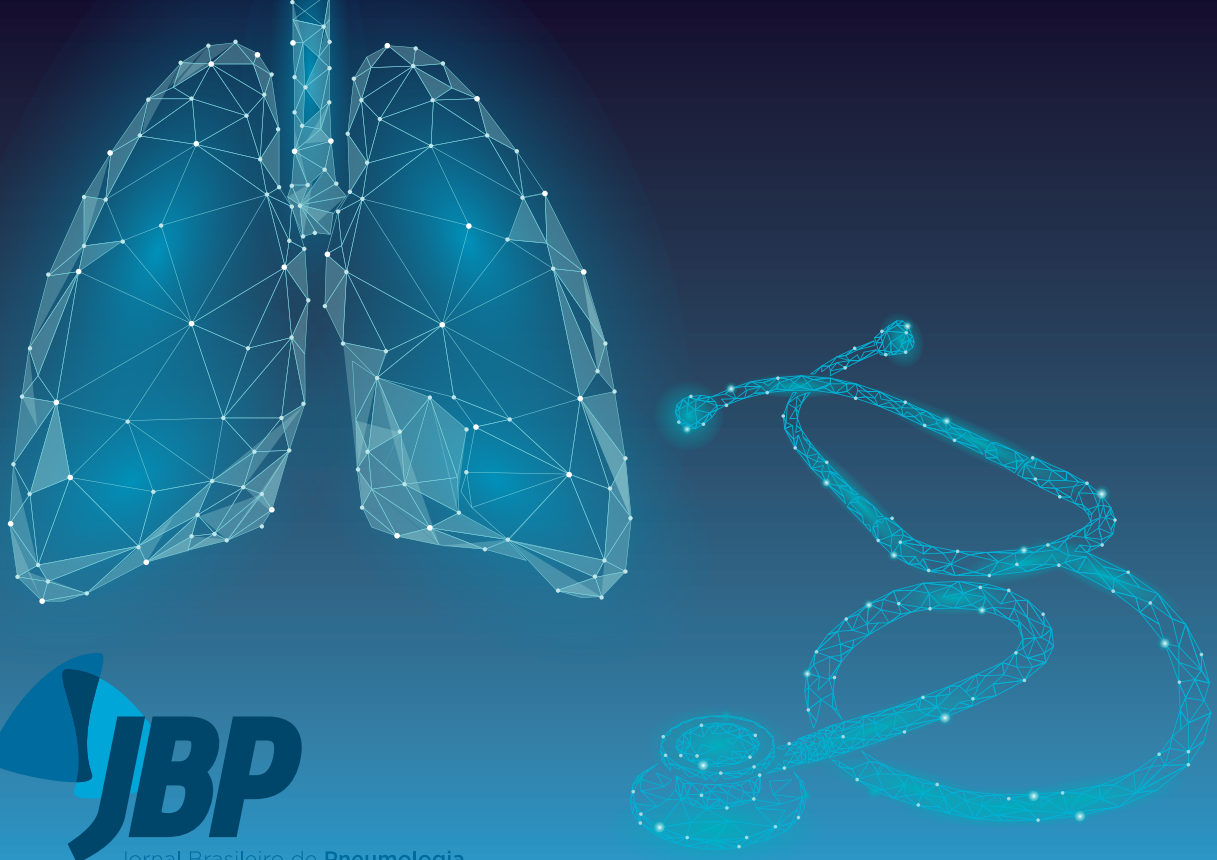
HIGHLIGHT

Tuberculosis mortality in Brazil

Microbiology in pulmonary nontuberculous mycobacterial infections

Latent tuberculosis infection and rheumatic diseases



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Tuberculosis series 2019

Denise Rossato Silva[1,a], Giovanni Battista Migliori[2,b], Fernanda Carvalho de Queiroz Mello[3,c]

The World Health Organization (WHO) End TB Strategy aims to end the global tuberculosis epidemic by 2030. Targets include a 90% reduction in tuberculosis mortality and an 80% reduction in tuberculosis incidence. Despite remarkable progress, with advances in disease detection and improvement in treatment success rates, tuberculosis is still common in several countries. Although the rates of tuberculosis incidence and tuberculosis-related mortality are declining worldwide, the disease continues to be an important public health issue.[1] In Latin America, the incidence rate has fallen by 1.7% per year since 2000, considerably less than the 5.3% annual decline needed in order to achieve the targets proposed in the WHO End TB Strategy.[2,3] In celebration of World TB Day, on March 24th, this issue of the JBP features several articles focusing on tuberculosis, to offer an overview of the various aspects of tuberculosis control.

To achieve its proposed targets, the WHO End TB Strategy has three pillars. One of the pillars (Pillar 2) is "bold policies and supportive systems", which includes regulatory frameworks for case notification.[1] In this issue of the JBP, an ecological time-series study[4] conducted in the city of Juazeiro, in the Brazilian state of Bahia, reported the behavior of the epidemiological indicators of tuberculosis. The results show the persistence of the disease burden in the municipality, identifying the local problems to be addressed and underscoring the importance of constant monitoring of epidemiological indicators.

Early diagnosis of tuberculosis is one of the components of Pillar 1 of the WHO End TB Strategy ("integrated, patient-centered care and prevention"). In our tuberculosis series, the diagnosis of tuberculosis is addressed in four original articles.[5-8] Since its introduction in 2010, the molecular test for *Mycobacterium tuberculosis* and its resistance to rifampin (Xpert MTB/RIF assay) is increasingly used as the initial diagnostic test for tuberculosis in many countries.[9,10] Two of the articles in our series addressed the use of the Xpert MTB/RIF assay.[5,6] In the first,[5] a retrospective study conducted at a tertiary referral center, the authors showed that the Xpert MTB/RIF assay is a highly accurate method of detecting tuberculosis and rifampin resistance in sputum, BAL fluid, and tracheal aspirate samples. In the second article,[6] the sensitivity and specificity of the Xpert MTB/RIF assay were evaluated in a population of indigenous Brazilians. That is an extremely important study, because it is the first to assess the performance of the test in such a population. Both articles emphasize that is essential to determine the effect of the Xpert MTB/RIF assay on the diagnosis of tuberculosis under programmatic conditions in Brazil.[11]

Improving the coverage and quality of diagnosis for individuals infected with drug-resistant tuberculosis is also relevant. In a cohort study conducted at a referral center for tuberculosis in the state of São Paulo, Brazil, between 2006 and 2010,[7] the authors found that early detection of infection with a drug-resistant strain of *M. tuberculosis* was associated with higher cure rates in patients without comorbidities and in patients with a higher body weight at the beginning of treatment (in comparison with the cure rates observed for those without comorbidities and for those with a lower body weight at the beginning of treatment). In another study conducted in the state of São Paulo,[8] the authors evaluated the diagnosis of multidrug-resistant tuberculosis (MDR-TB) using the GenoType MTBDR*plus* assay, version 2.0, which detects concomitant resistance to rifampin and isoniazid. The GenoType MTBDR*plus* assay has many advantages over phenotypic drug susceptibility testing, including excellent accuracy, reduced time to diagnosis, and fewer false results.

Another component of Pillar 1 of the WHO End TB Strategy is tuberculosis treatment. It is well-know that subtherapeutic concentrations of first-line antituberculosis drugs may contribute to treatment failure, relapse, acquired resistance, and death.[12] In a letter to the editor included in our tuberculosis series,[13] the investigators described the serum levels of pyrazinamide, as measured by HPLC, in 46 patients. They demonstrated that, at least in their sample, the therapeutic regimen in use in Brazil provides adequate exposure to pyrazinamide.

Yet another component of Pillar 1 of the WHO End TB Strategy is the preventive treatment of persons at high risk. Within that context, this issue of the JBP features an article focusing on the aspects related to latent tuberculosis infection in patients with rheumatologic diseases, especially those using tumor necrosis factor inhibitors, addressing the definition of latent tuberculosis infection, as well as the prevalence of the disease, the mechanisms involved in its pathogenesis, the medications in use, the screening criteria, its diagnosis, and its treatment.[14]

Even after adequate treatment and a microbiological cure, the sequelae of pulmonary tuberculosis can cause persistent symptoms, impairing lung function and quality of life. In a review article, Tiberi et al.[15] described the management of severe tuberculosis cases and their

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sequelae, discussing the importance of pharmacological and nonpharmacological interventions in the affected patients.

Finally, Pillar 3 of the WHO End TB Strategy is "intensified research and innovation".[1] In Latin America, there is a need to identify priorities in tuberculosis research and to increase the number of publications based on local data.[2,3] In this issue of the journal, Migliori et al.[16] report the results of a systematic review that identified studies on tuberculosis, drug-resistant tuberculosis and MDR-TB, published in priority countries of Latin America (Brazil, Peru, Mexico, Colombia, and Argentina). The authors found that the level of scientific production was highest in Brazil, Mexico, and Peru. They also found that there is still a lack of publications based on local data, showing that international collaborations would be quite helpful in scaling up scientific production in Latin America. The findings of that systematic review underscore the importance of building a pan-Latin American scientific network for research on tuberculosis. A regional network would enable the creation of more opportunities for collaborative research projects. In addition, scientific networks facilitate the recruitment of patients and allow the inclusion of patients from different settings. Furthermore, collaborations have overall positive effects on the number and quality of scientific manuscripts produced. Therefore, future perspectives include further collaboration incorporating relevant topics into the research agenda. The impact of international collaborations on the scientific landscape of Latin America has demonstrated the importance of a global approach to addressing the challenges of tuberculosis control.

We believe that this tuberculosis series, dedicated to the celebration of World TB Day, highlights the relevant advances in our understanding of many topics related to tuberculosis. It is important to focus on the three pillars of the WHO End TB Strategy, which was proposed in order to achieve the goal of ending the global tuberculosis epidemic.

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Management and outcomes of severe childhood tuberculosis in the pediatric intensive care setting: can we identify best practices?

Svetlana Velizarova[1,a], Natalia Gabrovska[1,b], Albena Spasova[1,c], Ben Marais[2,3,d], Elizabeth Page Harausz[4,e], Simon Tiberi[5,6,f], Giovanni Battista Migliori[7,g], Davide Manissero[8,h]

Tuberculosis continues to be a clinical and public health priority. In 2017, there were an estimated 10.0 million new cases of tuberculosis and 1.3 million deaths from the disease, approximately 1.0 million of the cases and 195,000 of the deaths occurring in children under 15 years of age.[1,2] Tuberculosis is now recognized as one of the top 10 leading causes of death in children under 5 years of age living in areas with a high tuberculosis incidence. Historically, attention has been focused on pulmonary tuberculosis in adults, because it is the most infectious form. Adults with pulmonary tuberculosis transmit the disease through infectious aerosol particles, which are typically produced when a patient with cavitary lung disease coughs. Young children are less likely to have cavitary lung disease and are therefore not considered to pose a major transmission risk.[3-6] It is also more difficult to diagnose tuberculosis in children, and the burden of disease is therefore generally underestimated.

Recently, there has been increased awareness of the global burden of tuberculosis in children, resulting in more attention being given to pediatric tuberculosis from a clinical, public health, and research perspective.[1,2,7-11] Improving the accuracy of the diagnosis of tuberculosis in children is made more difficult by the paucibacillary nature of their disease; the fact that the symptoms and signs they present, as well as the radiological findings, are often nonspecific; and the inability of immunodiagnostic tests (interferon-gamma release assays and tuberculin skin tests) to differentiate between infection with *Mycobacterium tuberculosis* and active disease. Expanding the evidence available in the scientific literature to support quality diagnostic and treatment guidelines specific to pediatrics is a core issue,[1,2,7-11] as is the need to increase the availability of the child-friendly drug formulations that are now available through the Global Drug Facility of the international Stop TB Partnership.[12]

Despite the extensive morbidity and mortality caused by the severe forms of tuberculosis in children (tuberculous meningitis and miliary tuberculosis), scarce attention has been paid to the optimal management of tuberculosis in children admitted to the ICU. Ongoing research and reviews are attempting to explore and define best practices in adult tuberculosis cases treated in the ICU.[13] The admission of adults with tuberculosis to the ICU is most often due to extensive pulmonary involvement, leading to ARDS, life-threatening hemoptysis, or lung surgery.[14] In immunocompromised adults, neurological deterioration due to tuberculous meningitis might be another reason for ICU admission. Approaches to recognizing and managing such presentations have been discussed in the literature.[15] Overall, the mortality in ICU patients with tuberculous is quite high, usually exceeding 50%.[16]

We drew upon our experience at the National Pediatric Reference Department for Respiratory Infections, in Sofia, Bulgaria, to identify research feasibility and gaps in the treatment of severe pediatric tuberculosis. Between 2015 and 2018, five children with tuberculous meningitis were admitted to the hospital, with a mean age of 1.2 years (range, 0-3 years) and a mean body weight of 9.6 kg (range, 6-13 kg). Two had non-HIV immunosuppression and no other major comorbidities. On the basis of cultures (of cerebrospinal fluid, in 3 cases, and gastric aspirate, in 2), all five children had a confirmed diagnosis of infection with drug-susceptible strains of *M. tuberculosis*; one patient was also smear positive on Ziehl-Neelsen staining. All of the children had non-cavitary pulmonary involvement on chest X-ray, bilateral disease suggestive of miliary tuberculosis being seen in two. None of the children had respiratory distress severe enough to require ventilatory support, and none developed any signs of sepsis. Routine treatment with isoniazid, rifampin, ethambutol, and pyrazinamide was initiated an average of 4.4 days after presentation (range, 1-16 days). The mean time to culture conversion, based on monthly cultures, was 43.2 days (range, 40-46 days). In all five children, a clinical cure was achieved after 10-12 months of treatment with an all-oral

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regimen. Although the evidence from those five clinical cases is limited, it provides an encouraging picture in terms of the potential outcomes of severe childhood tuberculosis treated in the ICU. Nevertheless, the cases described represent a small number of children with a limited range of tuberculosis disease manifestations. In addition, none of those children received a treatment regimen that was optimized to penetrate the central nervous system. Therefore, there is a need for a more comprehensive review of all pediatric patients with tuberculosis who are admitted to the ICU. Although children with miliary tuberculosis have disseminated disease, they often do not appear critically ill, no cases of children presenting with classic sepsis or septic shock having been documented. However, more information is needed in order to accurately describe the full range of manifestations of severe tuberculosis in children. Similarly, there have been only a few reports of ARDS in children with acute pneumonia-like symptoms secondary to tuberculosis, which can be easily confused with acute bacterial coinfection.[17] More insight is necessary in order to describe the epidemiology, presentation, and management of such cases in pediatric ICUs.[18]

Experience from adult cases demonstrates the complexity of the pharmacokinetics and pharmacodynamics of antituberculosis drugs in critically ill patients. Absorption of oral antituberculosis drugs may be reduced by 70% due to gastroparesis, intestinal paralysis, pharmacological prophylaxis against gastric ulcers, and altered gut microbiota. Suboptimal drug levels, in blood and affected tissues, can be attributed to several factors, including impaired circulation efficiency, with fluid accumulation, and glomerular hyperfiltration, with increased renal clearance.[19,20] Although this experience seems to be transferable to pediatric cases, no specific documented evidence is available for such cases. There are other concerns unique to children, including variable drug distribution and metabolism among different age groups.[21] The need to ensure adequate blood and tissue levels of the available oral and intravenous tuberculosis drug formulations, as well as to provide optimal supportive care, are key factors in managing such complex cases of tuberculosis. There are still few pharmacokinetic data for very young or critically ill children treated with first- or second-line tuberculosis drugs, and much more remains to be done.

The paucity of evidence on childhood tuberculosis in the context of ICU care of critically ill patients calls for a collective effort to determine the magnitude of this unmet medical need, as well as for the development of best practice guidelines. Although we have described a few cases of tuberculous meningitis requiring ICU care, there is a need for a more comprehensive overview of the full spectrum of the disease and its clinical presentations, as well as the best management approaches, in children who require ICU admission for severe tuberculosis. That can be achieved only in collaborative multicenter observational studies using standard data collection tools and a shared data platform.

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Nodular reversed halo sign

Edson Marchiori[1,a], Bruno Hochhegger[2,b], Gláucia Zanetti[1,c]

A 59-year-old female patient presented with fever and dry cough. A chest CT scan showed a reversed halo sign (RHS) with nodular walls in the right upper lobe (Figure 1).

The RHS is a CT finding defined as a focal, round area of ground-glass attenuation surrounded by a partial or complete rim of consolidation. It was initially described as a relatively specific sign of cryptogenic organizing pneumonia. However, subsequent studies have identified the RHS in a broad spectrum of diseases, including infectious and noninfectious conditions. Several recent studies have highlighted RHS characteristics that are very useful for establishing a diagnosis. The halo can be smooth or nodular and contain ground-glass attenuation, small nodules, or normal lung parenchyma. In patients presenting with active granulomatous disease and the RHS, the ring portion or the inner area of the halo can have a nodular appearance. The ring of consolidation has a nodular appearance in most patients presenting with proven active granulomatous disease and the RHS. Histopathological examination of specimens from such patients shows granulomas within the RHS, a pattern that is not seen in patients diagnosed with organizing pneumonia. Therefore, an RHS with a nodular appearance is a useful finding because it indicates the presence of active granulomatous disease (probably resulting from infection or sarcoidosis) rather than organizing pneumonia.[1]

In patients with post-primary pulmonary tuberculosis, well-recognized CT findings include centrilobular or airspace nodules, branching linear/nodular opacities (the tree-in-bud pattern), areas of consolidation, cavitations, bronchial wall thickening, miliary nodules, tuberculomas, calcifications, parenchymal bands, interlobular septal thickening, ground-glass opacities, paracicatricial emphysema, and fibrotic changes. Clusters of small nodules have also been described as a possible manifestation of pulmonary tuberculosis on CT scans. The identification of imaging patterns suggestive of active tuberculosis has long been recognized as playing an important role in public health and appropriate patient management.

The RHS is increasingly recognized as a valuable imaging finding in several lung diseases. When used in combination with clinical evaluation, careful analysis of the morphological characteristics of the RHS can narrow the differential diagnosis. In patients with pulmonary infection, a nodular RHS indicates the presence of active granulomatous disease (particularly tuberculosis). Our patient was diagnosed with pulmonary tuberculosis, and the diagnosis was confirmed by sputum culture. In conclusion, a nodular RHS is a CT finding suggestive of pulmonary tuberculosis.

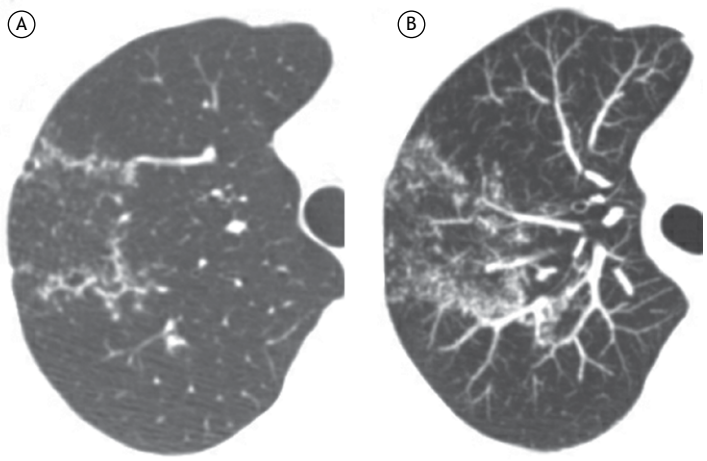

Figure 1. In A, axial CT image of the right upper lobe showing a reversed halo sign with nodular walls and nodules inside the halo. In B, reformatted axial image (maximum intensity projection) showing the nodules in greater detail.

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Loss to follow-up and missing data: important issues that can affect your study results

Juliana Carvalho Ferreira[1,2,a], Cecilia Maria Patino[1,3,b]

Investigators used data from the São Paulo State Tuberculosis Control Program database to evaluate the association between the place of diagnosis and treatment outcomes. They reported that 25% of new tuberculosis cases were diagnosed in emergency facilities and that, in comparison with patients diagnosed in outpatient settings, they were more likely to have unsuccessful treatment outcomes, including loss to follow-up (in 12%) and death (in 10%).[1]

LOSS TO FOLLOW-UP AND TYPES OF MISSING DATA

To study associations of an exposure (in cohort studies) or of an intervention (in randomized controlled trials) with clinical outcomes prospectively, investigators collect exposure/intervention information at study entry and then follow study participants over time and collect outcome data. If follow-up is incomplete or interrupted, leading to missing data at the end of the study, this could impact the internal validity of the study. Participants with missing data, compared with those with complete data, may differ systematically, for example when loss to follow-up is related to the death of participants.

Missing data occur for multiple reasons: 1. the variable of interest is not measured by the research team (e.g., forgetting to measure weight at baseline); 2. the study participant misses a scheduled study visit or test; or 3. the variable is measured but the team fails to register the variable value on the data collection form. However, loss to follow-up, where patient data is not available until the end of the follow-up period, is the most critical mechanism of missing data, because it might include missing outcome data, which is crucial to answer the research question.

Missing data are classified as missing completely at random (MCAR), when missingness is not related to the exposure, covariates, or the outcome; missing at random (MAR), when missingness is related to the exposure or confounders, but not the outcome; and missing not at random (MNAR), when missingness might be related to the outcome (Chart 1).[2] Loss to follow-up and missing data can threaten the internal validity of a study even if the mechanism is MCAR, in which we consider that the remaining participants are a random sample of the initial study population, because the study power will be decreased. If the mechanism is MAR, adjustments and imputation methods can be used, but that might introduce biases to the study. If the mechanism is MNAR, there is a serious risk of biased results.[3]

HOW TO DEAL WITH LOSS TO FOLLOW-UP AND MISSING DATA

The best strategy to avert missing data is to prevent loss to follow-up. Designing the study carefully, training staff, implementing data quality procedures, and developing mechanisms to retain and contact participants are key. Additionally, there are statistical methods available to deal with missing data, but these procedures should be planned a priori and with consultation of a biostatistician.[3] However, there are situations when investigators might not overcome problems related to missing data because the mechanism of missingness is MNAR. In that case, losses to follow-up of 20%, for example, can result in serious biases and, therefore, should not be considered "acceptable". Remember, missing data are common and best practices include thinking about it early on when defining the research question and writing the protocol.

Chart 1. Types of missing data and strategies to minimize them.

Type of missing data	Example	Strategies to minimize missing data
MCAR	Participant moves to another state and abandons the study; a test result is lost at the lab	Develop standardized collection forms; monitor data quality; keep participant contact information up to date
MAR	In a cohort of COPD patients, participants with mild disease are more likely to abandon the study because they are asymptomatic	Offer benefits and incentives to retain participants; regularly contact participants; conduct a pilot study to identify risk factors for loss to follow-up; and develop strategies to overcome them
MNAR	Loss to follow-up is higher among tuberculosis patients who have serious adverse events due to tuberculosis drugs than among patients who tolerate treatment, and treatment nonadherence is related to death	Offer adequate support for study participants; develop strategies to retain participants with a high risk of loss to follow-up; and develop alternative methods to measure the outcome even for participants lost to follow up

MCAR: missing completely at random; MAR: missing at random; and MNAR: missing not at random.

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Measuring slow vital capacity to detect airflow limitation in a woman with dyspnea and a preserved FEV_1/FVC ratio

Danilo Cortozi Berton[1,a], José Alberto Neder[2,b]

THE CLINICAL SCENARIO

A 42-year-old woman with a body mass index (BMI) of 51.2 kg/m² was referred to the respiratory clinic for investigation of progressive breathlessness which had worsened in the past 5 years—her score on the modified Medical Research Council scale at presentation was 3 (i.e., she reported having to stop for breath after walking for only a few minutes). She was a never smoker, and her dyspnea had been mainly attributed to obesity.

THE UNDERLYING PHYSIOLOGY

Spirometry showed a mild-to-moderate, proportional reduction in FEV_1 and in FVC, which resulted in a preserved FEV_1/FVC ratio (Figure 1A). According to the latest American Thoracic Society/European Respiratory Society algorithm for the interpretation of pulmonary function tests, the decision to label this pattern as restrictive or obstructive depends on measurements of TLC.[1] The authors of that document also stated that, although FVC is often used in the abovementioned ratio, it is preferable to use the largest available VC, whether it is that obtained during inspiration, that obtained during slow expiration, or that obtained during forced expiration. However, the FVC may underestimate the "true" maximal VC due to early closure of the small airways at low lung volumes. That is especially true in the presence of increased small airway compressibility or collapsibility.[2] It follows that a "pseudo-normal" FEV_1/FVC ratio might occur in patients with obstructive ventilatory disorder, provided there is a large difference between the slow vital capacity (SVC) and the FVC. Nevertheless, there are few reference values for SVC. It is possible that FVC decreases with aging at a faster rate than does SVC[3]; that is, a low FEV_1/SVC ratio might simply reflect the physiological effects of senescence. Therefore, there are potential advantages and disadvantages to using SVC rather than FVC in the FEV_1/VC ratio. A large recent study analyzing 13,893 consecutive adults with preserved FEV_1/FVC ratios and TLCs shed new light on this controversial issue.[4] The authors reported the following: one in every five subjects presented with a low FEV_1/SVC ratio ("discordant" subjects); most subjects showing obstruction only according to the FEV_1/SVC ratio were highly likely to have airway disease and dysfunction according to a cluster of clinical and physiological variables; regardless of the gender of the subject, the variables age < 60 years, BMI > 30 kg/m², and FEV_1 > 70% of the predicted value were all associated with "discordance"; and "discordant" subjects ≥ 70 years of age showed no other evidence of airway disease or dysfunction.

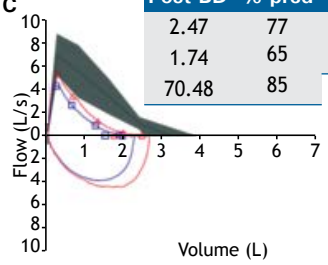
OVERVIEW

Due to time and operational constraints, most pulmonary function testing laboratories in primary care still perform only the forced expiratory maneuver.[5] Given the results of the abovementioned study,[4] adding the SVC maneuver may represent a simple strategy to reveal an obstructive ventilatory defect that was missed by determination of the FEV_1/FVC ratio in a non-elderly (< 60 year-old) obese subject with a high pre-test probability of airway disease. A positive flow or volume response to inhaled bronchodilator administration might also prove useful to reveal airflow limitation in these subjects. Considering the risk of overdiagnosis of obstruction in the elderly (individuals > 70 years of age), it seems prudent to avoid using SVC in the FEV_1/VC ratio in this subpopulation. A case-by-case approach should be applied in subjects 60-70 years of age.

A	Pre BD	LLN	ULN	Z score	% pred
FVC (L)	1.95	2.55	3.83	-1.94	61
FEV_1 (L)	1.51	2.05	3.27	-1.89	57
FEV_1/FVC	77.54	74.66	91.66	-0.66	93

B	Pre BD	LLN	ULN	Z score	% pred
Slow VC (L)	2.50	2.55	3.83	-1.08	78
FEV_1/Slow VC	60.38				

C



Post BD	% pred	Δ L	Δ %
2.47	77	0.52	26.68
1.74	65	0.23	15.14
70.48	85		

Figure 1. Baseline FVC (panel A) and SVC (panel B). Flow-volume loops before and after acute administration of albuterol (blue and red lines, respectively) are shown in panel C, as are the post-bronchodilator values and variations (Δ) in relation to the baseline values. BD: bronchodilator; LLN: lower limit of normality; ULN: upper limit of normality; and % pred: percentage of the predicted value.

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OUTCOME

In the case presented here, involving a middle-aged obese woman with a preserved FEV_1/FVC ratio (Figure 1A), airflow limitation was detected on the basis of the SVC (Figure 1B). A positive volume response to inhaled albuterol further supported the diagnosis of an obstructive ventilatory defect (Figure 1C). In fact, the patient reported a noticeable improvement in her shortness of breath after starting twice-daily treatment with inhaled formoterol plus budesonide.

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Primary bacillary resistance in multidrug-resistant tuberculosis and predictive factors associated with cure at a referral center in São Paulo, Brazil

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Study carried out at Universidade Federal de São Paulo, São Paulo (SP) Brasil.

ABSTRACT

Objective: To identify transmitted or primary resistance among cases of multidrug-resistant tuberculosis and predictive factors for cure in multidrug-resistant tuberculosis after the first treatment. **Method:** Descriptive study of a cohort from 2006 to 2010, in a reference unit of tuberculosis in São Paulo, Brazil. The data were obtained by the revision of medical records. Clinical criteria were used to classify transmitted and acquired resistance. Extended primary resistance was also defined, in this study, as cases initially treated with a standardized scheme, but with no therapeutic success, and the pre-treatment drug susceptibility test (DST) showed presence of resistance. **Results:** 156 patients with multidrug-resistant tuberculosis and their respective sputum samples were eligible for the study. Only 7% of the patients were positive for the human immunodeficiency virus (HIV). Previous treatment occurred in 95% of the sample. The cure rate after the first treatment was 54%. The median bacteriological conversion time of those who healed was one month. Bacillary resistance was considered acquired resistance in 100 (64%) and transmitted resistance in 56 (36%). By logistic regression, patients who presented primary multidrug-resistant tuberculosis (*odds ratio*—OR = 6,29), without comorbidity (OR = 3,37) and with higher initial weight (OR = 1.04) were associated with cure after the first treatment. **Conclusion:** The early detection of bacillary resistance and appropriate treatment are in favor of healing. Thus, it is crucial to know exactly the primary resistance rate avoiding the use of inadequate treatments, amplification of bacillary resistance and its transmission.

Descriptors: Multidrug-resistant tuberculosis; Drug resistance; Treatment outcome.

INTRODUCTION

The appearance of multidrug-resistant tuberculosis (MDR-TB) in the world has become a public health issue. Therefore, the strategic adjustments to raise the cure rate and the measurements to prevent the dissemination of the disease must be adopted fast.[1]

Bacillary multi-resistance is a biological phenomenon that can be considered as iatrogenic and multifactorial, considering that the exposure of Mycobacterium tuberculosis to drugs during the treatment of the disease causes selective pressure, which favors the permanence of resistant bacillary lineages.[2]

Factors that contribute with the existence of bacillary resistance are deficient tuberculosis-control programs, hard access to the system, lack of or delayed diagnosis, little adherence to treatment, low healing percentage, which leads to persistent transmission, and increasing number of individuals with latent infection (TBLI) with resistant bacillus, who might become sick.[1]

Drug-resistance is classified as: natural resistance, which appears during the process of bacillary multiplication; primary resistance, in patients who were never treated for tuberculosis, infected by previously resistant bacilli; and, finally, resistance acquired by patients with initially sensitive tuberculosis, who became resistant after exposure to the drugs.[3]

The World Health Organization (WHO), in 2015, reported that the susceptibility test (ST) for the diagnosis of bacillary resistance was carried out in only 58% of the patients who had been previously treated for TB, and in 12% of new cases, therefore missing the chance of an early diagnosis of the resistance.[4] In 2012, the institution had already estimated that 3.7% of the new cases, and 20% of the previously treated for TB in the world, were cases of MDR-TB. In some regions, that proportion was even higher.[5,6]

In Brazil, it is formally recommended to perform culture and phenotypic ST for all cases of retreatment, however, this rule is not always fulfilled.[7] Therefore, the magnitude of the problem is not totally known, which makes it difficult to assess the reality of the situation. [3,7] Even if no phenotypic ST is completely accurate,[8] its importance is undeniable.[9] The complexity of these patients associated with the difficulty in management, both aggravated by the association of the coinfection

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tuberculosis and the human immunodeficiency virus (HIV) – which was 10.4% in Brazil, in 2013 —,[7] turns this process into a huge challenge.

The knowledge of the characteristics and peculiarities of these patients with MDR-TB, in some regions of the country, constitutes an important database for the elaboration of new measures of control, diagnosis and therapeutic proposals.

São Paulo is the second state of the country in number of MDR-TB cases. In the study period, from 2006 to 2010, 453 cases were notified, of which 190 (42%) were registered and treated at Instituto Clemente Ferreira, which is a reference outpatient clinic for MDR-TB of the State Health Secretariat in São Paulo.[10,11]

This study aimed at identifying the extended primary resistance among cases of MDR-TB and predictive factors, among demographic, clinical and radiological variables, associated with the cure of MDR-TB after the first treatment.

METHOD

Study population

In Instituto Clemente Ferreira, 190 patients were diagnosed with phenotypic ST, and, according to the definition by the WHO,[12] patients with MDR-TB were those showing resistance to isoniazid and rifampicin, from January 2nd, 2006, to December 31st, 2010.

This project was approved by the Research Ethics Committee of Universidade Federal de São Paulo (UNIFESP), and conducted according to the ethical principles established by the Declaration of Helsinki.

Inclusion criteria

- Aged 18 years or more;
- Both genders;
- Diagnosis of MDR-TB according to the WHO;[12]
- Treatment of MDR-TB in Instituto Clemente Ferreira.

Exclusion criteria

- Initial resistance to quinolone and/or initial resistance to an injectable second line drug;
- Previous use of quinolone for the treatment of tuberculosis.

Study protocol

Descriptive study of a cohort nested in a structured database for the follow-up of adult patients with MDR-TB.

The following was pointed in the medical chart:

- Demographic variables, such as sex, age at the time of phenotypic ST, positive for MDR-TB, age of tuberculosis onset, hospitalization;
- Social variables, such as freedom deprivation, homeless patients;
- Clinical history, such as interval of time between date of the first possible episode of drug-sensitive tuberculosis and the onset of MDR-TB (first episode of TB/TB-MDRTB), number of previous treatments, number of previous treatments with first line drugs, and other treatments, time of culture negativation, contacts with tuberculosis (sensitive or resistant), primary resistance and acquired resistance.

Regarding the clinical data, the following were analyzed: initial weight, presence of comorbidities referred to in the medical chart (HIV, diabetes mellitus and hepatopathy), changes in the thoracic x-ray (lesion in the lung and cavity, uni or bilateral), besides the results of several cultures from each patients, their phenotypic STs and identification of the mycobacterium using the phenotypical or molecular method, during the follow-up period, without a pre-established periodicity. The phenotypic STs were carried out in the automated system Bactec MGIT Sire kit (SAT/Sire), and, for second line drugs, the methodology to determine the minimum inhibitory concentration (MIC). The non-performance of ST for second line drugs together with the first line was occasional.

Phenotypic TSs occurred in the microbiology laboratory of Instituto Clemente Ferreira and in Instituto Adolfo Lutz, both reference centers maintained under rigorous quality control.

At the end of the first MDR-TB treatment, that is, 18 months, the initial outcome events were assessed: abandonment, failure, cure and death, according to the definitions by the WHO[13] and the Ministry of Health.[3]After observing the records in the medical chart, bacillary resistance was categorized in:

- extended primary resistance: in our study, it was defined in three different situations: patients who had never been treated for tuberculosis; individuals who had been treated for less than 30 days,[3] and those who were never treated for tuberculosis who began the first treatment with first line drugs (isoniazid, rifampicin, ethambutol and pyrazinamide); however, with unsatisfactory clinical, radiological and microbiological evolution, and posterior confirmation of the MDR-TB pattern, in a sputum culture collected before the treatment began;[8,14]
- acquired resistance: established in patients with initially drug-sensitive tuberculosis who became resistant after exposure to drugs.[3,12]

The treatment plan for MDR-TB was the one established by the National Tuberculosis Control Program (PNCT), of the Ministry of Health, for the study period.[3]

Statistical analysis

Concerning the convenience sample, the categorical variables were expressed in absolute and relative frequencies (percentage), and numerical variables were expressed in mean, standard deviation (SD), medians and interquartile range.

For the analysis of demographic, clinical and radiological characteristics, the following were used: χ^2 and Fisher's exact tests, or the ANOVA One-Way test, with Bonferroni post-hoc test, and the Kruskal-Wallis

test with Mann-Whitney post-hoc test, depending on the nature of the variable's distribution.

A univariate logistic regression analysis was used to know the association of the factors that favor the cure among the demographic, clinical and radiological variables, and the ones that presented $p < 0.10$ were selected to test the multiple logistic regression model, using the Stepwise method.

The 5% value was considered for rejecting the nullity hypothesis in all tests, and the Statistical Package for the Social Sciences (SPSS), version 19.0, was used for statistical calculations.

RESULTS

From 2006 to 2010, in Instituto Clemente Ferreira, 531 sputum samples with presence of bacilli resistant to rifampicin and isoniazid, from 190 patients, were isolated using the phenotypic ST.

Nine patients were excluded (12 samples) due to transfer from the health unit; nine patients (25 samples), for previous use of second line injectable drug; and 16 patients (34 samples), for phenotypic ST showing pattern of pre-XDR TB, that is, in vitro bacillary resistance to rifampicin and isoniazid, added to the resistance to quinolone or a second line injectable drug (amikacin, kanamycin or capreomycin),[15] and with pattern of extensively resistant tuberculosis (XDR-TB), defined as in vitro bacillary resistance to rifampicin and isoniazid associated with resistance to both drugs, to quinolone and to a second line injectable drug.[12]

Therefore, 156 patients and their respective sputum samples were eligible for the study (Figure 1).

In this cohort, composed of patients with MDR-TB, mean age was 39.5 years (SD ± 12.5), most were male (60%), and mean basal weight was 57.2 kg (SD ± 11.8). The first diagnosis of tuberculosis was reported with mean age of 35.8 years (SD ± 13.2). Hospitalization caused by tuberculosis was mentioned by 1/3 of the patients. Only 11 individuals (7%) were freedom deprived at some point because of the disease, and six (4%) were homeless (Table 1). Only 6% of the patients were treatment naive.

Table 1 presents the median of the number of previous treatments with first line drugs. Also, the median time of evolution from the first episode of tuberculosis to the diagnosis of MDR-TB and time for negativation of the sputum in patients who were cured were described.

Most patients (72%) denied having previous contact with individuals with tuberculosis. On the other hand, 19 (12%) confirmed their contact with a drug-sensitive tuberculosis patient, whereas 25 (16%) mentioned the contact with a person with MDR-TB.

Of the 156 patients, 100 (64%) and 56 (36%) were classified as acquired and primary resistance, respectively (Table 1).

A higher proportion of patients without a lung cavity was associated with cure, whereas in the event of abandonment and failure, the association was higher with the presence of a lung cavity (uni or bilateral) ($p = 0.008$). The presence of unilateral parenchymal lesion was associated with cure ($p = 0.040$) (Table 2).

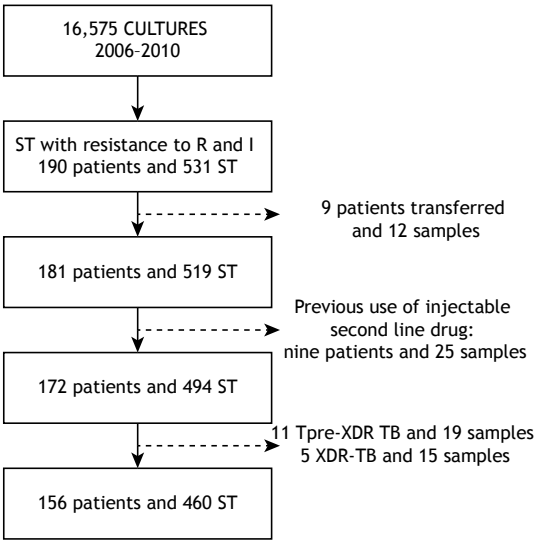

Figure 1. Flowchart of the study sample about multi-drug resistant tuberculosis (MDR-TB). ST: sensitivity test; pre-XDR TB: pre-extensively drug-resistant tuberculosis; XDR-TB: extensively drug-resistant tuberculosis.

Cure was associated with treatment naïve patients, whereas the negative events (failure and abandonment) were associated with patients who had been treated previously ($p < 0.001$) (Figure 2).

Among the individuals who healed, 56% had primary resistance, and 79% of the individuals in the failure group, and 86% in the abandonment group had acquired resistance ($p < 0.001$).

Multiple analysis was used to assess the association of the factors that favored the cure adjusted for probable confounding variables. Therefore, after the adjustment of the model for time between the date of the first supposedly drug-sensitive tuberculosis episode and the onset of MDR-TB (first episode tuberculosis/TB-MDRTB), and lack of bilateral cavity, the patients who presented with higher initial weight (odds ratio – OR = 1.04), without comorbidity (OR = 3.37), and with primary MDR-TB (OR = 6.29) were associated with cure at the end of the first treatment for MDR-TB (Table 3).

DISCUSSION

In four years of analysis in Instituto Clemente Ferreira, 16,575 sputum cultures for Mycobacterium tuberculosis were performed, of which only 3.2% showed resistance to the rifampicin-isoniazid binomial, which is lower in relation to specific regions of the world.[5,16]

The sample of 156 patients with MDR-TB was mainly composed of male patients, with mean age of 39 years.

In groups of patients in a special situation, such as the ones who required hospitalization, both for the

Table 1. Demographic characteristics of the 156 patients with multi-drug resistant tuberculosis (MDR-TB) included in the study.

Variable	
Age (years) mean ± SD	39.5 ± 12.5
Age of onset of the disease (years) mean ± SD	35.8 ± 13.2
Male n (%)	94 (60)
Weight (kg)	57.2 ± 11.8
Hospitalization n (%)	50 (32)
Freedom deprived n (%)	11 (7)
Homeless n (%)	06 (4)
T. drug-sensitive TB – MDR-TB (Years) Md [IQR]	1.0 [0.5 - 2.5]
Previous treatments	
Number of treatments Md [IQR]	2.0 [1 - 3]
n (%) previous plan with first line drugs	
Treatment naive	09 (06)
1	53 (34)
2	49 (31)
3 or more	45 (29)
Time for negativation (months) Md [IQR]	1 [1 - 2]
Contact with TB n (%)	
No contact	112 (72)
Contact drug-sensitive TB	19 (12)
Contact with MDR-TB	25 (16)
Resistance n (%)	
Acquired	100 (64)
Primary	56 (36)

SD: standard deviation; n: number; T.: time; TB: tuberculosis; Md: median; IQR: interquartile range.

Table 2. Clinical and radiological characteristics of patients regarding the initial outcome events.

Variables n = 156	Initial outcome					
	A (23)	F (37)	C (85)	D (11)	P	Total n (%)
Previous treatments n (%)	23 (96)	37 (100)	77 (91)	10 (91)	0.25§	146 (94%)
Number of treatments Md [IQR]	3 [2-3]	3 [2-3]	1 [1-2]	2 [1-3]	< 0.001¥	2 [1-3]
Contact TB n (%)					0.64§	
No contact	18 (78)	29 (78)	56 (66)	9 (82)		112 (72)
Drug-sensitive TB	01 (4)	04 (11)	13 (15)	1 (9)		19 (12)
MDR-TB	04 (18)	04 (11)	16 (19)	1 (9)		25 (16)
Parenchymous lesion					0.040	
Unilateral	02 (9)	04 (11)	25 (30)	03 (30)		34 (22)
Bilateral	21 (91)	33 (89)	59 (70)	07 (70)		120 (78)
Cavity					0.008	
Absent	00 (0)	01 (03)	15 (18)	03 (30)		19 (12)
Unilateral	11 (50)	15 (40)	45 (54)	04 (40)		75 (49)
Bilateral	11 (50)	21 (57)	24 (28)	03 (30)		59 (39)
Comorbidities	11 (50)	13 (35)	23 (28)	10 (91)	< 0.001	57 (37)
Diabetes mellitus	02 (9)	04 (11)	13 (16)	01 (9)	0.77	20 (13)
Hepatitis	02 (9)	01 (3)	03 (4)	01 (9)	0.59	07 (5)
HIV	04 (19)	03 (9)	00 (0)	03 (37)	< 0.001	10 (7)

n: number of patients; Md: median; TB: tuberculosis; MDR-TB: multi-drug resistant tuberculosis; HIV: human immunodeficiency virus; A: abandonment; F: failure; C: cure; D: death; §: χ^2 test (complemented by partitioning); ¥: Kruskal-Wallis.

severity of the disease or for their social condition, those who were freedom deprived[17,18] at some point because of the disease and those who were homeless were a minority, but elevated the complexity of conduct, considering the clinical and the social points of view. These groups require special care, since they are exposed to locations with high incidence of tuberculosis, with higher chances of exogenous reinfection, that is, primary resistance, which may require specific strategic.[19,20]

The median time between the first diagnosis of tuberculosis and the confirmation of resistance

compatible with the definition of MDR-TB was one year, creating the possibility of using different treatments with first line drugs,[21] perpetuating the transmission of the drug-sensitive and/or drug-resistant bacillus. This scenario confirms the identification of the fragilities of the tuberculosis control program and the management of unfavorable post-treatment events.[22]

Only 1/3 reported contact with tuberculosis, and not specifically with patients who had MDR-TB; however, the report of contact with MDR-TB was prevalent. These findings remain similar with time in the institution where the previous analysis was carried out by Melo et al.[23]

For 89% of the patients in this study, negativation occurred in up to six months. This results suggests the benefit of creating criteria for the early diagnosis of treatment failure, thus preventing the maintenance of an inadequate plan, and the consequent increase of bacillary resistance.[24]

It was amazing to find the proportion of 36% of primary MDR-TB in this sample, once, in Brazil, the rate of this descriptor is very low, around 4%.[3,11,23,25-27] It is worth to remember that many patients who showed resistance came from other services and were, afterwards, assisted at the reference center mentioned here, which characterizes that the percentage of the general population is lower than what is shown in the sample.

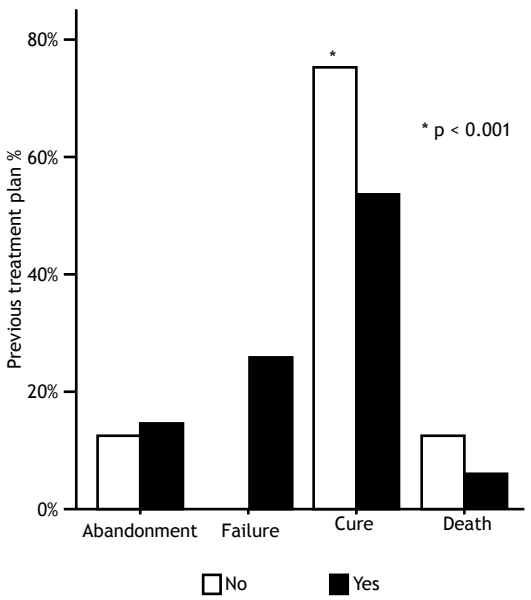

Figure 2. Association of the number of previous treatments of patients with multi-drug resistant tuberculosis (MDR-TB) with first line drugs in relation to the initial outcome events: χ^2 test added to the partitioning χ^2 test. *: significance.

The referred rate of primary resistance, of 4%, was based on the classic definition,[3,8] the most applied one, however, it may underestimate its presence,[8,14,28] since the ST is not universally offered before the beginning of the first therapeutic plan for tuberculosis.[26,29]

Therefore, the definition applied in this study was the extended primary resistance. The following cases were added to the classic definition group: patients who had never been treated for tuberculosis, but started the first treatment with a triple or basic plan, as established by the PNCT, Ministry of Health, supposedly patients with tuberculosis for bacilli who are sensitive to first line drugs, however, with unsatisfactory clinical, radiological and microbiological evolution who were not healed, with posterior confirmation of compatible bacillary resistance with MDR-TB pattern, using the phenotypic ST, in a sample collected before the beginning of the treatment.[8,14,30-32]

This extended definition could only be proposed because in the institution where this study took place, before the beginning of any treatment, the sputum culture for Mycobacterium tuberculosis and phenotypic ST[33] for first line drugs was routine for all of the patients. In case MDR-TB was confirmed, ST was performed for second line drugs.

According to this orientation from PNCT, only eight patients (5%) denied having undergone previous treatment with anti-tuberculosis drugs. In this scenario, there were possibly patients who could be cases of undiagnosed primary MDR-TB. Unfortunately, the maintenance of this recommendation, from our point of view, leads to the delayed diagnosis of bacillary resistance, and the use of an inadequate treatment plan, which can increase resistance, and, consequently, worsen the clinical conditions, besides the maintenance of transmission of multi-drug resistant bacilli.[26,30,34,35]

In thise sense, Günther et al., in a study carried out in 16 European countries, also reported high rates of MDR-TB, probably due to the transmission of the multi-drug resistance bacillus, because 52.4% of the patients had not undergone a previous treatment.[33]

In Brazil, a study performed in Espírito Santo showed primary resistance rate of 19.3%.[36] With molecular method resources, a similar situation occurred in a study conducted in São Paulo, with patients with

Table 3. Risk factors associated with cure, after 18 months of treatment, for the 156 patients with multi-drug resistant tuberculosis (MDR-TB): -2-Log-likelihood 138.271.

Variable	Odds Ratio (OR)	95%CI	P
Initial weight	1.04	0.99-1.08	0.072
T. Drug-sensitive TB - MDR-TB	1.03	0.89-1.18	0.716
No comorbidity	3.37	1.41-8.09	0.006
No bilateral cavity	2.60	1.14-5.91	0.023
Primary MDR-TB	6.29	2.35-16.79	< 0.001

T. time; TB: tuberculosis; 95%CI: 95% confidence interval.

HIV[37] and in Minas Gerais. Dantas et al. reported cases of MDR-TB for primary transmission in 20 to 30% of the patients.[28]

Such a variability is also owed to the existence of different tools to define the same parameter. Therefore, associating genotyping methods of Mycobacterium tuberculosis makes it possible to explore the dynamic transmission of the bacillus, besides distinguishing the disease by exogenous reinfection or endogenous reactivation.[33,36,38]

The broad use of rapid molecular test (Xpert RIF/MTB) as the first approach should collaborate with a quick diagnosis and the adequate conduct for bacillary resistance.

It is very important to distinguish primary resistance from acquired resistance, since there may be implications in the strategy to control the transmission of the multi-drug resistant bacillus.

Also, it is essential to perform a phenotypic ST for all cases of tuberculosis before the treatment, because, without it, it is impossible to assess and take adequate and accurate measures for transmission control. This characterization can be an indicator of efficiency of the tuberculosis control program, helping with the adjustments and the development of tools to control the MDR-TB.[32,34]

In conclusion, after the adjustment for confounding variables, the multiple analysis showed that patients with MDR-TB with primary resistance, without comorbidities and higher initial weight were the ones with higher chances of cure after the initial 18-month treatment. Logistic regression confirmed the expected in the daily clinic. Therefore, early diagnosis and adequate treatment in patients with good clinical conditions favor the cure.[21,38,39]

The treatment plan for MDR-TB in Brazil is standardized by PNCT, and is composed, in the study period, by five drugs, accounting for 18 to 24 months, according to the time for culture negativation and radiological clinical assessment. In the first six months, the following are administered: amikacin, levofloxacin or ofloxacin, ethambutol, terizidone, and pyrazinamide); in the following 12 months, ofloxavin or levofloxacin, ethambutol and terizidone.[6] In some situations, an alternative plan for MDR-TB was used and composed of three or four first line and second line drugs (all of them had ofloxacin), or, as aforementioned, with first line drugs. This plan changed in 2017. Currently, the plan established by the Ministry of Health for Brazil is different, according to the institution's information note.[40]

Study limitations are the fact that it is retrospective and carried out in a single center, where the information was taken from the charts of patients assisted by different physicians, so the data was provided by the patient. However, all relevant information was described in a structured, previously established report, in order to minimize possible bias. Besides, even if occasionally, phenotypic ST was not performed for first line or second line drugs concomitantly for all patients in the beginning of the treatment for MDR-TB, which could lead to more precision in primary resistance rate.

Studies with prospective design and phenotypic and genotypic ST should add more accurate data to this study.

The conclusion is that primary resistance among cases of MDR-TB was higher, and the predictive factors associated with healing after the first treatment ere: presence of primary MDR-TB, lack of bilateral cavity and lack of comorbidity after the adjustment for the initial weight of the patient, and time between drug-sensitive tuberculosis and diagnosis of MDR-TB.

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Smoking and pulmonary tuberculosis treatment failure: a case-control study

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ABSTRACT

Objective: To determine the association between smoking and pulmonary tuberculosis treatment failure. **Methods:** This was a case-control study conducted at the Brazilian Institute for Tuberculosis Research in the city of Salvador, Brazil, between 2007 and 2015. We evaluated 284 patients treated for pulmonary tuberculosis, comparing 50 cases of treatment failure with 234 control cases in which the final outcome was cure. **Results:** Treatment failure was attributed to smoking and age rather than to gender, income, level of education, alcohol consumption, or marital status. Therefore, even after adjustment for age, the risk of treatment failure was 2.1 times (95% CI: 1.1-4.1) higher among the patients with a history of smoking. In addition, being over 50 years of age was found to increase the likelihood of treatment failure by 2.8 times (95% CI: 1.4-6.0). **Conclusions:** Smoking and aging are both associated with pulmonary tuberculosis treatment failure. Therefore, as part of a tuberculosis control program, health personnel should be prepared to offer strategies to promote smoking cessation and should be more careful with older patients.

Keywords: Tobacco use disorder; Tuberculosis; Treatment failure.

INTRODUCTION

Smoking and tuberculosis are public health problems worldwide.[1-3] Tuberculosis is considered one of fastest growing infectious diseases. According to data from the World Health Organization (WHO), there were 6.3 million reported new cases of tuberculosis, as well as 1.3 million reported deaths from the disease, in 2016.[3] Smoking is directly responsible for 6 million deaths worldwide each year[1] and is a risk factor for diseases with high mortality, such as pulmonary tuberculosis.[1,4] When both diseases act together, they increase their impact, and the product of this interaction can be considered a synergistic epidemic, or syndemic.[5]

Within the syndemic framework, it is estimated that smoking is responsible for 20%[6] of the burden of tuberculosis and that smoking will be responsible for a total of 18 million new cases and 40 million deaths in the 2010-2050 period.[7,8] Smoking is also associated with severe forms of tuberculosis in terms of sequelae and with poor tuberculosis treatment outcomes, such as recurrence and death.[6,9] That is due to the fact that the components of tobacco smoke cause numerous pathophysiological changes in the respiratory system, promoting local inflammatory and immunological changes, thus inhibiting cell growth and the action of some chemical mediators of innate immunity.[10,11] In addition, adherence to tuberculosis treatment has been found to be poor among patients who smoke during treatment, especially those who are male.[12,13]

Poor tuberculosis treatment outcomes include treatment failure, defined by the WHO as ". . . sputum smear or culture . . . positive at month 5 or later during treatment."[14] Treatment failure lengthens the period during which patients are infectious, and such patients may harbor resistant bacilli.[15,16] Although there have been few studies focusing on this type of poor outcome, it has been proposed that smoking doubles the risk of pulmonary tuberculosis treatment failure.[17,18] However, it remains unclear which variables can change the outcome of tuberculosis treatment; alcohol consumption, poverty, and gender should be considered potential confounders.[18,19] Therefore, the objective of this study was to determine the risk of pulmonary tuberculosis treatment failure in smokers and to analyze the potential confounders described in the literature.

METHODS

This was an unmatched case-control study including patients who received pulmonary tuberculosis treatment

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between 2007 and 2015 at the José Silveira Foundation *Instituto Brasileiro para Investigação da Tuberculose* (IBIT, Brazilian Institute for Tuberculosis Research), a referral center for tuberculosis control and treatment in the city of Salvador, Brazil.

We included patients over 15 years of age who were diagnosed with pulmonary tuberculosis on the basis of a positive sputum smear or culture and who received treatment for drug-susceptible tuberculosis for 6 months, additional sputum smears being performed at months 2, 4, and 6 of treatment. We excluded patients with diabetes mellitus, HIV/AIDS, or comorbidities associated with drug abuse during treatment, as well as patients who dropped out of treatment or who did not comply with their follow-up visits.

Definitions of cases and controls

Cases were defined as pulmonary tuberculosis patients who experienced treatment failure. Treatment failure was defined by the criteria of the Brazilian National Guidelines for the Control of Tuberculosis[20]: remaining smear positive at the end of treatment; initiating treatment with a strongly positive (++ or +++) result and continuing to have this result up until month 4 of treatment; or having an initial positive result followed by negative results, then having new positive results for 2 consecutive months after month 4 of treatment. Controls were defined as pulmonary tuberculosis patients who, at the end of treatment, were classified as cured.

All cases in the IBIT database (MV System) that met the inclusion criteria were selected. Controls were selected by using the Predictive Analytics Software package, version 18.0 (SPSS Inc., Chicago, IL, USA) as a random number generator from the IBIT database. We considered a case/control ratio of 1:4. Sociodemographic and microbiological data were extracted from the medical records of each selected patient. Laboratory test results were obtained from the IBIT database.

Definition of exposure

Patients were categorized, by smoking status, as follows: current smokers—those who, at the time of diagnosis and during treatment, reported smoking; former smokers—those who, at the time of diagnosis, reported having stopped smoking and who did not smoke during treatment; and never smokers—those who had not smoked before being diagnosed and still did not smoke when they were classified as cured.

In addition to data on the exposure variable, we collected data on variables that could have an effect on tuberculosis treatment outcomes. We identified the following variables in the literature[18,19]: patient income; history of alcohol consumption; gender; age; level of education; and marital status.

Sample size and statistical analysis

Between 2007 and 2015, a total of 2,437 patients were diagnosed with pulmonary tuberculosis and treated at the IBIT. Our initial sample size calculation indicated that 60 cases and 240 controls were required in order to achieve a confidence interval of 95% and a power of 80%. We based our calculation on a previous study, conducted by Tachfouti et al.,[17] in which treatment failure was found to be associated with smoking (OR = 2.25).

Data analysis was performed with the Predictive Analytics Software package, version 18.0 (SPSS Inc.). Categorical variables were described as absolute and relative frequencies; age and income were stratified into categories. The smoking variable was grouped into two categories, current smokers and former smokers being grouped together and compared with never smokers, because the risk of having poor tuberculosis treatment outcomes is similar in current and former smokers.[21] Pearson's chi-square test was used in order to determine the statistical difference among the variables. We calculated ORs as a measure of association between treatment failure and the variables of interest, using a confidence interval of 95%. Values of $p < 0.05$ were considered statistically significant.

Covariates (gender, age, income, level of education, marital status, and alcohol consumption) were analyzed with the use of the Mantel-Haenszel test to determine whether any of them behaved as an effect-modifying factor in the association between smoking and treatment failure. We employed multivariate binary logistic regression analysis to adjust the effect of this association and the covariates, using a combined backward-forward procedure. The final logistic regression model included variables that changed the OR by at least 10%.

Ethical aspects

This study was approved by the Research Ethics Committee of the Federal University of Bahia Climério de Oliveira Maternity Hospital (CAAE protocol no. 51244415.4.0000.5543). All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

RESULTS

We included 284 patients (50 cases and 234 controls), 180 (63.3%) of whom were male. Of those 284 patients, 97 (34.1%) were current or former smokers. The mean age was 40.5 ± 14.7 years. The number of cases of treatment failure was higher among patients over 50 years of age (OR = 3.4; 95% CI: 1.8-6.4; Table 1 and Figure 1). The risk of treatment failure was 2.5 times higher (95% CI: 1.3-4.6) among the current or former smokers than among the never smokers (52.0% vs. 30.3%). However, gender, alcohol consumption, level of education, income, and marital status had similar distributions between the groups (Table 1).

After being adjusted for age, the OR for pulmonary tuberculosis treatment failure in current or former smokers was 2.1 (95% CI: 1.1-4.1; Table 2).

Table 1. Characteristics of the study population, including cases (of treatment failure) and controls, at the José Silveira Foundation Brazilian Institute for Tuberculosis Research, Salvador, Brazil, 2007-2015.

Characteristic	Cases (n = 50) n (%)	Controls (n = 234) n (%)	OR	(95% CI)
Male gender	33 (66.0)	146 (62.4)	1.17	(0.62-2.22)
Age, years				
< 50	26 (52.0)	184 (78.6)	Reference values	
< 30	8 (16.0)	74 (31.6)		
31-50	18 (36.0)	110 (47.0)		
> 50	24 (48.0)	50 (21.4)	3.40	(1.80-6.42)
Being in a stable relationship[a]	37 (74.0)	179 (76.5)	0.87	(0.43-1.76)
≤ 8 years of schooling	32 (64.0)	138 (59.0)	1.24	(0.66-2.33)
Income ≤ the Brazilian NMW[b]	23 (46.0)	127 (54.3)	0.72	(0.39-1.32)
Alcohol consumption[c]	29 (59.2)	122 (52.1)	1.33	(0.71-2.49)
Smoking status				
Current or former smoker	26 (52.0)	71 (30.3)	2.49	(1.34-4.63)
Current smoker	20 (40.0)	37 (15.8)	3.67	(1.83-7.34)
Former smoker	6 (12.0)	34 (14.5)	1.19	(0.45-3.16)
Never smoker	24 (48.0)	163 (69.7)	Reference values	

NMW: national (monthly) minimum wage. [a]Being in a stable relationship (marital status option) was defined as cohabiting or being married. [b]880 Brazilian reals (245 US dollars) in 2015. [c]Alcohol consumers were defined as patients who reported having consumed alcohol on a regular basis before or after being diagnosed with tuberculosis and were compared with patients who reported never having consumed alcohol.

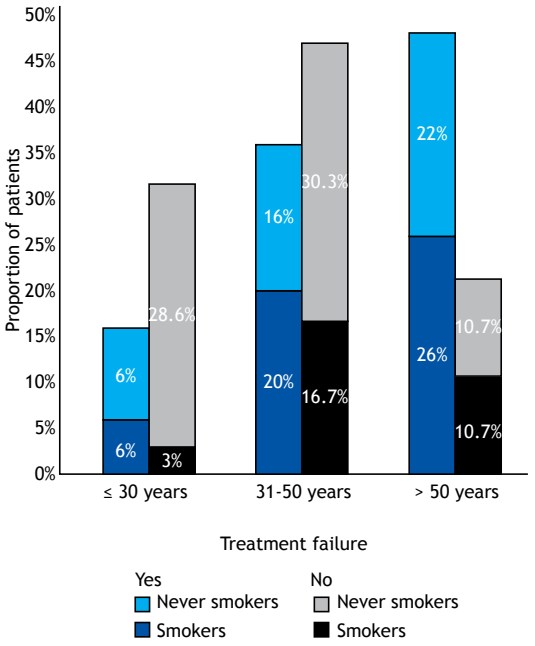


Figure 1. Distribution of the patients in whom treatment failed and those who were cured, by age and smoking status, at the José Silveira Foundation Brazilian Institute for Tuberculosis Research, Salvador, Brazil, 2007-2015.

Stratified analysis showed that being a woman and being a current smoker collectively increase the risk of treatment failure (OR = 6.0; 95% CI: 1.5-23.3 vs. OR = 3.1; 95% CI: 1.3-7.3 for being a man and a current smoker; Table 3). However, the homogeneity tests were not significant, and, during multivariate adjustment, the gender variable was not found to be a determining factor in the model.

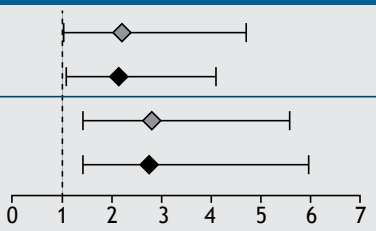
DISCUSSION

In the group of patients who were treated at the IBIT and selected for inclusion during the 8-year study period, we found that being a current or former smoker increased the risk of pulmonary tuberculosis treatment failure. That association persisted even after adjustment for potential confounders. We also found that, among the patients in whom treatment failed, the proportion of smokers was higher in the > 50-year age group. Previous studies have identified an association between treatment failure and smoking.[9,17] However, such studies have grouped treatment failure with other poor outcomes, such as treatment nonadherence and death, rather than measuring it separately.[9] In the Tachfouti et al. study,[17] which was conducted in Morocco, the model was adjusted for age and monthly income, and the risk of treatment failure was found to be higher in patients with a low income and in those who smoked. Of the covariates in our study (age, gender, income, level of education, marital status, and alcohol consumption), age had the greatest effect in the final model. Data stratification by age showed a higher proportion of current smokers over 50 years of age in the case group. This result is similar to that reported in the study conducted in China by Wang et al.,[22] in which the risk of treatment failure was shown to increase with age. However, that could be related to the fact that the prevalence of tuberculosis in the > 50-year age group is high in China and in Brazil.[23]

It is estimated that 20% of the incidence of pulmonary tuberculosis can be attributed to smoking.[6] In the patient cohort from which the cases of this study were extracted, the overall prevalence of smoking

Table 2. Multiple logistic regression of associations of smoking and age with the risk of pulmonary tuberculosis treatment failure at the José Silveira Foundation Brazilian Institute for Tuberculosis Research, Salvador, Brazil, 2007-2015.

Variable	Model	OR (95% CI)
Somoking[a]	Saturated	2.2 (1.1 - 4.7)
	Final	2.1 (1.1 - 4.1)
Age > 50 years	Saturated	2.8 (1.4 - 5.6)
	Final	2.2 (1.4 - 6.0)



[a]Before and during treatment. Saturated model adjusted for smoking, age, gender, level of education, marital status, income, and alcohol consumption. Final model adjusted for smoking and age.

Table 3. Associations between smoking and pulmonary tuberculosis treatment failure, by gender, among cases (of treatment failure) and controls at the José Silveira Foundation Brazilian Institute for Tuberculosis Research, Salvador, Brazil, 2007-2015.

Gender	Smoking status	Cases (n = 50) n (%)	Controls (n = 234) n (%)	OR	95% CI
Male	Current or former smoker	19 (57.6)	55 (37.7)	2.25	1.04-4.83
	Current smoker	15 (45.5)	31 (21.2)	3.15	1.37-7.25
	Former smoker	4 (12.1)	24 (16.4)	1.08	0.28-3.48
	Never smoker	14 (42.4)	91 (62.3)	Reference values	
Female	Current or former smoker	7 (41.2)	16 (6.8)	3.15	1.04-9.53
	Current smoker	5 (29.4)	6 (6.8)	6.00	1.54-23.35
	Former smoker	2 (11.8)	10 (11.4)	1.43	0.19-7.04
	Never smoker	10 (58.8)	72 (81.8)	Reference values	

at the time of diagnosis of pulmonary tuberculosis was 15.8%. That is higher than the prevalence of smoking in Brazil and in the city of Salvador, which was 12.0% and 5.1%, respectively, in 2016.[24] Other authors have also found a high prevalence of smoking among tuberculosis patients.[25,26]

There have been few studies addressing the associations of gender and smoking with poor pulmonary tuberculosis treatment outcomes, because the trend is to use age-matched controls or to select only men.[27-30] There is a disconnect between studies showing that gender is not associated with poor tuberculosis treatment outcomes[31,32] and those showing that being male is the strongest predictor of a poor outcome.[33,34] In our stratified analysis, female smokers were at greater risk of treatment failure than were male smokers, although the difference was not statistically significant, perhaps because of the small number of women in our sample. One likely explanation is related to the context or the socioeconomic profile of women, in terms of variables such as malnutrition, access to health care services, and stigma.[35,36] In addition, there is a trend toward gender equality in smoking prevalence rates with the passing of the epidemic.[37-39] We believe that this association should be further explored in future studies and that reporting of poor tuberculosis treatment outcomes by gender should be encouraged.

Although our study covered a relatively long period of time (8 years), its major limitation is that the number of available cases was small and, consequently, the sample size was smaller than the required sample size calculated, because treatment failure is an uncommon event, as was observed in a cohort in the state of Pernambuco, Brazil, in which the reported treatment failure rate was 2.1%.[40] However, the final number of patients included was large enough to demonstrate the association and achieve the major objective. Another important limitation is that inherent to retrospective studies, which is the use of clinical records to try to obtain data, because complete data were not available for all patients. Nevertheless, it was possible to adjust the model for the main variables described in the literature as potential confounders,[6,19] and we managed to use other data sources in addition to clinical records.

Smoking is a modifiable risk factor that has a major impact on pulmonary tuberculosis. To meet the objective of reducing the prevalence of smokers in the general population, in order to reduce the risk of pulmonary tuberculosis and poor treatment outcomes such as treatment failure, it is necessary to devise tuberculosis control strategies that include anti-smoking interventions, such as offering smoking cessation treatment at the time of diagnosis, as a means of achieving success in the treatment of tuberculosis.

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Pulmonary nontuberculous mycobacterial infections: presumptive diagnosis based on the international microbiological criteria adopted in the state of São Paulo, Brazil, 2011-2014

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ABSTRACT

Objective: Pulmonary nontuberculous mycobacterial infections are caused by nontuberculous mycobacteria (NTM), the microbiological diagnosis of which involves the isolation and identification of the same species in at least two sputum samples, one BAL fluid sample, or one lung biopsy sample. The objective of the present study was to determine the frequency at which the various NTM species are identified among selected individuals and in potential cases of pulmonary nontuberculous mycobacterial infection. **Methods:** This was a retrospective analysis of the data on species isolated from respiratory specimens collected from 2,843 individuals between 2011 and 2014. Potential NTM infection cases were identified on the basis of the international microbiological criteria adopted in the state of São Paulo. **Results:** A total of 50 species were identified using the molecular method PCR-restriction enzyme analysis. Samples collected from 1,014 individuals were analyzed in relation to the microbiological criteria, and 448 (44.18%) had a presumptive diagnosis of pulmonary nontuberculous mycobacterial infection, the species identified most frequently being, in descending order, *Mycobacterium kansasii*, *M. abscessus*, *M. intracellulare*, *M. avium*, and *M. szulgai*. **Conclusions:** Although various NTM species were identified among the individuals studied, those presumptively identified most frequently on the basis of the microbiological criteria adopted in the state of São Paulo were the ones that are most commonly associated with pulmonary nontuberculous mycobacterial infection worldwide or in specific geographic regions.

Keywords: Nontuberculous mycobacteria/classification; Mycobacterium infections, nontuberculous/diagnosis; Lung.

INTRODUCTION

Nontuberculous mycobacteria (NTM) are *Mycobacterium* spp. other than *M. leprae* and those that constitute the *M. tuberculosis* complex. NTM are widely distributed in nature, and some have been associated with human diseases that are characterized by pulmonary/extrapulmonary manifestations that are collectively known as nontuberculous mycobacterial infections. In addition to differences in virulence among NTM, several host factors play a determinant role in the development of clinical manifestations of pulmonary nontuberculous mycobacterial infection, including structural lung changes caused by previous tuberculosis, cystic fibrosis, COPD, silicosis, and pneumoconiosis, as well as conditions that affect the immune system, such as alcoholism, smoking, and treatment with immunosuppressive drugs.[1-3]

The diagnosis of pulmonary nontuberculous mycobacterial infection is highly complex and requires a combination of clinical, radiological, and microbiological findings, as established by the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) in 2007.[4] The clinical presentation of pulmonary nontuberculous mycobacterial infection is variable and nonspecific, and radiological features include cavitary and nodular lesions. The microbiological diagnosis of pulmonary nontuberculous mycobacterial infection involves the isolation and identification of the same NTM species in at least two sputum samples, one BAL fluid sample, or one lung biopsy sample. Although the ATS/IDSA did not specify the time interval between the first and second sputum sample collections in an official statement published in 2007,[4] it had previously been reported that a 12-month interval is required for positive culture results.[5]

Because pulmonary nontuberculous mycobacterial infections are difficult to diagnose and because reporting is not compulsory, it is difficult to establish epidemiological markers; however, evidence suggests that the prevalence of pulmonary nontuberculous mycobacterial infections has steadily increased in recent years.[3,6] The two NTM species that originally constituted the *M. avium complex* (MAC), namely *M. avium* and *M. intracellulare*,[7] as well as *M. abscessus*, are the NTM species that are most commonly associated with pulmonary nontuberculous mycobacterial infection worldwide, whereas others appear

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to be more common in certain geographic regions.[3] In Brazil, studies employing the ATS/IDSA criteria[4] have shown that the aforementioned species and *M. kansasii* are the most prevalent causes of pulmonary nontuberculous mycobacterial infection in the states of Rio de Janeiro[8] and Rio Grande do Sul.[9]

The frequency at which the various NTM species are identified in individuals with pulmonary nontuberculous mycobacterial infection has been determined on the basis of the ATS/IDSA microbiological criteria alone.[10-12] The present study is a retrospective analysis of the data on NTM species isolated from respiratory specimens in a referral laboratory in the state of São Paulo, Brazil, between 2011 and 2014. Our objective was to determine the frequency at which the various NTM species are identified among selected individuals and in potential cases of pulmonary nontuberculous mycobacterial infection on the basis of microbiological criteria.

METHODS

The *Instituto Adolfo Lutz* (IAL, Adolfo Lutz Institute) is a referral laboratory in the state of São Paulo. In addition to the IAL headquarters (a referral center for tuberculosis and nontuberculous mycobacterial infections in the city of São Paulo, Brazil), there are twelve regional laboratories strategically located throughout the state of São Paulo. Mycobacterial cultures are performed in the regional laboratories and in approximately 80 others (either public laboratories or laboratories affiliated with the Brazilian Unified Health Care System), and all positive cultures are sent to the IAL headquarters for identification. All patient data provided by the regional laboratories and the results of isolate identification are recorded in a laboratory information management system (LIMS). For the present study, the results of isolate identification for the period between 2011 and 2014 were entered into Microsoft Excel 2007 spreadsheets (one for each year), data on NTM species isolated from respiratory specimens being subsequently selected and entered into a new Microsoft Excel 2007 spreadsheet.

In the new spreadsheet, the first record of each individual was selected in order to identify patients for inclusion in the study ("selected patients"), and new spreadsheets were created for each NTM species identified during the study period. Patient laboratory records were reviewed in order to identify patients meeting the requirements for analysis of the ATS/IDSA microbiological criteria for the diagnosis of pulmonary nontuberculous mycobacterial infection,[4] all data being entered into the aforementioned spreadsheets. Subsequently, spreadsheets were created for those who met the requirements for analysis of the ATS/IDSA diagnostic criteria ("analyzed patients") and those who had a presumptive diagnosis based on the microbiological criteria ("identified patients") for each NTM isolate.

All NTM species were identified by PCR-restriction enzyme analysis (PRA), which consists of amplification of a 441-bp fragment of the *hsp65* gene, followed by digestion of the amplified product with two restriction enzymes (BstEII and HaeIII) and analysis of the fragments separated by agarose gel electrophoresis. The various NTM species have different restriction patterns, which can be used for species identification by means of an algorithm.[13]

The present study was approved by the Technical and Scientific Advisory Committee and the Research Ethics Committee of the IAL (CTC no. 07-G/2014).

RESULTS

Between 2011 and 2014, a total of 5,392 NTM were isolated from respiratory specimens from a total of 3,883 individuals. The *hsp65* PRA method failed to identify the NTM species in 881 patients (22.69%), isolates being phenotypically characterized as slowly growing mycobacteria (n = 630) or rapidly growing mycobacteria (n = 251). A restriction pattern suggestive of mixed culture (i.e., indicative of the presence of NTM and *M. tuberculosis* complex) was found in samples from 159 patients (4.09%). Therefore, 2,843 patients were selected for inclusion in the study.

Of the 2,843 patients selected for inclusion in the study, only 1,014 (35.67%) met the requirements for analysis of the ATS/IDSA microbiological criteria[4] for the diagnosis of pulmonary nontuberculous mycobacterial infection. A total of 50 species were identified, and Table 1 shows the frequency at which they were identified among selected patients and analyzed patients. In both groups of patients, 6 NTM species were most common, the proportions for analyzed/selected patients being as follows, in descending order: *M. abscessus*, *M. kansasii*, *M. avium*, *M. fortuitum*, *M. intracellulare*, and *M. gordonae*.

As can be seen in Table 2, 448 (44.18%) of the 1,014 patients who were analyzed in relation to the microbiological criteria were identified as potential cases of pulmonary nontuberculous mycobacterial infection. The remaining 566 had one culture with NTM growth and, subsequently, no mycobacterial growth on culture. Among the patients who were identified as potential cases of pulmonary nontuberculous mycobacterial infection, the NTM species identified most frequently were *M. kansasii* (66.50%) and *M. abscessus* (64.71%), followed by *M. intracellulare* (60.69%) and *M. avium* (46.97%). *M. gordonae* and *M. fortuitum* were found in a considerable number of analyzed patients. However, among those who were identified as potential cases of pulmonary nontuberculous mycobacterial infection, they were found in only 20.62% and 13.68%, respectively.

A total of 19 NTM species were identified among the 568 patients who had only one positive culture (the others being negative) and who therefore were not presumptively diagnosed with pulmonary nontuberculous mycobacterial infection. The 19 NTM species identified were as follows: *M. asiaticum*, *M. chitae*, *M. flavescens*, *M. florentinum*, *M. gastri*, *M. genavense*, *M. goodii*, *M. heidelbergense*, *M. immunogenum*, *M. kumamotonense*,

Table 1. Frequency and proportion of nontuberculous mycobacterial species among the patients selected for the study and those analyzed in relation to the microbiological criteria for the diagnosis of pulmonary nontuberculous mycobacterial infections in the state of São Paulo, Brazil, 2011-2014.

Species	Patients, n (%)		Analyzed/selected ratio, %
	Selected (n = 2,843)	Analyzed (n = 1,014)	
Mycobacterium avium	519 (18.26)	198 (19.53)	38.15
M. kansasii	453 (15.93)	197 (19.43)	43.49
M. intracellulare	423 (14.88)	145 (14.30)	34.28
M. gordonae	374 (13.16)	97 (9.57)	25.94
M. fortuitum	310 (10.90)	117 (11.54)	37.74
M. abscessus	219 (7.70)	119 (11.74)	54.34
M. peregrinum	143 (5.03)	35 (3.45)	24.48
M. chelonae	96 (3.38)	19 (1.87)	19.79
M. mucogenicum	66 (2.32)	16 (1.58)	24.24
M. lentiflavum	46 (1.62)	9 (0.89)	19.57
M. simiae	26 (0.91)	7 (0.69)	26.92
M. szulgai	19 (0.67)	11 (1.08)	57.89
M. asiaticum	16 (0.56)	6 (0.59)	37.50
M. florentinum	15 (0.53)	1 (0.10)	6.67
M. chitae	14 (0.49)	4(0.39)	28.57
M. goodii	14 (0.49)	5 (0.49)	35.71
M. nonchromogenicum	12 (0.42)	6 (0.59)	50.00
M. neoaurum	8 (0.28)	2 (0.20)	25.00
M. parascrofulaceum	8 (0.28)	2 (0.20)	25.00
M. novocastrense	7 (0.25)	1 (0.10)	14.29
M. xenopi	6 (0.21)	3 (0.30)	50.00
M. terrae	5 (0.18)	1 (0.10)	20.00
M. immunogenum	4 (0.14)	1 (0.10)	25.00
M. heidelbergense	3 (0.11)	1 (0.10)	33.33
M. kumamotonense	3 (0.11)	1 (0.10)	33.33
M. nebraskense	3 (0.11)	3 (0.30)	100.00
M. celatum	2 (0.07)	1 (0.10)	50.00
M. flavescens	2 (0.07)	1 (0.10)	50.00
M. gastri	2 (0.07)	1 (0.10)	50.00
M. parmense	2 (0.07)	1 (0.10)	50.00
M. sherrisii	2 (0.07)	1 (0.10)	50.00
M. brisbanense	2 (0.07)	0	-
M. monacense	2 (0.07)	0	-
M. triviale	2 (0.07)	0	-
M. genavense	1 (0.04)	1 (0.10)	100.00
M. triplex	1 (0.04)	1 (0.10)	100.00
Others[a]	13 (0.46)	0	-

[a]Thirteen species for which only one patient was selected: *M. arupense, M. aubagnense, M. branderi, M. brumae, M. conceptionense, M. farcinogenes, M. hiberniae, M. kubicae, M. montefiorense, M. scrofulaceum, M. senegalense, M. shimoidei*, and *M. wolinskyi*.

M. mucogenicum, *M. nebraskense*, *M. neoaurum*, *M. nonchromogenicum*, *M. novocastrense*, *M. parmense*, *M. terrae*, *M. triplex*, and *M. xenopi*.

DISCUSSION

The identification of NTM species is extremely important because the clinical relevance and antimicrobial treatment of pulmonary nontuberculous mycobacterial infections are determined on the basis of the characteristics of the NTM species. Although the molecular method used in the present study (i.e., the *hsp65* PRA method) is more rapid and specific than phenotypic methods, some of its limitations include the fact that PCR inhibitors interfere with the sample and the fact that the available algorithm does not define the restriction patterns of the species. In the present study, the *hsp65* PRA method failed to identify the NTM isolates from 22.68% of the selected patients, the species being phenotypically characterized. Esparcia et al.[14] used the same algorithm that was used in the

Table 2. Species of nontuberculous mycobacteria isolated from respiratory specimens from patients analyzed in relation to microbiological criteria for the diagnosis of pulmonary nontuberculous mycobacterial infections, as well as frequency of patients identified as potential cases of pulmonary nontuberculous mycobacterial infection in the state of São Paulo, Brazil, 2011-2014.

Species	Number of patients		
	Analyzed	Identified	%
Mycobacterium kansasii	197	131	66.50
M. abscessus	119	77	64.71
M. intracellulare	145	88	60.69
M. avium	198	93	46.97
M. szulgai	11	5	45.45
M. peregrinum	35	8	22.86
M. chelonae	19	4	21.05
M. gordonae	97	20	20.62
M. goodii	5	1	20.00
M. simiae	7	1	14.29
M. fortuitum	117	16	13.68
M. lentiflavum	9	1	11.11
Others[a]	55	3[b]	5.45
Total	1,014	448	44.18

[a]Twenty-one species (for 15, only 1 or 2 patients were analyzed). [b]*M. celatum*, *M. parascrofulaceum*, and *M. sherrisii.*

present study and reported that the *hsp65* PRA method failed to identify 32 (23.88%) of 134 NTM samples.

Although a wide variety of NTM species were identified in the present study, the most frequently identified species among selected and analyzed patients were, in descending order, *M. avium*, *M. kansasii*, *M. intracellulare*, *M. gordonae*, *M. fortuitum*, and *M. abscessus*. In a study conducted in the state of Rio Grande do Sul in the 2003-2013 period, the most frequently identified species were *M. avium*, *M. kansasii*, and *M. intracellulare*, followed by *M. abscessus*, *M. fortuitum*, and *M. gordonae*.[9] Between 1993 and 2011 in the state of Rio de Janeiro, the most frequently identified species was *M. kansasii*, followed by MAC, *M. abscessus*, and *M. fortuitum*.[8] It is of note that *M. abscessus* was the most frequently identified species among analyzed patients in the present study. This might be due to the fact that *M. abscessus* is resistant to many antimicrobial agents[3]—a fact that impedes patient clinical improvement—as well as to the fact that health professionals have limited knowledge of the relevance of *M. abscessus*.

Less than 40% of the selected patients in the present study met the requirements for analysis of the microbiological criteria for the diagnosis of pulmonary nontuberculous mycobacterial infection. This is probably due to the fact that the clinical specimens obtained after NTM identification were examined in laboratories where the IAL-LIMS is not used, screening therefore being impossible when the results were negative. However, in many patients for whom NTM cultures were performed in laboratories where the IAL-LIMS is used, no subsequent specimens were sent for analysis. This is probably due to loss to follow-up and a lack of knowledge on the part of the health care team regarding the need for multiple sputum examinations for laboratory diagnosis of pulmonary nontuberculous mycobacterial infection.

Given that NTM are widely distributed in nature, the isolation of NTM from a clinical specimen can represent colonization, infection, or pseudoinfection. Colonization is defined as the presence of NTM in the host microbiota without clinical manifestations, whereas infection is defined as the presence of NTM in the host microbiota with clinical manifestations. When neither colonization nor infection can be unequivocally confirmed, the possibility of pseudoinfection should be considered, pseudoinfection being usually caused by contamination during clinical specimen handling. There have been reports of outbreaks of nosocomial colonization and pseudoinfection with NTM primarily due to contaminated potable water supplies and inadequately disinfected medical equipment.[15]

In the present study, 14 NTM species led to a presumptive diagnosis of pulmonary nontuberculous mycobacterial infection based on microbiological criteria. The number of analyzed patients and the proportion of patients identified as potential cases of pulmonary nontuberculous mycobacterial infection were highest for the NTM species on which the ATS/IDSA criteria were based,[4] i.e., *M. avium*/*M. intracellulare* (MAC), *M. kansasii*, and *M. abscessus*. As in the present study, *M. fortuitum*, *M. peregrinum*, *M. chelonae*, and *M. szulgai* led to a presumptive diagnosis of pulmonary nontuberculous mycobacterial infection in a study conducted in Japan and employing microbiological criteria.[12] The fact that individuals with one positive culture[16] or more than one positive culture[17] for *M. fortuitum* were monitored for months and did not progress to lung disease was attributed to the fact that *M. fortuitum* is a low-virulence organism. *M. gordonae* is also considered to be a low-virulence strain and has been associated with several outbreaks of nosocomial mycobacterial pseudoinfection.[18]

Winthrop et al.[19] found that 183 (86%) of 214 patients with a presumptive diagnosis of pulmonary nontuberculous mycobacterial infection based on the ATS/IDSA microbiological criteria[4] had a confirmed diagnosis of pulmonary nontuberculous mycobacterial infection after analysis of clinical and radiological features, the authors having concluded that the ATS/IDSA microbiological criteria[4] are highly predictive of pulmonary nontuberculous mycobacterial infection. According to the ATS/IDSA, patients who are suspected of having pulmonary nontuberculous mycobacterial infection but do not meet the diagnostic criteria should be monitored until the diagnosis is firmly established or excluded.[4] Extensive clinical and laboratory monitoring of NTM isolates from clinical specimens obtained from patients with respiratory symptoms allows assessment of the relevance of NTM species, especially with regard to potential risk factors.

To our knowledge, ours is the first study in Brazil to determine the frequency at which the various NTM species are identified in a large number of individuals in a large geographic area rather than in a large number of respiratory specimens. The present study has limitations inherent to all retrospective studies, including missing information on patient medication use when multiple cultures are performed, the lack of which is minimized by innate NTM resistance to antimicrobials. Although various NTM species were identified in the individuals studied, those presumptively identified most frequently on the basis of the international microbiological criteria used in the state of São Paulo were the ones that are most commonly associated with pulmonary nontuberculous mycobacterial infection worldwide or in specific geographic regions. Health professionals should know more about pulmonary nontuberculous mycobacterial infections. There is a need for prospective multidisciplinary studies in which all of the diagnostic criteria established by the ATS/IDSA[4] are used and all of the characteristics of study participants are determined.

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Performance of diagnostic tests for pulmonary tuberculosis in indigenous populations in Brazil: the contribution of Rapid Molecular Testing

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ABSTRACT

Objective: To evaluate the accuracy of rapid molecular testing as a diagnostic tool and estimate the incidence of smear-positive pulmonary tuberculosis among the indigenous population. **Methods:** This is an epidemiological study based on secondary data. We calculated the incidence of smear-positive pulmonary tuberculosis between January 1st, 2011 and December 31, 2016, and the performance of bacilloscopy and rapid molecular testing in diagnosing pulmonary tuberculosis compared to sputum culture (standard test). **Results:** We included 4,048 cases of indigenous people with respiratory symptoms who provided sputum samples for analysis. Among them, 3.7%, 6.7%, and 3.7% had positive results for bacilloscopy, sputum culture, and rapid molecular testing, respectively. The mean incidence of pulmonary tuberculosis was 269.3/100 thousand inhabitants. Rapid molecular testing had 93.1% sensitivity and 98.2% specificity, compared to sputum culture. Bacilloscopy showed 55.1% sensitivity and 99.6% specificity. **Conclusions:** Rapid molecular testing can be useful in remote areas with limited resources and a high incidence of tuberculosis, such as indigenous villages in rural regions of Brazil. In addition, the main advantages of rapid molecular testing are its easy handling, fast results, and the possibility of detecting rifampicin resistance. Together, these attributes enable the early start of treatment, contributing to reduce the transmission in communities recognized as vulnerable to infection and disease.

Keywords: Tuberculosis; Molecular diagnostic testing; Diagnostic tests, routine; Indians, South American.

INTRODUCTION

Tuberculosis (TB) is a severe infectious disease that affects millions of people worldwide each year, causing losses to society, especially in developing countries. In 2016, an estimated 10.4 million people fell ill with TB, 1.8 million died, and 480 thousand new cases of multidrug-resistant TB (MDRTB) were reported.[1,2]

Early diagnosis and the timely start of drug therapy, associated with the search for respiratory symptomatic patients (RSPs), are considered key actions to control the disease. These actions combined have the potential to block the transmission chain and, consequently, reduce incidence and mortality rates, in addition to preventing the emergence of drug-resistant cases.[3]

The most common diagnostic tests for pulmonary TB are bacilloscopy and sputum culture. Bacilloscopy is more used for being simple, fast, and low-cost; however, its sensitivity is low, and it fails to diagnose approximately 50% of suspicious cases – particularly those that have a small bacillary load.[4]

On the other hand, sputum culture, both in solid medium (Löwenstein-Jensen and Ogawa-Kudoh) and liquid medium (MGIT – mycobacteria growth indicator tube), is considered the standard diagnostic test, as it detects 70 to 90% of cases, and has virtually 100% specificity. Nonetheless, cultures in solid medium must be incubated at 37°C and observed weekly until colonies appear. In positive cases, the minimum time for diagnosis is approximately 14 days. In negative cases – when no colonies grow –, the observation period can reach 60 days (incubation period of the microorganism).[4] The long waiting time for a conclusive culture result postpones the start of specific treatment, leading to a delay in interrupting the transmission chain, and negatively contributing to control the disease.[5]

To reduce the time to diagnosis and start of treatment, the World Health Organization (WHO) approved and recommended the use of rapid molecular testing (RMT) GeneXpert®, in 2010 (CEPHEID AB Röntgenvägen 5 SE-171 54 Solna Sweden) . RMT is a nucleic acid amplification test used to detect the *Mycobacterium tuberculosis* complex (MTBC). Its main advantages are fast results (approximately 2 hours) and identification of patients with resistance to rifampicin – one of the primary drugs in the standard treatment regimen.[3]

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After TB control programs incorporated RMT in their routines, several validation studies have been conducted.[6] A systematic literature review revealed that its sensitivity ranged from 72.5% to 98.2% in samples with negative and positive bacilloscopy, respectively, and its specificity was near 99.0%. Also, RMT is easy to handle, safe with regard to biosafety, and not susceptible to cross-contamination.[6]

In Brazil, RMT validation studies have analyzed different scenarios and contexts; however, until now, no work was conducted among indigenous populations, recognized as vulnerable to illness.

The objective of this work was to evaluate the accuracy of RMT as a diagnostic tool among the indigenous population.

METHODS

Study area and population

The study was carried out using records of indigenous people with productive cough for 2 weeks or more – now considered RSPs –,[7] identified in the routine of the Health Care Service for Indigenous People at Polo Base Amambai, part of the Special Indigenous Health District of Mato Grosso do Sul (*Distrito Sanitário Especial Indígena Mato Grosso do Sul* – DSEI/MS).

This Polo Base is located in the city of Amambai, 350 km from the capital Campo Grande, in the southern region of the state of Mato Grosso do Sul. According to the last national census, conducted in 2010, Amambai had 34,730 inhabitants;[8] 12,916 of them were indigenous people, and most belonged to the Guarani-Kaiowá ethnicity.

Polo Base Amambai is responsible for the care of indigenous people living in the villages Amambai, Limão Verde, Taquaperi, Guassuty, and Jaguari, distributed in three different cities (Amambai, Aral Moreira, and Coronel Sapucaia) located in the region of the international border with Paraguay.

We chose this location because it concentrated the largest number of TB cases among indigenous people living in Mato Grosso do Sul[9] and for having a laboratory that performs bacilloscopy since 2004 and sputum cultures since 2006. In the second half of 2014, the laboratory of Polo Base Amambai received one piece of equipment that performed RMT from the Ministry of Health.

One of the goals of multidisciplinary indigenous health teams (*equipes multidisciplinares de saúde indígena* – EMSI) that work at DSEI/MS is to find RSPs, either by active search in the community or by care in community health centers in the villages. According to the recommended guidelines for TB control in Brazil,[7] once RSPs are identified in the community, two sputum samples must be collected. In our study area, the samples collected in the EMSI routine are sent to the laboratory of Polo Base Amambai, where they are used for bacilloscopy and RMT. Next, they are seeded in Ogawa-Kudoh medium, and stored in an oven at 37º C. Samples from media with colony growth are sent to the Central Public Health Laboratory of Mato Grosso do Sul (*Laboratório Central de Saúde Pública do Mato Grosso do Sul* – LACEN/MS) to identify the MTBC and perform drug sensitivity tests. Macroscopic and microscopic examinations differentiated MTBC from nontuberculous mycobacteria (NTM). Also, the laboratory used an immunochromatographic assay to detect the presence of MTP64 in MTBC and a medium with p-nitrobenzoic acid to inhibit MTBC growth.

Study Design

We conducted a cross-sectional observational epidemiological study, which estimated the incidence of smear-positive pulmonary TB, according to the village of residence and year of notification. In addition, we analyzed the accuracy (sensitivity and specificity) of sputum bacilloscopy (January 1st, 2011 to December 31, 2016) and RMT (July 1st, 2014 to December 31, 2016), adopting the culture in Ogawa-Kudoh medium as the standard test.

Inclusion and exclusion criteria

We included all RSPs among the indigenous population who provided a sputum sample and excluded those who did not live in the coverage area of Polo Base Amambai and who had bacilloscopy and/or RMT performed to monitor the treatment of a previously diagnosed TB.

Data source

We consulted the records from the RSP investigation book from EMSI, the results of the tests performed in the laboratory of Polo Base, and population data from the demographic module of the Indigenous Health Care Information System (*Sistema de Informação da Atenção à Saúde Indígena* – SIASI). In addition, we checked the test results by consulting the Management System of Laboratory Environment (*Sistema Gerenciador de Ambiente Laboratorial* – GAL) at LACEN/MS.

Study variables

We investigated the RSP distribution according to gender, age group (0 |- 10 years; 10 |- 20; 20 |- 40; 40 |- 60; 60 and +), village of residence, and test results – sputum bacilloscopy (positive, negative, and not performed); RMT (positive, negative, and not performed), and sputum culture (positive, negative, not performed, NTM, contaminated, and no results).

Data analysis

We analyzed the data in the software Statistical Package for the Social Sciences, version 20.0 (SPSS Inc., Chicago, IL, USA). First, we assessed data completeness; next, we corrected the missing data by consulting GAL-LACEN/MS. Subsequently, we calculated incidence rates of smear-positive pulmonary TB by village and year of diagnosis. The numerator represented TB cases confirmed by sputum culture,

and the denominator, the population at risk in each village, from year to year.

Using 2000 and 2010 census data related to indigenous ethnicity from the Brazilian Institute of Geography and Statistics (*Instituto Brasileiro de Geografia e Estatística* – IBGE) as a reference, we calculated the annual growth rate (α) for the city of Amambai, adopting the geometric progression method, according to the following formula:

$$\propto=\{[(P_2/P_1)\hat{}(1/10)]-1\}$$

in which P_1 corresponds to the population surveyed by IBGE in 2000; and P_2, the population surveyed in 2010.

Afterward, we estimated the populations from 2011 to 2016. Population data by village were collected from the SIASI demographic module.

We conducted a descriptive data analysis, and calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the first and second samples, and of them combined after sputum bacilloscopy and RMT.

Ethical Aspects

This study is part of the project Social Inequalities and Tuberculosis: Transmission, Living Conditions, and Interfaces between Biomedicine and Traditional Indigenous Medicine, approved by the Research Ethics Committee of Escola Nacional de Saúde Pública and the National Research Ethics Committee (reports 354,060 and 650, 820).

RESULTS

There were 4,496 RSPs registered, from which 448 were excluded for not living in the researched villages, leaving 4,048 subjects for analysis. On average, 674 RSPs were identified annually (Table 1), corresponding to a proportion of 4.8% in the study period. The Guassuty and Limão Verde villages had the greatest percentages of RSPs, with 9.1% and 6.7%, respectively.

Except for the Limão Verde village, which had a slightly higher number of men among the RSPs (51.3%), in other villages, most RSPs consisted of women (54.7%). The mean RSP age was 37.4 years (standard deviation: 20.5; ranging from zero to 99 years). In the Taquaperi village, 7.0% of RSPs were children aged < 10 years (Table 2).

The mean annual incidence rate of pulmonary TB was 269.3/100 thousand inhabitants in the region. However, we found significant variations among the villages. In the Jaguari village, the mean incidence rate was 428.2/100 thousand, but it reached 2,420.5/100 thousand in 2012. The Amambai village had the lowest mean incidence rate (218.6/100 thousand).

Bacilloscopy was the most used test (86.7%). However, it was also the one that presented the lowest positivity (131/3,509). In 2015 and 2016, this test had the lowest proportions of performance (35.4% and 39.2%, respectively).

In turn, sputum culture was performed in 83.3% of tests, having a mean positivity of 6.7% (225/3,370) in the period. The Jaguari village showed the highest positivity (7.5% or 10/134). We underline that sputum culture was not used in 42.9% (267/663) of RSP samples in 2011. In addition, 12.0% of samples (405/3,370) were contaminated and 28 NTM cases (0.8%) were detected.

From 2014 to 2016, 557 RMT were carried out with a mean positivity of 3.7% (70/1,987). In 2016, positivity reached 5.6% (34/610). RMT also revealed two cases with an indeterminate pattern of rifampicin resistance, but both showed negative culture results. On the other hand, a case of rifampicin resistance detected by RMT was confirmed by LACEN/MS.

After assessing all tests together (considering the first and second samples), bacilloscopy sensitivity and specificity were 55.1% and 99.6%, respectively. PPV was 91.5%, and NPV was 96.7%. RMT reached 93.1% sensitivity and 98.3% specificity. PPV and NPV were 88.5% and 99.0%, respectively (Table 3).

The results of the first sputum sample revealed that bacilloscopy had 46.4% sensitivity, 99.7% specificity, 90.6% PPV, and 97.0% NPV, while RMT presented 95.3% sensitivity, 98.5% specificity, 87.2% PPV, and 99.5% NPV.

The result analysis of the second sample showed that bacilloscopy sensitivity increased to 79.9%, and specificity decreased to 97.9%, while PPV was 92.7%, and NPV, 92.9%. RMT sensitivity and specificity dropped to 86.7% and 93.7%, respectively, with 92.9% PPV and 88.2% NPV.

DISCUSSION

Our findings revealed that incidence rates in the region of Polo Base Amambai are extremely high, and the number of RSPs was greater than the expected in non-indigenous populations in Brazil.[7] RMT showed high sensitivity and specificity in detecting cases, both in the first and second sputum samples in the villages investigated. In a scenario in which TB presents high endemic levels for over a decade,[9,10] and technical, financial, and qualified human resources are scarce, RMT proved to be an excellent tool for a correct and early diagnosis, contributing to the timely start of treatment. In theory, these characteristics have the potential to block the transmission chain of the disease among this population, known for their vulnerability to TB.

The mean incidence rates revealed here were nearly eight times higher than those registered in the Brazilian population in 2016 (32.4/100 thousand inhabitants).[11,12] Incidence rates above the national average have been reported among the indigenous populations of Mato Grosso do Sul [9,10,13,14] and in the North region of the country, [15-20], leaving no doubt that TB represents a serious public health issue among these populations. It is noteworthy that the incidence rates presented in this study concern only cases of smear-positive

Table 1. Proportion of respiratory symptomatic patients (RSPs), incidence of smear-positive pulmonary tuberculosis in the villages, and diagnostic tests (bacilloscopy, rapid molecular testing, and sputum culture) performed in RSPs.

	2011		2012		2013		2014		2015		2016		Total	
RSPs in the villages	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Amambai	204	2.7	271	3.5	321	4.1	443	5.5	370	4.4	258	1.7	1,867	3.9
Taquaperi	236	7.9	212	6.9	173	5.5	123	3.8	143	4.2	139	3.1	1,026	5.3
Limão Verde	130	8.3	117	7.3	94	5.7	91	5.3	96	5.5	152	3.6	680	6.7
Guassuty	39	6.8	106	17.8	57	9.3	53	8.4	37	5.7	49	11.6	341	9.1
Jaguari	14	3.9	41	11	46	12	14	3.5	7	1.7	12	14.4	134	5.7
Total	623	4.8	747	5.6	691	5	724	5.1	653	4.5	610	0.7	4,048	4.8
Incidence of pulmonary tuberculosis														
Amambai	11	148.1	24	313.7	18	228.4	16	197.1	20	239.3	16	185.8	105	218.6
Taquaperi	10	334.4	4	129.9	6	189.1	10	306.1	12	356.6	13	375	55	284.4
Limão Verde	5	320.3	4	248.8	7	422.7	8	469	8	455.3	11	607.9	43	425.9
Guassuty	7	1213.2	1	168.3	2	326.7	0	0	1	154	1	149.5	12	321.5
Jaguari	1	277	9	2,420.50	0	0	0	0	0	0	0	0	10	428.2
Total	34	263.2	42	315.7	33	240.8	34	240.9	41	282	41	273.8	225	269.3
Bacilloscopy														
Positive	26	4.2	24	3.2	27	3.9	28	3.9	19	2.9	7	1.1	131	3.2
Negative	575	92.3	702	94	653	94.5	681	94.1	403	61.7	364	59.7	3,378	83.4
Not performed	22	3.5	21	2.8	11	1.6	15	2.1	231	35.4	239	39.2	539	13.3
Total	623		747		691		724		653		610		4,048	
Rapid molecular testing														
Positive	-	-	-	-	-	-	6	0.8	30	4.6	34	5.6	70	3.5
Negative	-	-	-	-	-	-	29	4	270	41.3	188	30.8	487	24.5
Not performed	-	-	-	-	-	-	689	95.2	353	54.1	388	63.6	1430	72
Total	-	-	-	-	-	-	724		653		610		1987	
Culture														
Positive	34	5.5	42	5.6	33	4.8	34	4.7	41	6.3	41	6.7	225	5.6
Negative	252	40.4	540	72.3	473	68.5	547	75.6	449	68.8	441	72.3	2702	66.7
Not performed	267	42.9	91	12.2	111	16.1	77	10.6	88	13.5	44	7.2	678	16.7
Nontuberculous mycobacteria	1	0.2	7	0.9	10	1.4	1	0.1	3	0.5	6	1	28	0.7
Contaminated	69	11.1	67	9	64	9.3	65	9	72	11.1	68	11.1	405	10
No results	0		0		0		0		0		10	0.2	10	0.2
Total	623		747		691		724		653		610		4048	

Table 2. Age group and gender of RSPs identified in the villages

	Amambai		Taquaperi		Limão Verde		Guassuty		Jaguari		Total	
Age group, years	n	%	n	%	n	%	n	%	n	%	n	%
0-10	83	4.4	72	7	29	4.3	16	4.7	1	0.7	201	5
10-20	324	17.4	150	14.6	114	16.8	26	7.6	34	25.4	648	16
20-40	752	40.3	424	41.3	302	44.5	132	38.7	64	47.8	1674	41.4
40-60	390	20.9	212	20.7	146	21.5	74	21.7	20	14.9	842	20.8
60 and +	318	17	168	16.4	88	13	93	27.3	15	11.2	682	16.9
Total	1,867		1,026		679		341		134		4,047	
Gender												
Female	1,071	57.4	537	52.3	331	48.7	202	59.2	73	54.5	2,214	54.7
Male	796	42.6	489	47.7	349	51.3	139	40.8	61	45.5	1,834	45.3
Total	1,867		1,026		680		341		134		4,048	

pulmonary TB, that is, those bacteriologically confirmed by sputum culture.

According to the epidemiological bulletin of the Ministry of Health, sputum culture was performed in only 36.6%

Table 3. Performance of bacilloscopy and rapid molecular testing (RMT) compared to sputum culture (standard test) in the first and second sputum samples, and in both samples combined

All samples							
RMT performance				Bacilloscopy performance			
RMT	Positive	Negative	Total	Bacilloscopy	Positive	Negative	Total
Positive	54	7	61	Positive	97	9	106
Negative	4	401	405	Negative	79	2,344	2,423
Total	58	408	466	Total	176	2,353	2,529
Sensitivity	93.1%	(83.5-97.2)		Sensitivity	55.1%	(47.7-62.2)	
Specificity	98.3%	(96.5-99.2)		Specificity	99.6%	(99.3-99.8)	
Positive predictive value	88.5%			Positive predictive value	91.5%		
Negative predictive value	99.0%			Negative predictive value	96.7%		
First sample							
Positive	41	6	47	Positive	58	6	64
Negative	2	385	387	Negative	67	2,195	2,262
Total	43	391	434	Total	125	2,201	2,326
Sensitivity	95.3%	(84.5-98.7)		Sensitivity	46.4%	(38.0-55.1)	
Specificity	98.5%	(96.7-99.3)		Specificity	99.7%	(99.4-99.9)	
Positive predictive value	87.2%			Positive predictive value	90.6%		
Negative predictive value	99.5%			Negative predictive value	97.0%		
Second sample							
Positive	13	1	14	Positive	38	3	41
Negative	2	15	17	Negative	10	132	142
Total	15	16	31	Total	48	135	183
Sensitivity	86.7%	(62.1-96.2)		Sensitivity	79.9%	(65.7-88.2)	
Specificity	93.7%	(71.7-98.9)		Specificity	97.9%	(93.7-99.2)	
Positive predictive value	92.9%			Positive predictive value	92.7%		
Negative predictive value	88.2%			Negative predictive value	92.9%		

of notified TB cases in Brazil, in 2015.[21] Similar results were reported in indigenous groups from other regions of the country, among which both bacilloscopy and sputum culture were underused.[15-17,22-24]

However, in our study population, the situation was quite different, given that bacilloscopy, sputum culture, and RMT were widely used in the RSP investigation, contributing to elucidate the alarming TB scenario in the region and revealing the importance of these tests for effective identification of the disease. Our findings are consistent with those reported in developed countries, such as Australia, Canada, and the United States, where sputum culture is recommended and performed in more than 90% of suspected TB cases.[25-27]

Perhaps due to the high number of tests conducted in non-ideal laboratory conditions, more than 10% of the samples sent for culture were contaminated. This rate is higher than the recommended by the TB and other mycobacteria laboratory surveillance.[4] Nonetheless, we emphasize that other locations have shown similar results.[28-30] The contamination could result from adverse weather conditions and inadequate storage and transportation of the samples from the villages to the laboratory. Despite this limitation, the laboratory of Polo Base Amambai had an excellent performance, being able to detect a significant number of cases in the period.

The substantial number of NTM cases diagnosed in the period is striking. However, some authors[31-34] warn about the need to establish strict criteria to diagnose these infections, as they can cause severe clinical manifestations. For this reason, it is essential to repeat the insulation of the same agent in at least two sequential samples, in addition to determining a clinical and laboratory correlation before the NTM diagnosis. Unfortunately, our study could not establish this correlation and identify the species detected.

After evaluating the performance of bacilloscopy, we found that sensitivity was lower than 50% in the first sample and reached almost 80% in the second. This finding reinforces the need to collect two samples: one in the first visit to the health service and the other in the following morning, regardless of the outcome of the first.[7]

The RMT assessment revealed promising results regarding both sensitivity and specificity. The results shown here agree with those reported in other locations.[6,35-38] We underline that this is the first time that the use of RMT was evaluated among the indigenous populations in Brazil.

With respect to costs, Pinto et al.[39] revealed that two sequential bacilloscopies – as recommended by the National Tuberculosis Control Program – cost approximately one RMT. Thus, we can assume that RMT is useful not only in providing accurate, cost-effective, and fast results but also in generating knowledge about rifampicin resistance and being good at estimating the presence of MDRTB.[3]

In spite of the evidence presented here, we need to consider some limitations. Our study analyzed data produced by the health service; therefore, a larger contingent of RSPs not identified by EMSI might exist in the community. Moreover, possible errors when filling in the records could have hidden positive results, underestimating the incidence and/or changing the accuracy of the diagnostic tests evaluated. Our group minimized these issues and increased the internal validity of the study by consulting the GAL-LACEN/MS to confirm the bacilloscopy and sputum culture results of the indigenous people evaluated.

Another limitation was the inclusion of RMT in the laboratory routine of Polo Base Amambai only in the second half of 2014, different from bacilloscopy and sputum culture, which had data available since 2011. This fact limited the analysis of a broader time-series. Also, the contamination rate of the sputum culture was relatively high, which might have hidden new cases and underestimated the incidence rates presented here.

In spite of the limitations mentioned, we believe that our results illustrate the epidemiological situation of TB among the indigenous people from the coverage area of Polo Base Amambai. In addition, the estimated RMT sensitivity and specificity in the region were similar to those reported in the specialized literature, indicating the potential of this diagnostic tool among vulnerable populations in Brazil.

Lastly, RMT can be extremely useful in remote areas, where resources are limited, and access is difficult, such as the indigenous villages in the interior of the country. In these environments, which have a high incidence of TB according to several authors, setting up a laboratory to perform sputum culture that complies with the biosafety levels required by legislation is hard. Considering this scenario, the main advantages of RMT are its easy handling, fast results, and the possibility of detecting rifampicin resistance. Together, these attributes can promote the timely start of treatment with appropriate drug regimens, contributing to reducing the transmission in communities recognized as vulnerable to illness.

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Treatment compliance of patients with paracoccidioidomycosis in Central-West Brazil

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ABSTRACT

Objective: To evaluate the treatment compliance of patients with paracoccidioidomycosis. **Methods:** We studied 188 patients with paracoccidioidomycosis admitted to a tertiary referral hospital in the Central-West Region of Brazil from 2000 to 2010, to assess their compliance to treatment. In order to be considered compliant, patients needed to present two established criteria: (1) receive medicines from the pharmacy, and (2) achieve a self-reported utilization of at least 80% of the dispensed antifungal compounds prescribed since their previous appointment. **Results:** Most patients were male (95.7%), had the chronic form of the disease (94.2%), and were treated with cotrimoxazole (86.2%). Only 44.6% of patients were treatment compliant. The highest loss to follow-up was observed in the first 4 months of treatment ($p < 0.02$). Treatment compliance was higher for patients with than for those without pulmonary involvement (OR: 2.986; 95%CI 1.351-6.599), and higher for patients with than without tuberculosis as co-morbidity (OR: 2.763; 95%CI 1.004-7.604). **Conclusions:** Compliance to paracoccidioidomycosis treatment was low, and the period with the highest loss to follow-up corresponds to the first four months. Pulmonary paracoccidioidal involvement or tuberculosis comorbidity predicts a higher compliance to paracoccidioidomycosis therapy.

Keywords: Paracoccidioidomycosis; Treatment adherence and compliance; loss to follow-up; mycoses

INTRODUCTION

Paracoccidioidomycosis (PCM) is a systemic mycosis caused by *Paracoccidioides brasiliensis* and *Paracoccidioides lutzii* that is endemic in Latin America.[1] Cases diagnosed in other countries are usually imported ones involving people who moved from Latin America.[1,2] In Brazil, the mortality rate for PCM is 1.45 per 1 million inhabitants, making it the eighth most common cause of mortality among chronic recurrent infectious and parasitic diseases.[3] The fatality rate is between 5 and 27%.[3,4] The main therapeutic challenges of PCM are its long duration, the high frequency of relapses and sequelae, and comorbidities. The most frequently observed comorbidities are endemic diseases such as tuberculosis, intestinal helminthiases, and cigarette smoking.[5,6] PCM treatment continues until an apparent cure is reached; the term "apparent cure" is preferred to that of "cure" because it is impossible to confirm the eradication of the fungus from the affected individual.[7] Treatment duration depends on host immunity, virulence of the isolate, *Paracoccidioides* sp inoculum size, and the antifungal compound used. The duration of the treatment is usually maintained until the recovery of cell-mediated immunity[8,9] to avoid reactivation of the fungal cells, which can persist as latent foci.

Compliance, i.e. the act of taking medications as prescribed, is a highly complex clinical behavior, and its evaluation remains problematic.[10] Non-compliance to chronic disease therapy is a problem faced by many health services and is the main obstacle to cure.[11] It may result in disease progression and, when caused by an infectious agent, resistant microorganisms. Long-term treatment of PCM can lead to a non-compliance.[5,12-14]

The purpose of this study was to assess the factors associated with treatment compliance for patients with PCM, aiming to design interventions to improve the care and quality of life for these patients.

METHODS

This study was conducted at the *Hospital Dia Professora Esterina Corsini* at the *Universidade Federal de Mato Grosso do Sul*, which is a reference center for PCM. All patients with active PCM admitted between January 2000 and December 2010 were invited to participate in the present investigation. Only confirmed cases, characterized by the presence of clinical manifestations compatible with PCM and the identification of typical *Paracoccidioides* spp. yeast forms using direct mycological

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or histopathological examination, were included in this study. Patients who died at the beginning of their treatment were excluded. This study used data collected prospectively by a standardized form with demographic, epidemiological, and clinical data. The same physician supervised the patients throughout the study period.

The antifungal compounds used for initial treatment were selected considering severity of disease, possibility of gastrointestinal absorption of the drug, and the presence of associated diseases. For some cases of severe forms, the dose of conventional amphotericin B 0.5 to 0.7 mg/kg/day was indicated. Intravenous trimethoprim-sulfamethoxazole combination (cotrimoxazole) was prescribed at a dose of 800mg/160mg every 8 hours for at initial treatment of some severe cases. For most of the cases, cotrimoxazole was administered at a dose of 1,200 mg/240 mg every 12 hours or itraconazole 200mg once at day was indicated. For complementary treatment, cotrimoxazole was prescribed at a dose of 800mg/160mg every 12hours, while itraconazole was prescribed at the same dose as during initial treatment.

The follow-up regimen included re-evaluation after 1 month of treatment, then every 2 months thereafter until clinical cure was reached. Clinical cure was defined as the disappearance of the previously observed symptoms and normalization of the erythrocyte sedimentation rate. Patients were then evaluated every 2 to 3 months until serological cure by immunodiffusion.[7] Treatment with itraconazole was maintained for at least 1 year, whereas the cotrimoxazole was continued for at least 2 years. After this period, the drug was suspended if the patient had reached and maintained serological cure for 6 months. Patients whose initial serology was unreactive or not performed used the time for treatment above described. All the antifungal compounds were provided to the patients at no cost.

Treatment compliance was defined by the presence of two conditions: (1) appointment compliance of, at least 80% of appointments during the follow-up period; and (2) medication compliance, having pharmacy confirmed drug dispensing and the patient reported the administration of at least 80% of the antifungal doses prescribed since their previous appointment.

Cigarette smoking, alcoholism and tuberculosis were included in the analysis as co-morbidities. We defined smokers as those who reported smoking one or more cigarettes per day, while alcohol users were those who reported regularly drinking alcohol, and tuberculosis was defined by the identification of acid-fast bacilli in histopathological examination, sputum smears, or culture of clinical specimens.

Statistical analysis was performed using Epi Info™ 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA). Categorical variables were expressed as percentages and were compared using the Chi-squared test, or the Fisher's exact test. The Cochran Q test was used to compare percentages in dependent populations. Odds ratios (OR) and 95% confidence intervals (95%CI) were used to assess the association of the variables with compliance. Confounding effects were minimized by performing binary logistic regression adjusting for potential confounders identified in the analysis. These potential confounders are variables that were found to have $p \leq 0.20$. The level of significance used in the analysis was 0.05.

This study was approved by the Ethics Committee of the *Universidade Federal de Mato Grosso do Sul* (CAE 05200812.0.0000.0021).

RESULTS

A total of 188 patients were initially enrolled in the study, with a mean of 17 cases per year. As 4 patients (2.1%) died at the beginning of the initial therapy and were excluded from the study, 184 cases were analyzed (Figure 1). The patients' ages ranged from 4 to 94 years (median: 48 years). The male-to-female ratio was 22.5:1.0. In all, 82.4% of patients were rural workers, 93.1% were cigarette smokers, and 81.9% had alcoholism. The median time between onset of symptoms and diagnosis was 5 months, ranging from 1 to 120 months. Sequelae characterized by fibrotic scars occurred in 77 (41%) patients, with predominance in the lungs, where it was detected in 66 (35.8%) patients, by thorax radiography or computed tomography (CT scan). Other sites with fibrotic scars were observed in adrenal glands (8 patients; 4.3%) by CT scan; in the larynx (6 patients; 3.3%) by laryngoscopy, in the mouth (4 patients; 2.2%) and skin (3 patients; 1.6%) through physical examination.

Eighty-six patients completed the treatment, 45 (52.3%) of them had serological cure; at admission, 21 (24.4%) patients showed no reagent serology at admission, and in 20 (23.2%) the serological evaluation was not carried out.

This study shows that only 82 (44.6%) patients were treatment compliant. The main loss to follow-up was observed in the first 4 months of treatment, which was showed by the decrease in the prevalence of the appointments. This prevalence was stable until the 12th month of follow-up, when a new decrease could be observed (Figure 2).

Demographic and epidemiological characteristics were not associated with treatment compliance; however pulmonary paracoccidioidal involvement and PCM tuberculosis co-infection were associated with higher treatment compliance, 2.99 and 2.76 times more, respectively (Tables 1 and 2).

DISCUSSION

The profile of patients with PCM included in this study was in line with the characteristics most frequently reported in the literature – middle-aged males, whose professional activity was associated with intense contact with soil, as well as increased prevalence of the chronic form of the disease.[12-14] Poor treatment

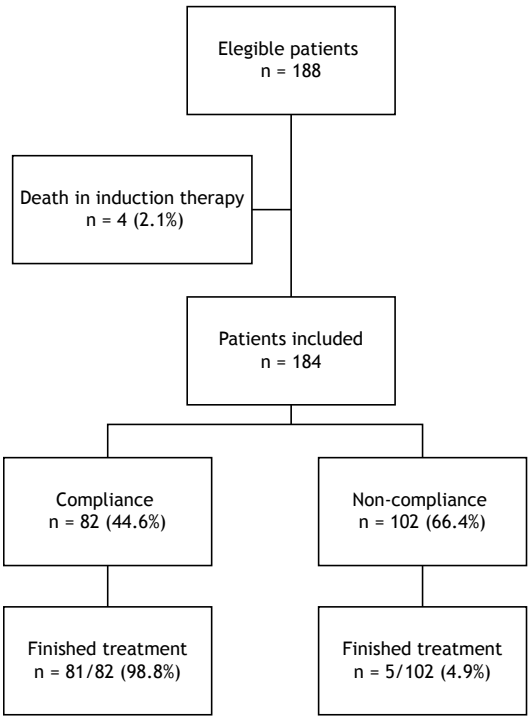

Figure 1. Flowchart of treatment compliance of 188 patients with paracoccidioidomycosis.

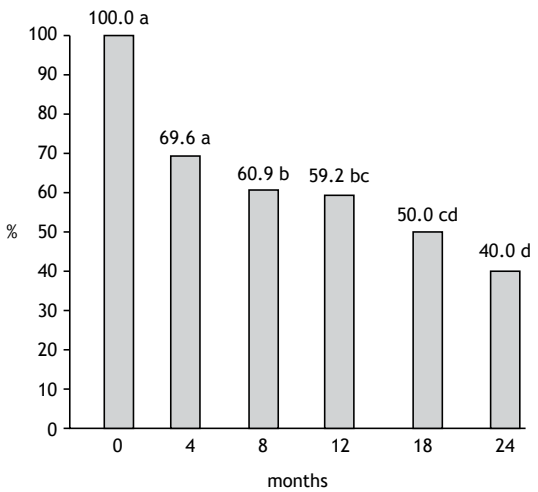

Figure 2. Follow-up of clinical appointments of 184 patients with paracoccidioidomycosis. Cochran Q test. Frequencies followed by the same letters do not differ ($p > 0.05$); frequencies followed by different letters indicate statistically significant differences ($p \leq 0.05$).

compliance was also observed in our study. In developing countries, treatment compliance for chronic conditions often reaches only 20%, leading to negative health statistics and very high costs to society, government, and families of patients.[11]

In contrast, a Brazilian study carried out in 2008 reported a global attendance of scheduled follow-ups, and treatment compliance of 88.2%.[10] In that study, the average individual clinic attendance compliance (ICAC) of 132 patients, with at least 10 routine appointments was 90.3%, with 85.6% of patients attending at least 80% of these clinical evaluations. Mean (± standard deviation) ICAC did not vary with clinical presentation: 90.8 ± 11.9% for patients with the chronic form of the disease and 88.2 ± 9.9% for those with the acute/subacute form ($p > 0.05$). It should be noted that this service has different characteristics from the other care centers in the country, with a greater emphasis on patient follow-up.

In this same study, the antifungal compliance (appropriate sulfonamide serum levels) of 95 patients treated with trimethoprim-sulfamethoxazole combination therapy was 42.1%, showing that patient self-reporting, in order to assess medication-taking, tends to overestimate compliance.[10] Blood level monitoring of sulfonamides in PCM[15] and of itraconazole[16] in systemic mycosis have been described, however they has not been used to assess compliance in the routine clinical setting, in contrast to other diseases, such as epilepsy. We believe that assessing antifungal therapeutic levels is a feasible strategy, and could be useful in controlling PCM treatment.

In our study, the highest loss to follow-up rate occurred in the first 4 months of treatment. Compliance to prolonged treatments for chronic infectious diseases is a clinical challenge. Patients with tuberculosis also present high rates of discontinuation of treatment, despite presenting different demographic and epidemiological profile of patients with PCM. A study on tuberculosis showed a similar time frame for the discontinuation of the treatment, the median time of which was 4.0 (range 0.5 to 28.9) months.[17] A low perception of disease severity may be a risk factor for treatment abandonment.[18] In patients with PCM, clinical improvement or clinical cure (characterized respectively by a decrease or disappearance of the symptomatology, such as dyspnea and skin/mucous membrane lesions) gives the patient the impression of being "cured".[7] This time period is critical, during which treatment compliance should be the focus; this should be emphasized during the follow-up appointments.

In our study, demographic and epidemiologic variables, such as sex, age range, residence, and rural work activities, were not associated with compliance to a therapeutic regimen, which is in line with previous reports.[10] The geographic location of the patients, i.e. metropolitan or rural areas, may have an effect on accessibility to medical care; however, it did not affect treatment compliance in our study. The World Health Organization considers illiteracy, poor economic conditions, and unemployment to be determinants of poor compliance to treatment of chronic comorbidities.[10,11] However, in our study, no association between education level and treatment compliance was observed.

Our study also showed no association between alcohol consumption and treatment compliance.

Table 1. Demographic, clinical, therapeutic, co-morbidities and treatment compliance among 184 patients with paracoccidioidomycosis.

Variables	Compliance	Non-compliance	p-value
Sex/male*	80/82 (97.6)	96 /102 (94.1)	0.557
Age rage, years†			
00-19	1/82 (1.2)	3/102 (2.9)	0.390
20-29	1/82 (1.2)	3/102 (2.9)	0.390
30-39	12/82 (14.6)	15/102 (14.7)	0.425
40-49	26/82 (31.7)	36/102 (35.3)	0.496
50-59	26/82 (31.7)	25/102 (24.5)	0.179
≥60	16/82 (19.5)	20/102 (19.6)	0.433
Resident in Campo Grande‡	35/82 (42.7)	38/101 (37.6)	0.487
Rural worker‡	32/79 (40.5)	38 /91 (41.8)	0.869
Cigarette smoker	76/82 (92.7)	97/101 (96.0)	0.347
Alcoholism‡	73/81 (90.1)	79/95 (83.2)	0.180
Education†			
Illiterate	8/79 (10.1)	16/87 (18.4)	0.097
Elementary school	57/79 (65.8)	57/87 (65.5)	0.452
Middle school	13/79 (16.5)	10/87 (11.5)	0.239
High school/college	6/79 (7.6)	4/87 (4.6)	0.245
Tuberculosis (co-infection)‡	15/82 (18.3)	6/100 (6.0)	0.010
Clinical form*			
Chronic	79/82 (96.3)	96/102 (94.1)	0.733
Acute/subacute	3/82 (3.7)	6/102 (5.9)	
Severity‡			0.389
Mild	18/82(21.9)	29/102(28.4)	
Moderate	54/82(65.9)	57/102(55.9)	
Severe	10/82(12.2)	16/102(15.7)	
Organs involved‡§			
Lungs	72/79 (91.1)	69/92 (75.0)	0.006
Oral mucous membrane infection	59/81 (72.8)	80/101 (79.2)	0.315
Lymph node enlargement	29/63 (46.0)	32/81 (39.5)	0.432
Antifungal compound‡			
Cotrimoxazole	72/83 (86.7)	90/95 (94.7)	0.063
Itraconazole	11/83 (13.3)	5/95 (5.3)	

Results are presented as ratios: number of cases for each variable/number of patients evaluated in each group (compliance and non-compliance). Data in parenthesis are presented as percentages. Rural workers were defined as such if they performed rural work at present, or in the past. *Fisher's exact test; †comparison between independent proportions; ‡Chi-square test; §possibly more than one organ involved.

Using a different approach, another study showed that alcohol intake over 50 mg/day had no impact on age at the onset of illness.[6] However, in our clinical practice, wives and children of patients have reported treatment withdrawal due to alcohol abuse, which is directly related to disease development.[6] This apparent contradiction may be due to the use of a dichotomous variable for frequent alcohol intake in this study, rather than a measurement of its amount.

An analysis of the association between compliance and the organs or systems affected by PCM revealed that pulmonary involvement, identified by radiography or CT scan, is a positive factor for compliance. These pulmonary symptoms are speculated to motivate the patients to attend follow-up appointments. Moreover, the respiratory symptoms can cause greater limitation in daily life, leading patients to be more concerned about their treatment.[19]

Compliance to therapeutic follow-up was also higher for patients with PCM-tuberculosis co-infection. The highest rate of follow-up loss occurred in the first 4 months, when patients with PCM and tuberculosis comorbidity were still on tuberculosis treatment. This finding may be related to the efforts enacted by the National Tuberculosis Control Program in Brazil, with community health workers involved in a directly observed treatment strategy.[20] Additionally, many campaigns have emphasized the importance of never abandoning tuberculosis treatment, which could have influenced the patients' behavior in not abandoning their PCM treatment as well.

Table 2. Variables influencing treatment compliance in 184 patients with paracoccidioidomycosis

Variable	Compliance	Non-compliance	Total	COR (95%CI)	AOR (95%CI)
Paracoccidioidal pulmonary lesion				3.43 (1.383-8.506)	2.986 (1.351-6.599)
Yes	72	69	141		
No	07	23	30		
All	79	92	171		
Tuberculosis co-infection					
Yes	15	06	21	3.51 (1.295-9.514)	2.763 (1.004-7.604)
No	67	94	161		
All	82	100	182		

AOR: adjusted odds ratio; 95%CI: 95% of confidence interval; COR: crude odds ratio.

The use of itraconazole could be associated with higher compliance rates due to its easier treatment regimen (2 pills/day for itraconazole and 4 to 6 pills/day for cotrimoxazole) and shorter treatment duration.[21] However, our study showed no difference between medications, possibly due to the small number of patients who used itraconazole. As it was reported in the Brazilian literature, the most commonly used antifungal compound to treat PCM is cotrimoxazole.[14,22] Cotrimoxazole is still the most used drug to treat PCM in Brazil because it is given free of charge to the patients through the public health system.

Treatment is considered completed after at least 12 months, if itraconazole was used, or 24 months if cotrimoxazole was used, and until the patient reaches and sustains serological cure for 6 months.[23] Therefore, immunodiffusion is very useful in the follow-up of patients, and when they do not present a significant decrease in the serological titres, it is suspected that they are not complaining to the treatment. In many patients, the serological cure criteria could not be used because serological examination was not performed or was negative at admission. About one-fifth of the patients had negative serology at admission, and this could be explained by the use of antigen obtained from *P. brasiliensis* strain B339 for serological test in our service.

The lack of standardization in antigen production for PCM serology makes the control of cure difficult in some services. The recent molecular identification of several species of fungi of the genus *Paracoccidioides* revealed different antigenic compositions among the species.[24] As the serological reactions are specific, patients with PCM caused by *P. lutzii* produce antibodies that are rarely detected by reactions in which the antigen used was isolated from *P. brasiliensis* species. As the highest concentration of P. lutzii was described in the Center-West Region of Brazil, it is possible that our serological negative cases at admission were caused by *P. lutzii*. Recently, a *P. lutzii* antigen has been identified and isolated,[25] which is not yet available for the vast majority of the clinical services. The same work of antigen identification and antigen validation for double immunodiffusion reactions should be done for all new recognized species.

It should be noted that research is being done to identify biomarkers in the serum of patients with PCM that can confirm this disease in its different stages – active untreated disease, active disease under treatment, clinical cure and serological cure, and when it is the case, PCM relapse.[26]

Some limitations of this study are the absence of objective parameters to evaluate compliance, such as follow-up of the serum levels of the antifungal compounds. More than a century after the first report by Adolfo Lutz,[27] PCM continues to be a relevant public health issue, especially regarding its treatment.

Our results showed the importance of the treatment compliance evaluation in patients with PCM, whose clinical cure is reached much before the immunological recovery. Thus, treatment compliance should be routinely performed in every appointment, including with the support of the social service, to call the patients who did not came to the appointment.

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Speeding up the diagnosis of multidrug-resistant tuberculosis in a high-burden region with the use of a commercial line probe assay

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ABSTRACT

Objective: To evaluate the rapid diagnosis of multidrug-resistant tuberculosis, by using a commercial line probe assay for rifampicin and isoniazid detection (LPA-*plus*), in the routine workflow of a tuberculosis reference laboratory. **Methods:** The LPA-*plus* was prospectively evaluated on 341 isolates concurrently submitted to the automated liquid drug susceptibility testing system. **Results:** Among 303 phenotypically valid results, none was genotypically rifampicin false-susceptible (13/13; 100% sensitivity). Two rifampicin-susceptible isolates harboured *rpo*B mutations (288/290; 99.3% specificity) which, however, were non-resistance-conferring mutations. LPA-*plus* missed three isoniazid-resistant isolates (23/26; 88.5% sensitivity) and detected all isoniazid-susceptible isolates (277/277; 100% specificity). Among the 38 (11%) invalid phenotypic results, LPA-*plus* identified 31 rifampicin- and isoniazid-susceptible isolates, one isoniazid-resistant and six as non-*Mycobacterium tuberculosis* complex. **Conclusions:** LPA-*plus* showed excellent agreement (≥91%) and accuracy (≥99%). Implementing LPA-*plus* in our setting can speed up the diagnosis of multidrug-resistant tuberculosis, yield a significantly higher number of valid results than phenotypic drug susceptibility testing and provide further information on the drug-resistance level.

Keywords: Tuberculosis, multidrug-resistant; Molecular diagnostic techniques; Microbial sensitivity tests; *Mycobacterium tuberculosis*.

INTRODUCTION

A major challenge to the effective control of tuberculosis (TB) worldwide is the occurrence of *Mycobacterium tuberculosis* complex (MTBC) strains showing resistance to both rifampicin (RIF) and isoniazid (INH), the two most effective first-line drugs in TB treatment.[1] This resistance profile, called "multidrug-resistant TB" (MDR-TB), leads to therefore less efficient drug regimens,[1] and is associated with treatment failures, relapses, and poor clinical outcomes.[2]

MDR-TB has called for an urgent development of rapid and accurate diagnostic testing, in order to start effective treatment earlier and reduce the spread of drug-resistant TB.[3,4] To that end, in 2008, the World Health Organization (WHO) endorsed the use of molecular assays for MDR-TB screening.[5] One of them, the GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany), is a line-probe assay that detects MTBC, as well as mutations and wild type sequences in the 81-base-pair hotspot region of the *rpo*B gene, in codon 315 of *kat*G gene, and in the promoter region of *inh*A gene.[6] MTBDR*plus* thus predicts MDR-TB by detecting resistance not only to RIF (*rpo*B gene) but also to INH (*kat*G and *inh*A genes). Although RIF resistance has been considered a surrogate of MDR-TB,[4,7] identifying INH resistance can be useful, mainly in high TB burden regions in which prevalence of MDR-TB is low,[4] as in Brazil, where 1.5% and 8.0% of the 82,676 TB cases reported in 2016 were estimated as primary and acquired MDR-TB, respectively.[8]

MTBDR*plus* has shown good accuracy and is now routinely used in many countries,[4] speeding up the MDR-TB diagnosis and reducing the laboratory demand for conventional drug susceptibility testing (DST). However, no studies using this test applied to isolates in the diagnostic workflow of a reference laboratory were conducted so far in Brazil.

We aimed to prospectively evaluate the performance of the MTBDR*plus* assay applied to MTBC cultures in comparison to phenotypic DST in a high-volume TB reference laboratory, as well as elucidate any discrepancies between the two methods.

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METHODS

Study site and population

This study was conducted in the Tuberculosis and Mycobacteriosis Core of *Instituto Adolfo Lutz* (IAL), a state governmental institution of São Paulo. The IAL is the TB and mycobacteriosis reference laboratory for São Paulo, responsible for 291 laboratories state wide with different infrastructures for processing the clinical material collected from in- and outpatients, covered by the publicly funded health care system. These laboratories perform acid-fast bacilli smear microscopy or the Xpert MTB/RIF assay (Cepheid, SUNV, CA, USA) implemented in 36 of them by the end of 2014. Eighty laboratories in this network perform cultures and send them to IAL, where DST is performed for patients at higher risk of drug-resistant TB. These include any smear-positive cases after 2 months on TB treatment, those who are contacts of known resistant TB patients, retreatment TB cases, and any immunosuppressed persons, alcohol abusers or illicit drugs users, healthcare workers, homeless individuals, indigenous, immigrants, inmates, inpatients, and prison officers.[9] In 2016, this laboratory framework in São Paulo virtually served a population of 44.85 million inhabitants with a TB incidence rate of 36.4 per 100,000.[10] IAL receives per year approximately 7,000 mycobacterial cultures to confirm identification and performs first-line DST on nearly 4,000 isolates.

For this study, a sample size (n) of 307 TB cases was calculated by using the formula $n=Z^2P(1-P)/d^2$[11] applied to an expected 15% (p=0.15) frequency of resistance to at least one of the anti-TB drugs, RIF or INH, and 95% of confidence interval (95%CI), with Z value of 1.96, with 4% precision (d=0.04).

Demographic and clinical data were collected from the Hospital Information and Management System and the TBWeb – Sistema de Controle de Pacientes com Tuberculose (TBWEB) of the state of São Paulo.

Identification of Mycobacterium tuberculosis complex isolates

Primary mycobacterial cultures referred to IAL in liquid mycobacteria growth indicator tube (MGIT) or on solid media were presumptively identified by observing growth and microscopic characteristics to differentiate MTBC from nontuberculous mycobacteria (NTM). Subsequent identification by phenotypic tests, including MPT64 protein detection, was carried out whenever needed, as already described.[12,13]

Phenotypic drug susceptibility testing

Presumptive MTBC isolates were subjected to DST on the automated BACTEC MGIT 960 system (Becton, Dickinson & Co., NJ, EUA),[14] using a modified protocol best suited to the IAL routine conditions.[13] Final concentrations were 0.1 µg INH mL^{-1} and 1.0 µg RIF mL^{-1}. In case of contamination or absence of growth, the respective primary culture was submitted to further speciation.

Genotype MTBDRplus version 2.0

This assay was prospectively performed on 341 isolates, one per patient, that were about to undergo MGIT DST. Cultures underwent DNA extraction on August and October 2014, a day before they entered the MGIT instrument for DST.

DNA extraction from liquid or solid cultures was done using Genolyse kit version 1.0 (Hain)[15] for no more than 23 isolates and a negative control at a time. MDRTB*plus* was carried out as explained elsewhere,[15] and the reactions detected on strips were visually interpreted with the aid of a cardboard template. In case of invalid results such as no signal with conjugate or any of the other control probes, and doubtful reactions as weak signals with the gene bands, the test was repeated using new DNA extraction.

Gene sequencing

Sanger sequencing was performed whenever results between MTBDR*plus* and phenotypic DST remained discordant upon repeating both tests. Isolates showing conflicting results for INH had the *mab*A-*inh*A regulatory region (positions -168 to 80, relative to codon) amplified and sequenced with primers mabA-inhAF and mabA-inhAR,[16] as well as the entire *inh*A and *kat*G genes by using the primer pairs inhA3 and inhA4, inhA3F and inhA5R, and the forward and reverse primers katG-P4, -P5, -P6, -P7 and -P8.[17] For isolates with RIF-discordant results, primers RPOB-1 and RPOB-2[18] were used to amplify and sequence a 350-bp fragment of *rpo*B encompassing the RIF resistance-determining region.

Single PCR included 12.5 µL of PrimeSTAR Max DNA Polymerase (Takara Bio, Shiga, Japan), 5 pmol of primers for *mab*A-*inh*A and *kat*G, 10 pmol of primers for *inh*A and *rpo*B, 2 µL of DNA template and PCR-grade water for a final volume of 25 µL. Amplification comprised 30 cycles of 98 °C for 10 seconds, 55 °C for 5 seconds, and 72 °C for 20 seconds. Amplimers purified with ExoSAP-it (Affymetrix, SCL, CA, USA) were sequenced with an ABI 3130xL Genetic Analyzer and the BigDye Terminator version 3.1 Kit (Applied Biosystems, FSTC, CA, USA). Sequences were aligned and analysed using the BioEdit v7.2.5 software[19] and the web-based MUBII-TB-DB[20] and BLAST[21] tools.

Turnaround time of results

The time taken to perform MGIT DST and MTBDR*plus* assays was recorded to calculate the mean time taken to complete the tests. Turnaround time (TAT) of results was calculated from the date oleic acid-albumin-dextrose-catalase (OADC) supplement and antimicrobial solutions were added to MGIT tubes to the date DST result reporting was available; and from the DNA extraction date to the date MTBDR*plus* result was written on the evaluation sheet.

Data analyses

The sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of the MTBDR*plus*

test were assessed for RIF and INH compared to the phenotypic DST. Agreement between both tests was calculated using kappa (κ) coefficient and the strength of agreement interpreted as poor (< 0.2), fair (> 0.2 ≤ 0.4), moderate (> 0.4 ≤ 0.6), good (> 0.6 ≤ 0.8) and very good (> 0.8 ≤ 1).[22] Two-tailed Fisher's Exact test was used for comparisons between proportions. Differences in TATs were evaluated using paired *t* test. The significance threshold was set at .05. Statistical analyses were performed using the web-based OpenEpi program.[23]

Ethical Statement

The Technical Scientific Council (CTC-IAL no. 98C/2012) and Research Ethics Committee (CEPIAL no. 207.606 dated Feb-21-2013) of IAL approved this study.

RESULTS

Phenotypic drug susceptibility testing

The results of MGIT DST, along with the demographic and clinical characteristics of the patients, are shown in Table 1. Most patients were men (80%), had pulmonary TB (93%) and no past history of TB treatment (65%). MDR-TB was observed only in previously treated pulmonary TB patients.

Figure 1 shows the study plan of the 341 isolates prospectively tested. Phenotypic DST provided interpretable results for 303 (89%) isolates, of which 276 (91%) were susceptible, 14 (5%) INH-monoresistant, 12 (4%) MDR, and one (<1%) was RIF-monoresistant.

For the 38 cultures with invalid DST results due to contamination (n=35) or absence of growth (n=3), subsequent speciation identified 23 MTBC, six mixed MTBC + NTM and four NTM cultures. Among the remaining five isolates, identification was not assessed due to insufficient growth of three primary cultures and to heavy contamination in two cases, both Ag MPT64-negative, reported as non-MTBC isolates.

Genotype MTBDRplus

All doubtful (n=9) and invalid (n=2) results became valid upon repeating the assay. MTBDR*plus* gave interpretable results for all 341 isolates (Figure 1). Among the 335 isolates identified as MTBC, there were 308 (92%) susceptible, 12 (3.6%) MDR, 12 (3.6%) INH-monoresistant and three (1%) RIF-monoresistant isolates.

MTBDR*plus* presented significantly higher interpretable results, providing information on 38 additional isolates (11%; 95%CI 8.1-14.8%; p<0.0001) for which no MGIT DST results were available (Figure 1). Among these isolates, the genotypic test identified 32 MTBC (31 susceptible and one INH-monoresistant) and six non-MTBC isolates. The one INH-monoresistant and seven susceptible isolates were later confirmed by MGIT DST, on a second isolate.

For RIF resistance prediction, MTBDR*plus* showed 100% sensitivity (13/13), 99.3% specificity (288/290) and 99.3% accuracy (301/303), as shown in Table 2. The test correctly detected INH resistance in 23/26 isolates (sensitivity 88.5%) and INH susceptibility in all 277 isolates (specificity 100%), with an overall diagnostic accuracy of 99.0% (300/303). PPV and NPV values were high for RIF resistance, INH resistance and MDR, ranging from 86.7% to 100%. The agreement between the genotypic and the phenotypic tests was very good (κ ≥ 0.91). To ascertain the test reproducibility, all the 31 repetitions confirmed the first results.

Discordances between tests

When the 303 valid results by the phenotypic test were compared to the genotypic test results, there were seven mismatches. After re-growing and re-examining these isolates, two of five initially INH-resistant isolates by the conventional DST matched the MTBDR*plus* results. The results of the five isolates that remained discrepant are summarized in Table 2. The two false-resistant RIF isolates had their *rpo*B mutations confirmed as His526Asn and Asp516Tyr by sequencing.

Table 1. Characteristics of the patients indicated for rifampicin and isoniazid susceptibility testing on August and October, 2014

Characteristics	Patients	RIF and INH susceptibility testing – BACTEC 960 MGIT system			
		Susceptible n = 276	Monoresistant n = 15*	Multiresistant n = 12	Invalid test n = 38
Age	37±13 (range 1-84)	37±13	44±15	35±12	38±15
Sex					
Male	272 (80)	225 (82)	10 (67)	8 (67)	29 (76)
Female	69 (20)	51 (18)	5* (33)	4 (33)	9 (24)
Clinical presentation					
Pulmonary	317 (93)	259 (94)	14 (93)	12 (100)	32 (84)
Pulmonary and extrapulmonary	15 (4)	12 (4)	0	0	3 (8)
Extrapulmonary	9 (3)	5 (2)	1* (7)	0	3 (8)
Past treatment history					
No history (new patient)	222 (65)	191 (69)	6 (40)	0	25 (66)
Retreatment	119 (35)	85 (31)	9* (60)	12 (100)	13 (34)

Age values expressed as mean ± standard deviation, and the other values as n (%). *One isolate is RIF-monoresistant and the others are INH-monoresistant. RIF: rifampicin; INH: isoniazid.

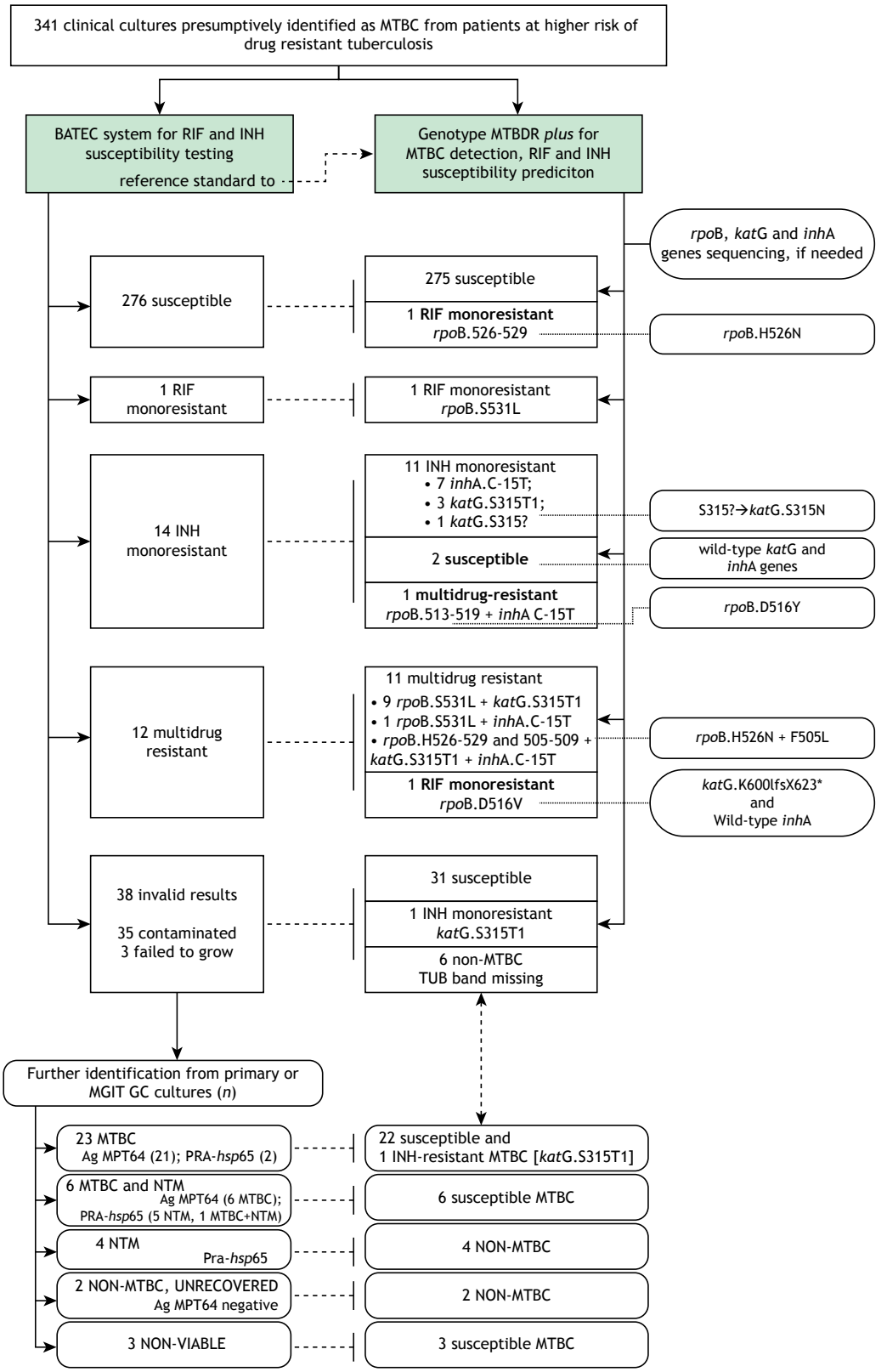

Figure 1. Flow outline of *Mycobacterium tuberculosis* complex (MTBC) isolates in this study. RIF: rifampicin, INH: isoniazid; MGIT GC: growth control tube in the BACTEC 960 system; Ag MPT64: detection of antigen MPT-64; PRA-*hsp*65: polymerase chain reaction and restriction-enzyme analysis of the *hsp*65 gene, NTM: nontuberculous mycobacteria. *katG*. S315T1: T1 means AGC→ACC exchange. *n.1798_1799insT, p.Lys600IlefsTGA623.

Table 2. Performance indices of the Genotype MTBDR*plus* for the detection of rifampicin, isoniazid and multidrug-resistant isolates, and discordances in comparison to phenotypic drug susceptibility testing by the BACTEC MGIT 960 system.

Genotype MTBDR*plus* compared to MGIT 960				Discordant results			
Test performance measure	n matching/ total	Rates	(95%CI)	n	Discordance	MTBDR*plus*	Gene sequencing
RIFAMPICIN							
Sensitivity	13/13	100%	(77.2-100)				
Specificity	288/290	99.3%	(97.5-99.8)	2	False-RIF^R	*rpo*B mut 526-529 *rpo*B mut 513-519	*rpo*B – His526Asn *rpo*B – Asp516Tyr
Accuracy	301/303	99.3%	(97.6-99.8)				
PPV	13/15	86.7%	(62.1-96.3)				
NPV	288/288	100%	(98.7-100)				
Agreement (k)	301/303	0.93	(0.81-1.04)				
ISONIAZID							
Sensitivity	23/26	88.5%	(71.0-96)	3	False-INH^S	*kat*G and *inh*A – WT	*kat*G and *inh*A - WT *kat*G and *inh*A - WT *kat*G - Lys600IlefsTGA623
Specificity	277/277	100%	(98.6-100)				
Accuracy	300/303	99.0%	(97.1-99.7)				
PPV	23/23	100%	(85.7-100)				
NPV	277/280	98.9%	(96.9-99.6)				
Agreement (k)	300/303	0.93	(0.82-1.05)				
MDR							
Sensitivity	11/12	91.7%	(64.6-98.5)	1*	False-INH^S	*kat*G and *inh*A - WT	*kat*G - Lys600IlefsTGA623
Specificity	290/291	99.7%	(98.1-99.9)	1†	False-RIF^R (MDR)	*rpo*B mut 513-519	*rpo*B - Asp516Tyr
Accuracy	301/303	99.3%	(97.6-99.8)				
PPV	11/12	91.7%	(64.6-98.5)				
NPV	290/291	99.7%	(98.1-99.9)				
Agreement (k)	301/303	0.91	(0.80-1.03)				

PPV: positive predictive value; NPV: negative predictive value; k: Cohen's kappa coefficient; MDR: multidrug resistance; 95%CI: 95% of confidence interval; RIF^R: resistance to rifampicin; INH^S: susceptibility to isoniazid; mut: mutation; WT: wild type; *the isolate is one of the isoniazid discordances in this table. †the isolate is a rifampicin discordant isolate in this table.

MTBDR*plus* failed to detect INH resistance in two phenotypically INH-monoresistant and one MDR isolates. Gene sequencing showed the first two isolates had neither *kat*G nor *inh*A gene mutations while the MDR isolate presented a T nucleotide insertion between positions 1,798 and 1,799 of *kat*G, leading to the Lys600Ile mutation and to a frameshift ending with a stop codon (TGA) at position 623 in the shifted reading frame.

Mutations in rpoB, katG and inhA genes

Ten different mutation profiles were identified among the 27 genotypically resistant isolates, as shown in Table 3. Regarding the *rpo*B gene mutations, the most frequent was Ser531Leu (11/15; 73%), mostly among phenotypically MDR isolates (10/12; 83%). His526Asn *rpo*B mutation alone was observed in one phenotypically susceptible isolate and in the only MDR isolate presenting double mutations in the *rpo*B gene and concurrent *kat*G and *inh*A mutations. Ser315Thr1 (AGC→ACC exchange) was the most frequent *kat*G mutation (14/16, 88%) and was harboured mostly by MDR isolates (10/12; 83%). The only mutation found in the *inh*A gene was C-15T (10; 100%), which was more frequent in INH-monoresistant isolates (8/12; 67%).

Turnaround time of results

For the MTBDR*plus* assay, two consecutive DNA extraction rounds comprising 11 isolates and one control each took about 3 hours. Amplification mix, thermo cycling, hybridization and interpretation of results in one round of 24 samples took 50 minutes, 1 hour and 50 minutes, 2 hours and 20 minutes, and 40 minutes respectively. Therefore, the average TAT from DNA extraction to reporting the results of 24 samples performed by one person alone was 8 to 9 hours overall.

TATs of both MGIT DST and MTBDR*plus* assays were compared using only valid results on conventional DST. The median TAT to reporting MTBDR*plus* results was 3 days (zero to 17 days), significantly shorter than that of MGIT DST (median 11 days, 7 to 78 days;

p<0.0001). Intervals > 9 days for 16 MTBDR*plus* results were due to temporary unavailability of the kit, and those > 23 days for 12 MGIT DST reports release were due to repetition of tests presenting growth failure or contamination. As shown in Figure 2, results by MTBDR*plus* were available much earlier than by MGIT, even though the test was performed by a single operator and in rounds of 24 isolates. By the 7th day, when the first three (1%) MGIT DST results were reported, there were already 231 (76%) MTBDR*plus* results available. The number of complete tests by the genotypic assay by the 9th day (287; 95%) was attained only on the 14th day by MGIT DST (285; 94%).

DISCUSSION

This study evaluates the use of the genotype MTBDR*plus* assay in the workflow of a routine TB laboratory in South-Eastern Brazil, where nearly 4,000 MTBC isolates from patients at high risk of drug-resistant TB in São Paulo undergo MGIT DST per year. The molecular assay was compared to the reference DST on MGIT 960, and discordant results between both methods were resolved by Sanger sequencing. The last IAL's annual reports estimated 91% valid first-line DST results, of which 91% of the isolates were RIF- and INH-susceptible, 4% were MDR, 4% were INH-resistant but RIF-susceptible and < 1% was RIF-resistant but INH-susceptible (data not shown), confirming that the study sample accurately reflected the population of isolates examined each year.

Our findings are in agreement with a review[24] that estimated pooled sensitivities and specificities for resistance prediction to RIF (91.3% and 97.1%) and INH (89.4% and 98.9%). Regarding the data from Brazil using the MTBDR*plus* in clinical isolates, our findings are comparable with a study[25] in the State of Minas Gerais, showing sensitivities of 93.3% for RIF, 83.3% for MDR or INH resistance detection, and 100% specificity for both drugs. A study on MDR-TB patients in Ribeirão Preto (SP), revealed 100% and 80% sensitivity in detecting RIF and INH resistance, respectively.[26]

In this study, *rpo*B Ser531Leu and *kat*G Ser315Thr mutations predominated, as in other settings.[25-28] Identifying the specific mutation associated with drug resistance, which is not possible by phenotypic methods, may provide additional information on the category of resistance and guide therapeutic decision,

Table 3. Mutation profiles in genes of the *Mycobacterium tuberculosis* complex associated to rifampicin (RIF) and isoniazid INH) resistance and phenotypic drug susceptibility testing by the BACTEC MGIT 960 system

n	Mutation pattern			Phenotypic results to RIF and INH
total = 27	*rpo*B	*kat*G	*inh*A	
1	His526Asn	WT	WT	Susceptible
1	Ser531Leu	WT	WT	RIF^R
7	WT	WT	C-15T	INH^R
1	Asp516Tyr	WT	C-15T	INH^R
1	WT	Ser315Asn	WT	INH^R
4	WT	Ser315Thr (G>C)	WT	3 INH^R; 1 ND
9	Ser531Leu	Ser315Thr (G>C)	WT	RIF^R - INH^R
1	Asp516Val	Lys600IlefsTGA623	WT	RIF^R - INH^R
1	Ser531Leu	WT	C-15T	RIF^R - INH^R
1	Phe505Leu + His526Asn	Ser315Thr (G>C)	C-15T	RIF^R - INH^R

WT: wild type; RIF^R: resistant to rifampicin; INH^R: resistant to isoniazid; ND: not determined due to contamination.

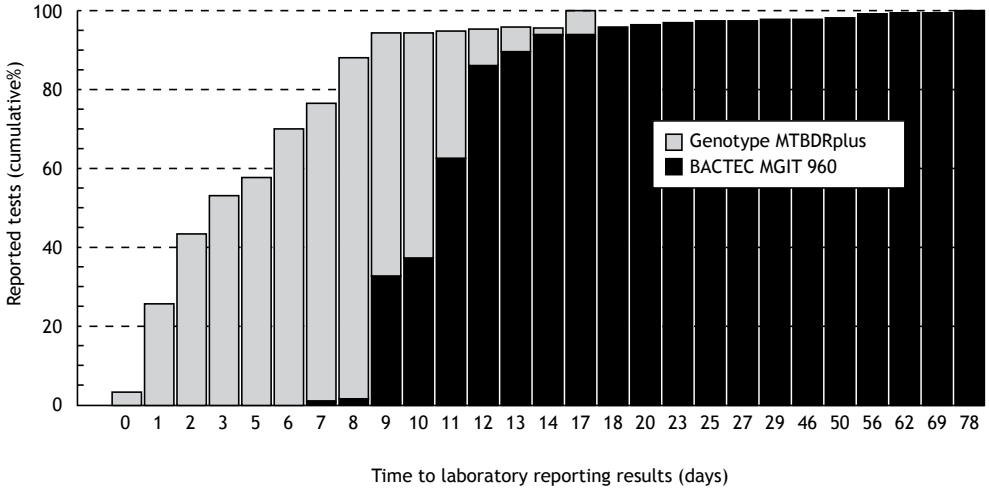

Figure 2. Time interval between the start of rifampicin and isoniazid susceptibility testing and laboratory reporting results.

as to the choice of the treatment regimen.[29] In this study, an isolate carrying the Asp516Val *rpo*B mutation, which was shown to confer resistance to RIF, but not rifabutin,[7,28] illustrates how genetic tests may help clinicians manage TB-resistant cases. Furthermore, the translation of genetic findings into clinical therapy has relevant implications in the use of INH for resistant TB, since this drug was shown to remain effective depending on the INH resistance-conferring mutation.[30] Usual-dose INH is effective when mutations occur solely in the *inh*A promoter region, and for mutations in *kat*G only, high-dose INH is still an option for most of patients.[30,31] These findings might explain the INH efficacy in the shorter regimen.

Some *rpo*B mutations detectable only by the absence of reaction with the wild type probes in the MTBDR*plus* may not be associated to RIF resistance.[7,28,32] This was the case of two phenotypically RIF-susceptible isolates in this study presenting mutations in *rpo*B codons 513-519 and 526-529, further identified by sequencing as Asp516Tyr and His526Asn, which have been shown not to be associated to RIF resistance.[7,28] As these are not true discordances with the phenotypic test, no false RIF resistance occurred in our study. The results above clearly demonstrate why in cases in which no reaction with the mutation probes occurs, sequencing or phenotypic DST must be performed to better interpret resistance. We did not find in the published literature the *rpo*B double mutation Phe505Leu and His526Asn seen in an isolate in this study. The MDR profile of that isolate, also harbouring *kat*G Ser315Thr and *inh*A C-15T mutations, is similar to the one recently described,[33] which presented Phe505Leu and Asp516Tyr, a RIF resistance-conferring double mutation.[32]

Sensitivity for INH resistance detection was lower, as expected, since it can arise from mutations other than those in codon 315 of *kat*G and in the regulatory region of *inh*A. According to Brossier et al.,[27] MTBDR*plus* may miss 8% to 21% of INH-resistant isolates. In this study, MTBDR*plus* missed 2/25 INH-resistant but RIF-susceptible isolates, and 1/12 MDR isolates. The MDR isolate misdiagnosed as RIF-resistant alone would have been submitted to first-line MGIT DST according to the IAL algorithm currently in use for isolates from Xpert-resistant samples. Therefore, the INH resistance of this isolate would be properly identified by MGIT DST. The true INH resistance of the other two false-negative INH results would probably be correctly detected during the follow-up of TB treatment.

Based on the sensitivity and specificity of the MTBDR*plus* and considering the prevalence estimates of INH resistance among 4,000 isolates received yearly at IAL, we estimated that this test would miss 34 of 292 INH-resistant isolates. On the other hand, MTBDR*plus* would provide additional information on RIF and INH susceptibilities of 375 MTBC from a total of 446 isolates with invalid results on MGIT DST, yearly.

The shorter TAT to complete the test makes MTBDR*plus* a more effective method. Most of the laboratory reports would be released before 1% of MGIT DST reports were available. Additionally, the workload on phenotypic DST performance would be drastically reduced, providing time to accommodate more exams. Therefore, not only would presumptive drug-resistant TB patients be given the opportunity to start treatment earlier with the most appropriate regimen, as observed in Ribeirão Preto,[26] but also we could extend access to at least one DST for all patients, as recommended by the WHO.[24] Moreover, MTBDR*plus* poses a smaller biohazard risk to the laboratory personnel than the conventional DST as it requires less manipulation of live cultures.

To the authors' knowledge, this is the first prospective study in Brazil assessing the usefulness of MTBDR*plus* in a reference TB laboratory serving the most populous Brazilian state. It provides information for the implementation of this test into the TB diagnostic algorithm in Brazil. However, the study has several limitations. First, the number of resistant isolates was not large enough to draw more sound conclusions on the frequency and pattern of mutations in our setting. Second, we did not measure the MIC of isolates presenting mutations, mainly the one carrying a combination of two *rpo*B mutations not described in literature. Finally, we did not investigate the presence of mutations in susceptible isolates, as we only sequenced isolates showing conflicting results or not completely identified by MTBDR*plus*.

In conclusion, the diagnostic accuracy of the MTBDR*plus* assay was excellent in detecting MTBC resistance to RIF and INH, and MDR. No phenotypically susceptible isolates were misidentified as MDR, nor were any MDR isolates incorrectly predicted as susceptible to both drugs. The advantages of the test, such as reducing the time to diagnosis, being easy to perform and yielding additional results otherwise invalid by the phenotypic DST preclude its disadvantages, notably the false-susceptible INH results. To accurately diagnose clinical resistance, the association of the nature of mutations with the level of phenotypic susceptibility must be carefully evaluated.

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Accuracy of a rapid molecular test for tuberculosis in sputum samples, bronchoalveolar lavage fluid, and tracheal aspirate obtained from patients with suspected pulmonary tuberculosis at a tertiary referral hospital

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ABSTRACT

Tuberculosis continues to be a major public health problem worldwide. The aim of the present study was to evaluate the accuracy of the Xpert MTB/RIF rapid molecular test for tuberculosis, using pulmonary samples obtained from patients treated at the Júlia Kubitschek Hospital, which is operated by the Hospital Foundation of the State of Minas Gerais, in the city of Belo Horizonte, Brazil. This was a retrospective study comparing the Xpert MTB/RIF test results with those of standard culture for *Mycobacterium tuberculosis* and phenotypic susceptibility tests. Although the Xpert MTB/RIF test showed high accuracy for the detection of *M. tuberculosis* and its resistance to rifampin, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.

Keywords: Tuberculosis/diagnosis; Molecular diagnostic techniques; Sputum; Bronchoalveolar lavage fluid.

Tuberculosis continues to be a major public health problem worldwide. It is estimated that there were 6.3 million new cases of tuberculosis worldwide in 2016, 1.7 million individuals having died from the disease, which is recognized by the World Health Organization (WHO) as the leading cause of death from infectious diseases worldwide.[1] In 2016, 66,796 new cases of tuberculosis were diagnosed and reported in Brazil.[2]

Early diagnosis and treatment of pulmonary tuberculosis are essential in reducing tuberculosis dissemination, morbidity, mortality, and costs.[3] In Brazil, 71.6% of all new tuberculosis cases in 2016 were confirmed by laboratory criteria, and, according to the latest WHO report, 41% of all multidrug-resistant tuberculosis cases in 2016 were diagnosed in Brazil.[1,2] However, conventional diagnostic methods have disadvantages such as low sensitivity and specificity (in the case of smear microscopy), as well as the considerable time required for obtaining test results (in the cases of culture and drug susceptibility testing).[4] Molecular diagnostic techniques have been reported as being more sensitive, specific, and rapid.[5]

In 2010, the WHO recommended the use of the Xpert MTB/RIF rapid molecular test for tuberculosis (Cepheid, Sunnyvale, CA, USA), a rapid and fully automated nucleic acid amplification test that detects *Mycobacterium tuberculosis* and its resistance to rifampin.[6] Although most studies validating the Xpert MTB/RIF test have shown promising results, showing good accuracy in sputum samples,[7] only a few have shown good accuracy in BAL fluid and tracheal aspirate (TA).[3,8-10]

The Brazilian National Ministry of Health has recently incorporated the use of the Xpert MTB/RIF test in some laboratories in Brazil, including the laboratory of the Júlia Kubitschek Hospital, which is operated by the Hospital Foundation of the State of Minas Gerais, a tertiary referral hospital for tuberculosis and drug-resistant tuberculosis in the city of Belo Horizonte, Brazil.[11] The objective of the present study was to evaluate the accuracy of the Xpert MTB/RIF test in sputum samples, BAL fluid, and TA obtained from patients with suspected pulmonary tuberculosis at the aforementioned hospital.

This was a retrospective descriptive study. We compared the Xpert MTB/RIF test results with those of standard culture for *M. tuberculosis* in a total of 534 samples in the period between December of 2014 and November of 2015. Of those samples, 238 were sputum samples, 199 were BAL fluid samples, and 97 were TA samples. Culture was considered the standard method for detecting *M.*

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tuberculosis. Antimicrobial susceptibility testing (AST) was considered the standard method for detecting resistance to rifampin. Samples with insufficient growth for mycobacterial species identification were excluded, as were contaminated samples and samples with growth of nontuberculous mycobacteria.

Culture was performed on Löwenstein-Jensen medium after decontamination by the sodium lauryl sulfate method.[12] Species identification and AST by the proportion method[13,14] or by an automated culture system (BACTEC Mycobacteria Growth Indicator Tube [MGIT] 960; Becton Dickinson, Sparks, MD, USA) were performed in the Ezequiel Dias Foundation state referral laboratory. The Xpert MTB/RIF test was performed in accordance with the manufacturer instructions.[15]

Sensitivity, specificity, positive predictive value, negative predictive value, accuracy, and agreement were calculated with the Minitab software, version 17 (Minitab Inc., State College, PA, USA) and the GraphPad Prism software, version 7 (GraphPad Software Inc., San Diego, CA, USA).

The study project was approved by the Research Ethics Committee of the Hospital Foundation of the State of Minas Gerais (Ruling no. 1,764,672).

Culture was positive for *M. tuberculosis* in 15.2% of the samples (81/534), and the Xpert MTB/RIF test was positive for *M. tuberculosis* in 19.9% of the samples (106/534). Table 1 shows the overall accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis*, whereas Table 2 shows the accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis* for each sample type.

AST was performed in 60 isolates from 81 cultures that were positive for *M. tuberculosis*. Of those 60 isolates, 9 were found to be resistant to rifampin by AST and the Xpert MTB/RIF test. With regard to susceptibility to rifampin, there was agreement between the two test methods for 49 of the 60 isolates and disagreement for 2 (resistance to rifampin by the Xpert MTB/RIF test and susceptibility to rifampin by AST in 1 and susceptibility to rifampin by the Xpert MTB/RIF test and resistance to rifampin by AST in 1). The accuracy of the Xpert MTB/RIF test in detecting resistance to rifampin is shown in Table 1.

In 25 patients, the Xpert MTB/RIF test results were positive but culture results were negative. Of those 25 patients, 9 had a history of tuberculosis, 4 had been receiving treatment at the time of testing, and 1 underwent testing for disease control (the test having therefore been incorrectly requested). In 4 patients, there was no history of tuberculosis and treatment was not initiated, the outcome being defined as mycobacteria other than tuberculosis. In the remaining 7 patients, it was impossible to evaluate clinical history.

Sensitivity was higher in the present study than in other studies (82-93%), whereas specificity was similar (96-100%).[3,7-9] The negative predictive value of the Xpert MTB/RIF test was found to be high, meaning that the test can rapidly rule out tuberculosis in patients suspected of having the disease.

With regard to the accuracy of the Xpert MTB/RIF test in detecting *M. tuberculosis* for each sample type, the results were similar except for the positive predictive values for sputum and BAL fluid samples, which were lower because of a higher number of discordant (false-positive) results between the two.

Of the 25 patients in whom there was disagreement between the Xpert MTB/RIF test results and culture results (i.e., positive Xpert MTB/RIF test results and negative culture results), 14 had a history of tuberculosis, with 4 being under treatment at the time of testing; this shows the importance of effective communication between the laboratory and clinical staff, given that the Xpert MTB/RIF test amplifies DNA originating from live or dead bacilli.[3] In a report published one year after the implementation of the Xpert MTB/RIF test in Brazil, 50% of the monitors stated that the test request forms used in their states lacked important information—such as whether the individual under investigation for tuberculosis has any risk factors for the disease—thus making it difficult to select the most appropriate tools for diagnosis.[16]

In patients without active disease but with dead bacilli in their lungs from previously treated active tuberculosis, the Xpert MTB/RIF test results can remain positive for up to five years; therefore, conversion to negative is not a suitable marker of treatment success. In such cases, the diagnosis of tuberculosis should be made exclusively by sputum smear microscopy and sputum culture; the Xpert MTB/RIF test can be used only to identify early resistance to rifampin.[17,18] Further studies are needed in order to quantify this better and determine predisposing factors.[17]

Table 1. Diagnostic accuracy of the Xpert MTB/RIF test for detecting *Mycobacterium tuberculosis* and its resistance to rifampin.[a]

Variable	Detection of MTB	Detection of resistance to RIF
Sensitivity (%)	100 (100-100)	100 (100-100)
Specificity (%)	94.5 (92.4-96.6)	98.0 (94.2-101.8)
PPV (%)	76.4 (68.3-84. 5)	90.0 (76.0-103.1)
NPV (%)	100 (100-100)	100 (100-100)
Accuracy (%)	95.3 (93.5-97.1)	98.3 (95.1-101.6)
Kappa*	0.84 (0.78-0.90)	0.94 (0.82-1.06)

MTB: *Mycobacterium tuberculosis*; RIF: rifampin; PPV: positive predictive value; and NPV: negative predictive value. [a]Values expressed as n (95% CI). *The criteria for kappa were as follows: < 0.20, poor; 0.21-0.40, weak; 0.41-0.60, moderate; 0.61-0.80, good; and > 0.80-1.00, very good.

Table 2. Diagnostic accuracy of the Xpert MTB/RIF test for different types of pulmonary samples.

Variable	Sputum (n = 238)	Bronchoalveolar lavage fluid (n = 199)	Tracheal aspirate (n = 97)
Sensitivity (%)	100 (100-100)	100 (100-100)	100 (100-100)
Specificity (%)	92.8 (89.1-96.4)	95 (91.8-98.2)	97.5 (94.0-100.9)
PPV (%)	75.9 (64.9-86.9)	67.9 (50.6-85.2)	90.0 (76.0-103.1)
NPV (%)	100 (100-100)	100 (100-100)	100 (100-100)
Accuracy (%)	94.1 (91.1-97.1)	95.5 (92.6-98.4)	97.9 (95.1-100.8)
Kappa*	0.83 (0.74-0.91)	0.78 (0.65-0.92)	0.93 (0.84-1.02)

PPV: positive predictive value; and NPV: negative predictive value. [a]Values expressed as n (95% CI). *The criteria for kappa were as follows: < 0.20, poor; 0.21-0.40, weak; 0.41-0.60, moderate; 0.61-0.80, good; and > 0.80-1.00, very good.

In the 4 patients who had no history of tuberculosis and who were classified as having mycobacteria other than tuberculosis in the present study, the results should be interpreted in a clinical context. In some cases, the Xpert MTB/RIF test can be more sensitive than conventional culture. The high sensitivity of the Xpert MTB/RIF test can be explained by the analytical detection limit, which is 131 colony-forming units/mL, being as high as 10 colony-forming units/mL in some samples.[9]

It has been hypothesized that false-positive results are due to residual persistent DNA from dead *M. tuberculosis* in lung tissue, expectorated because of another lung disease and thus leading to false-positive results for active tuberculosis.[17]

Of 25 positive Xpert MTB/RIF test results and negative culture results, 12 showed very low cycle threshold values (> 28 cycles) and 11 showed low values (23-28 cycles). These values represent a low concentration of *M. tuberculosis* complex DNA in our sample.[3] Further studies are needed in order to interpret these results in conjunction with patient clinical evaluation and the presence of other diseases.

Positive Xpert MTB/RIF test results and negative culture results might be related to technical manipulation issues, such as drastic decontamination procedures, temperature fluctuations in the incubator, and improper clinical sample storage.[12]

With regard to rifampin resistance, 2 samples showed disagreement between the Xpert MTB/RIF test and AST: resistance to rifampin by the Xpert MTB/RIF test and susceptibility to rifampin by AST in 1 and susceptibility to rifampin by the Xpert MTB/RIF test and resistance to rifampin by AST in 1. Resistance to rifampin is primarily due to mutations in the *rpoB* gene; however, rare mutations can occur outside the target region, and rifampin resistance cannot be detected unless 65-100% of the DNA population in the sample is mutant.[4,19] In addition, mixed infections can lead to false-negative or false-positive results. Heteroresistance is defined by the presence of susceptible and resistant populations of *M. tuberculosis* and has been reported as a possible cause of discordant AST results.[20]

One of the limitations of the present study is that sputum smear microscopy was not performed in parallel with the Xpert MTB/RIF test, because the samples were evaluated under routine laboratory conditions. In addition, it was impossible to analyze sociodemographic, clinical, and imaging data. Furthermore, it was impossible to study the impact of the Xpert MTB/RIF test on the time from diagnosis to treatment.

The results of the present study can contribute to improving the laboratory diagnosis of tuberculosis in sputum samples, BAL fluid, and TA; however, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.[3,17,18] In addition, it is important that test request forms be filled out correctly and include information on why the test is being requested (i.e., for diagnosis or follow-up), as well as patient history of tuberculosis treatment (i.e., previous tuberculosis treatment or no previous tuberculosis treatment) and risk factors for tuberculosis.[16]

Although the Xpert MTB/RIF test showed high accuracy for the detection of *M. tuberculosis* and its resistance to rifampin, attention must be given to the clinical status of the patient, in relation to the test results, as well as to the limitations of molecular tests.[3,17,18] Further studies are needed in order to evaluate the impact of the Xpert MTB/RIF test on patients and society in different settings (primary care, secondary care, and tertiary care) in the five regions of Brazil.

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Trends in tuberculosis mortality in Brazil (1990-2015): joinpoint analysis

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ABSTRACT

The objective of this study was to analyze trends in the tuberculosis mortality rate in Brazil (1990-2015) in an ecological time-series analysis. The indicators were obtained from the Brazilian National Ministry of Health. A joinpoint regression model was applied for the temporal analysis, with a level of significance of 5%. During the period in question, there was a trend toward a reduction in mortality in the country as a whole ($p < 0.001$) and in each of its five regions. The states with the highest tuberculosis mortality rates were Rio de Janeiro (7.0/100,000 population) and Pernambuco (5.0/100,000 population). Eleven states and the Federal District of Brasília showed downward trends. Only the state of Alagoas showed a significant increase ($p < 0.001$). The temporal behavior observed indicates that tuberculosis continues to be a major public health problem in Brazil.

Keywords: Tuberculosis/epidemiology; Tuberculosis/mortality; Mortality/trends; Epidemiologic studies.

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis*. It is estimated that one fourth of the world population is infected with *M. tuberculosis*.[1] In 2017 alone, an estimated 10 million people developed tuberculosis and 1.3 million died from the disease. Currently, tuberculosis is the leading cause of death among infectious diseases worldwide.[2] Also in 2017, a total of 72,770 new cases of tuberculosis were reported in Brazil, translating to an incidence of 35.0 cases/100,000 population. Although the Northeast and Southeast accounted for the highest number of cases (18,884 and 33,769, respectively), in terms of disease incidence, the North ranked first (46.6/100,000 population), followed by the Southeast (38.8/100,000 population).[3,4]

This spatial heterogeneity is even more marked when rates are analyzed state by state. The state of Amazonas had the highest incidence rate in 2017 (74.7/100,000 population), whereas the state of Tocantins had the lowest (10.0/100,000). In addition, ten states had a higher incidence rate than the national average in that same year.[4]

Concern about the global epidemiological scenario has led to the development of a new global strategy for fighting tuberculosis, known as End TB Strategy.[5] Proposed by the World Health Organization and approved by the World Health Assembly in 2014, this strategy proposes targets of a 90% reduction in tuberculosis incidence and a 95% reduction in tuberculosis mortality by 2035.[5]

Being one of the countries with the highest incidence of tuberculosis and being in two of the three groups of priority countries—ranking 20th regarding the burden of disease and 19th regarding the tuberculosis/HIV coinfection, Brazil has formulated the "*Plano Nacional pelo Fim da Tuberculose como Problema de Saúde Pública*" (Brazilian National Plan to End Tuberculosis as a Public Health Problem).[6] This plan is based on three pillars of action: integrated, patient-centered care and prevention; bold policies and supportive systems; and intensified research and innovation.[6]

In addition to the commitments made nationally and internationally, the relevance of studies of tuberculosis mortality lies in the fact that tuberculosis is a preventable disease.[2,5,6] Early diagnosis and appropriate treatment are imperative in this regard, because, in countries with universal health care system coverage, the proportion of people who die from tuberculosis may be less than 5%.[2] In this regard, tuberculosis mortality also indicates deficiencies in the health care system.[2,5]

In this scenario, the study of mortality rate trends may contribute to the management of the national plan, providing supports for public health decision making, such as identification of the most vulnerable regions and of weaknesses in the disease surveillance system. Therefore, the objective of the present study was to analyze trends in the tuberculosis mortality rate in the Brazilian regions and states for the period 1990-2015.

This was an ecological time-series analysis. Mortality data were extracted from the Brazilian National Ministry of Health Mortality Database, considering codes A15 to A19 of the International Classification of Diseases, 10th revision. The following equation was utilized to calculate the indicator: number of deaths/population for the reference year × 100,000. A joinpoint regression model was applied for the temporal analysis. This model tests

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whether a multi-segmented line is statistically better to describe the temporal evolution of a dataset than is a straight or less-segmented line.[7] The model allows us to detect the indicator trend (whether stationary, upward, or downward) and the points where the trend changes, allowing us to calculate the annual percent change (APC) and the percent change over the entire study period, known as average annual percent change. For each trend detected, we used a 95% CI and a level of significance of 5%. The analyses were performed with the Joinpoint Regression Program, version 4.5.0.1 (National Cancer Institute, Bethesda, MD, USA).

The tuberculosis mortality rate in Brazil between 1990 and 2015 ranged from 2.2/100,000 population (in 2014) to 3.8/100,000 population (in 1994). The regression model indicated three temporal behaviors: the first one, a stationary behavior between 1990 and 1998 (APC: 0.53; 95% CI: −0.3 to 1.3; p = 0.2); the second one, a decreasing behavior between 1998 and 2003 (APC: −5.81; 95% CI: −8.0 to −3.6; p < 0.001); and the third one, also a decreasing behavior (APC: −1.88; 95% CI: −2.3 to −1.4; p < 0.001). Analysis of the entire study period showed a significant downward trend in the mortality rate in Brazil (APC: −1.9; 95% CI: −2.4 to −1.4; p < 0.001), which decreased from 3.6 deaths/100,000 population in 1990 to 2.3/100,000 population in 2015, translating to a mean rate of 3.0/100,000 population in the period in question (Figure 1).

Analysis by macro-region showed that the highest mortality rates were observed in the Southeast (3.5/100,000 population) and Northeast (3.0/100,000 population). In the temporal analysis, all five regions exhibited a statistically significant decreasing behavior, with the Southeast showing the largest percent reduction (APC: −2.7; 95% CI: −3.1 to −2.2; p < 0.001) and the Northeast showing the smallest percent reduction (APC: −0.5; 95% CI: −0.9 to −0.01; Figure 1 and Table 1).

Analysis by state showed that the highest tuberculosis mortality rates were observed in Rio de Janeiro (7.0/100,000 population) and in Pernambuco (5.0/100,000 population). Analysis of the temporal model showed that 11 states and the Federal District of Brasília exhibited a downward trend. Of those 11 states, 4 are in the North, 1 is in the Northeast, 3 are in the Southeast, 2 are in the South, and 1 is in the Central-West, as is the Federal District of Brasília. In contrast, 14 states exhibited a stationary pattern, of which 3 are in the North, 7 are in the Northeast, 1 is in the Southeast, 1 is in the South, and 2 are in the Central-West. Only the state of Alagoas exhibited an upward trend (APC: 1.0; 95% CI: 0.2-1.8; p < 0.001; Table 1).

The downward trend observed in the tuberculosis mortality rate in Brazil is consistent with the global temporal pattern. Between 2000 and 2015, tuberculosis mortality worldwide decreased by 29% in HIV-negative individuals and by 44% in HIV-positive individuals. However, this reduction is far from what is recommended in the End TB Strategy, whose targets are a 35% reduction in tuberculosis mortality by 2020 and a 90% reduction by 2035.[8] This is, therefore, a bold goal that indicates the size of the challenge faced by Brazil in reaching it.

Currently, tuberculosis/HIV coinfection is one of the most important factors contributing to tuberculosis mortality worldwide.[8] Therefore, reducing tuberculosis mortality should also involve public policies aimed at HIV detection in the population and adherence to antiretroviral therapy, given that maintaining immunological competence is essential in order not to develop tuberculosis, as well as in order to prevent death from tuberculosis.[5,6,9]

Another factor that can significantly change the trends in tuberculosis mortality in Brazil is the implementation of the "*Protocolo para vigilância do óbito com menção de tuberculose nas causas de morte*" (Protocol for the surveillance of deaths with any mention of tuberculosis as a listed cause of death).[10] The purpose of this tool is to investigate deaths with any mention of tuberculosis among patients who were not reported to the Brazilian Tuberculosis Case Registry Database, reducing underreporting.[10] Therefore, the investigation of deaths, as recommended in the protocol, may result in an increase in tuberculosis mortality and incidence rates, reflecting a more realistic epidemiological scenario.

One of the most important factors in determining the risk of tuberculosis mortality is the direct influence of treatment dropout.[9] It is estimated that, in 2035, the tuberculosis mortality rate in Brazil will be 1.17/100,000 population without there being a change in the treatment dropout rate.[8] However, a 5% reduction in the treatment dropout rate would result in even lower mortality. Therefore, the tuberculosis mortality rate would be 0.94/100,000 population rather than 1.17/100,000 population, making it possible to achieve the global goal (i.e., a tuberculosis mortality rate < 1/100,000 population).[11] The adoption of measures that will result in reduced treatment dropout is a key imperative for reducing tuberculosis mortality.

In the analyses by state, our findings corroborate those of studies conducted in the states of São Paulo,[12] Paraná,[13] and Santa Catarina.[14] What those studies have in common is the fluctuation in mortality rates, characterized by a period of increase followed by successive periods of decline, with a stationary behavior in the last years of the time series. However, differences in rates are observed when comparing states by region. This discrepancy might reflect the influence of social inequities that act as social determinants of health and increase the risk of tuberculosis mortality.[15] In states in the North and Northeast, where there still is persistent social vulnerability, reducing mortality becomes an even greater challenge. In addition to macro-regional social issues, there are also issues related to health care itself, such as health care coordination and epidemiological surveillance activities, resulting in difficulties in tuberculosis control by states and cities.[16-18]

Tuberculosis mortality can be influenced by the degree of integration between epidemiological surveillance

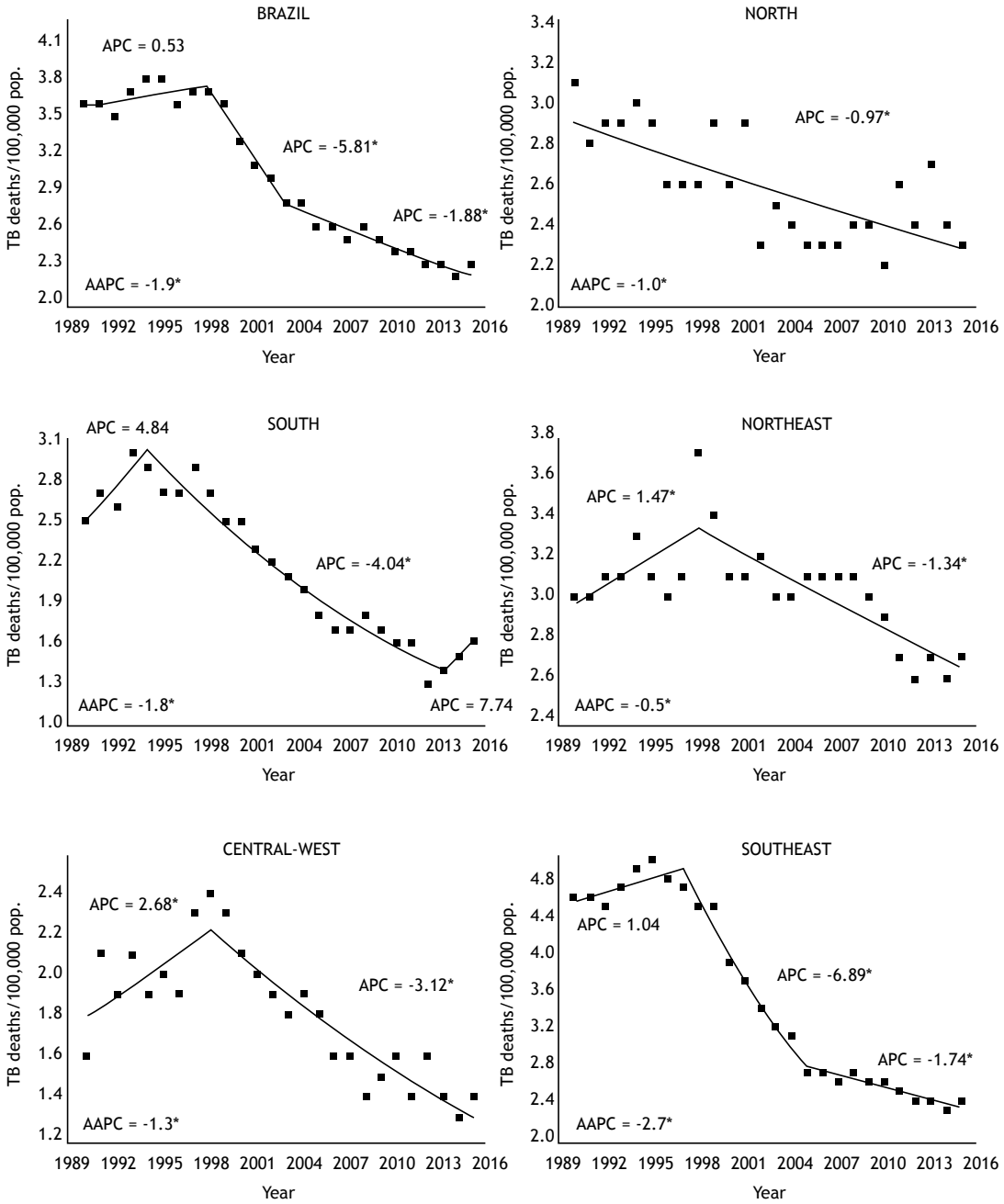

Figure 1. Temporal evolution of mortality rate due to tuberculosis in Brazil and its regions, 1990-2015. Parameters used in the joinpoint analysis—minimum: 0; maximum: 4; selection of the model: permutation test with 4,499 replications; significance of 5%; and error autocorrelation based on data. TB: tuberculosis; pop.: population; APC: annual percent change; and AAPC: average annual percent change. *p < 0.05.

activities and the care provided, especially with regard to primary care.[17,18] Therefore, the gap between these two components of the health care system can explain the disparities observed in state trends. In our study, the states with the highest mortality rates also showed the largest percent reductions when compared with those with the lowest rates, whose trends were stationary.

It should be noted that the present study has limitations, especially with regard to the poor quality of mortality records, including among regions. Inadequate completion of death certificates, resulting in a large number of garbage codes; difficulties in carrying out epidemiological investigations of deaths classified as having ill-defined causes; and the lack of personnel trained to perform surveillance activities are common problems found throughout Brazil, although the North and Northeast are the most affected regions.[19] Therefore, the number of deaths may be higher than that observed, especially in those regions where health surveillance

Table 1. Trends in the tuberculosis mortality rate (per 100,000 population) in all Brazilian states. Brazil, 1990-2015.

Region/state	Mortality/100,000 population			Period	APC (95% CI)	AAPC (95% CI)
	1990	2015	1990 to 2015			
BRAZIL	3.6	2.3		1990-1998	0.5 (-0.3 to 1.3)	-1.9* (-2.4 to -1.4)
			3.0	1998-2003	-5.8* (-8.0 to -3.6)	
				2003-2015	-1.9* (-2.3 to -1.4)	
North	3.1	2.3	2.6	1990-2015	-1.0* (-1.3 to -0.6)	-1.0* (-1.3 to -0.6)
RO	4.4	1.4	2.5	1990-2015	-4.1* (-4.9 to -3.4)	-4.1* (-4.9 to -3.4)
AC	3.7	2.2	3.6	1990-2015	-3.7* (-5.1 to -2.3)	-3.7* (-5.1 to -2.3)
AM	3.8	3.3	3.5	1990-2015	-0.4 (-0.9 to 0.1)	-0.4 (-0.9 to 0.1)
RR	3.9	0.8	2.5	1990-2015	-9.2* (-14.4 to -3.8)	-9.2* (-14.4 to -3.8)
PA	3.0	2.6	2.5	1990-1996	-6.0* (-10.0 to -1.7)	-0.6 (-1.7 to 0.5)
				1996-2015	1.1* (0.4 to 1.9)	
AP	2.5	1.8	1.9	1990-2015	-1.8* (-3.1 to -0.4)	-1.8* (-3.1 to -0.4)
TO	0.3	0.5	1.1	1990-1992	126.2 (-25.3 to 585.3)	4.4 (-4.3 to 13.8)
				1992-2015	-2.4 (-4.8 to 0.0)	
Northeast	3.0	2.7	3.0	1990-1998	1.5* (0.3 to 2.7)	-0.5* (-0.9 to -0.01)
				1998-2015	-1.3* (-1.7 to -1.0)	
MA	1.9	2.2	2.3	1990-2009	3.1* (1.9 to 4.4)	1.1 (-0.7 to 2.9)
				2009-2015	-5.1 (-11.5 to 1.8)	
PI	2.6	1.4	2.1	1990-1996	-10.1* (-17.9 to -1.5)	-1.1 (-7.4 to 5.5)
				1996-1999	27.3 (-25.9 to 118.6)	
				1999-2015	-2.3* (-4.3 to -0.2)	
CE	2.0	2.3	2.8	1990-1992	31.5 (-5.8 to 83.6)	1.0 (-4.7 to 7.0)
				1992-1995	-13.5 (-38.0 to 20.8)	
				1995-1998	17.9 (-15.5 to 64.6)	
				1998-2015	-2.1* (-3.3 to -1.0)	
RN	2.0	1.9	2.2	1990-2015	-1.0 (-2.2 to 0.3)	-1.0 (-2.2 to 0.3)
PB	1.7	2.2	2.0	1990-2015	1.1 (-0.2 to 2.5)	1.1 (-0.2 to 2.5)
PE	4.8	4.5	5.0	1990-1998	2.6* (0.7 to 4.4)	-0.3 (-1.7 to 1.1)
				1998-2013	-3.0* (-3.7 to -2.2)	
				2013-2015	9.1 (-7.4 to 28.6)	
AL	2.5	2.3	2.7	1990-2015	1.0* (0.2 to 1.8)	1.0* (0.2 to 1.8)
SE	2.3	2.0	2.0	1990-2015	0.0 (-0.8 to 0.9)	0.0 (-0.8 to 0.9)
BA	3.8	2.7	3.2	1990-2015	-1.6* (-1.9 - -1.2)	-1.6* (-1.9 to -1.2)
Southeast	4.6	2.4	3.5	1990-1997	1.0 (-0.0 to 2.1)	-2.7* (-3.1 to -2.2)
				1997-2005	-6.9* (-7.9 to -5.9)	
				2005-2015	-1.7* (-2.3 to -1.1)	
MG	2.4	1.1	1.8	1990-2011	-2.5* (-3.0 to -2.1)	-3.3* (-4.2 to -2.4)
				2011-2015	-7.2* (-12.3 to -1.8)	
ES	2.3	1.9	2.4	1990-1993	11.1 (-2.9 to 27.1)	-0.9 (-2.7 to 1.0)
				1993-2005	-5.3* (-7.0 to -3.6)	
				2005-2015	1.1 (-1.0 to 3.3)	
RJ	9.1	5.0	7.0	1990-1995	3.2 (-0.01 to 6.5)	-2.1* (-2.9 to -1.3)
				1995-2005	-6.1* (-7.3 to -4.9)	
				2005-2015	-0.6 (-1.7 to 0.5)	
SP	4.1	2.0	3.2	1990-1999	0.8 (-0.1 to 1.7)	-3.0* (-3.6 to -2.4)
				1999-2005	-10.3* (-12.2 to -8.3)	
				2005-2015	-1.8* (-2.6 to -1.1)	
South	2.5	1.6	2.2	1990-1994	4.8 (-0.6 to 10.5)	-1.8* (-3.3 to -0.3)
				1994-2013	-4.0* (-4.6 to -3.5)	
				2013-2015	7.7 (-8.9 to 27.4)	

APC: annual percent change; AAPC: average annual percent change; RO: Rondônia; AC: Acre; AM: Amazonas; RR: Roraima; PA: Pará; AP: Amapá; TO: Tocantins; MA: Maranhão; PI: Piauí; CE: Ceará; RN: Rio Grande do Norte; PB: Paraíba; PE: Pernambuco; AL: Alagoas; SE: Sergipe; BA: Bahia; MG: Minas Gerais; ES: Espírito Santo; RJ: Rio de Janeiro; SP: São Paulo; PR: Paraná; SC: Santa Catarina; RS: Rio Grande do Sul; MS: Mato Grosso do Sul; MT: Mato Grosso; GO: Goiás; and DF: Distrito Federal (Federal District of Brasília).*p < 0.05.

Table 1. Continued...

Region/state	Mortality/100,000 population			Period	APC (95% CI)	AAPC (95% CI)
	1990	2015	1990 to 2015			
PR	2.0	1.1	1.9	1990-1998	3.8* (0.9 to 6.7)	-2.5* (-4.2 to -0.7)
				1998-2012	-7.0* (-8.3 to -5.8)	
				2012-2015	3.6 (-8.9 to 17.9)	
SC	1.1	0.8	1.1	1990-2015	-2.1* (-2.8 to -1.3)	-2.1* (-2.8 to -1.3)
RS	3.6	2.6	3.0	1990-1993	4.8 (-3.1 to 13.3)	-1.3 (-3.7 to 1.2)
				1993-2006	-4.1* (-5.0 to -3.2)	
				2006-2009	2.7 (-12.2 to 20.2)	
				2009-2013	-6.4 (-13.4 to 1.3)	
				2013-2015	13.9 (-2.6 to 33.2)	
Central-West	1.6	1.4	1.8	1990-1998	2.7* (0.2 to 5.2)	-1.3* (-2.2 to -0.4)
				1998-2015	-3.1* (-3.9 to -2.4)	
MS	2.4	1.8	2.8	1990-2015	-1.3* (-2.2 to -0.3)	-1.3* (-2.2 to -0.3)
MT	2.2	2.2	3.0	1990-1998	7.0* (2.7 to 11.4)	-0.4 (-1.9 to 1.0)
				1998-2015	-3.8* (-5.0 to -2.5)	
GO	0.9	1.1	1.2	1990-1993	16.9 (-2.3 to 39.8)	0.1 (-2.0 to 2.3)
				1993-2015	-2.0* (-2.8 to -1.1)	
DF	1.9	0.5	1.0	1990-2015	-5.9* (-7.5 to -4.3)	-5.9* (-7.5 to -4.3)

APC: annual percent change; AAPC: average annual percent change; RO: Rondônia; AC: Acre; AM: Amazonas; RR: Roraima; PA: Pará; AP: Amapá; TO: Tocantins; MA: Maranhão; PI: Piauí; CE: Ceará; RN: Rio Grande do Norte; PB: Paraíba; PE: Pernambuco; AL: Alagoas; SE: Sergipe; BA: Bahia; MG: Minas Gerais; ES: Espírito Santo; RJ: Rio de Janeiro; SP: São Paulo; PR: Paraná; SC: Santa Catarina; RS: Rio Grande do Sul; MS: Mato Grosso do Sul; MT: Mato Grosso; GO: Goiás; and DF: Distrito Federal (Federal District of Brasília).*p < 0.05.

faces operational problems due particularly to the lack of (human, financial, and material) resources.[19]

Although many advances have been observed in tuberculosis control in recent years,[20] the temporal behavior of tuberculosis mortality rates that was observed during the period in question and the gap among states confirms that tuberculosis continues to be a major public health problem in Brazil. Therefore, regional and local strategies that can reduce tuberculosis mortality are needed. We advocate that, in order to reduce the problem, broad strategies of intervention, focused particularly on the social determinants of health, are needed.

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REVIEW ARTICLE

International collaboration among medical societies is an effective way to boost Latin American production of articles on tuberculosis

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Study carried out at the Centro de Investigación, Prevención y Tratamiento de Infecciones Respiratorias – CIPTIR – Monterrey, México, and the WHO Collaborating Centre For Tuberculosis and Lung Diseases, Tradate, Italia.

ABSTRACT

Objective: Most studies of tuberculosis originate from high-income countries with a low incidence of tuberculosis. A review of the scientific production on tuberculosis in Latin American countries, most of which are low- or middle-income countries (some with high or intermediate tuberculosis incidence rates), would improve the understanding of public health challenges, clinical needs, and research priorities. The aims of this systematic review were to determine what has been published recently in Latin America, to identify the leading authors involved, and to quantify the impact of international collaborations. **Methods:** We used PubMed to identify relevant manuscripts on pulmonary tuberculosis (PTB), drug-resistant tuberculosis (DR-TB), or multidrug-resistant tuberculosis (MDR-TB), published between 2013 and 2018. We selected only studies conducted in countries with an annual tuberculosis incidence of ≥ 10,000 reported cases and an annual MDR-TB incidence of ≥ 300 estimated cases, including Brazil, Peru, Mexico, Colombia, and Argentina. Articles were stratified by country, type, and topic. **Results:** We identified as eligible 395 studies on PTB and 188 studies on DR/MDR-TB—of which 96.4% and 96.8%, respectively, were original studies; 35.5% and 32.4%, respectively, had an epidemiological focus; and 52.7% and 36.2%, respectively, were conducted in Brazil. The recent Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project boosted the production of high-quality articles on PTB and DR/MDR-TB in Latin America. **Conclusions:** Most of the recent Latin American studies on tuberculosis were conducted in Brazil, Mexico, or Peru. Collaboration among medical societies facilitates the production of scientific papers on tuberculosis. Such initiatives are in support of the World Health Organization call for intensified research and innovation in tuberculosis.

Keywords: Tuberculosis, pulmonary; Tuberculosis, multidrug-resistant; Latin America.

INTRODUCTION

The World Health Organization (WHO) has estimated that, in 2017, there were 9.0-11.1 million new cases of active tuberculosis and 1.2-1.4 million tuberculosis-related deaths, indicating that tuberculosis is now the leading cause of infection-related death worldwide and is among the ten leading causes of death from any cause.[1] The WHO Region of the Americas, which is managed by the Pan American Health Organization, includes the United States and Canada, both of which have a low incidence of tuberculosis, whereas the incidence of tuberculosis ranges from low to high in Latin American and Caribbean countries, which are mainly low- to middle-income countries with limited resources allocated to health care and research.[1,2]

Scientific societies such as the *Asociación Latinoamericana de Tórax* (ALAT, Latin American Thoracic Association) and the *Sociedade Brasileira de Pneumologia e Tisiologia* (SBPT, Brazilian Thoracic Association) are both active in promoting training, continued medical education, and research that is useful in the fight against tuberculosis. The influence of those societies reaches most of the countries in Latin America. Recently, they have joined forces with the European Respiratory Society (ERS) to develop initiatives against tuberculosis in several fields, including research.[3,4] Such initiatives are collectively known as the ALAT/ERS/SBPT project. Because no specific funds were otherwise available for the task, the project included data collection, the creation of new databases, the ordering of existing databases, and the design of studies, as well as the writing/translation of the articles produced and the facilitation of their submission to peer-reviewed journals.

As clearly mentioned by the WHO and included in Pillar 3 of its "End TB Strategy",[5,6] research is crucial to promoting better clinical and public health initiatives.

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By reviewing who and what has recently been published in Latin America on the subject of active tuberculosis and measuring the impact of international collaboration on the production of scientific evidence, we could gain a better understanding of what aspects should be targeted in order to address the WHO recommendations.

For the purposes of this article, we included five of the six Latin American countries that report more than 10,000 cases of tuberculosis annually. Those countries are, in decreasing order of tuberculosis incidence, Brazil, Peru, Mexico, Colombia, and Argentina.[1-4] Collectively, they reported a total of 160,683 cases in 2016, as shown in Table S1 of the supplementary file (available online at http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=60).

Countries approaching the goal of tuberculosis elimination (defined as less than 1 case per million population) need to focus on specific interventions such as managing latent tuberculosis infection, and countries with a higher tuberculosis incidence need tuberculosis control activities focused on active pulmonary tuberculosis (PTB).[3,4,7-9] Therefore, we decided to limit our review to articles dealing with active tuberculosis.

The epidemiological diversity of Latin American countries was recently captured in two important documents related to the region, both published jointly by the Pan American Health Organization and the WHO: the 2013 Strategic Plan of the Pan American Health Organization[10]; and the 2014 Plan of Action for the Prevention and Control of Tuberculosis.[11] It is expected that research priorities will be aligned with the priorities and resources available in each country. The Brazilian National Plan to End Tuberculosis as a Public Health Problem is an example of that.[12] Further new information emerging from local studies is needed in order to increase the overall scientific production in Latin America.

The primary aim of this review was to identify the main areas of tuberculosis research conducted in the Latin American countries with the highest rates of active PTB, drug resistant-tuberculosis (DR-TB), and multidrug-resistant tuberculosis (MDR-TB). Secondary aims were to identify the Latin American researchers leading the production of tuberculosis research and to evaluate the impact that recent international collaborations among medical societies have had on the overall scientific output in the region.

METHODS

This study focused on the local scientific contributions of Brazil, Peru, Mexico, Colombia, and Argentina related to PTB and MDR-TB. Those five countries, all of which are middle-income countries, have the highest scientific production rates in Latin America. In each of those countries, the annual tuberculosis incidence is ≥ 10,000 reported cases and the annual MDR-TB incidence is ≥ 300 estimated cases, as shown in Table S1 of the supplementary file (available online at http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=60). Those are also the only countries that have participated in the ALAT/ERS/SBPT project.

Although Haiti ranks fourth in Latin America in terms of the incidence of tuberculosis, we decided not to include it in the regional analysis of this review, for a number of reasons. As a low-income country, Haiti receives long-term external financial support for research (mainly from United States government agencies). In addition, Haiti has not been involved in any studies related to the ALAT/ERS/SBPT project. Those two conditions would make it difficult to evaluate the spontaneous research contribution of the country.

Inclusion criteria

We selected peer-reviewed articles written in English, Spanish, or Portuguese by authors (corresponding authors or not) working in any of the five Latin American countries under study (Brazil, Peru, Mexico, Colombia, and Argentina). We used PubMed to identify any relevant manuscripts, published between January 1, 2013 and April 19, 2018, authored by Latin American researchers. To attribute a given article to a given country, the first selection criterion was the country of the corresponding author, followed by that of the first author and then that of each of the other authors, based on the affiliations as they appeared in the original manuscript. Manuscripts with authors whose main affiliation was in a high-income country (e.g., the United States, Canada, or a country in Europe) were not considered if no Latin American affiliations were listed.

We performed our searches in two steps, using the following search terms: "pulmonary tuberculosis" OR "pulmonary TB", to retrieve articles related to PTB (step 1); and "multidrug-resistant tuberculosis" OR "multidrug-resistant TB" OR "MDR-TB" OR "drug-resistant tuberculosis" OR "drug-resistant TB", to retrieve articles specifically focused on DR/MDR-TB (step 2). Most of the MDR-TB-related manuscripts were retrieved in the first step. Articles related to extrapulmonary tuberculosis were excluded, because the focus of this review was to identify the scientific production related to the transmissible form of tuberculosis (drug-susceptible or drug-resistant PTB).

We included full-text original articles, review articles, editorials, letters, correspondence containing original data, and case reports containing new information. To ensure the quality of the publication, we included only articles that were published in journals that had an impact factor in the year of publication. Basic research studies were included if they involved patients with PTB. Case reports containing no new information were excluded, as were editorials/letters containing no original data.

Studies that were not related to the ALAT/ERS/SBPT collaborative project were analyzed separately. We then drew comparisons between the articles that were related to the project and those that were not,

those comparisons being limited to articles published in 2016, 2017, or the first quarter of 2018.

Data analysis

The articles were first separated into two groups: those related to PTB; and those related to DR/MDR-TB. They were then stratified by country and type of manuscript—articles containing original data (full manuscripts, short reports, or letters), editorials, and review articles—as well as by topic (epidemiology/research, biochemistry/diagnosis, treatment/outcomes, or genetics/immunology/vaccines). Two of the authors, working independently, evaluated the manuscripts. Any disagreements were resolved by consensus.

For each country, the authors publishing the most articles, either on PTB or on DR/MDR-TB, were identified. For each of those authors, a complete bibliometric analysis was performed, including the overall number of publications, the h-index, and the number of citations. The articles related to the ALAT/ERS/SBPT project (and therefore their authors) were not considered in the main analysis, although they were considered in the comparative analysis. The study was conducted in accordance with the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines.[13]

RESULTS

For the period from January 2013 to April 2018, a total of 803 manuscripts were identified (Figure 1): 532 on PTB and 271 on DR/MDR-TB. Of those 803 manuscripts, 583 were deemed eligible for further analysis: 395 on PTB and 188 on DR/MDR-TB.

Of the 395 articles on PTB, 137 were excluded, for the following reasons (Figure 1A): being a case report or letter containing no new information (n = 45); being authored by individuals not working in one of the Latin American countries specified (n = 34); not focusing on tuberculosis (n = 25); and having been published in a journal that had no impact factor in the year of publication (n = 33). The annual number of articles on PTB unrelated to the ALAT/ERS/SBPT project was rather stable (Table 1): 60 in 2016; 90 in 2017; and 17 in the first quarter of 2018.

As can be seen in Figure 2A, the country contributing the greatest number of articles on PTB was Brazil, which accounted for 208 (52.7%) of the 395 articles, followed by Mexico, with 79 (20.0%), Peru, with 57 (14.4%), Colombia, with 29 (7.3%), and Argentina, with 22 (5.6%). Table 2 describes the types of articles published in Latin America, the largest proportion being original studies, which accounted for 96.4% (381 articles). The most common topic studied in those articles was epidemiology/research (in 35.5%), followed by genetics/immunology/vaccines (in 29.9%), biochemistry/diagnosis (in 23.5%), and treatment/outcomes (in 11.1%).

Of the 188 articles on DR/MDR-TB, 82 were excluded, for the following reasons (Figure 1B): being a duplicate or a case report/letter containing no new information (n = 11); being authored by individuals not working in one of the Latin American countries specified (n = 28); not focusing on DR/MDR-TB or focusing on animal tuberculosis (n = 32); and having been published in a journal that had no impact factor in the year of publication (n = 12). The annual number of articles on DR/MDR-TB unrelated to the ALAT/ERS/SBPT project was rather stable (Table 1): 32 in 2016; 41 in 2017; and 10 in the first quarter of 2018. As can be seen in Figure 2B, Brazil was the country contributing the greatest number of articles on DR/MDR-TB, accounting for 68 (36.2%) of the 188 articles, followed by Peru, with 53 (28.2%), Mexico, with 33 (17.6%), Argentina, with 20 (10.6%), and Colombia, with 14 (7.4%). Table 2 shows that the largest proportion of articles on DR/MDR-TB were original studies, which accounted for 96.8% (182 articles). In terms of topics, the most common was epidemiology/research (in 32.4%), followed by treatment/outcomes (in 30.9%), biochemistry/diagnosis (in 27.1%), and genetics/immunology/vaccines (in 9.6%).

The bibliometric analysis of the top authors per country for the PTB and DR/MDR-TB categories are summarized in Table 3. For both categories, it is evident that the Brazilian Tuberculosis Research Network plays a leading role in Brazil,[14-19] whereas the Peruvian Partners in Health Research Network and Harvard University play major roles in Peru.[20-23] In Argentina, Mexico, and Colombia, most of the basic research studies are conducted at a few high-level institutions, often in collaboration with other countries within and outside of Latin America.

Table 4 presents the comparative analysis of studies related and unrelated to the ALAT/ERS/SBPT project. A total of 289 articles were published in the comparison period (2016-2018). Studies related to the ALAT/ERS/SBPT project accounted for 13.5% of those articles overall, specifically accounting for 9.7% of the articles on PTB and 20.1% of the articles on DR/MDR-TB (Figure 3). All of those articles were published in journals with an impact factor. The contributions of the authors who published within the ALAT/ERS/SBPT project are summarized in Table 5.

DISCUSSION

The aims of this systematic review were to determine which were the main areas of research on PTB and DR/MDR-TB conducted recently in Brazil, Peru, Mexico, Colombia, and Argentina, to identify the Latin American authors involved in that research, and to quantify the impact of collaboration among international medical societies. It is difficult to evaluate the quantity and quality of the scientific production of the selected countries, because there is no benchmark or gold standard comparator.

In a recent bibliometric analysis, Sweileh et al.[24] evaluated the studies on MDR-TB published worldwide

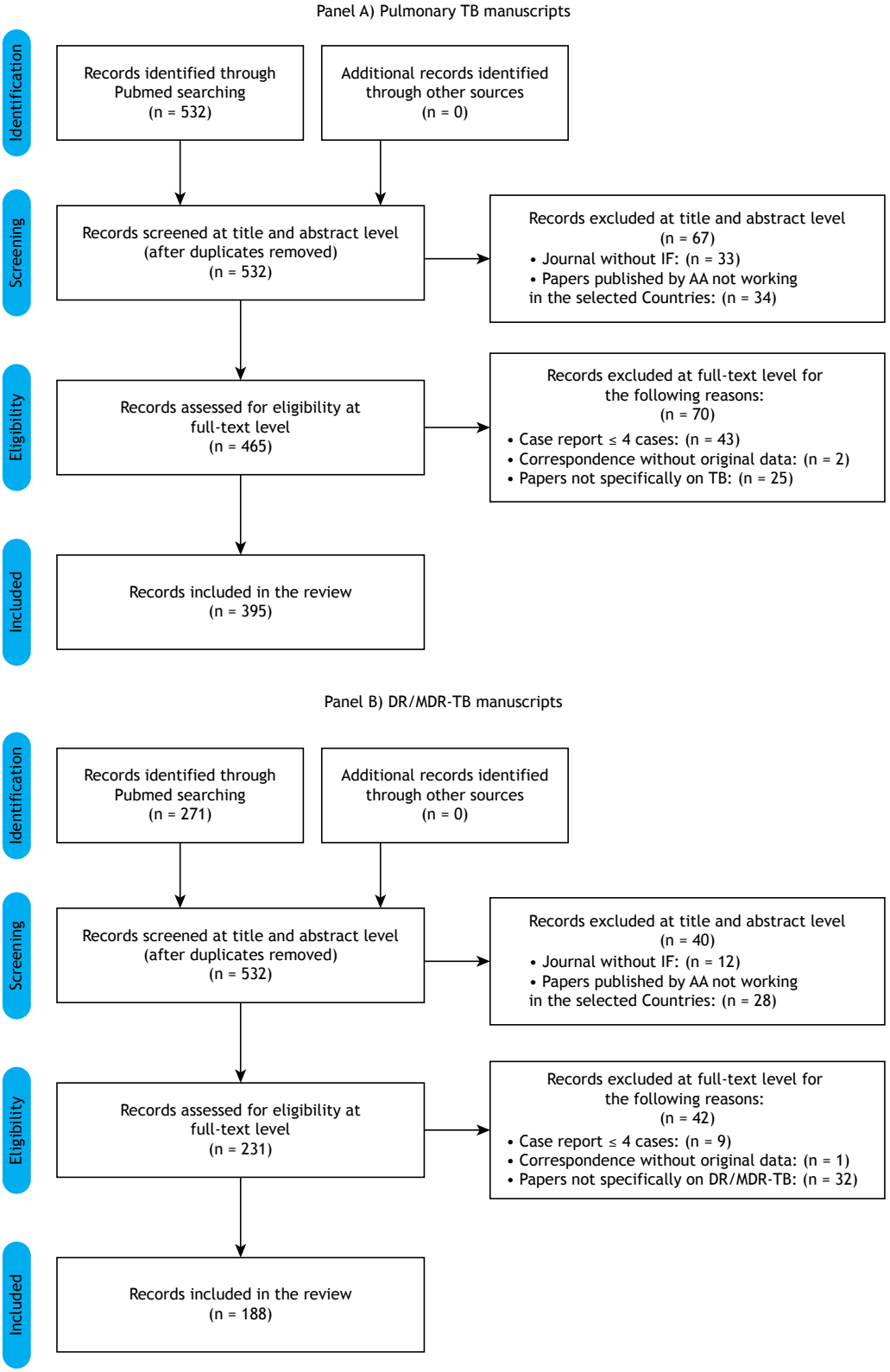


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses 2009 flow diagram of the process of selecting manuscripts on pulmonary tuberculosis (TB, panel A) and on drug-resistant/multidrug-resistant tuberculosis (DR/MDR-TB, panel B) unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project and authored by researchers working in Brazil, Mexico, Peru, Colombia, or Argentina. IF: impact factor.

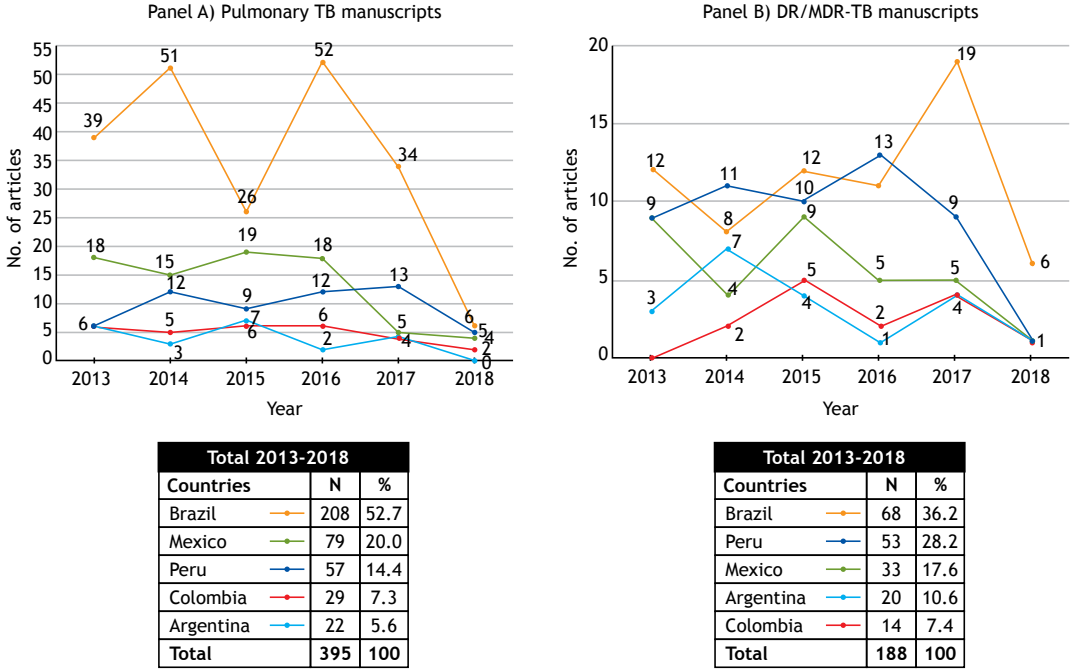
between 2006 and 2015. The authors found that the number of studies on tuberculosis and MDR-TB increased from 4,460 and 279, respectively, in 2013 to 4,711 and 342, respectively, in 2016. They also ranked countries by their level of scientific production on the topic of MDR-TB: Peru ranked 13th, with 69 articles; Brazil ranked 18th, with 51; Mexico ranked 24th, with 36; Argentina ranked 31st, with 29; and Colombia ranked 37th, with 14. In the worldwide bibliometric analysis, original articles accounted for 71.3% of the articles, whereas review articles accounted for 9.6% and editorials accounted for 3.8%. Despite the methodological differences between that study and ours (the former having used the Scopus database, having focused on MDR-TB, and not having limited the searches to journals with an impact factor), the overall production in the Latin American countries included in our study is quantitatively consistent with that reported by those authors. Given the continuous increase in the number of articles published over time, the number of articles published per year is comparable between the two studies. In terms of the types of articles, our findings were also similar: original articles were the most common type of articles, followed by review articles and editorials. One difference was related to the proportional distribution of the article types, original articles accounting for 96.5% of the articles identified in our study, compared with only 71.3% in the study conducted by Sweileh et al.[24] In the latter study, one Latin American author (Becerra MC, from Peru) was among the top 20 authors publishing research on MDR-TB, having authored 29 articles during the period evaluated. That same author also ranked highly in our study. Nine of the top-ranked authors from the five countries we studied had an h-index ≥ 20 (50 being the highest), confirming that high-quality research groups are active in the region. However, it should be borne in mind that some of the most highly ranked authors conduct research on a wide spectrum of tropical diseases other than tuberculosis and that their h-indices therefore reflect their overall scientific production.

Table 1. Articles unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project, 2013-2018.

Year	Pulmonary TB	DR/MDR-TB	Total
2013	75	33	108
2014	86	32	118
2015	67	40	107
2016	90	32	132
2017	60	41	114
2018[a]	17	10	44
Total	395	188	583

TB: tuberculosis; and DR/MDR-TB: drug-resistant/multidrug-resistant tuberculosis. [a]Only articles published in the first quarter of 2018.

In the present study, we considered the articles in which at least one of the authors had an affiliation in one of the five Latin American countries selected. Therefore, articles in which the affiliation was outside those countries (e.g., Harvard University rather than the Peruvian Partners in Health Research Network for studies conducted in Peru) were not counted. As previously mentioned, we did not consider studies conducted in Haiti, because research projects in



Total 2013-2018		
Countries	N	%
Brazil	208	52.7
Mexico	79	20.0
Peru	57	14.4
Colombia	29	7.3
Argentina	22	5.6
Total	**395**	**100**

Total 2013-2018		
Countries	N	%
Brazil	68	36.2
Peru	53	28.2
Mexico	33	17.6
Argentina	20	10.6
Colombia	14	7.4
Total	**188**	**100**

Figure 2. Results of the searches for manuscripts on pulmonary tuberculosis (TB, panel A) and on drug-resistant/multidrug-resistant tuberculosis (DR/MDR-TB, panel B) unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project, by country.

Table 2. Manuscript types and topics of the articles unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project, 2013-2018.[a]

Manuscript characteristic	Pulmonary TB		DR/MDR-TB	
Type	n	%	n	%
Original article[b]	381	96.5	182	96.8
Review article	11	2.8	4	2.1
Editorial	3	0.8	2	1.1
Total	395	100	188	100
Topic	n	%	n	%
Epidemiology/research	140	35.4	61	32.4
Biochemistry/diagnosis	93	23.5	51	27.1
Treatment/outcomes	44	11.1	58	30.9
Genetics/immunology/vaccines	118	29.9	18	9.6
Total	395	100	188	100

TB: tuberculosis; and DR/MDR-TB: drug-resistant/multidrug-resistant tuberculosis. [a]Included only the first quarter of 2018. [b]Included all articles containing original data (full manuscripts, short reports, and letters).

Table 3. Top authors, in each country, of articles on pulmonary tuberculosis or drug-resistant/multidrug-resistant tuberculosis that were unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project, as determined in the bibliometric analysis.

Country Author	Articles on pulmonary TB or DR/MDR-TB	H-index[a]	Cited documents[a]
Argentina			
Bottasso, Oscar A	11	22	158
Ritacco, Viviana	11	26	94
López, Beatriz	10	15	43
Sasiain, María del Carmen	9	17	68
Bay, María Luisa	8	14	40
Brazil			
Kritski, Afrânio Lineu	27	28	186
Maciel, Ethel Leonor Noia	16	17	98
Rossetti, Maria Lúcia Rosa	15	20	86
Dietze, Reynaldo	14	35	133
Trajman, Anete	14	17	77
Colombia			
Marín, Diana	8	4	17
Robledo, Jaime	8	18	70
Arbeláez, María Patricia	6	11	34
Barrera, Luis Fernando	5	15	32
García, Luis Fernando	5	28	108
Mexico			
Hernández-Pando, Rogelio	32	50	312
Mata-Espinosa, Dulce	20	9	32
Marquina-Castillo, Brenda	14	9	27
Barrios-Payán, Jorge	12	8	26
García-García, Lourdes	11	28	115
Zenteno-Cuevas, Roberto	11	8	32
Peru			
Contreras, Carmen	20	12	46
Lecca, Leonid	19	8	38
Becerra, Mercedes C	18	31	112
Coronel, Jorge	16	12	39
Calderon, Roger	12	6	18
Gotuzzo, Eduardo	12	52	401
Seas, Ramos Carlos	12	22	117

TB: tuberculosis; and DR/MDR-TB: drug-resistant/multidrug-resistant tuberculosis. [a]Data from the Scopus citation database.

Table 4. Comparative table showing studies related and unrelated to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project.

Year	PTB			DR/MDR-TB			PTB and DR/MDR-TB	
	Total	ALAT/ERS/SBPT		Total	ALAT/ERS/SBPT		Total	ALAT/ERS/SBPT
		Unrelated	Related		Unrelated	Related		Related
2016	92	90	2	40	32	8	132	10 (7.5%)
2017	63	60	3	50	41	9	113	12 (10.6%)
2018[a]	30	17	13	14	10	4	44	17 (38.6%)
Total	185	167	18 (9.7%)	104	83	21 (20.1%)	289	39 (13.5%)

PTB: pulmonary tuberculosis; DR/MDR-TB: drug-resistant/multidrug-resistant tuberculosis; ALAT: *Asociación Latino Americana de Tórax* (Latin American Thoracic Association); ERS: European Respiratory Society; and SBPT: *Sociedade Brasileira de Pneumologia e Tisiologia* (Brazilian Thoracic Association). [a]Only articles published in the first quarter of 2018.

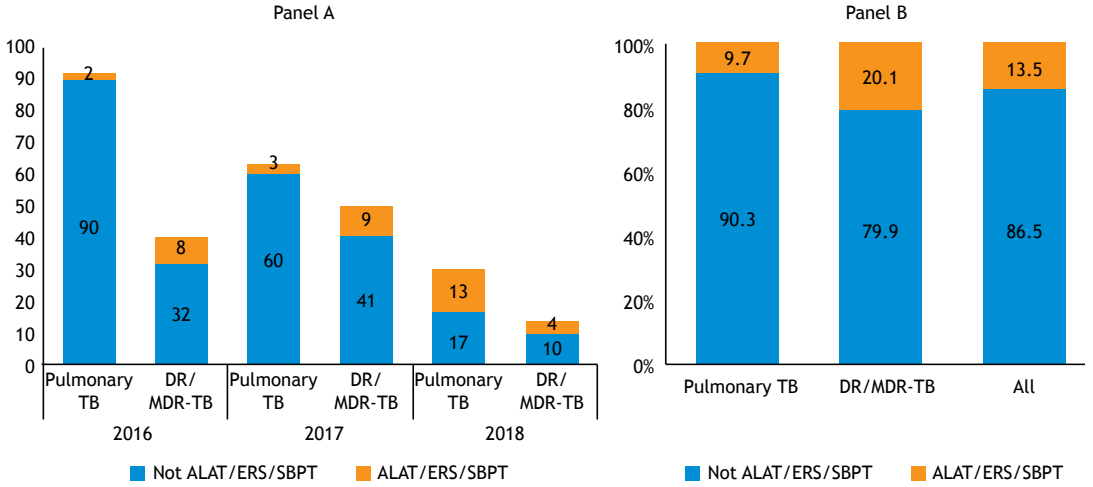


Figure 3. Impact of a collaborative project on the production of articles on pulmonary tuberculosis (TB) and drug-resistant/multidrug-resistant tuberculosis (DR/MDR-TB) published in the 2016-2018 period*. Panel A compares the number of articles per year, and panel B presents the overall contribution of the project. ALAT: *Asociación Latinoamericana de Tórax* (Latin American Thoracic Association); ERS: European Respiratory Society; and SBPT: *Sociedade Brasileira de Pneumologia e Tisiologia* (Brazilian Thoracic Association). *Included only the first quarter of 2018.

that country are mainly funded by the United States government and because Haiti has not yet participated in any of the studies published as part of the ALAT/ERS/SBPT project.

An indirect way of evaluating the quality of studies is by looking at the number and proportion of articles accepted in peer-reviewed journals with an impact factor. In the present study, articles were excluded if they were published in journals without an impact factor, as was the case for only 33 (7.7%) of 428 articles on PTB and only 12 (6.0%) of 200 articles on DR/MDR-TB. That suggests that the vast majority of such articles produced in Latin America have been published in high-quality journals.

The results of our study show how international collaborations are able to boost the quality and quantity of scientific production in Latin America. Examples of such collaborations are that between the Partners in Health initiative of Harvard University and the Peruvian National TB Program consortium,[20-23] as well as the internal collaboration in Brazil within the Brazilian Tuberculosis Research Network, which has allowed several international collaborations to be developed on the foundation of a well-designed national research plan.[14-19]

The ALAT/ERS/SBPT project allowed a research network involving five Latin American countries and Italy to be established in collaboration with the Maugeri Scientific Institute (Tradate, Italy). Particularly relevant are the scientific collaborations within this project involving four institutions in Mexico—the National Institute of Respiratory Diseases, in Mexico City; the Center for the Investigation, Prevention, and Treatment of Respiratory Infections, at the University Hospital of Monterrey; the Autonomous University of Nuevo León, in San Nicolás de los Garza; and the Mexican National TB Program—as well as four institutions in Brazil—the Oswaldo Cruz Foundation, in the city of Rio de Janeiro; the Federal University of Rio Grande do Sul, in Porto Alegre; the Brazilian Tuberculosis Research Network; and the Brazilian National Tuberculosis Control Program. Thus, the ALAT/ERS/SBPT project not only involved three scientific medical associations but also worked with three universities and two national tuberculosis programs, with no funding at all.

Table 5. Summary of all of the authors publishing studies related to the Latin American Thoracic Association/European Respiratory Society/Brazilian Thoracic Association collaborative project between 2016 and 2018.[a]

Country	Author(s)	n
Argentina	Palmero DJ	2
	González Montaner P	1
Brazil	Arbex MA, Dalcolmo M	8
	Silva DR	7
	Mello FCQ	6
	Bonini EH, Carvalho ACC	5
	Kritski AL	3
	Alves TG, Borga L, Braga JU, Dockhorn F, Fandinho F, Rabahi MF, Rocha JL	2
	Arakaki-Sanchez D, Arbex FF, Augusto VM, Barbosa MS, Beraldi-Magalhães F, Cardoso CAA, Cordeiro-Santos M, Dias NJD, Ferreira MD, Galesi VM, Kawakame Pirolla G, Martire TM, Neves CPD, Pereira GR, Sant'Anna CC, Sanchez DA, Souza AB	1
Colombia	Torres-Duque CA	4
	Fuentes Z	2
Mexico	Rendon A	12
	Muñoz-Torrico M	11
	Salazar-Lezama MA	6
	Pérez-Padilla R	3
	Carrillo-Alduenda JL, Flores-Vergara H, García-Sancho C, Gayoso R, Martínez-Mendoza D, Torres-Cruz A, Villareal-Velarde H	2
	Martínez-Orozco JA, Millán MJM, Narváez-Diaz LA, Saavedra Herrera N, Segura-Del Pilar M	1
Peru	Alarcón VA	4
	Alarcón E, Manga S, Varga-Vasquez D	3
	Bayona J, Becerra MC, Perales R, Reaño M	1

[a]Included only the first quarter of 2018.

According to Sweileh et al.,[24] the Harvard University Partners in Health initiative and the Maugeri Scientific Institute are both in the top 10 most active institutions worldwide in terms of the number of published articles on MDR-TB.[3-6,25-59] Additional examples of scientific collaboration identified in our study are those on basic research involving leading institutions in Mexico, Argentina, and Colombia, which are often funded by other international partners.[60-64]

It is noteworthy that the ALAT/ERS/SBPT project not only boosted the quality and quantity of scientific production in Latin America but also encouraged young investigators to publish for the first time (improving their academic records) while consolidating the publication records of several senior experts (Table 4). In addition, as previously mentioned, the collaboration did not receive any specific funding from any group or medical society.

Although comprehensive in its design, our study has several limitations. First, it covered only five Latin American countries, all with a high incidence of tuberculosis, notably excluding Haiti and thus not covering the whole WHO Region of the Americas. Therefore, the total scientific production in the region was not covered in this study. In addition, we chose to use "pulmonary TB" rather than "TB" as a search term, thus omitting a certain number of potentially relevant publications. However, that approach was useful in order to limit the number of papers dealing with extrapulmonary cases and including tuberculosis in absence of a main focus on the disease (there were several articles on animal tuberculosis or in which tuberculosis was mentioned only in the discussion without any data being provided). Nevertheless, the overall scientific production on tuberculosis in the region was likely underestimated. Furthermore, a direct comparison between the manuscripts that were related to the ALAT/ERS/SBPT project and those that were not was formally possible only for those dealing with DR/MDR-TB. Moreover, although the main collaborations were described and the essential bibliometric analysis was performed, a detailed analysis of the scientific collaborations and of the citation counts of the articles identified was outside the scope of our study.

In conclusion, although we have shown that the scientific production in Latin America is of high quality, the number of publications seems low in comparison with that reported for other regions.[24] We find it surprising that the national tuberculosis programs in the countries evaluated, despite having access to a large amount of data, have sponsored few published articles. More support is necessary in order to scale-up the existing research efforts in Latin America, which would strengthen the capacity of national tuberculosis programs to use their data to improve the prevention, diagnosis, and treatment of drug-susceptible and drug-resistant PTB in the respective countries, as well as to overcome the funding limitations and the language barriers.[24]

International collaboration among medical societies should be promoted as a proven effective way to boost scientific production in the field of tuberculosis in Latin America. Despite a lack of funding, such collaborations

could support Pillar 3 (the intensified research and innovation portion) of the WHO "End TB Strategy".

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Managing severe tuberculosis and its sequelae: from intensive care to surgery and rehabilitation

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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) continue to challenge physicians and public health specialists. Global treatment outcomes continue to be unsatisfactory, positive outcomes being achieved in only 54% of patients. Overall outcomes are even worse in patients infected with highly resistant strains. Treating MDR-/XDR-TB is difficult because of frequent adverse events, the long duration of drug regimens, the high costs of second-line drugs, chronic post-infectious sequelae, and loss of organ function. Ongoing research efforts (studies and trials) have various aims: increasing the rates of treatment success; understanding the potentialities of new and repurposed drugs; shortening the treatment duration; and reducing the rates of adverse events. It is hoped that better access to rapid diagnostics, increased awareness, and treatments that are more effective will reduce the rate of complications and of lung function impairment. This article aims to discuss the management of severe tuberculosis (defined as that which is potentially life threatening, requiring higher levels of care) and its sequelae, from intensive care to the postoperative period, rehabilitation, and recovery. We also discuss the nonpharmacological interventions available to manage chronic sequelae and improve patient quality of life. Because the majority of MDR-/XDR-TB cases evolve to lung function impairment (typically obstructive but occasionally restrictive), impaired quality of life, and low performance status (as measured by walk tests or other metrics), other interventions (e.g., smoking cessation, pulmonary rehabilitation, vaccination/prevention of secondary bacterial infections/exacerbations, complemented by psychological and nutritional support) are required.

Keywords: Extensively drug-resistant tuberculosis; Tuberculosis, multidrug-resistant; Critical care; Smoking cessation.

INTRODUCTION

Tuberculosis, also known as the "white plague", continues to be a public health priority. In its most recent Global Tuberculosis Report,[1] the World Health Organization (WHO) estimated that 1.6 million tuberculosis-related deaths occurred in 2017. In addition, half a million cases of multidrug-resistant tuberculosis (MDR-TB, defined as infection with a strain of *Mycobacterium tuberculosis* that is resistant to at least isoniazid and rifampin) have been reported worldwide, and 8.5% of those were cases of extensively drug-resistant tuberculosis (XDR-TB, defined as infection with an MDR-TB strain that is also resistant to fluoroquinolones and at least one second-line injectable drug).

Although recent studies have demonstrated that higher tuberculosis treatment success rates are achievable,[2] the overall rate of treatment success among MDR-TB patients worldwide is currently below 55%, treatment success rates being lower than 20% in difficult-to-treat cases in which the resistance profile is XDR or beyond.[1,3] Drug abuse, smoking, and alcohol dependence can further aggravate outcomes.[4-9] Treating MDR-/XDR-TB is challenging because of frequent adverse events, the lengthy duration of costly second-line drug regimens, and the fact that patient management is often onerous,[4-9] not to mention the financial and social impact of the illness on the affected individuals and their families. There are ongoing research efforts (studies and trials) with a variety of aims[1,4,10-12]: increasing treatment success rates; understanding the potentialities of new and repurposed drugs; shortening the treatment duration; and reducing the rate of adverse events.

This paper aims to provide an overview of the management of tuberculosis in the intensive care setting, the role of adjunctive surgery, and the rehabilitation of patients affected by tuberculosis. The manuscript can be read in its entirety—as a pathway from admission to intensive care, surgical intervention (when indicated), rehabilitation, and recovery—or in its separate units. An additional aim is to remind the reader that patients

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are seldom entirely back to their former selves when they have completed the prescribed drug therapy regimen, because a long rehabilitation/convalescence phase typically ensues. Therefore, the importance of nonpharmacological interventions (e.g., pulmonary rehabilitation with supervised exercises, chest/breathing exercises, expectoration techniques, vaccination, and smoking cessation) should be explored in surviving patients in order to improve the functional residual capacity, limit end organ damage, and minimize the chronic sequelae of tuberculosis.

METHODS

We searched for articles in English, Spanish, or Portuguese, published between November 1, 2014 and June 1, 2018, on Google, Google Scholar, PubMed, and ClinicalTrials.gov. The following search terms were used: "tuberculosis"; "MDR-TB"; "XDR-TB"; "severe tuberculosis"; "intensive care and tuberculosis"; and "tuberculosis and surgery". Targeted searches were also performed for articles dealing with pulmonary rehabilitation, smoking cessation, or quality of life. The WHO definitions are used throughout the manuscript.[13,14]

TUBERCULOSIS IN THE INTENSIVE CARE SETTING

Despite the success of curative therapy, a significant proportion of tuberculosis patients are hospitalized every year, 1-3% requiring admission to the ICU for close monitoring or organ support.[15] The indications for ICU admission include the following: complications of tuberculosis (including respiratory failure and conditions requiring surgical interventions, such as hemorrhage, pneumothorax, and pleural effusion); the severe forms of tuberculosis (e.g., tuberculous meningitis with impaired consciousness requiring intubation) or severe clinical manifestations of comorbidities (e.g., liver disease, renal disease, and uncontrolled diabetes); and life-threatening events resulting from adverse reactions to antituberculosis drugs (e.g. organ failure, severe seizures, and anaphylaxis).

The most common reasons for admission to the ICU are acute respiratory failure (in > 90%), septic shock (in 20-34%), and multiorgan failure (in 34-44%).[15-17] Other causes include renal failure (in 10%), neurological disorders (in 20%), and meningeal tuberculosis (in 20%).[15,17] In addition, patients with tuberculosis can be admitted to the ICU for extrapulmonary manifestations of the disease, including spinal, pericardial, bone marrow, hematological, and genitourinary disease,[17] as well as for bacterial coinfections, antituberculosis drug toxicity, thromboembolic complications, or pulmonary hemorrhage.[16-19] Patients with tuberculosis can also become critically ill because of factors indirectly related to their tuberculosis, such as diabetic ketoacidosis, alcohol withdrawal, and electrolyte imbalances.[17,18] ICU admission due to HIV coinfection occurs in 68.7% of tuberculosis patients in high-incidence countries and in 40% of those in low-incidence countries.[15]

Most tuberculosis patients who are admitted to the ICU have an established diagnosis of tuberculosis, although there are some who do not present with the typical clinical or radiological signs of tuberculosis. In such cases, a high index of suspicion is required in order to make the diagnosis. A diagnosis of tuberculosis should be suspected in patients who are contacts of tuberculosis patients and in those who have risk factors for the disease.[20] The possibility of reactivation of latent tuberculosis infection due to stress or immunosuppression should also be considered.[21]

The management of tuberculosis in the ICU is daunting given the frequent complexity and poor outcomes associated with the disease. For cases of infection with drug-susceptible strains of *M. tuberculosis*, the WHO guidelines recommend standard quadruple therapy with rifampin, isoniazid, ethambutol, and pyrazinamide.[22] It is also necessary to seek advice from clinicians with expertise in managing the treatment of MDR-TB, XDR-TB, and tuberculosis involving coinfection with other pathogens.[23,24]

The mode of delivery of antituberculosis drugs in the ICU setting depends mainly on intestinal absorption, which can be delayed or altered due to gastroparesis, intestinal paralysis, enteral nutrition, edema due to hypoalbuminemia, and critical illness-associated changes in the gut microbiota.[25] In addition, the pharmacokinetics of antituberculosis drugs can be altered during critical illness.[26] In an observational case series of critically ill tuberculosis patients who received quadruple therapy administered via a nasogastric tube, therapeutic blood levels were achieved in only 30%.[27] Although rifampin is available in an intravenous formulation in some countries (not currently in Brazil), other antituberculosis drugs generally are not. In most cases, antituberculosis drugs are administered parenterally.[20,25] Furthermore, although some drugs can adhere to the nasogastric tube (e.g., rifampin), they can be administered intravenously to achieve and maintain therapeutic blood levels, which makes them more efficacious.[25] The fact that first-line drugs are rarely available in an intravenous formulation results in the widespread use of second-line drugs such as fluoroquinolones and aminoglycosides.

In the ICU, corticosteroids are frequently administered in conjunction with antituberculosis drugs. There is evidence that adjuvant treatment with corticosteroids reduces mortality in non-HIV-infected patients with tuberculous meningitis or pericarditis.[28] In a recent meta-analysis, corticosteroids were reported to reduce mortality in all forms of tuberculosis, with a more pronounced effect in patients with a severe form of the disease, such as miliary tuberculosis.[29,30]

Due to the variable pharmacokinetics and pharmacodynamics of antituberculosis drugs, it is essential to remain vigilant regarding drug toxicity and interactions, by carrying out active drug safety

monitoring. This is especially true for rifampin, which will interact with many drugs used in the ICU setting because of its effect of inducing cytochrome P450.[21,25]

Among tuberculosis patients requiring admission to the ICU, mortality is > 50%, ranging from 20% to 70%.[15,17,31,32] Mortality is even higher among patients on mechanical ventilation, one study reporting a mortality rate of 80% in tuberculosis patients with ARDS who required mechanical ventilation.[15] Risk factors for mortality include ARDS, multiorgan failure, sepsis, mechanical ventilation, renal replacement therapy, a high Acute Physiology and Chronic Health Evaluation II score, and a high Sequential Organ Failure Assessment score.[17,24,31,32] We find it interesting that diabetes and HIV have not been associated with increased mortality, possibly because they are overshadowed by the aforementioned risk factors.[17,24,25,31,32]

Tuberculosis patients admitted to the ICU are a heterogeneous population, and the complexities of tuberculosis exacerbate that heterogeneity. Mortality remains high, and the disease is associated with considerable morbidity. Most of the studies on the topic have been case reports and retrospective analyses. There is therefore a need for prospective studies in this area, especially regarding rapid diagnostic methods, novel treatments (including the use of immunomodulatory agents and host-directed therapies), and intensification of tuberculosis treatment.

ADJUNCTIVE SURGERY IN THE MANAGEMENT OF TUBERCULOSIS

Historically (before the development of antituberculosis drugs), surgery was the only treatment available for tuberculosis. Surgical procedures could be grouped into those that artificially collapse the lung and those in which the affected tissue is excised. Procedures in the first group include lung collapse by artificial induction of pneumothorax and thoracoplasty involving the removal of a rib or ribs (to collapse the lung cavity).[33] Procedures in the second group are more widely accepted by the medical community and include the following[33]: wedge resection, first described by Tuffler in 1891; pneumonectomy, first described by Lilienthal in 1933; and lobectomy, first described by Freedlander in 1935. The advent of combination therapy for tuberculosis, in 1952, allowed the infection to be eradicated through noninvasive means and subsequently reduced the number of operations performed in the affected patients.[33-35]

Over the last few decades, the emergence of drug-resistant *M. tuberculosis* strains has reduced success rates for treatment with drug therapy alone and has increased the number of tuberculosis patients who require surgery. Scarring and fibrous tissue can protect bacteria from the host immune response, allowing them to continue to replicate, thus preventing the eradication of infection and driving the development of drug-resistant mutations. Surgical removal of the affected tissue allows the antimicrobial therapy to penetrate the remaining lung more effectively and eradicates the foci of bacillary growth.[36] The largest case series to date was published in 2018 by Giller at al.,[37] who documented 5,599 thoracic surgical procedures in tuberculosis patients treated in Russia during a 17-year period. The authors reported an overall mortality rate of 0.1%, also reporting treatment success rates of 93.0% and 92.1% in patients with MDR-TB and XDR-TB, respectively.

Most surgical procedures in tuberculosis patients have been performed on a case-by-case basis, and current evidence is therefore from observational studies with a paucity of reliable data on the indications, individual procedure outcomes, and cure rates related to surgery used in combination with an antituberculosis treatment regimen. Consequently, the WHO issued a consensus statement in 2014,[34] followed by an update of the MDR-TB treatment guidelines in 2016,[38] with a section focusing on the role of surgery in tuberculosis treatment. The WHO consensus statement referenced a 2013 systematic review conducted by Marrone et al.,[39] who found the overall success rates of pulmonary resection in combination with antituberculosis therapy to be 88-92%, with a reduction in overall all-cause mortality, when that treatment combination is performed in appropriate settings on carefully selected patients. Evidence from a study conducted in Peru suggested that the addition of surgery can reduce the overall cost of MDR-TB treatment because it allows the duration of the treatment regimen to be shortened.[40]

The indications for surgery in patients with tuberculosis, as listed by Dara et al.,[35] are divided into three main sections: emergency—profuse lung hemorrhage and spontaneous tension pneumothorax; urgent—irreversible progression of disease despite tuberculosis therapy and recurrent or recalcitrant hemoptysis; and elective—localized cavities with persistent smear/culture positivity for *M. tuberculosis* after 4-6 months of directly observed antituberculosis therapy, MDR-/XDR-TB in which antituberculosis treatment has failed, and complications of tuberculosis requiring surgical intervention, including pneumothorax (which can be spontaneous), pyopneumothorax, pleural emphysema (with or without bronchopleural fistula), and aspergilloma. The 2014 WHO consensus statement stipulated that patients should receive at least 4-6 months of an appropriate antituberculosis regimen before surgery and their suitability as surgical candidates should be assessed, ensuring adequate postoperative pulmonary functional residual capacity.[34] The procedure should be performed at a center with adequate facilities and by a highly-skilled surgeon with experience in tuberculosis. Because the mortality rate reported for lobectomy (2-3%) is lower than that reported for pneumonectomy (7-8%), the former is the preferred procedure. Postoperatively, patients should continue to receive antituberculosis

therapy for at least 4 months, depending on the characteristics of the underlying disease.

The most recent WHO MDR-TB guidelines, updated in 2016,[38] also recommend elective partial lung resection (lobectomy or wedge resection) in conjunction with an appropriate MDR-TB treatment regimen. That recommendation is based on three meta-analyses that collectively found treatment outcomes to be significantly better in patients treated with the combination of surgery and drug therapy than in those treated with drug therapy alone (81.9% vs. 59.7%; OR = 2.62, 95% CI: 1.94-3.54).[41-43] However, the guidelines also stressed the superiority of partial lung resection over pneumonectomy in achieving a cure, as well as in improving overall outcomes.

We believe that there is a role for surgery in the treatment of complicated tuberculosis infection, especially infection with drug-resistant strains, with the potential to shorten treatment duration and improve outcomes. Better retrospective data surveillance is needed in order to inform clinicians of the indications for surgery, the optimal surgical procedures, and their potential outcomes.

REHABILITATION

The sequelae of pulmonary tuberculosis (PTB) can cause significant pulmonary impairment and morbidity, particularly in young adults. Therefore, the completion of tuberculosis treatment might mark the beginning of a chronic respiratory disease. Unfortunately, there have been only a few studies addressing this issue, and most of them have assessed respiratory function only through the use of simple spirometry.

A history of PTB is undoubtedly related to lung function impairment and lung function test abnormalities.[44,45] Lung damage can occur in the bronchial airway, the lung parenchyma, or both. A number of population-based studies using spirometry and bronchodilator tests have demonstrated that individuals with PTB have airflow obstruction that does not respond to bronchodilator administration[46,47] Therefore, PTB is a well-recognized risk factor for the development of COPD in young adults with no history of smoking.[48] PTB has also been described as a frequent cause of bronchiectasis and tracheobronchial stenosis. At the parenchymal level, the severity can be quite variable: single or multiple cavities can be seen, with or without areas of scarring; or there can be areas of complete lung destruction. The presence of lung destruction confers a poor prognosis, especially if the destruction is extensive.[49] Such damage can also involve the pleura and promote the development of diffuse pleural fibrosis, resulting in restrictive lung disease. In addition to the variety of pulmonary abnormalities caused by PTB sequelae, the remaining areas of damaged lung increase the risk of further complications such as the development of aspergilloma and infection with nontuberculous mycobacteria.

There is still a need to carry out more studies to understand the pathophysiology of PTB sequelae, thoroughly assessing its impact on pulmonary function and patient quality of life. However, it is clear that such sequelae cause pulmonary impairment and contribute significantly to the burden of chronic respiratory diseases worldwide.[44,50] Therefore, it is necessary to perform a complete pulmonary evaluation (with imaging examinations and pulmonary function tests) at the end of treatment in PTB patients, as is done in patients with other chronic respiratory diseases, in order to improve their quality of life.

As a result of the lung destruction due to PTB, affected patients frequently have persistent respiratory symptoms, which limit their activities of daily living and reduce their quality of life.[51,52] Therefore, pulmonary rehabilitation at the end treatment is an appropriate measure. Pulmonary rehabilitation has been proven to improve the perception of dyspnea, exercise tolerance, and health-related quality of life in patients with COPD or other chronic respiratory diseases.[53,54] Although there are few data regarding its use in patients with PTB sequelae, some studies have suggested that it is beneficial for such patients.[55-59] It might even be possible to adapt the rehabilitation program to specific circumstances, so that it is made accessible to individuals in low-resource settings.[56] In addition to its role in the management of PTB sequelae, pulmonary rehabilitation can be a useful tool in the multidisciplinary management of surgical candidates, as well as in patients with severe tuberculosis who require ICU admission and long hospital stays, in order to decrease the risk of further respiratory complications and to prevent or reverse muscle atrophy.[60,61] The indications for pulmonary rehabilitation can include evidence of lung damage (resulting in obstructive or restrictive lung disease), exercise-induced oxygen desaturation, and impaired quality of life.

A recent review of the available literature on chronic sequelae after the completion of antituberculosis treatment[44] focused specifically on sequelae and their functional evaluation, as well as on lung destruction and the pulmonary interventions available (e.g., long-term oxygen therapy, ventilation, and respiratory therapy). The authors recommended that future studies not only evaluate the outcomes of antituberculosis drug therapy but also include a complete description of the pathophysiological status of the patients, including radiological aspects (Figure 1); spirometry findings and bronchodilator response (Figure 2); assessment of lung volumes by plethysmography (Figure 3); DLCO (Figure 4); arterial blood gases; six-minute walk distance; and quality of life (evaluated with validated tools such as the Saint George's Respiratory Questionnaire). If rehabilitation programs are implemented, it is essential to collect information on pre- and post-rehabilitation outcomes, as well as on the costs of the intervention.

Cigarette smoking is a definite risk factor for various pulmonary infections, including tuberculosis. A number

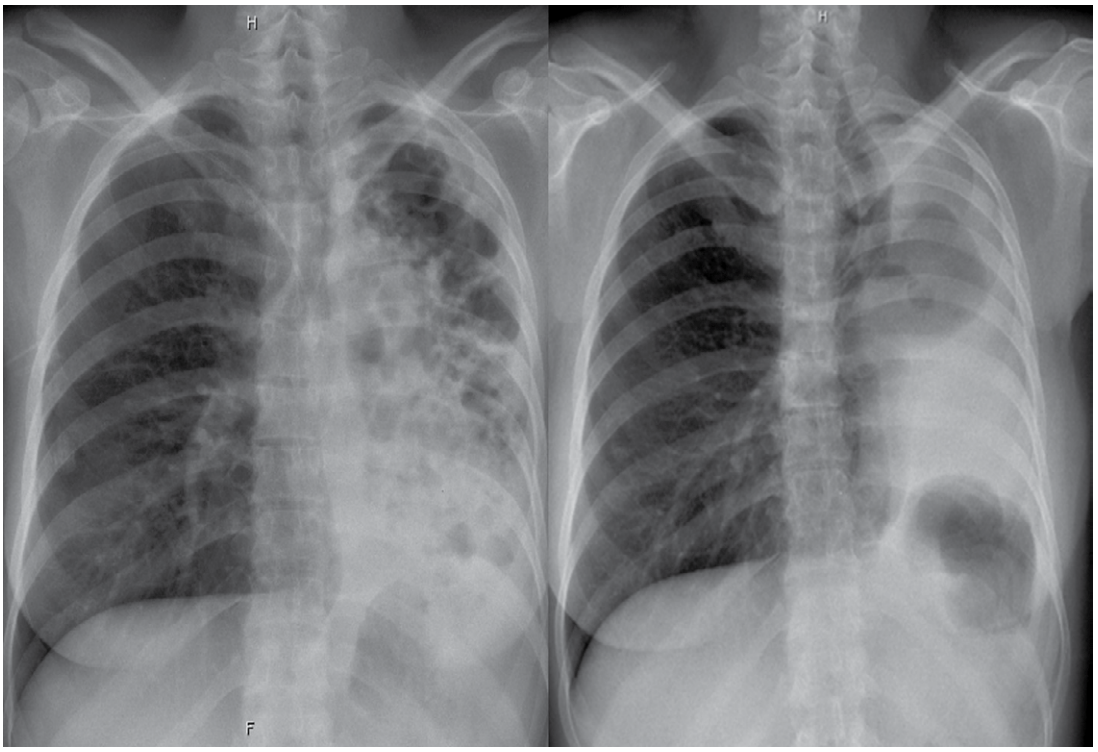

Figure 1. Pre- and post-treatment X-rays of a patient with multidrug-resistant tuberculosis, showing sequelae on the left side.

		Pred	A1	% (A1/P)	A2	% (A2/P)	D%(A2/A1)
Hora			02:21:51		03:13		
Fecha			15-06-11		15-06		
FVC	[L]	3.17	1.90	60	2.08	65	9
FEV_1	[L]	2.74	1.00	37	1.21	44	21
FEV_1 % FVC	[%]		52.76		58.42		11
MMEF 75/25	[L/s]	3.62	0.37	10	0.44	12	17
PEF	[L/s]	6.51	2.68	41	2.83	43	6
FET	[s]		7.37		8.51		15
V backextrapolation ex	[L]		0.02		0.04		159

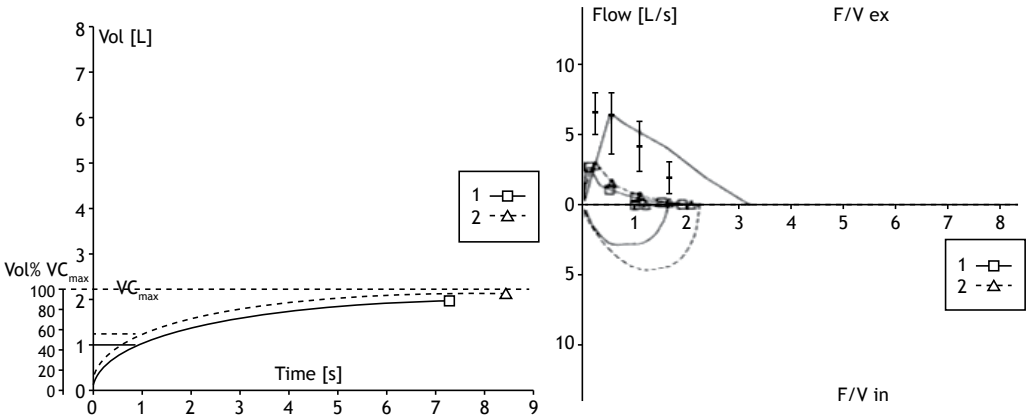

Figure 2. Spirometry findings in a patient with severe multidrug-resistant tuberculosis, at the end of antituberculosis treatment.

of population-based studies have shown that smoking increases the risk of developing latent or active tuberculosis.[62] In addition, smoking has been linked to adverse outcomes (treatment failure or death), relapse, and an increased risk of developing drug-resistant tuberculosis,[63] as well as to worsening of the initial pattern of drug resistance. A coherent smoking cessation approach, including a wide range of interventions (e.g., psychosocial and pharmacological interventions), has been proven to increase the treatment success rates in tuberculosis patients while decreasing the risk of further pulmonary complications.[64-66] Therefore, the WHO recommends integrating early and effective smoking cessation measures, starting at the primary health care level, into tuberculosis control plans.[67] Because of the similar risk posed by alcohol abuse, comparable interventions have also been recommended for individuals with alcohol dependence.[68,69]

FINAL CONSIDERATIONS

In conclusion, the existence of effective therapy notwithstanding, tuberculosis is frequently encountered in the ICU, and the disease represents a distinct challenge with poor outcomes. The emergence of drug-resistant strains of *M. tuberculosis* has recently restored thoracic surgery to a prominent position among interventions designed to combat tuberculosis. When combined with effective drug therapy, surgery has been associated with favorable outcomes in cases of MDR-/XDR-TB. There is recent evidence that chronic post-infectious sequelae of tuberculosis are common even after effective therapy, more so after surgery.[44] That suggests that the majority of tuberculosis cases will evolve to lung function impairment (typically obstructive but occasionally restrictive), impaired quality of life, and reduced performance status (as measured by walk tests or other metrics). Therefore, other interventions—pulmonary rehabilitation (including supervised exercise, chest/breathing exercises, and expectoration), smoking cessation therapy, and prevention (of secondary bacterial infections and exacerbations), complemented by psychological and nutritional support—are required in order to protect or restore functional residual capacity, thereby improving quality of life and slowing the progression to frailty.

		Pred	Best	A1	Act2	Act3	Act4	Act5	Act6	Act7	Act8	Act9	Act10	Act11	%(B/P)
Fecha			15-06-11												
Hora			02:48:10												
ITGV	[L]	2.60	3.04		3.03	3.07	3.02	3.06							117
RV	[L]	1.49	2.03		2.11	2.25	2.08	2.04							136
VC	[L]	3.15	2.04		1.92	2.04	2.00	1.97							65
IC	[L]	2.11	1.02		1.00	1.22	1.06	0.96							48
ERV	[L]	1.11	1.01		0.92	0.82	0.94	1.01							91
TLC	[L]	4.72	4.07		4.03	4.29	4.08	4.02							86
RV % TLC	[%]	31.9	49.94		52.3	52.5	51.1	50.9							157
R tot	[KPa * s/L]	0.30	1.02	1.05	0.98	1.03	0.98	0.97	1.02	0.96	1.03	1.07	1.08		341
R IN	[KPa * s/L]		0.72	0.74	0.66	0.74	0.74	0.71	0.64	0.61	0.76	0.68	0.74		
R EX	[KPa * s/L]		1.33	1.36	1.27	1.33	1.25	1.21	1.35	1.24	1.33	1.44	1.45		
Delta volue	ITGV [L]				0.17	0.05	0.17	0.09	0.18						
BF Res	[l/min]		90.63		88.2	93.8	88.2	90.9	93.8	93.8	96.8	88.2	90.9		

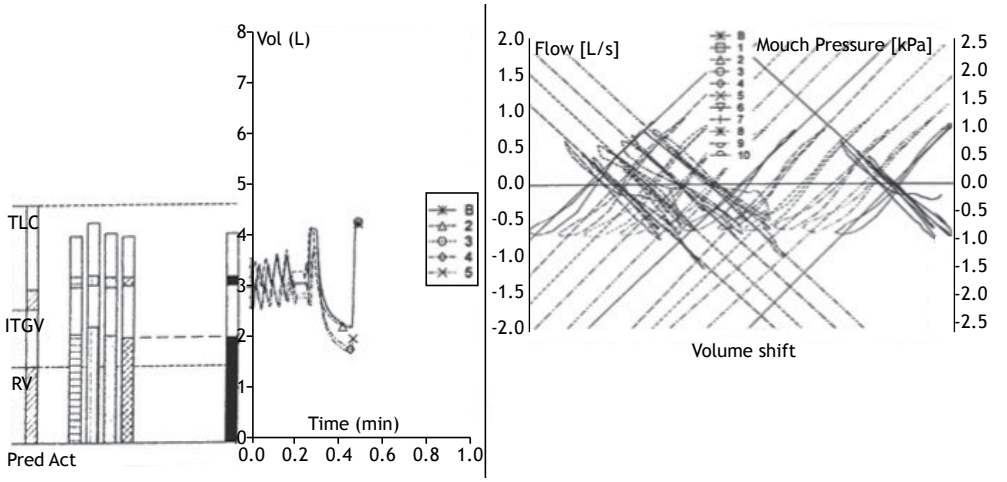

Figure 3. Plethysmography showing air trapping in a patient with multidrug-resistant tuberculosis, after the completion of antituberculosis treatment.

		Pred	Act1	Act2	Act3	A4	B	%(B/P)
Fecha			15-06-11					
Hora			03:13:56p.					
TLCOc SB	[ml/min/mmHg]	25.14	10.98»	11.86»			11.39»	45»
TLCOc/BSA	[mmol/min/kPa/m]		2.36	2.55			2.45	
TLCO/VA	[ml/min/mmHg/L]	5.33	3.95»	4.30»			4.12»	77»
RV-He	[L]	1.49	0.84	0.92			0.88	59
TLX-He	[L]	4.72	2.94	2.91			2.92	62
RV%TLC-He	[%]	31.88	28.67	31.56			30.12	94
VA	[L]	4.567	2.787	2.761			2.774	61
TA	[s]		10.80	11.10			10.95	
VIN	[L]	3.151	2.095	1.992			2.043	65
VC max (Spir)	[L]		2.21	2.21			2.21	
FI He	[%]		9.950	9.950			9.950	
FA He	[%]		5.898	5.702			5.800	
FI CO	[%]		0.300	0.300			0.300	
FA CO	[%]		0.096	0.087			0.092	
Hb	[g/100 ml]		13.50	13.50			13.50	
Discard vol	[L]		0.75	0.75			0.75	
Sample vol	[L]		0.60	0.60			0.60	
Insp. time	[s]		0.60	1.20			0.90	
Exp. time	[s]		0.20	0.20			0.20	
ATS Error codes			0	140			0	

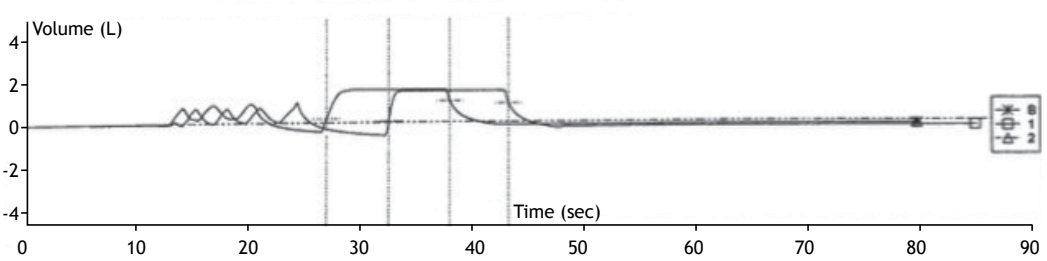

Figure 4. Severe (39%) reduction in DLCO in a patient with multidrug-resistant tuberculosis.

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Latent tuberculosis infection in patients with rheumatic diseases

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ABSTRACT

Most people infected by Mycobacterium tuberculosis (Mtb) do not have any signs or disease symptoms, a condition known as latent tuberculosis infection (LTBI). The introduction of biological agents, mainly tumor necrosis factor (TNF) inhibitors, for the treatment of immune-mediated diseases such as Rheumatoid Arthritis (RA) and other rheumatic diseases, increased the risk of reactivation of LTBI, leading to development of active TB. Thus, this review will approach the aspects related to LTBI in patients with rheumatologic diseases, especially those using iTNF drugs. For this purpose it will be considered the definition and prevalence of LTBI, mechanisms associated with diseases and medications in use, criteria for screening, diagnosis and treatment. Considering that reactivation of LTBI accounts for a large proportion of the incidence of active TB, adequate diagnosis and treatment are crucial, especially in high-risk groups such as patients with rheumatologic diseases.

Keywords: Tuberculosis; Latent tuberculosis; Tuberculin skin test; Anti-TNF therapy; Tumor necrosis factor-alpha; Rheumatoid arthritis.

INTRODUCTION

The most people infected by *Mycobacterium tuberculosis* (Mtb) has no signs or symptoms of the disease, a condition known as Tuberculosis Latent Infection (LTBI). According to the World Health Organization (WHO), about 2 to 3 billion people worldwide are infected by Mtb; including 5 to 15% will progress from LTBI to active symptomatic disease during their lifetime. The reactivation of LTBI is responsible for a large proportion of cases of tuberculosis (TB) active, which makes diagnosis and treatment crucial, especially in high-risk groups.[1-3]

The introduction of biological agents, especially tumor necrosis factor (iTNF) inhibitors, to treat immune-mediated diseases such as rheumatoid arthritis (RA) and other rheumatic diseases has increased the risk of developing TB.[4] The iTNF can promote the reactivation of TB to neutralize TNF, which protects the host against Mtb and plays a key role in granuloma formation which limits the extent of injury.[1,5,6]

Thus, the objective of this article is to review the aspects related to LTBI in patients with rheumatologic diseases, especially in those using iTNF drugs. For this purpose, it will be discussed the definition and prevalence of LTBI, the mechanisms associated with diseases and medications, as well as criteria for screening, diagnosis and treatment of LTBI.

DEFINITION AND MECHANISMS OF LTBI IN RHEUMATIC DISEASES

According to WHO, the LTBI is characterized by the presence of persistent immune response to Mtb without clinical evidence of active disease.[7] The chance of infection after exposure to TB bacillus is about 30% in healthy people, depending on the degree of exposure, infectivity of the index case, and the individual's immune factors. Approximately 5% of people cannot prevent the multiplication of bacillus and then develop the active disease soon after infection. Other 5% later become ill

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by reactivation of latent infection or as a consequence of a new exposure to the bacillus. Besides that, several factors may increase the risk of reactivation of TB, such as disease or immunosuppressive treatments used in rheumatic diseases.[8]

According to research conducted in patients with RA, even those who have never used iTNF have a risk of TB of two to ten times greater compared to the general population.[9-13] In one such study, which was a prospective population-based cohort,[9] in Sweden, demonstrated that Rheumatoid Arthritis (RA) patients not exposed to biological had a four-fold increased risk of TB compared to the general population, noting that the risk TB is independent of the use of iTNF and that probably is associated with immunosuppression linked to the disease and the use of other medications such as corticosteroids.

In any case, the use of iTNF is related to a risk of TB of 2 to 30 times greater, depending on the medication used and the place of study.[9-14] It is known that TNF plays a critical role in the host's response to infection, since it influences the transport of cells to the infectious focus, promoting the formation of granuloma capable of containing the disease progression, as well as increasing the phagocytic capacity of the macrophages and the death of viable intracellular bacteria. In addition, TNF is responsible for maintaining the structural integrity of the granuloma. Thus, the use of TNF antagonists leads to the resumption of mycobacterial growth within the granuloma, resulting in even its structural disintegration (Figure 1).[15,16]

Another class of medications used in the treatment of rheumatic diseases is not biological iTNF such as: Anti-interleukin-1 (IL-1), Anakinra (ANK), receptor inhibitor of the IL-6 tocilizumab (TCZ), Anti-CD20 Rituximab (RTX), stimulus blocker of abatacept T-lymphocytes (ATB), Anti-IL-12 and IL-23 Ustekinumab (UST), and Anti-IL-17 Secukinumab (SEC). According to data from controlled clinical trials and national registries, biological non-iTNF not has a negligible risk of TB reactivation. Thus, probably, in these cases, the tracking LTBI is not necessary and these medicaments are the safest option in patients with increased risk of reactivation of TB.[17]

DIAGNOSIS OF LTBI

For the diagnosis of LTBI, there is the Tuberculin Skin Test (TST), also known as the Mantoux test or Mendel–Mantoux test, and tests IGRA (*Interferon-Gamma Release Assay* in English). Both do not differentiate infection from active disease, so they are only used to diagnose LTBI.[7]

Tuberculin Test (TST)

The TST has been used for many years for the diagnosis of LTBI. Two to ten weeks after Mtb infection, T-lymphocytes become responsive to components of the bacillus, and tuberculin antigen injection triggers off a delayed hypersensitivity reaction. The tuberculin used in Brazil (PPD RT23-) is applied using the same technique and equipment recommended by WHO, intradermal in the anterior part of the left forearm, at a dose of 0.1 ml, equivalent to 2 TU (tuberculin units). The TST reading is performed 48 to 72 hours after application (with the possibility of extending for up to 96 hours), measuring the transverse diameter of the hardened area in millimeters and discarding the surrounding erythema. In patients who are candidates for the use of biologicals for rheumatologic diseases, the criterion ≥ 5 mm is used to indicate treatment of LTBI.[8,18]

False-positive results (TST positive and LTBI negative) may occur in individuals infected by non-tuberculous mycobacteria or vaccinated people with the Calmette-Guérin bacillus (BCG), especially if vaccination occurs after the first year of life, when BCG produces greater and more lasting reactions. However, the effect of neonatal BCG on TST decreases gradually in the first seven years of life.[19] Besides that, immunization with BCG does not have significant influence on the TST response after ten years.[20] In endemic countries, the positivity of the TST reflects most likely a high prevalence of LTBI than a result of vaccination with BCG.[19,20]

On the other hand, false-negative results (negative TST and positive ILTB) may occur in immunosuppressed patients receiving corticosteroids (doses of prednisone> 15 mg / day) and other immunosuppressants, a situation fairly common in individuals with rheumatologic diseases. Furthermore, in RA, T cell function is altered, which could result in inability to develop an appropriate response to TST.[21] The exact mechanism for this change is not fully elucidated, but it is known that in RA there is a decrease in the amount and function of regulatory T cells (CD4 + and CD25 +), and the number of CD4 + T cells determines the magnitude of response to TST.[22,23]

In fact, lower rates of positivity for TST have been described in patients with RA.[21,24-28] In a case control study[27] conducted in Turkey, the frequency of TST positivity in patients with RA (29.8%) was lower than in patients with ankylosing spondylitis (AE) (65.9%). In another study,[28] held in Brazil, the prevalence of TST positive in the control group was higher (33.3%) compared to RA patients (14.6%) ($p = 0.034$).

Besides these, another TST disadvantage is the possibility of booster effect. Individuals infected by Mtb may have the ability to react to TST reduced over time because of the loss of T-lymphocyte response from memory, which could cause a false negative result in a first TST. The booster effect occurs when the second TST, performed from one to three weeks after the first, has resulted ≥ 10 mm, with an increase of at least 6 mm in relation to the previous TST. Thus, whenever TST is repeated within three weeks, the results should be interpreted with caution so as not to confuse the booster effect with tuberculin turn and to have a false indication of LTBI. However, the booster effect indication research (TST repeat up in to three weeks) is currently restricted to health professionals,

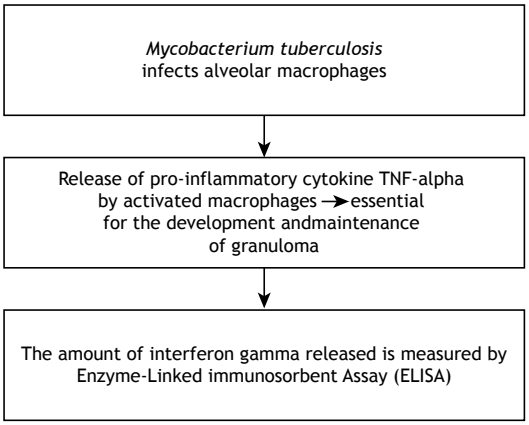

Figure 1. Effects of anti-TNF in granuloma formation.

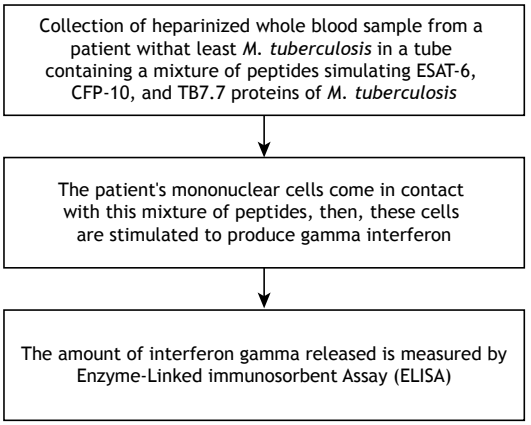

Figure 2. QuantiFERON-TB Gold in tube (QFT).

which were negative in the first TST for subsequent annual follow-up of tuberculin conversion.[8]

Despite its limitations, such as low sensitivity in immunosuppressed patients, cross reactions with BCG vaccination and infections by non-tuberculous mycobacterial, TST is still the most widely available test for clinical practice.[8]

IGRA test

The IGRA tests detect the production of interferon-γ (IFN-γ) derived from T in the peripheral blood activated by specific antigens. Nowadays, there are two commercial kits available: the QuantiFERON-TB Gold in tube (QFT), based on the ELISA method, and has been validated for use in Brazil, and the T-SPOT.TB, based on simplified enzyme immunoassay (ELISPOT). The QFT test uses the antigens of the RD1 region of Mtb, ESAT-6, CFP-10 and TB7.7 to measure the IFN-γ responses of patients (Figure 2) and is considered positive if the IFN value -γ (quantified in IU / mL) is above the upper limit of the test. And concerning the T-SPOT.TB test estimates the number of IFN-γ producing cells and is considered positive if the number of cells is greater than the negative control.

They have an excellent specificity (90-100%) for not being affected by BCG vaccination and, like TST, a good level of agreement, although discordant results may occur in up to 15% of the patients, depending on the population studied (HIV-positive people, cirrhotic, individuals with autoimmune diseases, transplanted and vaccinated by BCG).[29-31] False-positive and false-negative results may occur in IGRA tests because of mitogenic contamination of isolated cells.[32] However, a meta-analysis presented in 2016,[33] which included 11 studies, demonstrated that, in patients with previous BCG vaccination or corticosteroid use, IGRA tests would be the best choice to identify patients with LTBI because they have fewer false positive results and false negatives compared to TST.

The realization of the examination on a single patient visit and the speed of the results are other advantages of the IGRA tests. However, the high cost,[8] the need for personnel, specialized equipment, the possibility of conversion and reversal in serial tests are disadvantageous. The clinical significance of conversions and reversals in IGRA tests remains unclear, but it is known that they may be caused by the use of corticosteroids and iTNF, and this should be considered whenever the test is repeated.

The Chart 1 shows the comparison between TST and IGRA tests.

PREVALENCE OF LTBI IN RHEUMATIC DISEASES

The prevalence of LTBI in patients with rheumatic disease was evaluated in some studies,[1,36-40] usually by TST, variable results may be explained by differences in the selection of patients (which were included rheumatic diseases) or in the use of concomitant medications, especially corticosteroids.

The overall prevalence of LTBI was 13%, of which 4% in Rheumatoid Arthritis (RA), 26% in Ankylosing Spondylitis (AS) and 23% in Psoriatic Arthritis (PA), in a study performed in Fortaleza[37] with 157 patients who were candidates for infliximab. The limited value of TST in this population was questioned, but the use of corticosteroids was not described, which may have interfered with TST results. Another study, conducted in Recife, evaluated 48 patients with RA who used prednisone, with a average dose of 12.7 ± 6.7 mg / day, with a low prevalence of LTBI (14.6%). which can be explained at least partially by false-negative TST results, which may occur with doses > 15 mg prednisone.[8]

Two other studies (1.38), also conducted in Brazil, showed higher prevalences. One of them, performed in São Paulo,[38] evaluated 202 patients with RA, of which 32.7% had a diagnosis of LTBI. In addition, more than 80% of the patients selected used prednisone, but with a low dose (9.9 ± 5.3 mg / day). Another study, conducted in Porto Alegre,[1] included 176 patients with rheumatologic diseases (89 RA, 49 AS and 31 AP) and demonstrated a prevalence of LTBI of 29.5%, despite the use of prednisone by 46.6 % of

Chart 1. Comparison between TST and IGRA tests.

TST	IGRA
False-positive by BCG and non-tuberculous mycobacteria.	Results not affected by BCG vaccination and non-tuberculous mycobacteria.
False-negative immunosuppressants.	Limited data on immunosuppressed (although it appears to be less influenced by immunosuppression than TST) and in patients recently exposed to *Mycobacterium tuberculosis*.
• Sensitivity of 77% and specificity of 97% (not vaccinated with BCG). • Low and heterogeneous specificity - 35% to 78.6% (vaccinated with BCG).	• Sensitivity of 78% and specificity of 99% (not vaccinated with BCG).* • Specificity of 98% (vaccinated with BCG).*
Possibility of variability among observers.	No possibility of variability between observers.
Need for two visits (for reading the test).	Just a visit for blood collection.
More time consuming results.	Faster results.
Training needed to read the test result.	Need for qualified personnel and specialized equipment.
Low Cost	High Cost

TST: tuberculin skin test; IGRA: interferon-gamma release assay; BCG: Calmette-Guérin bacillus.[8, 34, 35] *Results for Quantiferon.

patients, with an average dose of 12.2 ± 8.4 mg / day. A similar prevalence was found in Peru (29.4%), with all patients receiving prednisone at doses of ≤ 7.5 mg / day.[36] In India, a region with a high prevalence of TB, Agarwal et al.[39] showed a 20.4% prevalence of LTBI, which was not affected by the use of methotrexate, but was lower in patients with recent corticosteroid use (3%) compared to patients who did not used in the last three months (25%) (p = 0.0002).

SCREENING RECOMMENDATIONS OF ILTB IN PATIENTS WITH RHEUMATOLOGIC DISEASES

The WHO recommends that all patients that will begin treatment with iTNF are systematically tested and, if indicated, treated for LTBI. For the diagnosis of LTBI, both TST and IGRA tests can be used. In those patients without signs and symptoms of TB, active and positive for TST or IGRA, a chest radiograph should be requested to rule out the TB active. If the radiograph is negative, treatment for LTBI is indicated.[7]

But the *Centers for Disease Control and Prevention* (CDC) recommends giving high priority to the treatment of LTBI in individuals using iTNF drugs (or who will use them) and having a positive IGRA test or TST ≥ 5 mm. For the diagnosis of LTBi, it is advisable to perform the IGRA tests in the case of people vaccinated with BCG, as well as the request for a chest radiograph whenever an IGRA or TST is positive. Individuals with fibrotic or nodular lesions consistent with old TB are candidates with high priority to the treatment of LTBI, after excluding active TB. In addition, sputum bacilloscopy is indicated in patients with IGRA or positive TST and abnormal radiography or who present with respiratory symptoms (even with normal radiography).[41]

The recommendation of the Brazilian Ministry of Health (MS) is that all people who will start using iTNF drugs will be investigated for LTBI. If one of the IGRA tests is positive or there is a TST ≥ 5 mm, the treatment of LTBI is indicated, as long as it excludes active TB.[8]

Finally, the *American College of Rheumatology* (ACR) recommends that screening be done to identify LTBI in all patients considered for treatment with biological agents, regardless of the presence of risk factors for LTBI. Both TST and IGRA tests can be used, however it is advisable to choose the latter in cases of BCG vaccinated patients. If TST is positive or the same for IGRA tests, it should be performed chest radiographs of patients. If the radiography is suggestive of active TB, sputum bacilloscopy should be performed to rule out TB. Since patients with RA may have false-negative results of the TST or IGRA tests due to immunosuppression, negative results should not be interpreted as excluding the possibility of LTBI. Thus, in patients with RA, risk factors for LTBI and negative initial tracing, the repetition of TST or IGRA tests is recommended between one and three weeks after the initial test. The annual screening is recommended in patients who live or work in places where there is a likelihood of exposure to TB, the duration of treatment with biological agents.[42,43] It has already been shown that approximately 1/3 of patients with negative baseline screening develop conversion into at least one screening test during treatment with iTNF.[44]

Patients who attested positive in TST or one of the IGRA may remain positive even after adequate treatment of LTBI or TB. In such cases, they require periodic monitoring of signs and symptoms of TB recurrence, since repeat testing is not useful in the diagnosis. In the ACR recommendations updated in 2015, the only modification is that patients receiving Tofacitinib should follow the same guidelines as patients using biologicals.[42,43]

TREATMENT OF LTBI

The ACR recommends that treatment with biological is started or restarted after a month of treatment of LTBI or in the case of active TB after the completed

TB treatment. In those patients using biologicals, it is suggested that these be suspended.[42,43]

The treatment regimens recommended for LTBI in patients with rheumatologic diseases follow the same norms as for the general population, always considering the presence of other comorbidities that may influence the therapeutic decision, in addition to monthly monitoring.[7,8]

The Isoniazid Monotherapy (INH) remains the most widely used treatment. It has already been evaluated in more than 20 randomized, placebo-controlled clinical trials, showing an average reduction in TB cases of 60%, reaching up to 90% efficacy if adherence to treatment is good. Several treatment durations have been studied, but the most commonly recommended are six and nine months.[45,46] On the other hand, the duration of protection ranges according to the duration of follow-up studies, being five years in most studies with isoniazid, although the protective effect is greater in the first year after treatment.[45]

Another form of treatment is rifampicin (RMP) by monotherapy, used for four months. It was evaluated in several small non-randomized clinical trials, demonstrating efficacy equal to or greater than six months of INH.[45,46] A randomized clinical trial[47] in Chinese patients with silicosis showed that RMP monotherapy, used for three months, was more effective than placebo, INH for six months and combination of INH with RMP for three months. Recently, another open, randomized and multicenter clinical trial, the RMP for four months was not lesser than INH for nine months[48] and also showed higher rates of completion of treatment. The RMP monotherapy is also considered an alternative for locations with high proportion of INH mono-resistance, in case of intolerance to INH and for those patients unlikely to complete six months of INH.[45,46]

The combination of RMP and INH for three / four months is also a possibility for LTBI treatment. A meta-analysis made in 2005,[49] including studies in Hong Kong, Spain, and Uganda, showed that the RMP plus INH regimen for three months had equal efficacy and adverse effects profile similar to INH regimens lasting 6 to 12 months. However, it has been shown that the incidence of hepatitis with this regimen is only lower than the INH regimens used for 12 months; when compared to INH for six to nine months; the incidence of hepatitis is significantly higher.[50]

The rifapentine (RPT) plus INH regimen, which has been used with a weekly dose for three months for a total of 12 doses and recommended since 2011 by the CDC, has been evaluated in at least three randomized controlled trials[51-53] and in a meta-analysis,[54] showing to be as effective as INH monotherapy, with higher rates of treatment completion and less hepatotoxicity. However, recent research[55,56] with adults aged 50 to 70 years, who were randomized to receive one of two short regimens containing rifapentine (three months of RPT plus INH or two months of RPT 600 mg twice a week, plus INH 600 mg) had to be discontinued prematurely due to the high rate of adverse events. Although studies have methodological limitations, these findings raise concerns about the use of RPT more INH in older adults and require a more careful evaluation. The CDC still suggests several layouts: INH for six or nine months, RIF for four months and RPT plus INH for three months.[41]

The WHO recommends the first choice of treatment for INH for six months. For locations with high incidence of TB, it suggests the most INH plus RPT layout for three months and, for locations with low incidence of TB, INH for nine months, RPT more INH for three months, RIF more INH for three / four months and RIF three / four-months.[7]

On the other hand, the recommendation of the Brazilian Ministry of Health (MH) for the treatment of LTBI is INH at a dose of 5 to 10 mg / kg / day up to a maximum dose of 300 mg / day in adults, lasting six to nine months; however, it is considered that the most important is the number of doses, and not only the time of treatment. It is also indicated the use of 270 doses, taken from 9 to 12 months, and 180 doses, taken between six and nine months, but only considered in individual cases after assessment of adherence. However, there is evidence that the use of 270 doses protects more than 180 doses.

The Ministry of Health (MH) also suggests RMP for four months as an option for individuals over 50 years of age, liver disease people, with contacts of INH mono-resilients and intolerance to INH, at the dose of 10 mg / kg / day of weight up to the maximum dose of 600 mg / day in adults. In this case, the use must be at least 120 doses, taken for four months, and may extend up to six months.[8] It is also considered that the most important is the number of doses, and not only the time of treatment.

The Chart 2 summarizes the main treatment layout.

CONCLUSIONS

In 5-10% of individuals with impaired immune response to Mtb, the LTBI can progress to active TB. Therefore, screening for LTBI is strongly recommended in patients with rheumatic diseases, especially before starting treatment with iTNF. The screening always involves the exclusion of active TB, and the recommendation of the test of choice (TST or IGRA) may vary according to BCG vaccination and the costs and availability of the tests. The first choice of treatment remains the INH for six to nine months, with the RMP for four months as a treatment option. Individuals living in areas with high incidence of TB should be tested annually for LTBI, the duration of the treatment iTNF.

Despite the diagnosis and treatment advances in this area, there are still some gaps in the knowledge of LTBI in patients with rheumatic diseases. There is a need for a diagnostic test with better performance and shorter treatment regimens and with fewer adverse effects. Besides that, it is important to assess the

Chart 2. LTBI treatment layouts.

Layouts	Dose	Duration	Observations
Isoniazid	5 to 10 mg / kg / day of weight up to the maximum dose of 300 mg / day in adults. It is recommended to use 270 doses.	6 a 9 months.	First choice of treatment, according to WHO and MH Brazil.
Rifampicin	10 mg / kg / day of weight up to the maximum dose of 600 mg per day in adults. It is recommended to use at least 120 doses.	4 months.	Option for individuals over 50 years old, liver disease, with mono-resilient contacts to INH and INH intolerance.
Isoniazid plus Rifapentine	One weekly dose: Isoniazid 15 mg / kg (maximum 900 mg). Rifapentine: 900 mg (> 50 kg).	3 months (Total of 12 doses).	Not available in Brazil.
Isoniazid plus Rifampicin	Isoniazid: 5 mg / kg / day (maximum of 300 mg / day). Rifampicin: 10 mg / kg / day (maximum of 600 mg).	3-4 months.	Hepatitis incidence greater than isoniazid for 6-9 months.

risk of resistance to the treatment of LTBI, especially in cases with low adherence. Finally, there are few studies that analyze the best follow-up strategy for these patients.

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Calcified intracavitary mass: a rare presentation of aspergilloma

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A 69-year-old woman presented with a 2-year history of recurrent episodes of hemoptysis, one of which was severe, leading to admission to an intensive care unit. She had a history of pulmonary tuberculosis treated 20 years previously. A chest X-ray (Figures 1A and 1B) and chest CT (Figures 1C and 1D) showed a thin-walled cavity, containing an ovoid calcified mass, in the upper right lobe. The patient underwent right upper lobectomy. Microscopic examination showed that the mass was a calcified capsule filled with abundant necrotic material, fungal hyphae, and birefringent calcium oxalate crystals. Cultures grew *Aspergillus niger*. The final diagnosis was pulmonary aspergilloma caused by *A. niger* and presenting as a calcified mass.

A fungus ball or aspergilloma is the most common cause of intracavitary nodules, generally resulting from fungal colonization of pre-existing lung cavities.[1] One feature of *A. niger* infection that is key for the diagnosis is the presence of calcium oxalate crystals, detected by pathological examination.[2,3] Some early reports of aspergillomas mentioned calcification, as identified on chest X-rays, which is related to the presence of calcium oxalate crystals. However, to our knowledge, there have been no reports of aspergilloma presenting as a calcified intracavitary mass identified on computed tomography scans.

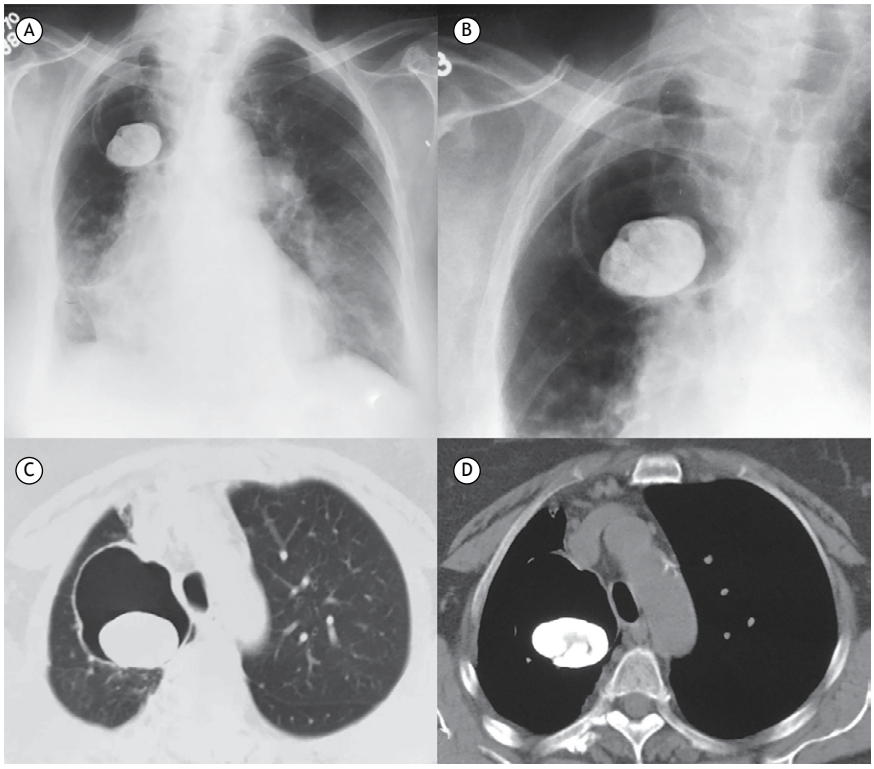

Figure 1. Chest X-ray (A), with a detailed view of the right upper lung region (B), showing a thin-walled cavity in the right upper lobe containing an ovoid calcified mass with a maximum diameter of about 4 cm. Chest CT with lung and mediastinal window settings (C and D, respectively), confirming the presence of the mass inside the cavity.

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Tuberculosis surveillance in an endemic area of northeastern Brazil. What do the epidemiological indicators reveal?

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TO THE EDITOR,

Tuberculosis is a chronic infectious disease, the etiologic agent of which is *Mycobacterium tuberculosis*, and continues to be a major public health problem in several countries.[1] In 2015, approximately 10.4 million new cases were detected worldwide, resulting in more than 1 million deaths.[2]

For the 2016-2020 period, the World Health Organization has listed three groups of priority countries for tuberculosis surveillance, on the basis of the incidence of tuberculosis (magnitude), tuberculosis/HIV coinfection, and multidrug-resistant tuberculosis. In total, 48 are considered priority countries, some of which are included in more than one group. Brazil is part of two priority groups, ranking 20th in the magnitude group and 19th in the tuberculosis/HIV coinfection group.[2]

Although Brazil has experienced a significant reduction in the incidence of tuberculosis in recent years, the problem is still far from being solved. In 2015, more than 63,000 new cases of tuberculosis were diagnosed, of which 6,800 were diagnosed in people living with HIV, and there were 4,500 tuberculosis-related deaths.[3,4]

This entire context indicates the need for regular surveillance of epidemiological indicators. Systematic disease monitoring allows the assessment of both the magnitude of the problem in a given area and the outcomes of activities, plans, and health care policies that may have an impact on the reduction in incidence and mortality rates.[5,6]

Therefore, the objective of the present study was to analyze the time trends of tuberculosis monitoring indicators in the city of Juazeiro, located in the state of Bahia, Brazil. To that end, we conducted an ecological time-series study. We included all new cases of tuberculosis diagnosed between 2006 and 2015 in residents of the city. Clinical data were obtained from the National Case Registry Database. The demographic data required to calculate the indicators were obtained from the Brazilian Institute of Geography and Statistics, using the 2010 census and the intercensal projections for the other years of the time series.

The following epidemiological indicators were selected:

Group 1 - Indicators of the impact of tuberculosis control activities

- Tuberculosis incidence rate/100,000 population
- Incidence rate of active pulmonary tuberculosis/100,000 population
- Tuberculosis mortality rate/100,000 population

Group 2 - Indicators of the outcome of tuberculosis control activities

- Proportion of tuberculosis/HIV coinfection
- Proportion of cured cases of tuberculosis
- Proportion of tuberculosis cases that dropped out of treatment
- Proportion of tuberculosis cases that received directly observed treatment
- Proportion of cases of tuberculosis retreatment
- Proportion of contacts of reported cases of tuberculosis who were examined

For the trend analysis, we used a linear regression model with a trend component (Y = b0 + b1X), where Y is the time series scale; b0 corresponds to the intersection between the line and the vertical axis; b1 corresponds to the slope of the line; and X is the time frame. Type I error was set at 5%. Statistical calculations were performed using the R software, version 2.15.0 (The R Foundation for Statistical Computing, Vienna, Austria).

Analysis of the indicators of the impact of tuberculosis control activities in the city studied revealed that there was no trend for change in the time behavior of any of the three indicators (Table 1). Between 2006 and 2015, the tuberculosis incidence rate ranged from 18.10 to 34.54 new cases/100,000 population, the incidence rate of active pulmonary tuberculosis ranged from 9.68 to 14.06 cases/100,000 population, and the tuberculosis mortality rate ranged from 0.46 to 2.48 deaths/100,000 population. The persistence of the disease burden over the time series suggests that the chain of transmission is active, indicating the persistence of the problem. Similar time behaviors were observed in São Paulo[7] and in Paraná.[8]

Analysis of the indicators of the outcome of tuberculosis control activities (Table 1) showed significant upward trends in four of the six parameters studied: tuberculosis/HIV coinfection; treatment dropout; directly observed treatment; and tuberculosis retreatment.

The outcome indicators show the weaknesses of the health care facilities in the city of Juazeiro, Brazil, in following up patients. The low cure rates, which are in contrast with

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Table 1. Indicators of the impact and outcome of tuberculosis control activities in the city of Juazeiro, Brazil, between 2006 and 2015.

Year	Tuberculosis incidence rate/100,000 population	Incidence rate of active pulmonary tuberculosis/100,000 population	Tuberculosis mortality rate/100,000 population	Proportion of TB/HIV coinfection, %	Proportion of cured cases of tuberculosis, %	Proportion of tuberculosis cases that dropped out of treatment, %	Proportion of tuberculosis cases that received DOT, %	Proportion of cases of tuberculosis retreatment, %	Proportion of contacts of reported cases of tuberculosis who were examined, %
2006	21.11	10.08	0.96	6.82	75.00	5.00	0.00	0.00	57.49
2007	27.18	14.06	2.81	3.45	69.00	7.00	48.28	13.79	56.56
2008	18.10	9.68	1.68	6.98	70.00	5.00	46.51	6.98	60.61
2009	27.47	12.30	2.05	7.46	78.00	4.00	37.31	7.46	81.91
2010	29.30	12.12	1.52	6.90	67.00	9.00	25.86	13.79	63.09
2011	34.54	9.01	1.50	10.14	58.00	9.00	18.84	21.74	65.41
2012	28.78	8.44	2.48	12.07	59.00	14.00	25.86	15.52	68.46
2013	18.63	9.78	1.40	22.50	75.00	8.00	27.50	15.00	81.89
2014	28.63	12.00	0.46	12.90	68.00	8.00	17.74	11.29	72.16
2015	24.73	10.53	1.83	29.63	52.00	13.00	22.22	12.96	70.32
Slope	0.01356	−0.01267	−0.04373	0.18262	−0.02446	0.09480	0.09480	0.09480	0.02740
p	> 0.05	> 0.05	> 0.05	< 0.001	> 0.05	< 0.05	< 0.05	< 0.05	> 0.05
Trend	Stationary	Stationary	Stationary	Upward	Stationary	Upward	Upward	Upward	Stationary
Graph									

TB: tuberculosis; and DOT: directly observed treatment.

the World Health Organization recommendation that at least 85% of cases should be cured, might be due to poor treatment adherence, which results in treatment dropout and in later need for retreatment, increasing the likelihood of drug resistance.[9] It is of note that cure is one of the major strategies for reducing morbidity and mortality from tuberculosis.

Treatment dropout, as well as poor contact investigation, contributes to the persistence of the chain of transmission. This scenario is a cause for even greater concern when we consider the growth in the proportion of patients coinfected with tuberculosis and HIV. However, the increase in the proportion of coinfection might be due to the fact that more patients are being tested, which represents a major advancement.[7] Similar results have been observed throughout Brazil, especially in the north and northeastern regions.[10]

What do the indicators presented here reveal then? Much more than showing the persistence of the disease in the city, they point out the local weaknesses and the urgent need for developing systematic activities that transcend the biological dimension of the disease and reach the subjects and their contexts of vulnerability, allowing patients themselves and civil society in general to engage in the fight against the disease.

At the same time, the provision of services that are less bureaucratic and more accessible to the community, with thorough and continuous treatment, seems to be an important way to overcome the problem. In this aspect, the emphasis is on strengthening primary health care. We conclude that, in addition to revealing important issues regarding the dynamics of the disease in the city, the epidemiological indicators presented here reinforce the importance of health surveillance itself in the monitoring of health problems. Limited access to diagnostic services suggests that the true incidence of the disease is even higher than that presented here.

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Serum pyrazinamide concentrations in patients with pulmonary tuberculosis

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TO THE EDITOR,

Tuberculosis is an important public health issue in Brazil. Although incidence rates have decreased and cure rates have increased in recent years in the country, there were 69,569 reported new cases of tuberculosis in 2017.[1,2] Exposing *Mycobacterium tuberculosis* to effective serum concentrations of chemotherapeutic agents is a requirement for maintaining and increasing the cure rates, as well as for reducing the risk of the emergence of drug-resistant bacilli.[3] The measurement of serum concentrations of chemotherapeutic agents is considered the gold standard for assessing *M. tuberculosis* exposure to such drugs. However, it has not been widely used in patients with tuberculosis, most studies having focused on measuring concentrations of rifampin and isoniazid.[4] There have been few studies investigating the serum concentrations of pyrazinamide achieved after the use of therapeutic doses of the drug, which might be due to the fact that pyrazinamide is used only in the intensive phase of tuberculosis treatment.

Pyrazinamide is a prodrug that requires conversion to its active metabolite, pyrazinoic acid, by pyrazinamidase, an *M. tuberculosis* enzyme. Its therapeutic efficacy depends on the serum concentrations achieved after administration of conventional doses; for tuberculosis treatment, doses should range from 20 to 50 μg/mL.[3,4] However, there is wide interindividual variation in serum pyrazinamide concentrations during the course of tuberculosis treatment, which requires that these concentrations be periodically monitored in studies of therapeutic efficacy.[4,5] In addition, there have been few studies investigating the influence of gender and treatment duration on serum pyrazinamide concentrations. Finally, there is lack of studies investigating *M. tuberculosis* exposure to pyrazinamide in Brazil. To clarify these issues, we assessed serum pyrazinamide concentrations in 46 patients over 18 years of age with a clinical and laboratory diagnosis (positive sputum smear and culture results) of tuberculosis caused by M. tuberculosis. Patients were selected from two health care clinics in the city of Belém, Brazil, where their antituberculosis treatment was initiated. We excluded cases of retreatment, treatment dropout, multidrug-resistant tuberculosis, and extrapulmonary tuberculosis, as well as pregnant women, breastfeeding women, individuals with comorbidities, and licit or illicit drug users.

Patients were treated with the standard regimen recommended by the Brazilian National Ministry of Health, which consists of fixed-dose combination tablets containing rifampin (150 mg), isoniazid (75 mg), pyrazinamide (400 mg), and ethambutol (275 mg); treatment duration and dose adjustment for patient weight were as per the guidelines.[2] Administration was once daily, preferably on an empty stomach, and in the presence of a family member. Clinical and laboratory follow-up lasted six months. Blood samples were collected before the initiation of therapy, as well as before and after drug administration (pre-dose and post-dose samples, respectively) on treatment days 30 and 60, which corresponded to the days when patients returned to the clinic to get their free monthly medication. On those days, patients reported to the clinic with an empty stomach for supervised administration of the dose by the project team. Serum pyrazinamide concentrations were measured by high-performance liquid chromatography.[6]

Serum pyrazinamide concentrations were expressed as median (interquartile range) and were compared between sample collection days (day 30 vs. day 60), as well as between genders, with the Mann-Whitney U test. The level of significance required in order to reject the null hypothesis was set at 5%. The study was approved by the Human Research Ethics Committee of the Federal University of Pará Center for Tropical Medicine (Protocol no. 1.591.019).

All 46 patients completed the clinical and laboratory follow-up and had a negative sputum smear at the end of the second month of treatment. None of the samples collected at patient inclusion (n = 46) showed measurable concentrations of pyrazinamide, which indicates that the drug had not been used recently. The time between drug administration and the collection of pre-dose samples on days 30 and 60 (n = 46 for both time points) ranged from 22 to 24 h; and the time between drug administration on days 30 and 60 and the collection of post-dose samples (n = 25 for both time points) ranged from 40 to 70 min. In the pre-dose samples, the median plasma concentrations of pyrazinamide were 3.75 μg/mL (0.3-10.9 μg/mL) and 2.7 μg/mL (0.4-10.7 μg/mL) on days 30 and 60, respectively. Serum pyrazinamide concentrations were similar at the two study time points (U = 890; p = 0.191).

Given the pharmacokinetic properties of pyrazinamide, such as its biological half-life of 8 to 11 h, the pyrazinamide concentrations present in pre-dose samples are of limited value in assessing exposure to the drug, because

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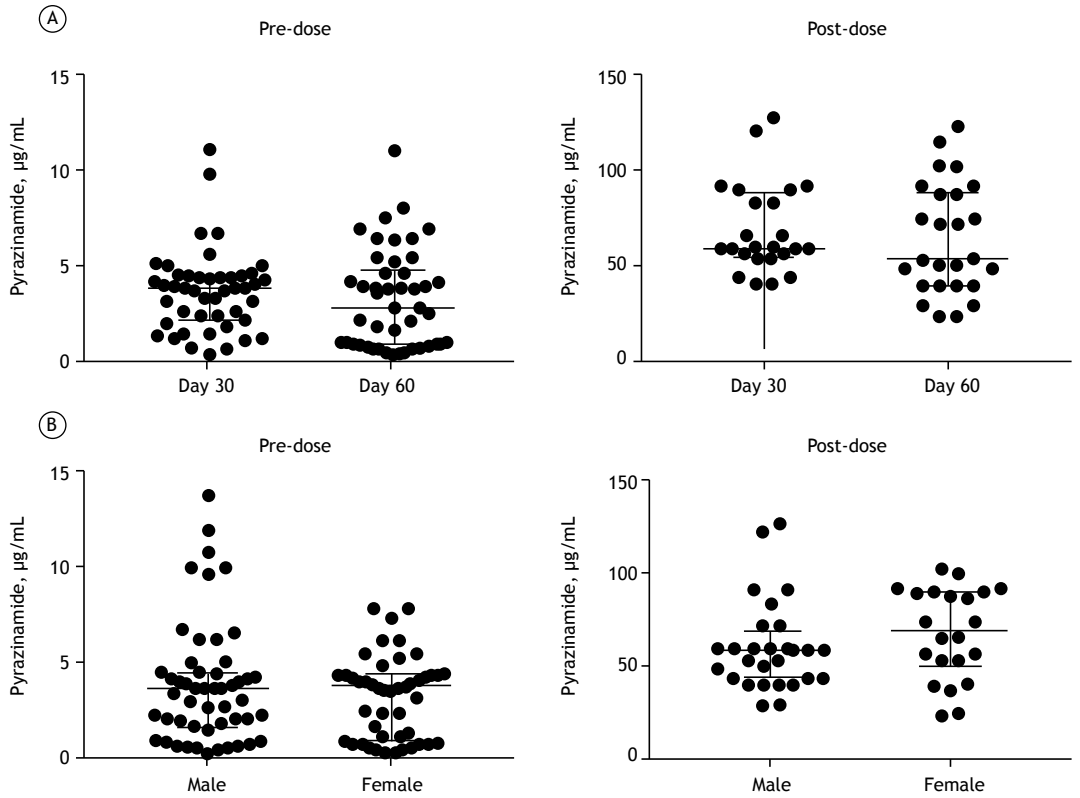

Figure 1. Serum pyrazinamide concentrations measured in blood samples collected before and after supervised drug administration (pre-dose and post-dose samples, respectively). Comparison between sample collection days (day 30 vs. day 60; in A) and between genders (in B). The longest horizontal line represents the median of the results, and the other horizontal lines represent the interquartile ranges.

they correspond to residual values.[5] In addition, pyrazinamide does not accumulate significantly in organic compartments, which can be verified by the low pyrazinamide concentrations present in pre-dose samples, as well as by the absence of significant differences in the serum pyrazinamide concentrations of samples collected on days 30 and 60.

Post-dose samples more reliably indicate bacillus exposure to drugs, especially drugs with a short biological half-life.[5] In addition, assessment of exposure to this type of drug can be performed on any treatment day.[5] In the present study, the median plasma concentrations of pyrazinamide were 59.5 µg/mL (40.3-127.0 µg/mL) and 53.6 µg/mL (23.4-122.7 µg/mL) on days 30 and 60, respectively (Figure 1A). Serum pyrazinamide concentrations were similar at the two study time points (U = 253; p = 0.254). These results are within the range of effective serum concentrations for pyrazinamide-susceptible *M. tuberculosis* strains (20.0-50.0 µg/mL).[3-5] In fact, serum concentrations below 20 µg/mL are considered low and suggest a change in the oral bioavailability of the drug, as well as being associated with increased treatment failure.[3-5] Therefore, the serum pyrazinamide concentrations found in the patients included in the present study indicate that the treatment regimen used in Brazil provides adequate bacillus exposure to the drug. This finding was corroborated by the fact that all patients were smear negative at the end of the intensive phase of treatment. There was a wide range of pyrazinamide concentrations in the pre-dose and post-dose samples. That finding has been reported in studies of pyrazinamide pharmacokinetics and has been related to variations in absorption and clearance. In addition, it is likely that the time between drug administration and the collection of blood samples contributed to the variation in the drug concentrations.[4,5] In fact, it has been reported that serum pyrazinamide concentrations measured in blood samples collected 2 h after its administration more reliably reflect the end of the absorption phase and show less interindividual variation.[3,5] However, it is likely that the time to sample collection in the present study did not significantly influence the assessment of *M. tuberculosis* exposure to the drug, given that the values found were similar to those reported in studies of other population groups.[3-5]

Gender influences serum concentrations of several chemotherapeutic agents because of differences in hormone concentrations and in body fat distribution.[5] The medians (interquartile ranges) of the serum pyrazinamide concentrations in the pre-dose samples from men (n = 25) and women (n = 21) were 3.7 µg/mL (0.3-13.9 µg/mL) and 3.8 µg/mL (0.3-7.9 µg/mL), respectively (Figure 1B). In the post-dose samples from men (n = 14) and women (n = 11), the medians (interquartile ranges) of the serum pyrazinamide

concentrations were 59 µg/L (29.5-127.0 µg/mL) and 70.1 µg/mL (23.4-102.5 µg/mL), respectively (Figure 1B). In the present study, gender did not significantly influence the pre-dose pyrazinamide concentrations (U = 112; p = 0.659) or post-dose pyrazinamide concentrations (U = 250; p = 0.261).

The main limitations of this study were the small sample size and the time of collection of the post-dose samples, which might have contributed to the variation in serum pyrazinamide concentrations among the patients. Another limitation is that the chemotherapeutic agents were administered under the supervision of the health care team only on the sample collection days; on the remaining days, they were administered under the supervision of a family member, which does not ensure strict adherence to the treatment regimen, although all of the patients had a negative sputum smear at the end of the second month of treatment.

In conclusion, the results of the present study confirm that the tuberculosis treatment regimen adopted by the Brazilian National Ministry of Health provides adequate *M. tuberculosis* exposure to pyrazinamide. In addition, they show that gender and the timing of blood sample collection did not influence serum pyrazinamide concentrations.

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Palmar telangiectasia is associated with the intensity of smoking

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TO THE EDITOR:

Palmar telangiectasias (PTs) usually involve the thenar and hypothenar areas. In the mid-20th century, they were described as cutaneous vascular manifestations, including vascular spiders and palmar erythema, during pregnancy.[1] There are various etiologies of PT[2-4]: they can have a primary cutaneous pathogenesis; they can be a manifestation of systemic, neoplastic, or infectious diseases; or they can be drug-induced. In 2012, PTs were reported in a patient with Graves' disease.[5] A more recent study suggested that PT is a cutaneous sign of smoking.[6]

In our clinical practice, we encounter a lot of smokers among our patients. That is not surprising, because tobacco smoke has components that are known to cause oxidative stress, as well as to reduce the innate and host immune responses, thus affecting cellular and humoral immunity. Smoking is considered a major risk factor for severe diseases such as lung cancer and COPD. However, we have not seen PT in every current smoker. The aim of this study was to determine the prevalence of PT among ill and healthy subjects, taking into consideration their general health and smoking status.

We recruited 236 adult subjects. We excluded individuals who had been taking system corticosteroids or epidermal growth factor receptor inhibitors, as well as those who had Graves' disease, those who were pregnant, and those who had been taking oral contraceptives. We asked the participants about their smoking status, defined as nonsmoker, current smoker, or former smoker (having quit smoking ≥ 1 year prior). We asked current and former smokers about the duration of their smoking (in years) and the number of cigarettes they smoked per day. It was possible to determine the total exposure to tobacco smoke (i.e., the total smoking history) by calculating pack-years (number of packs smoked per day multiplied by the number of years the person has smoked). During the physical examination, we paid special attention to presence of PT. We performed a statistical analysis to determine whether PT was associated with demographic characteristics, illness, and smoking intensity.

The study sample comprised 236 adults (116 men and 120 women) with a mean age of 60.87 ± 15.72 years—31 were healthy, and 205 suffered from at least one disease: COPD (n = 47); asthma (n = 5); pneumonia (n = 17); arterial hypertension (n = 38); pulmonary embolism (n = 18); lung cancer (n = 41); lymphadenopathy (n = 9); or other diseases (n = 30). Of the 236 individuals evaluated, 79 were current smokers and 157 were nonsmokers (comprising 56 former smokers and 101 never smokers). Pearson's chi-square test showed that the prevalence of PT among the smokers did not differ significantly from that observed among the patients diagnosed with any of the diseases listed above ($p = 0.132$). Using a t-test for equality of means, we found no significant difference between the current and former smokers in terms of the number of pack-years ($p = 0.048$). Although none of the never smokers had PTs, we found them in 65 (82.27%) of the 79 current smokers and in 23 (41.07%) of the 56 former smokers. A two-tailed t-test for independent samples showed a highly significant difference between the subjects with PT and those without in terms of the total exposure to tobacco smoke ($p \leq 0.001$). We also found a highly significant difference between the current and former smokers in terms of the prevalence of PT ($p \leq 0.001$).

Our study showed that the appearance of PT is strongly associated with the intensity of smoking. The lack of a standardized measure might explain previous discrepancies in terms of whether PT correlates with smoking history (pack-years), with the mean number of cigarettes smoked per day in current smokers,[6] or with the mean number of cigarettes smoked by former smokers.

Our findings of no PT in the never smokers and that PT existed not only in current smokers but also in former smokers with a history of a certain intensity of smoking might speak in favor of the importance of the components of tobacco smoke in the etiopathogenesis of PT. There is evidence that some subjects are more susceptible to smoking-related diseases. The high degree of variation in the number of pack-years associated with PT suggests that the appearance of PT in smokers could be influenced by individual susceptibility to the components of tobacco smoke.

We found that PT was not associated with gender, age, or any of the diseases from which the participants suffered. One previous study suggested that there is an association between PT and lung cancer.[3] Given the results of our study and the fact that smoking is a major risk factor for lung cancer, that association requires further analysis in studies with larger samples. It remains unknown whether smoking contributes to the appearance of telangiectasia and lung cancer; it is possible that both

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are consequences of smoking-related disturbances in homeostasis. Among the individuals evaluated in our study, 41 had lung cancer. PT was seen in only 24 of the 32 smokers. Finally, some of the smokers with PT suffered from smoking-related diseases other than lung cancer, such as COPD and cardiovascular disease.

PT in smokers should be the focus of detailed molecular biology research. Detected by simple inspection in clinical practice, PT might serve as indicator of disturbances in homeostasis and could be a useful marker in the primary prevention or early detection of serious smoking-related diseases.

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Reflections on the article "Correlation of lung function and respiratory muscle strength with functional exercise capacity in obese individuals with obstructive sleep apnea syndrome"

Andressa Silva Figueira[1,a], Marina Esteves[1,b], Luiz Alberto Forgiarini Júnior[2,c]

First, we would like to congratulate Carvalho et al. on their article published in the JBP entitled "Correlation of lung function and respiratory muscle strength with functional exercise capacity in obese individuals with obstructive sleep apnea syndrome" (OSAS),[1] a matter of extreme importance for all professionals working in this area; this clinical condition affects a large number of people, with a direct impact on their quality of life.

An important observation to be made about the aforementioned study[1] is that it has a cross-sectional design, which does not establish causality, that is, it is not possible to know whether the changes in lung function and respiratory muscle strength are due to either obesity or OSAS alone. Support for the first hypothesis may be evidenced in a study by Melo et al.,[2] who reviewed studies of lung function in obese individuals and observed reductions in total lung capacity and FVC, accompanied by a reduction in FEV_1; those were the key findings across all samples, suggesting that the presence of a restrictive respiratory pattern is associated with obesity. What can be perceived is that decreased lung capacity is already a characteristic of obesity, even in the absence of comorbid OSAS.[2] A study by Tassinari et al.,[3] cited by the study in question,[1] reported that no lung function or respiratory muscle impairment was observed in normal-weight OSAS patients and that there were similarities between that group of patients and healthy subjects.

Another limiting factor are the comorbidities in the sample,[1] such as type II diabetes mellitus; it has been reported in a study by Punjabi et al.[4] that, regardless of adiposity, sleep-disordered breathing is associated with impairments in insulin sensitivity and that using body mass index, which does not discriminate between muscle mass and adipose tissue, as an assessment variable may affect the results found regarding the comparison of the degree of obesity of each individual.

A finding that deserves comment is the lack of correlation between lung function and six-minute walk distance in that population,[1] because the six-minute walk test has been validated in obese subjects, and found to be reproducible, and it has been demonstrated that an 80 m increase in six-minute walk distance is related to clinical improvement. A factor that could possibly explain this finding was reported by Ucok et al.,[5] who compared individuals with OSAS and healthy individuals: individuals with lower maximal oxygen consumption values had premature leg fatigue. This disorder of the muscle metabolism is associated with high levels of lactic acid in the blood and with decreased ability to reduce these levels during exercise in patients with sleep disorders.[5]

It is incumbent upon us to emphasize that the results obtained are likely to be improved by using a different methodological design and by redefining the study population by limiting it to individuals without comorbidities, thus avoiding a possible selection bias.

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Authors' reply

Thays Maria da Conceição Silva Carvalho[1,a], Anísio Francisco Soares[2,b], Danielle Cristina Silva Climaco[3,c], Isaac Vieira Secundo[3,d], Anna Myrna Jaguaribe de Lima[2,e]

First of all, we would like to thank the authors of the letter above for their comments on our article entitled "Correlation of lung function and respiratory muscle strength with functional exercise capacity in obese individuals with obstructive sleep apnea syndrome" (OSAS).[1] In the article, we showed that the patients in our sample, which consisted of obese individuals with untreated OSAS, had reduced lung function, reduced inspiratory muscle strength, and reduced physical capacity. In addition, we found that, in these patients, reduced lung function, but not reduced respiratory muscle strength, was associated with reduced shuttle walk distance. However, no correlation was found between lung function or respiratory muscle strength and six-minute walk distance (6MWD).

With regard to the comment made by those authors regarding the study design, which is cross-sectional and observational and therefore is not the most appropriate for establishing a cause for the reduction in strength and lung function found in our patients, we agree with it. In fact, we consider this to be one of the limitations of our study. We suggested that, in order to establish causality accurately, studies with greater methodological rigor, such as randomized clinical trials, should be performed, given that our study investigated only one group of obese individuals with OSAS and there were no groups for subsequent comparisons. With regard to the presence of comorbidities, this is a common finding in patients with OSAS. The intermittent episodes of hypoxia and reoxygenation present in OSAS can promote oxidative stress associated with the release of inflammatory markers, contributing to the emergence of comorbidities and of consequences for peripheral and cardiorespiratory muscles, and this can directly affect exercise tolerance. As for the authors' remarks regarding the use of body mass index to assess obesity, we agree that this is not the most reliable method to classify obesity because it does not take body composition into account. Nevertheless, according to an editorial in the BMJ in 2018,[2] body mass index remains the most commonly used and widely accepted measure of obesity in adults and children, as well as having a strong correlation with gold standard measures of body fat.

Finally, regarding the comment on the lack of correlation between lung function and 6MWD, we would like to emphasize that the correlation between the two variables is not associated with the fact that the six-minute walk test has been validated in obese subjects and found to be reproducible, as highlighted in the letter above. What a lack of correlation tells us, from a statistical point of view, is that a change in the value of an independent variable (i.e., lung function) did not cause changes in the value of a dependent variable (i.e., 6MWD). Similar results have also been reported by Ferreira et al.,[3] who found no correlations between lung function and 6MWD when analyzing obese children and adolescents.

In conclusion, we would like to thank once again the authors of the letter above for the continuing discussion about the methodological aspects and results of our paper,[1] making it possible to broaden the debate on OSAS, a topic that is so current and important in the field of respiratory and sleep medicine.

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On page 416, where is written:

"**Chart 6.** Dosing, dosing schedule, and routes of administration of antibiotics that can be used in the treatment of community-acquired pneumonia."

It should be read (changes in bold type):

Chart 6. Dosing, dosing schedule, and routes of administration of antibiotics that can be used in the treatment of community-acquired pneumonia.

Drug	Route	Dose	Interval, h
Amoxicillin	**Oral**	**500 mg**	**8**
Amoxicillin/clavulanic acid	Oral	875/125 mg	**12**
Amoxicillin/clavulanic acid	**Oral**	**500/125 mg**	**8**
Amoxicillin/clavulanic acid	**Intravenous**	**1,000/200 mg**	**8***
Ampicillin/sulbactam	Intravenous	1.5/3.0 g	6-8
Azithromycin	Oral-intravenous	500 mg	24
Cefepime	Intravenous	2g	12
Cefotaxime	Intravenous	1-2 g	8
Ceftaroline	Intravenous	600 mg	12
Ceftriaxone	Intravenous	1 g	12
Ciprofloxacin	Oral	500-750 mg	12
Ciprofloxacin	Intravenous	400 mg	8-12
Clarithromycin	Oral	500 mg	12
Extended-release clarithromycin	Oral	1,000 mg	24
Clarithromycin	Intravenous	500 mg	12
Clindamycin	Oral	600 mg	**8-12**
Clindamycin	Intravenous	600 mg	8
Ertapenem	Intravenous	1 g	24
Imipenem	Intravenous	1 g	8
Levofloxacin	Oral	500-750 mg	24
Levofloxacin	Intravenous	**500-750 mg**	24
Linezolid	Oral-intravenous	600 mg	12
Meropenem	Intravenous	1 g	8
Moxifloxacin	**Oral-intravenous**	400 mg	24
Piperacillin/tazobactam	Intravenous	4.0/0.5 g	6-8
Vancomycin	Intravenous	500/1,000 mg	6/12

***Dosing schedule can be modified to every 6 hours in cases of severe infection.**

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After being approved by the Editorial Board, all articles will be evaluated by qualified reviewers, and anonymity will be preserved throughout the review process.

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Abstracts

2. Singer M, Lefort J, Lapa e Silva JR, Vargaftig BB. Failure of granulocyte depletion to suppress mucin production in a murine model of allergy [abstract]. Am J Respir Crit Care Med. 2000;161:A863.

Chapter in a Book

3. Queluz T, Andres G. Goodpasture's syndrome. In: Roitt IM, Delves PJ, editors. Encyclopedia of Immunology. 1st ed. London: Academic Press; 1992. p. 621-3.

Official Publications

4. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. WHO/Tb, 1994;178:1-24.

Theses

5. Martinez TY. Impacto da dispnéia e parâmetros funcionais respiratórios em medidas de qualidade de vida relacionada a saúde de pacientes com fibrose pulmonar idiopática [thesis]. São Paulo: Universidade Federal de São Paulo; 1998.

Electronic publications

6. Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Homepages/URLs

7. Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/

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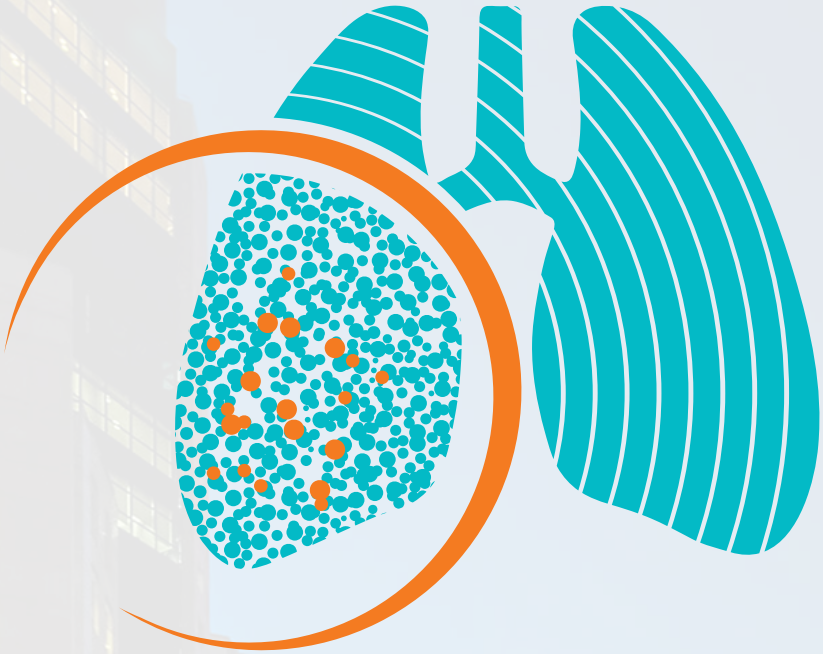

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