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HIGHLIGHT

Tuberculosis Series 2020

Brazilian guidelines for the pharmacological treatment of idiopathic pulmonary fibrosis

Multiple cavitary lung lesions: CT imaging findings

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EDITORIAL

Tuberculosis series 2020

Denise Rossato Silva[1], Fernanda Carvalho de Queiroz Mello[2], Giovanni Battista Migliori[3,4]

According to the World Health Organization (WHO), tuberculosis is the leading cause of death from a single infectious agent worldwide,[1] as well as being the leading cause of death among people living with HIV. In 2018, there were an estimated 10 million new tuberculosis cases worldwide, and 1.5 million people died from the disease. In the same year in Brazil, the incidence of tuberculosis was 45 cases/100,000 population, and the tuberculosis-related mortality rate was 2.3 deaths/100,000 population.[1] Therefore, in celebration of World TB Day, on March 24th, this issue of the JBP features several articles focusing on diverse aspects of tuberculosis control.

In 2018, 484,000 people worldwide developed tuberculosis that was resistant to rifampin, and 78% of those had multidrug-resistant tuberculosis (MDR-TB).[1] In December of 2019, the WHO issued a rapid communication promoting key changes in the treatment of MDR-TB,[2] and the first global report on the adverse events of antituberculosis drugs was published.[3,4] In this issue of the JBP, a review study[5] provides an overview of the management of MDR-TB in Brazil from 2004 to 2018, demonstrating the modifications in the national recommendations. Another study,[6] which investigated risk factors for MDR-TB, shows that previous treatment and cavitation on chest X-rays are associated with MDR-TB.

Between 2000 and 2018, 58 million lives were saved through effective diagnosis and treatment of tuberculosis. Chest X-ray is an essential tool for early detection of tuberculosis and has higher sensitivity for the diagnosis of pulmonary tuberculosis than does screening for tuberculosis symptoms.[7] Muller et al.[8] reported that the median time from the first chest X-ray to the diagnosis of tuberculosis in the emergency room of a tertiary care hospital was 2 days. Cavitation on a chest X-ray was an independent factor associated with an earlier diagnosis. Although hospitalization allows rapid management of cases and favors faster diagnosis, the unavailability of rapid diagnostic tests, such as the Xpert MTB/RIF rapid molecular test for tuberculosis, can result in unacceptable diagnostic delays.

Case detection and treatment are core elements of tuberculosis control, especially in prisons. The authors of a letter published in this issue of the JBP[9] described the bacteriological diagnosis of tuberculosis in the prison system in southern Brazil, where the prevalence of tuberculosis is 2,488 cases/100,000 population. They reported that the Xpert MTB/RIF test is available to only 13.6% of the primary care teams in prisons and that the number of health care workers (HCWs) is insufficient to meet the demand. Delays in the detection and treatment of tuberculosis cases must be minimized in order to improve tuberculosis control in prisons.

Nonadherence to treatment and loss to follow-up are associated with a longer duration of treatment in cases of drug-susceptible tuberculosis. In addition, the treatment success rate with longer regimens is low (approximately 50%) in cases of MDR-TB. Therefore, shorter regimens with existing or repurposed drugs could significantly improve tuberculosis management and treatment success rates.[1,10] A review study in this issue of the JBP[11] reports recent advances and findings of ongoing clinical trials aimed at shortening regimens for drug-susceptible tuberculosis and MDR-TB.

In addition to proper diagnosis and treatment, preventive treatment for tuberculosis, including infection control in healthcare settings, is a key component of the WHO End TB strategy.[12] In 2017, 9,299 tuberculosis cases were reported among HCWs, and Brazil accounted for 11% of those cases.[1] The authors of another letter published in this issue of the JBP described a prospective cohort study in which they evaluated latent tuberculosis infection (LTBI) among HCWs in primary care settings.[13] Using the QuantiFERON-TB Gold In-Tube Test (QFT; QIAGEN, Hilden, Germany), the authors found the prevalence of LTBI to be 23.3% among the HCWs evaluated. That was the first study[13] to evaluate the prevalence of LTBI in primary care HCWs in Brazil, highlighting the need for regular monitoring and screening of such HCWs.

Even after the appropriate treatment of tuberculosis, pulmonary sequelae can impair pulmonary function and quality of life.[14-16] A multicenter cross-sectional study, conducted in Brazil and included in this tuberculosis series,[17] compared pulmonary tuberculosis patients with and without previous lung disease, in terms of post-treatment spirometry changes. The authors showed that lung function impairment is common after treatment of pulmonary tuberculosis, regardless of whether the patient has a history of smoking or previous lung disease. They concluded that spirometry is advisable for patients who develop grade 2-4 dyspnea or major radiological alterations after treatment for pulmonary tuberculosis.

This tuberculosis series, dedicated to the celebration of World TB Day, spotlights several tuberculosis articles, with the objective of providing an overview of the diagnosis and treatment of tuberculosis. We hope that this series will lead to improved management of cases and new lines of tuberculosis research.

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In the time of strategies to end tuberculosis, prevention is better than treatment

Ana Paula Santos[1,2], Denise Rossato Silva[3], Fernanda Carvalho de Queiroz Mello[1]

In 2015, the World Health Organization (WHO) launched the End TB Strategy, which included among its goals a 95% reduction in the incidence of tuberculosis worldwide by 2030.[1] Achieving that ambitious goal will require early diagnosis and treatment of active disease, in order to interrupt the chain of transmission of *Mycobacterium tuberculosis* (Mtb), as well as preventive measures. The Brazilian National Plan to End Tuberculosis as a Public Health Problem,[2] proposed by the Brazilian National Ministry of Health, also has, among its priorities, preventive treatment against tuberculosis.

Latent tuberculosis infection (LTBI) occurs when a person is infected with Mtb but does not manifest active disease.[3] This condition has been considered a priority because it is key to achieving the goals of the WHO and the Brazilian National Ministry of Health. It is particularly important for health care professionals, who are susceptible to nosocomial Mtb infection and are at risk for LTBI.[4]

The WHO defines health care professionals as people engaged in the promotion, protection, improvement, and care of the health of the population in a given location.[5] The following factors have been associated with a higher risk of LTBI among primary health care professionals in Brazil[6]: being over 50 years of age; not having a BCG vaccine scar; having a history of smoking; working as a nurse, nursing technician, or community health agent; and not having used an N95 mask on a regular basis in the workplace.

In the article by Lima et al.,[7] published in this issue of the *Jornal Brasileiro de Pneumologia*, the authors used the QuantiFERON-TB Gold In-Tube assay (QFT-GIT; QIAGEN, Hilden, Germany) to assess the incidence of LTBI among primary health care professionals in two Brazilian cities with a high incidence of tuberculosis: Vitória and Manaus.

The QFT-GIT is an IFN-gamma release assay (IGRA) and quantifies, through an immunoenzymatic test (ELISA), the levels of this cytokine released by memory T lymphocytes after stimulation of a whole blood sample with Mtb-specific antigens; it is one of two alternatives for the diagnosis of mycobacterial infection, the other being the tuberculin skin test (TST).[3]

The main advantage of an IGRA over the TST is the fact that it is not influenced by previous BCG vaccination or by infection with nontuberculous mycobacteria, which gives the method high specificity. The other advantages are as follows: the simplicity of training for blood collection; absence of the reading bias present in the application of the TST by the health professional; direct testing of a biological sample, which reduces the risk of adverse effects; and the operational advantage of not requiring patients to return for the reading of the result. However, its high cost (in comparison with that of the TST), the need for blood collection, the fact that serial testing in not recommended, and the high frequency of indeterminate results, as well as the need for a well-equipped laboratory and careful handling of the samples to maintain lymphocyte viability, are considered limiting disadvantages.[8]

Lima et al.[7] found that the QFT-GIT conversion rate in the population studied was lower than that reported in other studies involving similar samples; that is, primary health care professionals in countries with a high incidence of tuberculosis.

In addition to the limitations described by the authors, such as the high proportional losses to follow-up and the absence of a gold standard for the diagnosis of LTBI, there is a lack of standardization of the QFT-GIT for conducting serial tests, in view of the possibility of spontaneous conversion and reversal. The QFT-GIT has also been criticized for having such a high cost, which limits its utility in countries with limited resources.

Data on the serial use of IGRAs are scarce, and it must be determined whether the identified conversions were true or whether they reflected only dynamic immunological processes, difficulties in the reproducibility of the test, or simply variations from person to person.[9] In a survey conducted in Canada, the number of QFT-GIT conversions was higher than expected and was not accompanied by a comparable number of TST conversions. In addition, occupational exposures were unrelated to the high number of QFT-GIT conversions, which suggests that these results do not reflect recent Mtb infection.[9]

Regarding the cost and effectiveness aspects, Loureiro et al.[10] found the QFT-GIT to be the test that correctly classified the largest number of individuals with LTBI among professionals at primary health care clinics in Brazil. However, the test had the lowest cost-effectiveness when the authors applied an analytical model, considering a hypothetical cohort.

The Lima et al. study[7] indicates a future application of QFT-GIT in health care professionals: that of identifying conversions after occupational exposure. However, it showed limitations, such as the high proportional losses to follow-up in the sample, as well as questions about the serial use of an IGRA and the use of a method that is still quite costly. However, at a time when the prevention of active tuberculosis has been exalted, the use of alternatives to the TST that are more specific for the identification of LTBI should be seen as imperative,

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especially in high-risk populations, who are potential links in the chain of disease transmission. Studies aimed at improving understanding of the serial results of the test, in accordance with the guidelines of the End TB Strategy[1] and the Brazilian National Plan to End Tuberculosis,[2] should be encouraged, as should proposals for improving adherence to the QFT-GIT protocols and for reducing its cost.

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CT-based radiomics of benign and malignant features in multiple cavitary pulmonary lesions

Erique Guedes Pinto[1], Diana Penha[2], Klaus Irion[3]

In this issue of the JBP, Giacomelli et al.[1] aimed to identify characteristics of pulmonary cavitary lesions on CT scans, which could allow the differentiation between benign and malignant features. Pulmonary cavities are a common diagnostic dilemma for radiologists because they cover a wide range of etiologies that, at first glance, may have similar morphological CT features. Possible differential diagnoses include infectious diseases, such as tuberculosis, fungal infections, and parasitic infections, as well as noninfectious diseases, such as malignant and autoimmune diseases.[2,3] Tuberculosis and aspergilloma are common benign causes of multiple cavitary lung lesions. Among malignant causes, metastases from extrathoracic malignancy are by far more common than primary lung cancer.[2,3]

Giacomelli et al.[1] found that the clinical presentation can be similar in patients with benign or malignant multiple cavitary nodules.[1] Therefore, radiological criteria indicating the nature of these lesions may be crucial, particularly in patients who are not candidates for invasive diagnostic procedures or in order to expedite treatment interventions.

The authors tested various radiological parameters and concluded that a greater number of cavities favors malignant etiologies.[1] In contrast, the presence of centrilobular nodules significantly correlates with benign etiologies. Their observations have also reinforced previous findings in Brazilian studies regarding the association of the reversed halo sign with nodularity and active pulmonary tuberculosis.[4-6] The finding that nodular walls or nodules within reversed halo sign lesions are highly suggestive of granulomatous diseases, especially tuberculosis, rather than of cryptogenic organizing pneumonia, is a significant contribution that allows radiologists to be confident about the nature of such lesions.[4-6] However, Franquet et al.[7] reported that centrilobular nodules and centrilobular branching opacities (tree-in-bud pattern) can also be identified in some metastatic tumors and in thrombotic microangiopathy. The latter is a rare and distinct form of tumor embolism, with widespread fibrocellular intimal hyperplasia of small pulmonary arteries and arterioles that is induced by tumor microemboli. The presentation can be similar to infectious bronchiolitis, with small centrilobular nodules and tree-in-bud opacities.[7]

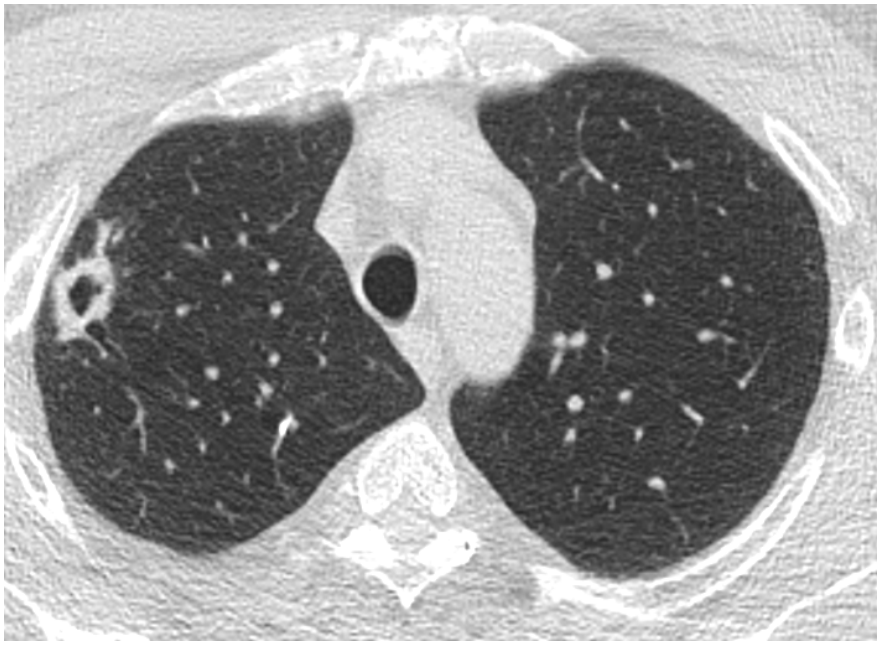

Figure 1. Axial CT scan at the level of the upper lobes with a lung window setting showing a cavitary lesion with an irregular and thick wall. A CT-guided biopsy was taken from the thick wall, and histological evaluation confirmed non-small cell adenocarcinoma.

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Another contribution of Giacomelli et al.[1] is the unexpected observation that the wall thickness and the diameter of the largest lesion, as well as its location, are not reliable discriminators between benign and malignant lesions is another contribution from their study.[1]

Lung neoplasms manifesting as cystic lung lesions are among the causes of a lack of correlation between wall thickness and the benign or malignant nature of cavitary lung lesions. These are forms of lung cancer in which the growth of cancerous cells occurs in the wall of a thin-walled cyst, usually in an asymmetric fashion to a focal or diffuse thickening of the walls.[8,9] Although malignancy can be the cause of the cyst, this tends to be diagnosed at a later stage, when asymmetric thickening of the walls is perceptible on CT (Figure 1).

The findings of Giacomelli et al.[1] can support future research on radiomics of lung lesions. Their study adds information to the findings of Beig et al.,[10] who also highlighted the importance of analyzing the internal structure and perinodular regions of lung lesions in order to distinguish between malignant and benign lung diseases.

This editorial acknowledges the contribution of that study[1] and aims to instigate further research and systematic reviews that can support the development of robust radiomics in thoracic imaging, which is crucial to provide an accurate and fast diagnosis, minimizing the variability of patient care.

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Pharmacological treatment of idiopathic pulmonary fibrosis: time to step out of the comfort zone?

Giacomo Sgalla[1], Luca Richeldi[1,2]

Over the last two decades, the efforts made by international respiratory societies worldwide to standardize diagnostic criteria of idiopathic pulmonary fibrosis (IPF) crucially enabled a deeper understanding of the pathogenetic mechanisms of the disease, thus leading to outstanding therapeutic achievements. After many years of disappointing trials, nintedanib and pirfenidone ultimately emerged as the first effective drugs in slowing down the rate of decline in lung function in these patients,[1,2] heralding the dawn of a new era in the management of IPF. The approval of the two drugs by regulatory agencies—pirfenidone received approval for use in Japan in 2008, in Europe in 2011, and in the USA in 2014, whereas nintedanib received approval for use in the USA in 2014 and in Europe in 2015—sanctioned the widespread use of antifibrotic treatment for IPF in clinical practice. The increasing amount of evidence from well-designed randomized clinical trials in the first half of the last decade warranted an update of the American Thoracic Society/European Respiratory Society/Japanese Respiratory Society/Latin American Thoracic Association (ATS/ERS/JRS/ALAT) evidence-based guidelines[3] for the management of IPF patients, which provided an unprecedented conditional recommendation in favor of the pharmacological treatment with either pirfenidone or nintedanib. Over the last years, further proof of safety and efficacy in the long term of both agents has been delivered by open-label extension studies and various real-life experiences.

In this issue of the *Jornal Brasileiro de Pneumologia*, the working group led by Baddini-Martinez provides a pragmatic, evidence-based set of recommendations for guiding the use of pharmacological therapies in IPF patients in Brazil.[4] The panel of experts focused on Patients of interest, Intervention to be studied, Comparison of intervention and Outcome of interest (PICO)-style questions related to seven types of treatment for IPF. According to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach, clinical outcomes (stratified as critical, important, and unimportant) and quality of available evidence were the major factors considered to express conditional or strong recommendations in favor of or against the investigated types of treatment. A peculiar methodological feature of these guidelines is represented by the search strategy: the choice to evaluate systematic reviews with meta-analyses instead of single studies facilitated a synthetic reporting of outcomes and allowed, up to a certain extent, indirect comparisons of treatments to be made. Unsurprisingly, the group of experts confirmed a conditional recommendation in favor of pirfenidone and nintedanib, which were found to be similarly effective in reducing the risk of occurrence of a > 10% decline in FVC as compared with placebo (estimated OR = 0.64 and 0.61, respectively). The use of anticoagulant therapy was strongly discouraged, corroborating the ATS/ERS/JRS/ALAT guidelines.[3] Likewise, phosphodiesterase-5 inhibitors received a conditional recommendation against use; importantly, the authors note that the combination of sildenafil with nintedanib also failed to prove greater benefit when compared with the use of nintedanib alone in a recent trial,[5] although that study could not be included in the current statement because it was published after the completion of the analyses for the present guidelines.[4] A small, although noteworthy, divergence from the 2015 ATS/ERS/JRS/ALAT guidelines[3] regards the recommendation on the use of antacids in IPF: the international guidelines[3] expressed a conditional recommendation for antacid treatment in all patients with IPF, whereas the present Brazilian guidelines[4] were unable to find articles suitable for developing recommendations for or against the pharmacological treatment of gastroesophageal reflux because of the low quality of the available evidence, mostly consisting of retrospective observational studies. The position taken by the Brazilian working group[4] finds support in a recent methodological study[6] showing how the findings of various studies on the impact of anti-reflux treatment in IPF are substantially biased by the time required to define treatment exposure, which inherently determines a survival advantage.

In conclusion, the Brazilian Thoracic Association statement not only represents a useful, practical guide for Brazilian physicians to approach IPF patients, but it also provides the most up-to-date standpoint on the use of pharmacological therapies that have been evaluated in the treatment of IPF over the last two decades. Now that the benefits of pirfenidone and nintedanib in IPF have been long proven, the scientific community will be soon called to pronounce on new emerging questions. Recent studies have provided compelling evidence of efficacy of current antifibrotic medications in other forms of interstitial lung diseases[7-9]; for example, in the future, starting antifibrotic therapy is likely to be extended to patients showing signs of disease progression irrespective of its etiology and classification. In this scenario, the identification of criteria indicating clinically meaningful disease progression will be critical to guarantee a timely and appropriate start of treatment, properly balanced by the risks related to potential adverse effects. Most importantly, the search

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for novel effective agents to halt IPF progression has brought to the first positive phase 2 trials in years[10-12]: hopefully, the next era of pharmacological treatment of IPF will offer the opportunity to combine different drugs in a more patient-tailored approach. So far, the approach of combining the two approved therapies for IPF has been surrounded by many uncertainties, and although a few open-label studies showed the lack of significant interaction between the two drugs and the relative safety of combined therapy, to date, there is insufficient evidence for respiratory societies or regulatory agencies to endorse it. Building on the design of many recent and ongoing IPF randomized trials that will allow for background therapy, the approach of associating a targeted, highly tolerable novel therapy on top of currently antifibrotic drugs might become more appealing and outclass the urgency to further prove the synergistic effect of the coadministration of nintedanib and pirfenidone.

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Ground-glass opacities associated with pulmonary cysts

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A 28-year-old man presented with a three-day history of fever, cough, and progressive dyspnea. Chest CT showed diffuse ground-glass opacities containing pulmonary cysts (Figure 1).

Ground-glass opacity is perhaps the most common abnormal pattern seen on chest CT scans of individuals with lung diseases, making it an extremely nonspecific finding. The differential diagnosis of pulmonary cysts is more limited, although it still encompasses a considerable number of diseases. When the two patterns coexist, the list of diagnostic possibilities is greatly reduced.

The association of ground-glass opacities with pulmonary cysts can occasionally be seen in desquamative interstitial pneumonia and hypersensitivity pneumonitis. However, in those diseases, cysts are rare and, when present, are typically few in number. A history of contact with birds or fungi can indicate a diagnosis of hypersensitivity pneumonitis.

Hemorrhagic pulmonary metastases and traumatic pulmonary pseudocysts can also have similar imaging aspects when accompanied by pulmonary hemorrhage. The clinical history is generally sufficient to raise the diagnostic suspicion of those two diseases.

The two conditions that most often manifest as an association of ground-glass opacities with pulmonary cysts are lymphocytic interstitial pneumonia and *Pneumocystis jirovecii* pneumonia (pneumocystosis).

Lymphocytic interstitial pneumonia is commonly seen in individuals infected with HIV, those infected with the Epstein–Barr virus, and those with immunodeficiency from other causes. Notable among the systemic diseases seen in immunocompromised individuals are Sjögren's syndrome and systemic lupus erythematosus. The main clinical manifestations of those two diseases include dyspnea, cough, weight loss, and chest pain. In most cases, CT shows a combination of ground-glass opacities, consolidation, peribronchovascular thickening, poorly defined nodules, and cysts. The cysts are few in number and are diffusely distributed.

Pneumocystosis is seen almost exclusively in immunocompromised patients (HIV-infected patients, bone marrow transplant recipients, and patients using immunosuppressants). Although the symptoms are insidious, including a dry cough, low-grade fever, and dyspnea, untreated cases can progress to respiratory failure and death. The combination of a positive medical history, physical examination findings consistent with the disease, lymphopenia, and elevated serum levels of lactate dehydrogenase facilitate the diagnosis. The fungus can be identified in sputum and bronchoalveolar lavage fluid. The findings on CT include extensive ground-glass opacities, with or without septal thickening. Cysts are relatively common and tend to have a predilection for the upper lobes. Pleural effusion is quite rare.

Our patient presented with a rapidly evolving pulmonary infection, hypoxemia, and elevated serum levels of lactate dehydrogenase. Given those circumstances, the most likely hypothesis was pneumocystosis. The patient underwent an HIV test, which was positive. Examination of the bronchoalveolar lavage fluid showed the presence of *P. jirovecii*, confirming the diagnosis.

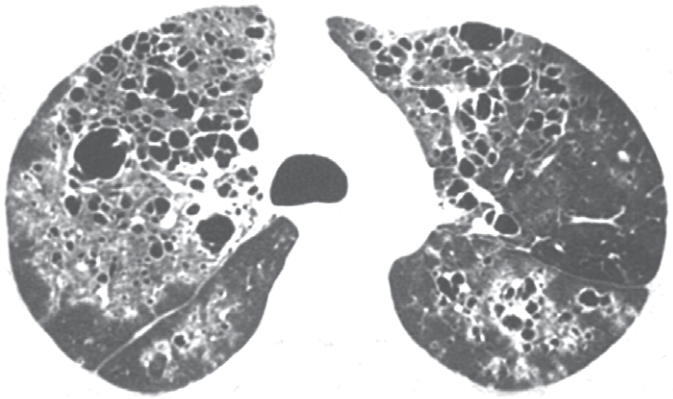

Figure 1. Chest CT. An axial slice at the level of the upper lobes, showing diffuse ground-glass opacities, in both lungs, containing multiple cystic formations.

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Estimating risk in clinical studies: odds ratio and risk ratio

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PRACTICAL SCENARIO

A retrospective cohort study evaluated the association between type of ventilatory support and mortality among adult patients with interstitial lung disease and acute respiratory failure.[1] In comparison with noninvasive ventilation (NIV), invasive mechanical ventilation (IMV) increased mortality, with an OR of 26.0 (95% CI: 5.9-116.6) and a risk ratio (RR) of 2.2 (95% CI: 1.7-2.9), as detailed in table 1.

In our example, we calculated the OR and RR to answer the study question. It is important to understand the difference between these two statistical methods for calculating risk, which one is more applicable to answer this research question, and how they are interpreted.

The OR is defined as the ***ratio of odds*** of an event occurring, estimated by calculating the ratio of the number of times that the event of interest occurs to the number of times that it does not (ratio of events to non-events) between exposed and unexposed groups.[2] The RR is defined as the ***ratio of probabilities*** of an event occurring (ratio of events to subjects) between exposed and unexposed groups (Table 1). A positive association (increased risk) between exposure and outcome implies that the OR or RR is > 1.0, and a negative association (decreased risk) implies that the OR or RR is < 1.0.

HOW THE RR AND OR SHOULD BE INTERPRETED

The RR expresses that the "risk of the event (e.g., mortality) is X times larger/smaller in the exposed group than in the unexposed group". That statement is easily interpreted because it deals with probabilities (which range from 0 to 1). However, the OR is expressed as the ratio of the odds of event X in the exposed group to the odds of the same event in the unexposed group. Although we often use ORs to estimate RRs, they are different and ORs are not as intuitive to grasp and are therefore often misinterpreted.

The RR is commonly (and most correctly) used in order to estimate the risk of an event in randomized controlled trials, cohort studies, and cross-sectional studies, because all of those study designs calculate absolute risk and the RR can therefore be estimated. The OR is utilized to estimate risk in case-control studies, where the prevalence/incidence of the outcome cannot be estimated, given that the numbers of individuals with and without the outcome (cases and controls, respectively) are fixed by the investigators. The OR is also commonly used in order to calculate risk in cohort studies and randomized controlled trials when a logistic regression statistical model is employed to adjust for confounders or test for effect modification. However, we should bear in mind the fact that ORs and RRs are not equivalent.

In comparison with an RR, an OR tends to overestimate the strength of the association between exposure and outcome. However, the degree of overestimation is negligible in studies in which the outcome of interest occurs rarely (typically in < 10% of the participants). In our example, the ratio of the odds of death in the IMV group to that in the NIV group is 26 to 1 (OR = 26.0). In contrast, the risk of death in patients on IMV is 95%, compared with 43% for those on NIV (RR = 2.2). That RR is interpreted as the risk of death being 2.2 times higher in the IMV group than in the NIV group. This large difference between OR and RR is explained by the high proportion of participants who died in our example (41%). The OR estimates the RR more accurately when the study outcome is rare.

Table 1. Calculation of odds ratios and risk ratios for our example of noninvasive ventilation versus invasive mechanical ventilation in interstitial lung disease.

Group	Death	Survival	Total	Odds	Risk
IMV	39 (a)	2 (b)	41	Odds of death in IMV $\frac{a}{b} = \frac{39}{2} = 19.5$	Risk of death in IMV $\frac{a}{a+b} = \frac{39}{41} = 0.95$
NIV	32 (c)	43 (d)	75	Odds of death in NIV $\frac{c}{d} = \frac{32}{43} = 0.74$	Risk of death in NIV $\frac{c}{c+d} = \frac{32}{75} = 0.43$
Total	71	45	116	OR for death between groups $\frac{a}{b}/\frac{c}{d} = \frac{a*c}{b*d} = \frac{19.5}{0.74} = 26.4$	RR for death between groups $\frac{a}{a+b}/\frac{c}{c+d} = \frac{0.95}{0.43} = 2.2$

IMV: invasive mechanical ventilation; NIV: noninvasive ventilation; and RR: risk ratio. Adapted from Güngör et al.[1]

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Identifying small airway dysfunction in asthma in clinical practice

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BACKGROUND

Small airways are defined as those with a diameter ≤ 2 mm.[1] There is a current focus on small airway dysfunction (SAD) in asthma and the techniques used in order to measure this.[1-3] Postma et al. reported that SAD is present across all severities of asthma and can be measured using different techniques, including lung volumes and oscillometry.[1,3] The case histories described here illustrate how these methods can be applied in a clinical setting to identify SAD in asthma.

CASE HISTORIES

Table 1 shows data from two patients with moderate to severe asthma (Global Initiative for Asthma classification system = 4) who attended our research centre for lung function assessment. Both were non-smoking females of a similar age with uncontrolled asthma; both had Asthma Control Questionnaire scores = 2.3. There were similar levels of fractional exhaled nitric oxide (21 and 12 ppb), FEV_1 (68% and 72% of the predicted value), and FEV_1/FVC ratio (0.61 and 0.70). Patient 1 demonstrated greater reversibility than patient 2 (420 mL and 22% and 240 mL and 12%, respectively).

Body plethysmography was used to assess lung volumes (Autobox 6200 DL; Sensormedics Corporation, CA, USA). Residual volume (RV) was increased (153% of predicted) in patient 1, indicating gas trapping due to SAD. There was no evidence of gas trapping in patient 2. Impulse oscillometry was used in order to measure airway resistance (Masterscreen IOS; Erich Jaeger, Hoechenberg, Germany), with peripheral airway resistance measured by resistance at 5 Hz minus resistance at 20 Hz (R5 – R20).[3] Patient 1 demonstrated a value of 0.23 kPa/L/s, whereas patient 2 had a much lower value of 0.01 kPa/L/s, indicating minimal peripheral airway resistance. These differences in R5 – R20 can be due to small airway inflammation, remodelling or bronchoconstriction.

Table 1. Demographic and clinical data.

Variable	Patient 1	Patient 2
Gender	Female	Female
Age, years	45	41
Smoking status	Never smoker	Never smoker
BMI, kg/m^2	31	26
GINA	4	4
ACQ-7	2.3	2.3
FeNO, ppb	21	12
FEV_1, L	1.87	2.03
FEV_1, % predicted	68	72
FEV_1/FVC ratio	0.61	0.70
Reversibility, mL	420	240
Reversibility, %	22	12
RV, %	153	100
R5 – R20, kPa/L/s	0.23	0.01

BMI: Body mass index; GINA: Global Initiative for Asthma; ACQ-7: Asthma Control Questionnaire 7; FeNO: fractional exhaled nitric oxide; RV: residual volume; R5: resistance at 5 Hz; and R20: resistance at 20 Hz.

CLINICAL MESSAGE

The evidence that SAD is present from mild to severe asthma[3] raises the practical issue of how to diagnose and monitor SAD in clinical practice. SAD was previously thought to be difficult to measure due to the inaccessible nature of the lung periphery.[1] However, these case studies show the potential value of RV and oscillometry measurements in clinical practice; the two cases presented here had very similar clinical characteristics based on spirometry and asthma control, but only one had evidence of significant SAD.

Although normal ranges for oscillometry measurements have yet to be firmly established,[4] the R5 – R20 value for patient 1 is beyond the threshold for SAD used in previous publications.[4,5] Establishing normal ranges for oscillometry is an important future consideration for this technique in clinical practice.

The clinical management of SAD can include the use of inhaled treatments with smaller particle sizes that target the small airways. The diagnosis of SAD may therefore lead to different clinical management. The cases presented here show that the use of RV and R5 – R20 measurements can facilitate the diagnosis of SAD. We believe that diagnosing SAD in asthma should not be overlooked as the opportunity for targeted treatment may be missed.

AUTHOR CONTRIBUTIONS

DS and JR designed the research. JR and NJ organised data collection. DS and NJ wrote the manuscript. JR reviewed and approved the manuscript.

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Multiple cavitary lung lesions on CT: imaging findings to differentiate between malignant and benign etiologies

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ABSTRACT

Objective: To determine the CT findings of multiple cavitary lung lesions that allow the differentiation between benign and malignant etiologies. **Methods:** We reviewed CT scans, including patients with two or more cavitary lung lesions. We evaluated the number of cavitary lesions, their location, cavity wall thickness, and additional findings, correlating the variables with the diagnosis of a benign or malignant lesion. **Results:** We reviewed the chest CT scans of 102 patients, 58 (56.9%) of whom were male. The average age was 50.5 ± 18.0 years. Benign and malignant lesions were diagnosed in 74 (72.6%) and 28 (27.4%) of the patients, respectively. On the CT scans, the mean number of cavities was 3, the mean wall thickness of the largest lesions was 6.0 mm, and the mean diameter of the largest lesions was 27.0 mm. The lesions were predominantly in the upper lobes, especially on the right (in 43.1%). In our comparison of the variables studied, a diagnosis of malignancy was not found to correlate significantly with the wall thickness of the largest cavity, lymph node enlargement, emphysema, consolidation, bronchiectasis, or bronchial obstruction. The presence of centrilobular nodules correlated significantly with the absence of malignant disease ($p < 0.05$). In contrast, a greater number of cavities correlated significantly with malignancy ($p < 0.026$). **Conclusions:** A larger number of cavitary lung lesions and the absence of centrilobular nodules may be characteristic of a malignant etiology. However, on the basis of our evaluation of the lesions in our sample, we cannot state that wall thickness is a good indicator of a benign or malignant etiology.

Keywords: Lung neoplasms/diagnosis; Lung diseases/diagnosis; Tomography, X-ray computed; Neoplasms.

INTRODUCTION

The differential diagnosis of multiple cavitary lung lesions is wide-ranging and includes infectious diseases such as tuberculosis, fungal infections, and parasitic infections, as well as noninfectious diseases such as malignant and rheumatic lesions.[1] A cavitary lesion may be caused by various pathological processes, including suppurative necrosis, caseous necrosis, and ischemic necrosis, and is defined as a gas-filled space, evidenced in multislice CT scans as an area of low-grade attenuation within a pulmonary consolidation, mass, or nodule.[2]

The imaging characteristics of lesions may increase diagnostic accuracy, especially when the initial clinical findings are indeterminate or inconsistent.[3,4] Simple chest X-ray and CT are the most commonly used chest imaging modalities, CT being more widely used and more sensitive in the detection of lung diseases and their characteristics, including shape, dimensions, wall thickness, location, and other aspects.[1,3]

The objective of the present study was to determine which CT findings of multiple cavitary lung lesions help differentiate between benign and malignant disease, on the basis of etiology.

METHODS

Study population

We retrospectively reviewed 102 consecutive chest CT scans performed at two university hospitals between 2012 and 2017. The search terms "cavitation" and "cavitary lesion" were used in searches of imaging systems and electronic reports (PACS; MV Informática, Recife, Brazil; and PixViewer; Pixeon, São Paulo, Brazil). This cross-sectional analytical study was approved by the Research Ethics Committee of the Santa Casa de Misericórdia in Porto Alegre (CAAE protocol no. 35917214.4.0000.5335).

The study included patients in whom CT scans of the chest showed two or more cavitary lung lesions during

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the study period. Patients without a defined etiological diagnosis and those with a single cavitary lesion were excluded.

Additional patient data, such as laboratory results, histological reports, and immune status, were obtained from electronic medical records (Tasy; Philips Clinical Informatics, Blumenau, Brazil, and Soul; MV Informática). The criteria for immunosuppression included corticosteroid therapy or chemotherapy in the four weeks prior to a CT scan, a history of organ transplantation, and a diagnosis of AIDS. Definitive diagnoses were obtained through histological study, sputum smear microscopy, or clinical/radiological follow-up. The diagnosis of tuberculosis was based on symptoms suggestive of the disease, a demonstrated response to treatment, and cultures of respiratory secretions testing positive for *Mycobacterium tuberculosis*, with or without lesion histology that indicated that diagnosis. Pulmonary abscesses were defined by the presence of a clinical history consistent with their diagnosis and the resolution of clinical and radiological findings after antimicrobial therapy, with or without postoperative histological analysis.

CT protocol

The images were obtained using two 64-slice multidetector CT scanners (LightSpeed VCT; GE Healthcare, Waukesha, WI, USA) with the following parameters: tube voltage: 120 kVp; tube current: 250 mA; rotation time: 0.8 s; and pitch: 1.375.

Analysis of images

The CT scans were evaluated by two radiologists with more than 10 years of experience, both of whom were blinded to the clinical status and data regarding the diagnosis of the patients. After independent analysis, the two radiologists reviewed the images with a third thoracic radiologist (with over 30 years of experience), who was likewise blinded, in order to reach a final consensus.

The number of cavitary lesions, their location, the measurement of the two largest lesions on two axes in the axial slice, and the wall thickness at its greatest point were evaluated, as were the dimensions of the largest and second largest lesions. We also evaluated associated findings such as the presence of mediastinal lymph node enlargement, centrilobular nodules, central acinar emphysema, bronchial obstruction, bronchiectasis, and parenchymal consolidation, as well as immunosuppression and the histopathological diagnosis of the lesions.

We acquired the images volumetrically and evaluated them with lung and mediastinal window settings, using specific filters. Measurements were taken in the axial plane with only a lung window setting in order to increase the reproducibility of the results. The characteristics obtained from the CT scans were then correlated with the diagnosis of malignancy.

A nodule was defined as an ill- or well-defined, round or irregularly shaped opacity with a diameter ≤ 3 cm. Mediastinal lymph node enlargement was defined as a lymph node with a short-axis diameter > 10 mm. Cavities were defined as gas-filled spaces, characterized as transparencies or areas of low-grade attenuation within pulmonary consolidations, masses, or nodules. Consolidation was defined as homogeneous opacification of the parenchyma with obscuration of the underlying blood vessels. To define these concepts, the recommendations of the Fleischner Society were used.[2]

Statistical analysis

The data are expressed as absolute and relative frequencies, mean ± standard deviation, or median (interquartile range [IQR]). The normality of the data was assessed with the Shapiro-Wilk test. The Mann-Whitney test was used in order to compare the means. The proportions were compared by using the chi-square test. We developed a multivariate statistical model using logistic regression expressed in ORs in relation to the tomographic factors associated with malignancy. Values of $p < 0.05$ were considered statistically significant for all analyses, which were performed with the IBM SPSS Statistics software, version 22.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

The study sample included 102 patients with at least two cavitary lesions on CT scans of the chest. The mean age was 50.5 ± 18.0 years, and 58 patients (57%) were male.

Benign and malignant lesions were diagnosed in 74 (72.6%) and 28 (27.4%) of the patients, respectively. Among the 74 patients with benign lesions, the diagnosis was tuberculosis in 50 (49.0%); lung abscess in 9 (8.8%); mycetoma in 6 (5.9%); septic embolism in 3 (2.9%); atypical mycobacteriosis in 2 (2.0%); atypical aspergillosis in 2 (2.0%); fusariosis in 1 (1.0%); and granulomatosis with polyangiitis in 1 (1.0%). Of the 28 patients with malignant lesions, 20 (71.4%) were diagnosed with extrapulmonary neoplasms, whereas 8 (28.6%) were diagnosed with pulmonary neoplasms. Table 1 shows the main etiological diagnoses of the lesions.

Of the 102 patients in the sample, 17 (16.7%) were immunocompromised. There were no statistically significant differences between the characteristics of the benign lesions and those of the malignant lesions, in the immunocompromised patients or in the immunocompetent patients ($p = 0.775$ for both).

Regarding the CT findings, the median number of cavities observed was 3 (IQR, 2-6), the median thickness of the largest lesion was 6 mm (IQR, 4-8 mm), and the median diameter of the largest lesion was 27 mm (IQR, 14-43 mm). With respect to the location of the lesions, they were more prevalent in the upper lobes, 44 (43.1%) being observed in the upper right lobe and 23 (22.5%) being observed in the upper left lobe. The most common imaging finding was that of centrilobular nodules, which were observed in slightly more than

half of the patients (53.9%), followed by consolidation, lymph node enlargement, bronchiectasis, bronchial obstruction, and emphysema (Table 2).

In the univariate analysis of the variables studied, there were no significant differences between the benign and malignant etiologies in relation to the mean wall thickness of the largest lesion, lymph node enlargement, emphysema, or bronchial obstruction. The presence of a centrilobular nodule correlated significantly with the absence of malignant disease ($p < 0.05$; Table 3).

In the multivariate analysis adjusted for age, diameter of the largest lesion, centrilobular nodules, consolidation, and bronchiectasis, a larger number of cavities correlated significantly with malignancy ($p < 0.026$; Table 3). Figure 1 shows the CT scans of 2 patients with multiple cavitary lesions of benign and malignant etiology, respectively.

Table 1. Frequencies of the pathologies associated with multiple cavitary lesions.[a]

Pathologies	(n = 102)
Tuberculosis	50 (49.0)
Neoplastic lesions of extrapulmonary origin	20 (19.6)
Bacterial abscess	9 (8.8)
Neoplastic lesions of pulmonary origin	8 (7.8)
Mycetoma	6 (5.9)
Septic embolism	3 (2.9)
Atypical mycobacteriosis	2 (2.0)
Atypical aspergillosis	2 (2.0)
Fusariosis	1 (1.0)
Granulomatosis with polyangiitis	1 (1.0)

[a]Values expressed in n (%).

DISCUSSION

In the present study, we showed that a larger number of cavitary lesions is associated with a malignant etiology, and that the presence of centrilobular nodules is associated with a benign etiology. In addition, we found that did not differentiate between benign and malignant lesions did not differ significantly in terms of the wall thickness of the lesion, the diameter of the largest lesion, or the location of the largest lesion. The most common benign pathology (seen in nearly 50% of the cases of benign lesion) was tuberculosis, followed by bacterial abscess, and most of the malignant lesions were extrapulmonary.

Cavitary pulmonary lesions are common in clinical practice and are widely used as a differential in diagnoses based on CT findings, including nonmalignant pathologies such as infectious processes (caused by mycobacteria, fungi, or parasites) and autoimmune diseases, as well as malignant pulmonary and extrapulmonary pathologies.[3,4] Clinical findings of a benign pathology can often be similar to findings common in neoplastic lesions, and the results of laboratory tests performed at symptom onset may be

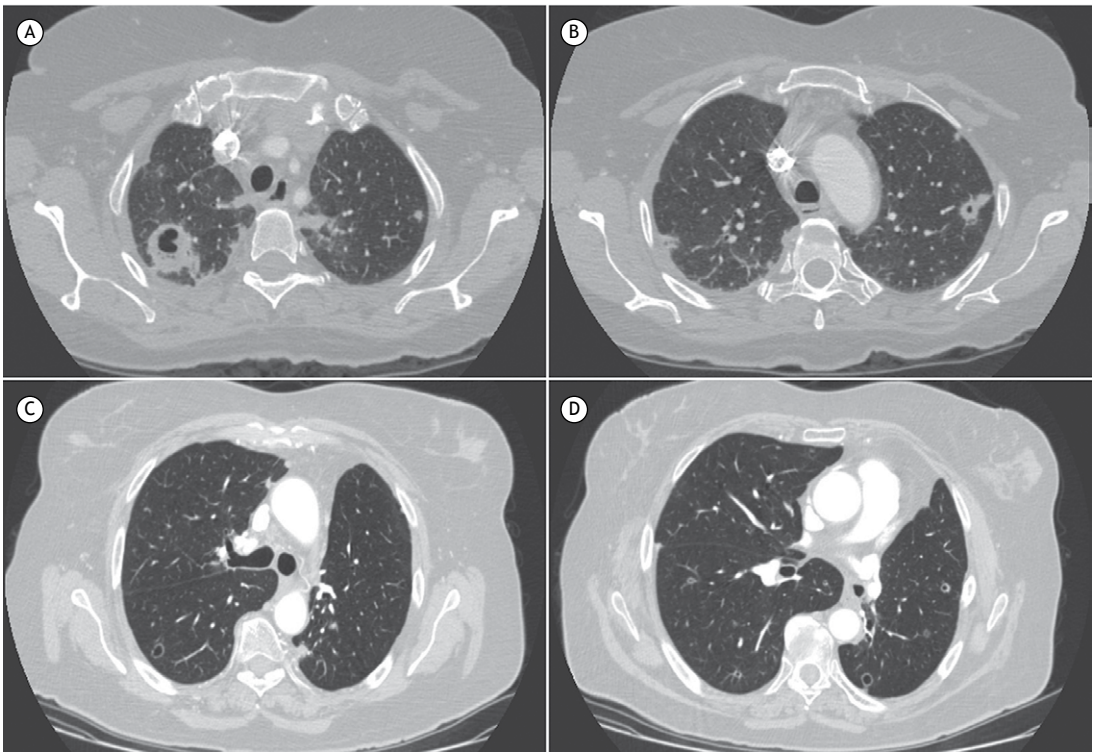

Figure 1. Axial CT scans of the chest of a 57-year-old female patient, showing two cavitary lesions with irregular walls and thickened upper lobes (in A and B). The final diagnosis was septic embolism. In C and D, CT scan of the chest of a 70-year-old female patient with multiple cavitary lesions, most of them thin-walled, apart from one thick-walled lesion in the lower left lobe. The final diagnosis was pulmonary metastases of a colorectal neoplasm.

Table 2. Characteristics of patients in accordance with the type of lesion.

Parameters	Total (n = 102)	Benign lesions (n = 74)	Malignant lesions (n = 28)	p
Male	58 (56.9)	43 (58.1)	15 (53.6)	0.680
Age, years	50 ± 18	47 ± 17	59 ± 16	0.001
Immunosuppression	17 (16.7)	13 (17.6)	4 (14.3)	0.775
Characteristics of the lesion				
Number of cavities	3 (2-6)	3 (2-6)	4 (2-9)	0.122
Wall thickness of the largest lesion, mm	6 (4-8)	6 (4-8)	4 (3-10)	0.242
Diameter of the largest lesion, mm	27 (14-43)	30 (17-48)	18 (9-39)	0.024
Location of the largest lesion				< 0.001
Upper right lobe	44 (43.1)	38 (48.6)	8 (28.6)	
Upper left lobe	23 (22.5)	22 (29.7)	1 (3.6)	
Lower right lobe	16 (15.7)	5 (6.8)	11 (39.3)	
Lower left lobe	9 (8.8)	6 (8.1)	3 (10.7)	
Middle lobe	6 (5.9)	2 (2.7)	4 (14.3)	
Lingula	4 (3.9)	3 (4.1)	1 (3.6)	
Imaging findings				
Centrilobular nodule	55 (53.9)	48 (64.9)	7 (25.0)	< 0.001
Consolidation	43 (42.2)	39 (52.7)	4 (14.3)	< 0.001
Emphysema	15 (14.7)	12 (16.2)	3 (10.7)	0.755
Lymph node enlargement	42 (41.2)	30 (40.2)	12 (42.9)	0.826
Bronchiectasis	23 (22.5)	21 (28.4)	2 (7.1)	0.032
Bronchial obstruction	16 (15.7)	13 (17.6)	3 (10.7)	0.547

[a]Values expressed in n (%), mean ± SD, or median (interquartile range).

Table 3. Univariate and multivariate analysis of factors associated with malignity.

Parameter	Univariate analysis		Multivariate analysis	
	ORs (95% CI)	p	ORs (95% CI)	p
Male	1.20 (0.49-2.89)	0.681		
Age, years	1.04 (1.00-1.08)	0.013	1.06 (1.01-1.10)	0.004
Immunosuppression	1.27 (0.37-4.34)	0.693		
Characteristics of the lesion				
Number of cavities	1.13 (1.00-1.27)	0.036	1.25 (1.02-1.52)	0.026
Wall thickness of the largest lesion, mm	1.01 (0.88-1.16)	0.826		
Diameter of the largest lesion, mm	0.97 (0.95-0.99)	0.045	0.98 (0.96-1.01)	0.451
Location of the largest lesion		0.792		
Upper right lobe	1.00			
Upper left lobe	0.20 (0.02-1.76)			
Lower right lobe	9.90 (2.66-36.7)			
Lower left lobe	2.25 (0.45-11.0)			
Middle lobe	9.00 (1.38-58.4)			
Lingula	1.50 (0.13-16.5)			
Imaging findings				
Centrilobular nodule	5.53 (2.06-14.8)	0.001	3.64 (1.07-12.2)	0.037
Consolidation	6.68 (2.09-21.2)	0.001	1.99 (0.49-8.03)	0.329
Emphysema	1.61 (0.41-6.24)	0.489		
Lymph node enlargement	0.90 (0.37-2.20)	0.833		
Bronchiectasis	5.15 (1.11-23.8)	0.036	2.27 (0.40-12.9)	0.353
Bronchial obstruction	1.77 (0.46-6.81)	0.403		

normal. Therefore, CT is of great value and contributes to the etiological recognition of pathologies, notably by taking into account the characteristics of lesions.[4-7] A previous study[8] described findings that can help differentiate between benign and malignant cavitary lesions; however, that study evaluated a population sample different than ours, in which granulomatous lesions (tuberculosis) were quite common.

Various authors have demonstrated the importance of the characteristics of cavitary lesions, together with

clinical findings and imaging results, in the differential diagnosis.[1,4,9] Woodring et al.[9,10] found that a cutoff point for wall thickness of > 15 mm was suggestive of a malignant lesion in X-rays, whereas Nin et al.[3] defined a cutoff point of > 24 mm for CT. In our study, the average wall thickness was 6 mm, and there were no statistical differences between benign and malignant lesions, which is similar to results reported previously in the literature.[8] One of the likely causes of there being such a large difference in wall thickness measurements is that the aforementioned studies[3,9,10] evaluated single pulmonary lesions, rather than multiple lesions as in our study. There were also variations in other aspects, such as the systemic conditions and pathophysiology of the formation of the lesions. It is of note that even thin-walled lesions can be related to a malignant etiology.[11]

In the present study, we observed a higher probability of malignancy in cases with a greater number of cavitary lesions, the multivariate analysis having been adjusted for age, diameter of the largest lesion, average wall thickness of the largest lesion, presence of centrilobular nodules, consolidation, and bronchiectasis. However, this is a finding that should be evaluated with caution, because, among other reasons, and as has been demonstrated in various studies,[1,3] malignant lesions, including secondary implants, may present as a single lesion or even as dozens of lesions.

The presence of centrilobular nodules was associated with a higher probability of nonmalignant etiology. This finding is in accordance with data in the literature, because centrilobular nodules are more commonly found in patients with mycobacteriosis,[12] as well as in those with infection caused by bacteria, fungi, parasites, or viruses,[13,14] than in those with malignant lesions.[8]

Our study has some limitations. First, given the retrospective nature of the study, we could not include all clinical and laboratory variables, because of the inconsistency of the collected data. Second, the profile of patients at a tertiary care hospital is not the same as that of outpatients in the community, which restricts to an extent the generalizability of the findings to the general population.

In conclusion, the present study demonstrated two characteristics of cavitary lesions on CT scans of the chest that may contribute to the etiological differentiation between benign and malignant lesions. A greater number of cavitary lesions and the absence of centrilobular nodules were associated with a higher probability of malignancy. However, the parameters wall thickness and location of the lesions were not capable of differentiating between benign and malignant lesions.

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ORIGINAL ARTICLE

Spirometry results after treatment for pulmonary tuberculosis: comparison between patients with and without previous lung disease: a multicenter study

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ABSTRACT

Objective: To compare patients with and without previous lung disease, in terms of the spirometry results after they had been treated for pulmonary tuberculosis (PTB) and cured, as well as to analyze risk factors related to functional severity. **Methods:** This was a cross-sectional, multicenter study conducted at four referral centers in Brazil. Patients were divided into two groups: those with a history of lung disease or smoking (LDS+ group); and those with no such history (LDS– group). Patients underwent spirometry (at least six months after being cured). Sociodemographic and clinical data were collected. **Results:** A total of 378 patients were included: 174 (46.1%) in the LDS+ group and 204 (53.9%) in the LDS– group. In the sample as a whole, 238 patients (62.7%) had spirometric changes. In the LDS+ group, there was a predominance of obstructive lung disease (in 33.3%), whereas restrictive lung disease predominated in the LDS– group (in 24.7%). Radiological changes were less common in the LDS– group than in the LDS+ group ($p < 0.01$), as were functional changes ($p < 0.05$). However, of the 140 (79.1%) LDS– group patients with a normal or minimally altered chest X-ray, 76 (54%) had functional changes ($p < 0.01$). The risk factors associated with functional severity in the LDS– group were degree of dyspnea ($p = 0.03$) and moderate or severe radiological changes ($p = 0.01$). **Conclusions:** Impaired pulmonary function is common after treatment for PTB, regardless of the history of lung disease or smoking. Spirometry should be suggested for patients who develop moderate/severe dyspnea or relevant radiological changes after treatment for PTB.

Keywords: Tuberculosis, pulmonary; Respiratory function tests; Airway obstruction/complications.

INTRODUCTION

Tuberculosis is recognized as a global public health problem, and its control has been a challenge in recent decades.[1] In 2015, Brazil met the millennium goals by decreasing the incidence of and mortality from tuberculosis. Among the so-called BRICS countries (Brazil, Russia, India, China, and South Africa), Brazil has the lowest tuberculosis incidence rate, although the prevalence of the disease remains steady.[2] However, there are still major obstacles to controlling tuberculosis in Brazil, such as impediments to investigating contacts, to making an early diagnosis, and to initiating antituberculosis therapy in primary care.[2]

Delayed diagnosis and the low effectiveness of antituberculosis therapy, which may be due to sociocultural factors associated with patients, physicians, and the organization of the health care system, promote the spread of the disease and increase the risk of progression of lung lesions.[3-5]

One of the aspects rarely addressed in the literature is the clinical and social impact of pulmonary sequelae among patients who have completed the treatment

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regimen. Pulmonary tuberculosis (PTB) can lead to chronic airflow obstruction, depending on the degree of anatomical distortion present, and to restricted ventilation caused by fibrotic scarring associated with reduced TLC. Greater lung injury, related to disability and impaired quality of life, has been associated with delayed diagnosis of tuberculosis, quantity of previous treatments, smoking, malnutrition, and a high bacillary load at the initiation of antituberculosis therapy.[6-10]

The functional changes resulting from PTB that are observed after treatment manifest as restrictive lung disease (RLD), obstructive lung disease (OLD), or mixed obstructive-restrictive lung disease (MORLD), regardless of the history of exposure to smoking.[11] The most commonly described functional change is OLD.[6,7,12] The prevalence of OLD after treatment for PTB is estimated to range from 15% to 77%,[10,12] whereas that of MORLD, the second most prevalent, is estimated to range from 9% to 34%.[9,12] At one center in Brazil, MORLD was reported to be the most common functional change, with a prevalence of 34%.[11] However, OLD was observed in 33% of the patients evaluated by Di Naso et al.[13] and in 49% of those evaluated by Nihues et al.[14] At another center, RLD was the most prevalent (in 41%).[15]

In Brazil, there have been few studies reporting the proportion of patients with radiological and functional changes after treatment for PTB, and in those few studies[9-15] there was no exclusion of patients with a history of lung disease or smoking prior to treatment for PTB. Therefore, given the prevalence of PTB in Brazil, it is relevant to have knowledge of the magnitude of spirometric changes in this population.

The objective of the present study was to compare patients with a history of lung disease or smoking and those with no such history, on the basis of the spirometry results after treatment for PTB, at one of four referral centers in Brazil, as well as to analyze risk factors related to functional severity in the patients with no history of lung disease or smoking.

METHODS

Study design

This was a cross-sectional, multicenter study involving non-probability purposive sampling. The study population consisted of 418 patients recruited between 2014 and 2015 from one of the following referral centers in Brazil: the Federal University of Minas Gerais *Hospital das Clínicas* (a tertiary referral center), in the city of Belo Horizonte; the Brazilian Institute for Tuberculosis Research (a secondary referral center), in the city of Salvador; the Dourados Municipal Program for Tuberculosis Control (a secondary referral center), in the city of Dourados; and the Thoracic Diseases Institute of the Federal University of Rio de Janeiro School of Medicine Clementino Fraga Filho University Hospital, (a tertiary referral center), in the city of Rio de Janeiro.

We included patients who were ≥ 18 years of age; had been diagnosed with PTB, confirmed by sputum smear microscopy or mycobacterial culture; had received a single treatment for PTB at one of the four referral centers; had been cured; and had undergone spirometry. We excluded patients whose spirometry results did not meet the *Sociedade Brasileira de Pneumologia e Tisiologia* (SBPT, Brazilian Thoracic Association) acceptability and reproducibility criteria[16] or who had a condition that made it impossible to perform the test or was a contraindication to it, such as hemoptysis, an altered level of consciousness, acute myocardial infarction or ischemic stroke in the last 3 months, uncontrolled systemic arterial hypertension, and aortic aneurysm.

All patients who had been cured of PTB were invited to participate in the study. Participants completed a standardized questionnaire that collected information on sociodemographic and clinical characteristics, such as gender, age, smoking, alcoholism, and comorbidities. In addition, the time from symptom onset to diagnosis of PTB was determined. Smokers were defined as individuals who had smoked at least 100 cigarettes (or equivalent) in their lifetime, and former smokers were defined as those who had quit smoking more than 12 months prior.[17] Alcoholism was assessed with the Cut down, Annoyed, Guilty, and Eye-opener questionnaire.[18]

Sociodemographic and clinical characteristics were categorized as follows: skin color, defined as White or Non-White; marital status, defined as married/living as married or other (widowed, separated/divorced, or single); level of education, defined as ≤ 9 or > 9 years of schooling; and self-reported comorbidities. Diagnoses of lung disease (asthma, COPD, bronchiectasis, interstitial lung disease, and silicosis) prior to treatment for PTB were reviewed by the pulmonologists involved in the present study, who followed the definitions proposed in the Global Initiative for Asthma guidelines and the Global Initiative for Chronic Obstructive Lung Disease guidelines, as well as in a national practice guideline.[19-21]

With regard to signs and symptoms, participants were evaluated for the presence of dyspnea, as classified by the modified Medical Research Council (mMRC) scale,[22] cough, expectoration, and wheezing. These data were obtained on the day of the spirometry.

Patients were divided into two groups: those with a history of lung disease or smoking prior to treatment for PTB (LDS+ group); and those with no such history (LDS− group). Spirometry was performed at least 6 months after the PTB had been cured.

Functional assessment

Spirometry was performed with a Koko spirometer (Pulmonary Data Service Inc., Louisville, CO, USA). All tests were performed and interpreted following the recommendations of the SBPT guidelines.[16]

Post-bronchodilator values were compared with pre-bronchodilator values and were expressed as absolute values and as a percentage of predicted values, according to Knudson et al.[23] for female and male patients under 20 and 25 years of age, respectively, and according to Pereira et al.[24] for patients older than that. The technicians who performed the tests were certified by the SBPT. Spirometry results were classified as follows: normal spirometry; OLD; OLD with reduced (F)VC; and RLD. The severity of obstruction was graded on the basis of FEV_1 and FEV_1/FVC, in % of predicted, as follows[16]: mild, ≥ 60%; moderate, 41-59%; and severe, ≤ 40%. The severity of restriction was graded on the basis of (F) VC, in percentage of predicted, as follows[16]: mild, ≥ 60%; moderate, 51-59%; and severe, ≤ 50%.

Radiological assessment

Chest X-rays were performed on dates close to those on which spirometry was performed, were evaluated by radiologists, and were classified by pulmonologists. Chest X-rays showing no changes were classified as normal. The remaining chest X-rays were classified as defined in the National Tuberculosis Association (NTA) classification[25]: NTA-I, or minimal; NTA-II, or moderately advanced (in one or both lungs, there may be lesions, the total extent of which should not exceed the total volume of one lung if the lesions are not confluent and should not exceed one third of the volume of one lung if they are confluent); and NTA-III, or far advanced (if the lesions are even more extensive).

Statistical analysis

The study variables were entered into an Excel database and were analyzed using the IBM SPSS Statistics software package, version 22.0 (IBM Corporation, Armonk, NY, USA). The distribution of the variables was assessed by the Kolmogorov-Smirnov test. Continuous variables are expressed as means and standard deviations or as medians and interquartile ranges, whereas categorical variables are expressed as absolute and relative frequencies. Clinical, demographic, radiological, and functional characteristics were compared between the two groups by using the chi-square test or the Student's t-test, as appropriate. The spirometric classifications of the LDS− group patients were analyzed, in terms of clinical, demographic, and radiological characteristics, with Pearson's chi-square test and were divided into two groups: no disease (normal spirometry results) or mild disease; and moderate or severe disease. Values of $p < 0.05$ were considered significant.

Ethical considerations

The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (Reference no. 14606113.7.0000.5149). All participating patients gave written informed consent.

RESULTS

Of the 418 patients initially recruited, 40 (10.5%) were excluded because the spirometry tests did not meet acceptability and reproducibility criteria. There were no significant clinical, demographic, or radiological differences between the patients who were included in the study and those who were excluded.

Therefore, the final sample comprised 378 patients, of whom 174 (46%) were in the LDS+ group and 204 (54%) were in the LDS− group. The characteristics of the patients are described in Table 1. The proportions of patients who were male, were > 49 years of age, had completed ≤ 9 years of schooling, had at least one comorbidity (systemic arterial hypertension, heart disease, or diabetes mellitus), were alcoholics, and had respiratory symptoms were significantly higher in the LDS+ group than in the LDS− group (Table 1).

Chest X-rays classified as normal or NTA-I were more common in the LDS− group than in the LDS+ group ($p < 0.01$; Table 1). Pre- and post-bronchodilator absolute and percentage of predicted FEV_1, $FEF_{25-75\%}$, FEV_1/FVC, and $FEF_{25-75\%}$/FVC were significantly higher in the LDS− group than in the LDS+ group (Table 2).

Of the 378 patients evaluated, 238 (62.7%) had spirometric changes. Normal spirometry was more common in the LDS− group ($p < 0.01$). As can be seen in Table 3, the most common spirometric changes in the LDS+ group were OLD, in 58 patients (33.3%) and OLD with reduced FVC, in 49 (28.2%), whereas the most common spirometric changes in the LDS− group were RLD, in 50 (24.7%) and OLD, in 42 (20.6%).

With regard to the severity of OLD and RLD, there was a predominance of mild disease in both groups. Among the patients who had OLD with reduced FVC, severe disease was more common in those in the LDS+ group, whereas moderate disease was more common in those in the LDS− group (Table 3).

Table 4 shows the radiological grading and spirometric classification of the LDS− group patients (n = 177). Of the 140 (79.1%) LDS− group patients whose chest X-ray was classified as normal or NTA-I, 76 (54.0%) had functional changes ($p < 0.01$).

Analysis of the classification of the LDS− group patients, based on spirometry results and clinical, demographic, and radiological characteristics, revealed that age > 49 years, mMRC grade 2-4 dyspnea, and radiological changes classified as NTA-II/III were associated with moderate and severe functional changes. There was no significant association between the time from symptom onset to diagnosis of tuberculosis and the severity of spirometric changes (Table 5).

DISCUSSION

The main finding of the present study is that, after having been treated for PTB and cured, 238

Table 1. Clinical, demographic, and radiological characteristics of patients treated for pulmonary tuberculosis (N = 378) who had a history of lung disease or smoking or who had no such history; 2014-2015.

Characteristic	LDS+ group (n = 174)		LDS− group (n = 204)		p
	n	%	n	%	
Gender					
Male	115	66.1	82	40.2	< 0,01
Female	59	33.9	122	59.8	
Age, years					
18-29	15	8.6	54	26.5	< 0.01
30-49	56	32.2	89	43.6	
> 49	103	59.2	61	29.9	
Skin color					
White	32	18.4	33	16.3	0.60
Non-White	142	81.6	169	83.7	
Marital status					
Married/living as married	106	61.3	100	51.0	0.05
Other[a]	67	38.7	96	49.0	
Level of education					
≤ 9 years of schooling	105	80.8	96	60.0	< 0.01
> 9 years of schooling	25	19.2	64	40.0	
HIV/AIDS					
Yes	8	6.0	5	3.3	0.30
No	126	94.0	145	96.7	
Systemic arterial hypertension					
Yes	57	33.3	34	16.7	< 0.01
No	114	66.7	170	83.3	
Heart disease					
Yes	13	7.5	3	1.5	< 0.01
No	161	92.5	201	98.5	
Diabetes mellitus					
Yes	25	14.6	15	7.6	0.03
No	146	85.4	182	92.4	
Chronic kidney disease					
Yes	10	5.7	11	5.4	0.88
No	164	94.3	193	94.6	
Cancer					
Yes	7	4.0	3	1.5	0.12
No	167	96.0	201	98.5	
Smoking					
No	25	14.4	204	100	< 0.01
Smoker	71	40.8			
Former smoker	78	44.8			
Alcoholism					
Yes	63	36.8	36	18.4	< 0.01
No	108	63.2	160	81.6	
Dyspnea, mMRC					
0-1	142	84.0	181	93.3	< 0.01
2-4	27	16.0	13	6.7	
Cough					
Yes	92	52.9	50	25.5	< 0.01
No	82	47.1	146	74.5	
Expectoration					
Yes	68	39.1	32	16.3	< 0.01
No	106	60.9	164	83.7	
Wheezing					
Yes	40	23.1	10	5.1	< 0.01
No	133	76.9	186	94.9	
Chest X-ray					
Normal/NTA-I	87	55.4	140	79.1	< 0.01
NTA-II/III	70	44.6	87	20.9	
Disease duration to diagnosis					
< 30 days	24	20.9	37	23.6	0.59
≥ 30 days	91	79.1	120	76.4	

LDS: history of lung disease or smoking; mMRC: modified Medical Research Council (scale); and NTA: National Tuberculosis Association (classification system).[25] [a]Widowed, separated/divorced, or single.

Table 2. Spirometric variables of patients treated for pulmonary tuberculosis (N = 378) who had a history of lung disease or smoking or who had no such history; 2014-2015.[a]

Variable	LDS+ group (n = 174)	LDS− group (n = 204)	p*
IC, L	2.29 ± 0.82	2.40 ± 0.68	< 0.01
IC, % of predicted	78.8 ± 23.9	82.5 ± 16.3	
Post-BD IC, L	2.44 ± 0.83	2.43 ± 0.60	0.04
VC, L	3.35 ± 1.05	3.37 ± 0.91	0.01
VC, % of predicted	83.9 ± 20.6	84.9 ± 1.5	
Post-BD VC, L	3.50 ± 0.97	3.45 ± 0.93	< 0.01
FVC, L	3.36 ± 1.13	3.31 ± 0.86	0.01
FVC, % of predicted	82.9 ± 20.4	84.3 ± 15.1	
Post-BD FVC, L	3.44 ± 1.11	3.34 ± 0.87	< 0.01
FEV_1, L	2.39 ± 1.00	2.66 ± 0.74	0.01
FEV_1, % of predicted	72.2 ± 24.2	82.2 ± 17.5	
Post-BD FEV_1, L	2.54 ± 1.03	2.74 ± 0.75	0.01
$FEF_{25-75\%}$, L/s	1.92 ± 1.28	2.69 ± 1.18	< 0.01
$FEF_{25-75\%}$, % of predicted	58.3 ± 35.3	80.5 ± 34.2	
Post-BD $FEF_{25-75\%}$, L/s	2.21 ± 1.40	2.95 ± 1.22	< 0.01
FEV_1/FVC, %	69.7 ± 14.2	80.4 ± 11.1	< 0.01
FEV_1/FVC, % of predicted	86.0 ± 16.8	97.0 ± 12.5	
$FEF_{25-75\%}$/FVC, %	54.8 ± 30.9	82.5 ± 35.2	< 0.01
$FEF_{25-75\%}$/FVC, % of predicted	67.8 ± 36.0	95.7 ± 39.8	

LDS: history of lung disease or smoking; IC: inspiratory capacity; and BD: bronchodilator. [a]Values expressed as mean ± SD. *Chi-square test.

Table 3. Spirometric classification of patients treated for pulmonary tuberculosis (N = 378) who had a history of lung disease or smoking or who had no such history; 2014-2015.[a]

Classification	LDS+ group (n = 174)		LDS− group (n = 204)		p*
	n	%	n	%	
Normal spirometry	48	27.6	92	45.8	0.01
Obstructive lung disease	58	33.3	42	20.6	0.99
Mild	44	75.9	37	88.1	
Moderate	13	22.4	4	9.5	
Severe	1	1.7	1	2.4	
Obstructive lung disease with reduced FVC	49	28.2	18	8.9	0.03
Mild	8	16.3	4	20.0	
Moderate	17	34.7	12	60.0	
Severe	24	49.0	4	20.0	
Restrictive lung disease	19	10.9	50	24.7	0.54
Mild	17	89.5	46	92.0	
Moderate	0	0.0	1	2.0	
Severe	2	10.5	3	6.0	

LDS: history of lung disease or smoking. [a]According to the recommendations of the Brazilian Thoracic Association guidelines.[16] *Chi-square test for linear association.

(62.7%) of the patients had spirometric changes. Comparison of the two groups revealed that functional and radiological changes were less common in the LDS− group. However, of the 140 (79.1%) LDS− group patients whose chest X-ray was classified as normal or NTA-I, 76 (54.0%) had functional changes. These data suggest that impaired pulmonary function after PTB is a major cause of chronic lung disease. The risk factors related to functional severity in this group were age > 49 years, mMRC grade 2-4 dyspnea, and radiological changes classified as NTA-II/III.

Investigation of changes in pulmonary function after completion of treatment for PTB has been gaining prominence in the literature.[6,7,9,11-15,26,27] In our study, the most common functional change in the LDS− group was RLD (24.7%). Cruz et al.[15] also described RLD as the main functional change after treatment for PTB; however, that study included patients who underwent more than one treatment for PTB.

In the literature, the reported frequency of OLD ranges from 15% to 77%.[6,7,9,12,28,29] The combination

Table 4. Comparison between spirometry results and chest X-ray results of patients treated for pulmonary tuberculosis who had no history of lung disease or smoking (n = 177); 2014-2015.

Spirometry results[b]	Chest X-ray results[a]							
	Normal (n = 68)		NTA-I (n = 72)		NTA-II (n = 31)		NTA-III (n = 6)	
	n	%	n	%	n	%	n	%
Normal spirometry	35	46.7	29	38.7	10	13.3	1	1.3
Obstructive lung disease	11	28.9	19	50.0	8	21.1	0	0.0
Restrictive lung disease	17	37.0	18	39.1	9	19.6	2	4.3
Obstructive lung disease with reduced FVC	5	27.8	6	33.3	4	22.2	3	16.7

[a]According to the National Tuberculosis Association (NTA) classification system.[25] [b]According to the recommendations of the Brazilian Thoracic Association guidelines.[16] Chi-square test; p = 0.01.

of PTB and airflow obstruction can occur regardless of the presence of smoking,[30] suggesting that the obstruction found in the LDS− group patients may be due to their PTB lesions. Some population-based studies have also described OLD as the most common functional change; however, those studies included smokers, former smokers, and patients with a history of lung disease,[28,29] similar to what was observed in the LDS+ group in the present study.

PTB can affect the airways and lung parenchyma, leading to mucosal edema, hypertrophy/hyperplasia of the mucous glands, increased mucus secretion, smooth muscle layer hypertrophy, and increased activity of metalloproteinases. Consequently, there can be changes in airway caliber (caused by stenosis, distortion, bronchiectasis, and emphysema), increased airway resistance, and reduced airflow.[31] In addition, fibrotic scarring can reduce lung compliance, leading to restriction.[27] This heterogeneity of lung lesions should be more extensively studied, especially with regard to pathophysiology and inflammatory phenomena.

Functional changes, which may persist after PTB has been cured, are associated with disability and reduced quality of life.[32,33] Lee et al.[4] stated that early diagnosis and prompt initiation of therapy translate to a lower likelihood of significant functional changes. In our study, there was no association between the time from symptom onset to diagnosis of PTB and the severity of spirometric changes, suggesting that PTB may lead to functional changes, regardless of the timing of the initiation of therapy, as also reported by Manji et al.[34]

Specifically in the LDS− group patients, dyspnea can be explained by structural (NTA-II and NTA-III) lesions leading to airway obstruction, with small airways involvement and bronchiectasis,[10] as well as destruction of the lung parenchyma. Other studies have identified an association between the extent of radiographic changes and changes in pulmonary function.[13,15,32,34-36] Those studies suggest that greater lesion extent translates to greater tissue damage, these sequelae being reflected in changes in pulmonary function. However, those studies did not assess the severity of spirometric changes.[13,15,32,34-36] Disease overlap could explain the higher frequency of symptoms (dyspnea, cough, expectoration, and wheezing) in the LDS+ group. Some limitations of the present study should be noted. The first limitation is that functional changes were not assessed longitudinally, rather being assessed 24 months after completion of the antituberculosis therapy. That could provide different results if the lesions have yet to stabilize.[37] The second limitation is the use of chest X-ray, a method that may underestimate the extent of sequelae. Chest CT can more accurately characterize the changes caused by PTB sequelae. However, in a recent study, chest CT and chest X-ray were in disagreement in only 7% of the examinations in patients with PTB sequelae.[26] In addition, chest X-ray is inexpensive and is available at tuberculosis treatment centers in Brazil, in contrast to chest CT, to which access is limited. Another limitation is that pulmonary function was assessed by spirometry only, without plethysmography or DLCO measurement. However, the equipment needed for those tests is unavailable at most centers in Brazil. Finally, the relationship between greater functional severity and being > 49 years of age was not investigated.

Functional changes constitute knowledge gaps that warrant further studies, especially studies aimed at determining whether airway obstruction due to PTB is a different entity from COPD due to exposure to cigarette smoke or smoke from wood-burning stoves, silicosis, etc.[38] In addition, further studies could assess responses to treatments such as the use of specific inhaled medication and pulmonary rehabilitation to improve patient quality of life.

In conclusion, impaired pulmonary function is common after treatment for PTB, regardless of the history of smoking or lung disease. Spirometry should be suggested for patients who develop mMRC grade 2-4 dyspnea or relevant radiological changes after treatment for PTB.

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Table 5. Clinical, demographic, and radiological characteristics of patients treated for pulmonary tuberculosis who had no history of lung disease or smoking (n = 204), by spirometry classification; 2014-2015.

Characteristic	No disease or mild disease		Moderate or severe disease		p
	n	%	n	%	
Gender					
Male	66	36.9	16	64.0	0.10
Female	113	63.1	9	36.0	
Age, years					
18-29	52	29.0	2	8.0	0.03
30-49	78	43.6	11	44.0	
> 49	49	27.4	12	48.0	
Skin color					
White	28	15.8	5	20.0	0.60
Non-White	149	84.2	20	80.0	
Marital status					
Married/living as married	86	50.0	14	58.3	0.24
Other[a]	86	50.0	10	41.7	
Level of education					
≤ 9 years of schooling	82	58.6	14	70.0	0.33
> 9 years of schooling	58	41.4	6	30.0	
Alcoholism					
Yes	32	18.5	4	17.4	0.90
No	141	81.5	19	82.6	
HIV/AIDS					
Yes	4	3.0	1	6.3	0.49
No	130	97.0	15	93.8	
Systemic arterial hypertension					
Yes	28	15.6	6	24.0	0.29
No	151	84.4	19	76.0	
Heart disease					
Yes	3	1.7	0		0.51
No	176	98.3	25		
Diabetes mellitus					
Yes	12	6.9	3	12.5	0.34
No	161	93.1	21	87.5	
Chronic kidney disease					
Yes	11	6.1	0	0.0	0.20
No	168	93.9	25	100.0	
Cancer					
Yes	2	1.1	1	4.0	0.26
No	177	98.9	24	96.0	
Dyspnea, mMRC					
0-1	162	94.7	19	82.6	0.03
2-4	9	5.3	4	17.4	
Cough					
Yes	42	24.3	8	34.8	0.28
No	131	75.7	15	65.2	
Expectoration					
Yes	26	15.0	6	26.1	0.18
No	147	85.0	17	73.9	
Wheezing					
Yes	7	4.0	3	13.0	0.07
No	166	96.0	20	87.0	
Chest X-ray					
Normal/NTA-I	128	82.1	12	57.1	0.01
NTA-II/III	28	17.9	9	42.9	
Disease duration to diagnosis					
< 30 days	34	24.3	3	17.7	0.54
≥ 30 days	106	75.7	14	82.4	

mMRC: modified Medical Research Council (scale); and NTA: National Tuberculosis Association (classification system). [a]Widowed, separated/divorced, or single.

AUTHOR CONTRIBUTIONS

All authors contributed to all stages of this study, including study conception and design, data acquisition and analysis, data interpretation, preparation and revision of the manuscript, and approval of the final manuscript.

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ORIGINAL ARTICLE

Association between the radiological presentation and elapsed time for the diagnosis of pulmonary tuberculosis in the emergency department of a university hospital

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ABSTRACT

Objective: To evaluate the radiological presentation of patients with pulmonary tuberculosis diagnosed in the emergency department and to investigate its association with the time to diagnosis. **Methods:** This was a prospective observational study involving patients diagnosed with pulmonary tuberculosis in the emergency department of a tertiary university hospital in southern Brazil. Chest X-rays taken on admission were evaluated by a radiologist. The various patterns of radiological findings and locations of the lesions were described. The main study outcome was the total time elapsed between the initial radiological examination and the diagnosis of tuberculosis. **Results:** A total of 78 patients were included in the study. The median time from chest X-ray to diagnosis was 2 days, early and delayed diagnosis being defined as a time to diagnosis < 2 days and ≥ 2 days, respectively. Sputum smear positivity was associated with early diagnosis (p = 0.005), and positive culture was associated with delayed diagnosis (p = 0.005). Early diagnosis was associated with the presence of sputum (p = 0.03), weight loss (p = 0.047), cavitation (p = 0.001), and consolidation (p = 0.003). Pulmonary cavitation was found to be an independent predictor of early diagnosis (OR = 3.50; p = 0.028). **Conclusions:** There is a need for tuberculosis-specific protocols in emergency departments, not only to avoid delays in diagnosis and treatment but also to modify the transmission dynamics of the disease.

Keywords: Tuberculosis/diagnosis; Emergency medical services; Radiography, thoracic.

INTRODUCTION

Tuberculosis is one of the most lethal communicable diseases. It is estimated that approximately 10 million people developed the disease in 2017, leading to 1.3 million deaths, with an additional 300,000 deaths in individuals who were coinfected with HIV.[1] There is a socioeconomic component, as evidenced by the fact that 90% of all tuberculosis cases are distributed among 22 developing countries, including Brazil.[2]

Early diagnosis and treatment are essential for tuberculosis control. In areas of high tuberculosis prevalence, early diagnosis can be defined as that occurring within the first two to three weeks after the onset of respiratory symptoms, whereas delayed diagnosis can be defined as that occurring four weeks or more after the onset of such symptoms.[3] Despite the fact that tuberculosis control programs have prioritized making the diagnosis at the primary health care level, many cases are still diagnosed at hospitals, especially public referral hospitals.[4] Porto Alegre has one of the highest tuberculosis incidence rates among all Brazilian cities, and the rate of in-hospital diagnosis of tuberculosis in the city is 39%.[5]

Imaging studies play an important role in the diagnostic evaluation of patients with suspected pulmonary tuberculosis (PTB).[6] There have been few studies evaluating the radiological findings related to patients diagnosed with PTB in the emergency department. One study showed that a radiological pattern other than the typical one (of apical infiltrates or cavitations) was associated with delayed clinical suspicion of tuberculosis.[7] In this context, it is important to analyze the radiological findings among patients diagnosed with PTB in the emergency department. We hypothesize that this information will alert physicians to the difficulties of diagnosing PTB, thus helping reduce the burden of hospitalization for tuberculosis.

This study aims to evaluate the radiological presentation of patients with PTB diagnosed in the emergency department and to investigate its association with the time to diagnosis.

METHODS

This was a prospective cohort study involving patients with active PTB who were diagnosed in the emergency

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department of a tertiary university hospital in the city of Porto Alegre, located in the state of Rio Grande do Sul, in southern Brazil. We assessed the radiological presentations and their association with the time elapsed between the initial radiological examination and the diagnosis of PTB.

All procedures were performed in accordance with the ethical standards of the local institution and with Brazilian National Health Council Resolution no. 466/12, as well as with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of the Porto Alegre *Hospital de Clínicas* (Registration no. 14-0130; Protocol no. 1686812800005327), and all participating patients gave written informed consent.

We included patients who were ≥ 14 years of age, were suspected of having active PTB, and had undergone radiological examination. Patients for whom chest X-rays were unavailable were excluded, as were those in whom the diagnosis of tuberculosis was unconfirmed, those already being treated for tuberculosis, those who had previously abandoned tuberculosis treatment, and those who had exclusively extrapulmonary tuberculosis (EPTB).

The diagnosis of PTB followed the criteria established in the Third Brazilian Thoracic Association Guidelines on Tuberculosis[2]: positive sputum smear microscopy (Ziehl-Neelsen staining) in two samples; positive sputum smear microscopy (Ziehl-Neelsen staining) in one sample and a positive result for *Mycobacterium tuberculosis* in one culture (in Löwenstein-Jensen medium); positive sputum smear microscopy (Ziehl-Neelsen staining) in one sample and radiological findings consistent with tuberculosis; a positive result for *M. tuberculosis* in one culture (in Löwenstein-Jensen medium) alone; or the presence of clinical, epidemiological, and radiological characteristics consistent with tuberculosis. Therefore, some subjects were initially diagnosed and treated on the basis of the presence of clinical, epidemiological, and radiological characteristics consistent with tuberculosis, the diagnosis subsequently being confirmed by a positive culture. In all cases, the diagnosis was also confirmed by the response to tuberculosis treatment. The diagnosis of EPTB was based on the results of clinical and laboratory tests indicating the location of the disease.

A researcher conducted the interview and clinical evaluation of all patients identified as having been diagnosed with tuberculosis. The demographic characteristics, clinical features of the disease, and the presence of comorbidities were determined in the clinical evaluation and by reviewing electronic medical records with a specific collection instrument. The following data were collected: date of admission to the emergency department; date of the first radiological examination; date of the diagnosis of tuberculosis; smoking status; symptoms; and comorbidities, including HIV infection and lung diseases. At the time of the interview, we asked the patients to estimate the time in days from the onset of any symptoms.

To avoid and prevent a conscious or unconscious interpretation bias, the chest X-rays taken at admission were evaluated by a radiologist who was a member of the research team and was blinded to the other clinical characteristics of the patients. The different patterns of radiological findings and the locations of the lesions were described by the radiologist and categorized as follows: suggestive of primary tuberculosis; suggestive of post-primary tuberculosis; indeterminate; or suggestive of another diagnosis (mentioned).

The main study outcome was the total elapsed time between the initial radiological examination and the diagnosis of PTB. Secondary endpoints were the correlations of HIV infection with the various radiological presentations and the correlations of the radiological presentation with the diagnostic test results.

Data were entered into a Microsoft Excel database, being processed and analyzed using the Predictive Analytics Software package, version 18.0 (SPSS Inc., Chicago, IL, USA). For the purpose of statistical analysis, patients were stratified into two groups, according to the time elapsed between the initial radiological examination and the diagnosis of PTB: early diagnosis, comprising those for whom the time to diagnosis was below the median for this variable; and delayed diagnosis, comprising those for whom the time to diagnosis was equal to or above the median. Quantitative data are expressed as mean ± standard deviation or as median (interquartile range). Qualitative data are expressed as absolute and relative frequencies. In the analysis of continuous variables with normal distributions, we used Student's t-tests for independent samples. In the analysis of continuous variables with non-normal distributions, we used the Mann-Whitney U test. For categorical variables, we used the chi-square test and, if necessary, Yates' correction or Fisher's exact test. All statistical tests were two-tailed, and the level of significance was set at 5%. We performed multivariate logistic regression in which the dependent variable was an acceptable time to diagnosis (early diagnosis) and the independent variables were demographic, clinical, and radiological characteristics that showed statistical significance in the univariate analysis. Potential predictors were selected for the final multiple regression model by the Enter method, based on clinical judgment, the analysis of non-collinearity, and statistical significance ($p < 0.1$), and the model was adjusted for gender and age. The sample size calculation was based on that employed in a previous study,[6] in which a radiological pattern other than the typical one (of apical infiltrates or cavitations) was associated with delayed clinical suspicion of tuberculosis. Considering a prevalence of atypical radiological patterns of 30%, with an amplitude of the confidence interval of 0.20 and a confidence level of 95%, we determined that it would be necessary to include 81 patients in the study.

RESULTS

Between September of 2014 and December of 2015, we evaluated 134 potential candidates for inclusion in

the study. Of those 134 individuals, 56 were excluded, for one of the following reasons: lack of confirmation of the diagnosis; exclusively EPTB; and previous abandonment of tuberculosis treatment. Therefore, the final sample comprised 78 patients.

Table 1 shows the descriptive characteristics of the sample. The median duration of symptoms was 47 days. In most individuals (52%), tuberculosis was diagnosed on the basis of a positive result on sputum smear microscopy for AFB. The median time elapsed from the initial chest X-ray to the diagnosis of PTB was 2 days (interquartile range, 0-58 days). The most common radiological findings were consolidation (in 67%) and reticular infiltrate (in 47%). It is noteworthy that 5% of the chest X-rays were classified as normal.

Table 2 shows the comparative analysis according to the time elapsed between the initial radiological examination and the diagnosis of PTB. The frequency of sputum smear positivity was higher in the early diagnosis group than in the delayed diagnosis group (75% vs. 40%; p = 0.005). There were also differences between those two groups regarding symptoms such as sputum production (64.3% vs. 36.0%; p = 0.03) and weight loss (89.3% vs. 66.0%; p = 0.047). As can be seen in Table 3, the proportion of patients in whom a chest X-ray showed cavitation was higher in the early diagnosis group than in the delayed diagnosis group (64.3% vs. 22.0%; p = 0.001), as was that of those in whom a chest X-ray showed consolidation (89.3% vs. 54.0%; p = 0.003).

Table 4 shows the multivariate logistic regression of factors associated with early diagnosis. The presence of cavitation on a chest X-ray was identified as an independent predictor of early diagnosis (OR = 3.50; 95% CI: 1.14-10.72; p = 0.028).

DISCUSSION

In this prospective observational study, we evaluated the association between the radiological presentation and the time to the diagnosis of PTB in the emergency department of a tertiary care hospital. We found a median elapsed time from the first chest X-ray to tuberculosis diagnosis of 2 days. Cavitation and consolidation were more common in the early diagnosis group than in the delayed diagnosis group, the presence of cavitation being found to be an independent predictor of early diagnosis.

There is no consensus on what is considered an acceptable delay in diagnosis. Previous studies have suggested that the time to diagnosis is related to health care services and the local epidemiology.[8] It has been demonstrated that the time to the diagnosis of tuberculosis is shorter at hospitals located in areas where the prevalence of the disease is high.[9] The in-hospital time to diagnosis is particularly important, especially in places with high tuberculosis incidence

Table 1. Characteristics of patients diagnosed with pulmonary tuberculosis.

Characteristic	(N = 78)
Age (years), mean ± SD	41.88 ± 16.45
Male gender, n (%)	47 (60.3)
Smoking status, n (%)	
Current smoker	32 (41.0)
Former smoker	20 (25.6)
HIV/AIDS, n (%)	33 (42.3)
Non-HIV-related immunosuppression, n (%)	9 (11.5)
Drug dependence, n (%)	31 (39.7)
Duration of symptoms (days), median (IQR)	30 (13-75)
Diagnostic criteria, n (%)	
AFB-positive smear	41 (52.6)
Positive culture	15 (19.2)
AFB-positive smear + positive culture	22 (28.2)
Days from chest X-ray to PTB diagnosis, median (IQR)	2 (1-7)
Chest X-ray findings	
None	4 (5.1)
Cavitation	29 (37.1)
Reticular infiltrate	37 (47.4)
Consolidation	52 (66.7)
Residual fibrosis	19 (24.4)
Miliary pattern	8 (10.3)
Pleural effusion	22 (28.2)
Bronchiectasis	1 (1.3)
Atelectasis	18 (23.1)
Hilar lymphadenopathy	3 (3.8)

IQR: interquartile range; and PTB: pulmonary tuberculosis.

Table 2. Comparative analysis of patient characteristics, by group (early and delayed diagnosis of pulmonary tuberculosis).

Characteristic	Group		p
	Early diagnosis* (n = 28)	Delayed diagnosis† (n = 50)	
Age (years), mean ± SD	39.21 ± 12.80	43.4 ± 18.1	0.241
Male gender, n (%)	18 (64.3)	29 (58.0)	0.762
Diagnostic criteria, n (%)			
AFB-positive smear	21 (75.0)	20 (40.0)	
Positive culture	1 (3.6)	14 (28.0)	0.005
AFB-positive smear + positive culture	6 (21.1)	16 (32.0)	
Smoking status, n (%)			
Never smoker	7 (25.0)	19 (38.0)	
Former smoker	7 (25.0)	13 (26.0)	0.412
Current smoker	14 (50.0)	18 (36.0)	
Symptoms, n (%)			
Asthenia	25 (89.3)	37 (64.0)	0.190
Cough	25 (89.3)	36 (72.0)	0.137
Sputum	18 (64.3)	18 (36.0)	0.030
Fever	19 (67.9)	31 (62.0)	0.786
Weight loss	25 (89.3)	33 (66.0)	0.047
Dyspnea	13 (46.4)	22 (44.0)	1.000
Hemoptysis	5 (17.9)	4 (8.0)	0.270
Night sweats	13 (46.4)	20 (40.0)	0.755
Chest pain	8 (28.6)	13 (26.0)	1.000
Drug dependence, n (%)	14 (50.0)	17 (34.0)	0.253
Alcohol dependence	7 (25.0)	9 (18.0)	0.658
HIV/AIDS	10 (35.7)	23 (46.0)	0.520
Immunosuppression, n (%)			
Corticosteroid use	2 (7.1)	6 (12.0)	0.704
Immunosuppressive therapy	1 (3.6)	5 (10.2)	0.408
Transplant recipient	0 (0.0)	4 (8.0)	0.291
Previous tuberculosis, n (%)	6 (21.4)	9 (18.0)	0.945
Chronic lung disease, n (%)			
COPD	1 (3.6)	6 (12.0)	0.411
Other	1 (3.6)	2 (4.0)	1.000
Malignancy, n (%)			
Lung cancer	0 (0.0)	1 (2.0)	1.000
Other	0 (0.0)	2 (4.0)	0.534

*< 2 days from the initial radiological examination to the diagnosis of pulmonary tuberculosis. †≥ 2 days from the initial radiological examination to the diagnosis of pulmonary tuberculosis.

rates, such as Porto Alegre, where the rate of in-hospital diagnosis of tuberculosis is nearly 40%,[6] because a delay in diagnosis has been associated with higher mortality.[9] Another study,[6] also conducted in Porto Alegre, reported a median time to diagnosis of 6 days. However, in that study, the sample also included patients with EPTB, which accounted for a significant delay in diagnosis.

In the present study, cavitation and consolidation on a chest X-ray were associated with early diagnosis. In fact, the presence of cavitation was found to be an independent predictor of a time to diagnosis < 2 days (i.e., early diagnosis). Cavitation is considered a typical radiological feature in PTB, having been shown to be associated with a shorter time to diagnosis.[7] The location of radiological findings did not differ significantly between the early diagnosis and delayed diagnosis groups, as was also the case for the presence of residual fibrotic alterations. In a study conducted in the same emergency department as our study,[5] residual fibrosis, which is suggestive of previous tuberculosis, was identified as a factor associated with a health care system-related diagnostic delay.

In our univariate analysis, sputum smear positivity was associated with early diagnosis and a positive culture was associated with delayed diagnosis. It is well known that smear-negative PTB is associated with a delay in diagnosis and, consequently, higher mortality.[5,10,11] In the present study, the presence of

Table 3. Comparative analysis of radiological findings, by group (early and delayed diagnosis of pulmonary tuberculosis).

Characteristic	Group		p
	Early diagnosis* (n = 28) n (%)	Delayed diagnosis† (n = 50) n (%)	
Chest X-ray findings			
Normal	0 (0.0)	4 (8.0)	0.291
Cavitation	18 (64.3)	11 (22.0)	0.001
Reticular infiltrate	16 (57.1)	21 (42.0)	0.294
Consolidation	25 (89.3)	27 (54.0)	0.003
Residual fibrosis	7 (25.0)	12 (24.0)	1.000
Miliary pattern	1 (3.6)	7 (14.0)	0.247
Pleural effusion	5 (17.9)	17 (34.0)	0.209
Bronchiectasis	0 (0.0)	1 (2.0)	1.000
Atelectasis	8 (28.6)	10 (20.0)	0.561
Lymphadenopathy	0 (0.0)	3 (6.0)	0.549
Location of lesions			
APUL	16 (57.1)	21 (42.0)	0.294
B6	9 (32.1)	14 (28.0)	0.900
AUL	12 (42.9)	14 (28.0)	0.278
Middle lobe	1 (3.6)	3 (6.0)	1.000
Lingula	1 (3.6)	2 (4.0)	1.000
Basal pyramid	8 (28.6)	11 (22.0)	0.709
Diffuse	7 (25.0)	13 (26.0)	1.000
Parenchymal damage			
None	0 (0.0)	4 (8.0)	0.432
Left lung	5 (17.9)	11 (22.0)	
Right lung	9 (32.1)	14 (28.0)	
Bilateral	14 (50.0)	21 (42.0)	
Radiologist conclusion			
Post-primary tuberculosis	16 (57.0)	21 (42.0)	0.332
Another diagnosis	6 (21.4)	11 (22.0)	
Undetermined	6 (21.4)	14 (28.0)	
Normal	0 (0.0)	4 (8.0)	

APUL: anterior and posterior upper lobe; B6: superior segment of the lower lobe; and AUL: anterior upper lobe. *< 2 days from the initial radiological examination to the diagnosis of pulmonary tuberculosis. †≥ 2 days from the initial radiological examination to the diagnosis of pulmonary tuberculosis.

sputum is associated with a shorter time to diagnosis, as previously demonstrated.[5,7]

Another symptom associated with early diagnosis was weight loss. Solari et al.[12] developed a clinical prediction rule, based on information obtainable on admission, to permit rapid identification of patients with PTB in emergency departments. The authors found that weight loss was an independent predictor of the diagnosis of tuberculosis. In addition, other studies have shown that weight loss is associated with a delay in diagnosis and in the initiation of treatment.[13,14] On the basis of the findings of our study, we may suppose that physicians have sufficient understanding of the importance of the association between weight loss and tuberculosis diagnosis, which could explain the association between this symptom and the early diagnosis of tuberculosis.

Drug dependence and HIV infection are major problems in Porto Alegre, where the incidence of tuberculosis/HIV coinfection is 25.2%, which is among the highest among cities in Brazil.[15] However, in the present study, neither HIV infection nor drug dependence showed an association with a delay in the diagnosis of PTB. Other studies have also shown that HIV infection is not associated with delayed diagnosis of PTB,[16,17] despite the possibility of atypical presentations of tuberculosis.[18,19] In addition, drug dependence has been shown to be associated only with patient-related delays and not with health care system-related delays.[5] Our finding that important clinical and radiological variables (asthenia, fever, night sweats, HIV infection, immunosuppression, a miliary pattern, and the location of the lesions) were not associated with the time to diagnosis should alert physicians to the difficulty of diagnosing PTB in the emergency department.

Our study has some limitations. It was conducted at a single center, we did not evaluate previous chest X-rays, and we did not investigate patient-related delays. In addition, we did not use imaging techniques that are more advanced, such as CT, because such

Table 4. Multivariate regression to identify factors associated with the time elapsed between the initial radiological examination and the diagnosis of pulmonary tuberculosis.

Variable	B	Wald	p	OR	95% CI
Age	0.014	0.660	0.416	1.010	0.98-1.05
Gender	0.309	0.305	0.581	1.360	0.45-4.09
Cavitation*	1.252	4.804	0.028	3.500	1.14-10.72
Consolidation	1.155	2.426	0.119	3.170	0.74-13.58
AFB-positive smear	1.608	2.054	0.152	4.990	0.55-44.98
Constant	−1.315	0.839	0.117	0.268	-

*Cavitation on a chest X-ray was identified as an independent predictor of early diagnosis (within the first 2 days after the initial radiological examination).

techniques are more costly and are not widely available. Nevertheless, our results underscore the message that a typical radiological presentation, particularly cavitation, is associated with a shorter time to PTB diagnosis.

In summary, we demonstrated a median elapsed time from the initial chest X-ray to the diagnosis of PTB of 2 days. We also found that cavitation was an independent predictor of early diagnosis. Reducing diagnostic delays may require greater awareness on the part of health care professionals and a review of health care facility practices. Specific strategies, such as the use of tuberculosis-specific protocols in the emergency department, should be developed, not only to expedite diagnosis and treatment but also to modify the transmission dynamics of tuberculosis.

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ORIGINAL ARTICLE

Factors associated with tuberculosis and multidrug-resistant tuberculosis in patients treated at a tertiary referral hospital in the state of Minas Gerais, Brazil

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Study carried out in the Júlia Kubitschek Hospital, Fundação Hospitalar do Estado de Minas Gerais, Belo Horizonte (MG) Brasil.

ABSTRACT

Objective: To evaluate the risk factors for the development of tuberculosis and multidrug-resistant tuberculosis (MDR-TB) in patients treated at a tertiary referral hospital. **Methods:** This was a cross-sectional study based on data obtained from patients treated at the Júlia Kubitschek Hospital, located in the city of Belo Horizonte, Brazil, between October of 2012 and October of 2014. We evaluated sociodemographic, behavioral, clinical, and radiological variables. The outcome considered to identify associations between tuberculosis and the explanatory variables was the treatment prescribed. To evaluate the associations between MDR-TB and the same explanatory variables, the change in MDR-TB treatment was considered. **Results:** The factors associated with tuberculosis were alcoholism, comorbidities, pulmonary cavitations, and a radiological pattern suggestive of tuberculosis. Cavitation and previous treatment for tuberculosis were associated with MDR-TB. **Conclusions:** Despite the significant progress made in the fight against tuberculosis, there is a need for coordinated actions that include social protection measures and patient support.

Keywords: Tuberculosis; Risk factors; Tuberculosis, multidrug-resistant.

INTRODUCTION

Tuberculosis continues to be a serious public health problem worldwide and, according to the World Health Organization (WHO), is the leading cause of death from diseases caused by a single infectious agent, ahead of HIV.[1] In 2017, 10 million new cases of tuberculosis were reported worldwide.[2]

In 2017, 79,222 new cases of tuberculosis were reported in Brazil[2] and 13,347 cases of retreatment were reported, corresponding to 16.1% of all cases reported in the same period.[1] The state of Minas Gerais recorded incidence and mortality rates of 15.8/100,000 population and 1.3/100,000 population, respectively, and 3,343 new cases of tuberculosis were reported.[1]

In 2017, 2,000 cases of drug-resistant tuberculosis were diagnosed. Those results were obtained by using the rapid molecular test for tuberculosis or drug susceptibility testing, with 1.5% of the total corresponding to new cases and 8.0% corresponding to cases of retreatment (relapse or readmission after treatment noncompliance).[2] In 2016, 752 cases of patients who were started on treatment for drug-resistant tuberculosis were reported in the Special Tuberculosis Treatment Database. Of those 752 patients, 177 (23.5%) had single-drug resistant tuberculosis, 330 (43.9%) had rifampin-resistant tuberculosis (detected by using the rapid molecular test), 49 (6.5%) had polyresistant tuberculosis, 193 (25.7%) had multidrug-resistant tuberculosis (MDR-TB), and no results were available for 3 (0.3%).[3]

Mycobacterium tuberculosis infection is associated with factors such as incarceration, smoking, alcoholism, a history of drug use, low body mass index (which is a risk factor and a sign of infection), diabetes mellitus (DM), hepatitis C, HIV/AIDS, and depression.[4] Although efforts to control the epidemic have reduced its mortality and incidence, there are several predisposing factors to be controlled to reduce the disease burden.[5]

The combination of tuberculosis and other comorbidities (multimorbidity), as well as some social habits, should be considered and evaluated in populations exposed to tuberculosis, because it may be a complicating factor in clinical treatment.[6] In recent years, it has been observed that active tuberculosis develops more frequently in patients with poor glycemic control and that, radiologically, patients with tuberculosis and DM have more extensive lesions and, more frequently, present multilobar disease and the presence of cavitation.[5,7]

Regarding social habits, observational studies have shown that exposure to smoking is associated with tuberculosis

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infection, active tuberculosis, and tuberculosis-related mortality.[5,8,9] In addition, although alcohol consumption is considered socially acceptable in most countries, it influences not only the incidence of tuberculosis but also its clinical evolution and outcomes.[5,10]

The WHO recognizes that the measures taken to combat tuberculosis should be applied at the primary, secondary, and tertiary levels of health care, including prisons. Clinical and operational research has recently indicated that such approaches are most effective when responding to local sociocultural characteristics, health service organization, and the types of community activities.[11] Within this context, the study and monitoring of factors related to tuberculosis exposure may be important tools to disrupt the maintenance of the tuberculosis transmission chain and to increase the impact of disease control program interventions.[2,5] Therefore, the aim of the present study was to evaluate the factors associated with tuberculosis and MDR-TB in patients treated at a tertiary referral hospital.

METHODS

Study design

This is a cross-sectional study based on data obtained from patients treated between October of 2012 and October of 2014 at the Júlia Kubitschek Hospital (JKH), a public general hospital that is a tertiary referral center for the treatment of tuberculosis and drug-resistant tuberculosis, located in the city of Belo Horizonte, which conducts educational and medical activities in the state of Minas Gerais, Brazil. Every month, the JKH Microbiology Laboratory receives approximately 200 samples from suspected tuberculosis patients, and, on average, 12 present a confirmed diagnosis of tuberculosis.

The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (*UFMG, Universidade Federal de Minas Gerais*) in 2013 (CAAE: 02232412.7.1001.5149) and by the Research Ethics Committee of the Minas Gerais State Hospital Foundation in 2012 (Ruling no. 018B / 2012).

Study population

We included patients ≥ 18 years of age with suspected tuberculosis (pulmonary or extrapulmonary) who were treated at the outpatient clinics, emergency rooms, and hospital wards of the JKH and who had their clinical samples sent to the JKH Microbiology Laboratory. All of the patients included gave written informed consent. Patients diagnosed with nontuberculous mycobacterial infection were excluded.

Measurements and procedures

The participants were interviewed with a standardized questionnaire, and missing data were located by consulting medical records. The questionnaires were applied by researchers from the Mycobacterial Research Group of the UFMG School of Medicine, all of who had been trained in its use. The questionnaire contained sociodemographic, behavioral, clinical, and radiological data. To assess alcoholism, we applied the **C**ut down, **A**nnoyed, **G**uilty, and **E**ye-opener (CAGE) questionnaire, which has been validated for use in Brazil.[12] The CAGE questionnaire, which comprises four questions, was utilized with a cutoff point of two affirmative answers suggesting positivity for alcohol abuse or dependence.[4]

The presence of pulmonary cavitation and the chest X-ray pattern were evaluated according to the following classification[13]: suggestive, defined as the presence of infiltrate in the upper lobes(s) or apical segment of the lower lobe; typical, defined as mediastinal enlargement or an enlarged hilar lymph node, together with a miliary pattern or pleural effusion; and atypical, defined as any other pattern.

The patients were interviewed at the time of suspicion of tuberculosis and at the end of treatment to assess the outcomes (cure, death, and treatment noncompliance or maintenance of treatment).

Outcome measures of the study: dependent variable

The outcome considered to identify associations between tuberculosis and the explanatory variables was the treatment prescribed. The cases of tuberculosis were diagnosed according to the Brazilian National Tuberculosis Control Program through clinical, epidemiological, mycobacteriological, radiological, histopathological, and complementary examinations.[13] To evaluate the association between drug-resistant tuberculosis and the same explanatory variables, the authors considered any change in the treatment prescribed for MDR-TB.

Outcome measures of the study: exposure factors

The variables used were grouped according to sociodemographic characteristics (age, gender, skin color, income, and education); behavioral characteristics (alcoholism, smoking, marital status, and homelessness); clinical characteristics (fever, cough, hemoptysis, dyspnea, expectoration, previous treatment for tuberculosis, and presence of comorbidities); and radiological characteristics.

To categorize the level of education, we established a dichotomous variable (< or ≥ 9 years of schooling). To categorize income, the third quartile was considered the cutoff point.

The comorbidities considered were alcoholism (assessed using the CAGE questionnaire),[4] DM, COPD, pulmonary silicosis, liver diseases, malignant neoplasms, diffuse lung disease, chronic kidney disease, HIV/AIDS, drug use, corticosteroid use, malnutrition, anemia, depression, asthma, and high blood pressure.

Statistical analysis

Databases were created with the 2003 version of Microsoft Excel. The first database included all patients

with suspected tuberculosis regardless of a positive or negative diagnosis for tuberculosis. A descriptive analysis was performed using frequency distribution of the categorical variables. For the continuous variables, the measures of central tendency and dispersion (mean and standard deviation) were evaluated. In the second database, only patients with a confirmed diagnosis of tuberculosis (sensitive or resistant) were selected. That second database was created to test the association between the explanatory variables and MDR-TB.

The magnitude of the association was expressed in ORs and 95% CIs. For all the analyses conducted, the level of significance was set at $p < 0.05$.

Variables with a $p \leq 0.20$ in the uncorrected chi-square test in the univariate analysis were manually selected to start the multivariate model via a stepwise regression selection procedure. Multivariate analysis was used in order to assess the association between exposure factors and the dependent variable. After the multivariate analysis, only the variables with a $p \leq 0.05$ were retained in the final model, and the collinearity of the variables inserted in this model was verified by calculating the generalized variance inflation factor. The variables were considered collinear when the coefficient was greater than 5.[14] The analyses were performed using the programs Epi Info, version 7, and RStudio, version 1.2.5019 (RStudio, Inc., Boston, MA, USA).

Sample size

The sample size was determined by considering a 10% margin of error, 95% CI, and a frequency of 50% to determine the population of patients who underwent mycobacteriology tests during the 2-year study. The minimum sample size was calculated to be 184 patients.

RESULTS

During the study period, we included 251 patients with suspected tuberculosis, of whom 176 (70.12%) were male. The mean age was 55.4 ± 15.7 years. The mean monthly income, in Brazilian reals (R$) was R$1,244.00 ± 1,151.06. The sociodemographic, behavioral, clinical, and radiological variables are presented in Table 1.

Of the 251 patients, 95 (38.6%) were diagnosed with tuberculosis. Of those patients, 71 (74.7%) were cured; 11 (11.6%) abandoned treatment; 3 (3.2%) died from tuberculosis; 4 (4.1%) died from other causes; 2 (2.1%) were receiving ongoing treatment; and 4 (4.2%) were lost to follow-up.

In the sample as a whole, the most common comorbidities were alcoholism, in 59 patients (23.5%); COPD, in 35 (13.9%); and type 2 DM, in 25 (10.0). Of the 11 patients (4.4%) with HIV/AIDS, only 1 presented coinfection with tuberculosis.

The presence of comorbidities, COPD, dyspnea, fever, alcoholism, pulmonary cavitation, and a radiographic pattern suggestive of tuberculosis were significantly associated with tuberculosis (Table 2).

The final multivariate model included four variables as independent factors associated with tuberculosis (Table 3): alcoholism, comorbidities, pulmonary cavitation, and a radiological pattern suggestive of tuberculosis. However, COPD and symptoms such as dyspnea and fever were not considered risk factors for tuberculosis after the multivariate analysis. The values of the collinearity coefficient were as follows: alcoholism = 1.210070; comorbidities = 1.206232; pulmonary cavitation = 1.147316; and a radiological pattern suggestive of tuberculosis = 1.135740. Therefore, there was no collinearity between the variables presented as factors associated with tuberculosis.

Of the 95 patients who were started on a tuberculosis treatment regimen of rifampin, isoniazid, pyrazinamide, and ethambutol, 12 (12.6%) switched to a standard regimen (due to secondary resistance as a result of treatment noncompliance in 11 patients; and due to primary resistance in 1). Of those 12 patients, 11 (91.6%) were classified as having MDR-TB via drug susceptibility testing, and the treatment was changed to a standardized resistance regimen in 1 (8.4%) because that patient reported having had contact with a family member with MDR-TB (primary resistance), as well as presenting clinical worsening, although the drug susceptibility testing did not show resistance to rifampin. Regarding the outcome of those 12 patients, 8 (66.7%) were cured, 2 (16.7%) were still undergoing treatment at the end of the study, and 2 (16.7%) abandoned treatment.

Table 4 presents the factors associated with MDR-TB. There was a significant association with previous treatment for tuberculosis and pulmonary cavitation. Multivariate analysis was not performed due to the limited number of patients with MDR-TB.

DISCUSSION

In the present study, behavioral determinants (especially alcoholism), suggestive radiological changes, and other potential risk factors were important for the development of tuberculosis. Pulmonary cavitation and previous treatment for tuberculosis were also shown to be associated with MDR-TB. Increasingly, it is necessary to prioritize investments in public policies that address behavioral and clinical factors, thereby promoting intersectoral coordination in the health care system, as well as supervised treatment and the encouragement of societal participation in tuberculosis control.

Most participants were male, and the mean age was 55.4 years, which was higher than that reported in other studies conducted in Minas Gerais.[15,16] This is probably due to the profile of the population treated at the JKH. Age, gender, skin color, income, and level of education were not found to be associated with tuberculosis and MDR-TB in the present study.

Alcoholism, when evaluated separately, was associated with tuberculosis. Despite being a risk factor for tuberculosis (OR = 3.70; 95% CI: 1.33-10.98), alcoholism

Table 1. Descriptive analysis of the sociodemographic, behavioral, clinical, and radiological characteristics of the patients in the study (N = 251).

Characteristic	n	%
Age, years		
18-40	41	16.3
≥ 41	210	83.7
Gender		
Male	176	70.1
Female	75	29.9
Skin color		
White	45	22.3
Non-White	157	77.7
Income		
≥ R$ 1,875.00	48	21.6
< R$ 1,875.00	174	78.4
Years of schooling		
< 9	76	31.0
≥ 9	169	67.0
Alcoholism		
Yes	59	24.1
No	185	75.8
Smoking status		
Current or former smoker	161	74.2
Never smoker	56	25.8
Marital status		
Single/separated/widowed	112	50.9
Married/living as married	116	49.1
Homeless		
No	238	94.8
Yes	13	5.2
Treatment prescribed for tuberculosis		
Yes	95	38.6
No	151	61.4
Change of treatment due to resistance		
Yes	12	12.6
No	83	87.4
Previous treatment for tuberculosis		
Yes	88	39.1
No	137	60.9
Presence of comorbidities		
Yes	183	74.1
No	64	25.9
Type 2 diabetes mellitus		
Yes	25	10.0
No	223	90.0
HIV/AIDS		
Yes	11	4.4
No	240	95.6
Pulmonary cavitation		
Yes	43	28.3
No	109	71.7
Chest X-ray pattern		
Suggestive/typical	89	54.9
Atypical	73	45.1
Fever		
Yes	95	40.2
No	141	59.7
Cough		
Yes	220	88.7
No	28	11.3
Hemoptysis		
Yes	69	29.6
No	164	70.4
Dyspnea		
Yes	160	65.6
No	84	34.4
Expectoration		
Yes	187	76.3
No	58	23.7

R$: Brazilian reals.

Table 2. Comparison between patients with a confirmed diagnosis of tuberculosis and those with suspected tuberculosis but without a confirmed diagnosis, by risk factors.

Risk factor	Tuberculosis[a,b]		OR (95% CI)	p
	Confirmed (n = 95)	Suspected (n = 156)		
Age, years				
18-40	14 (14.8)	24 (15.9)	0.91 (0.44-1.87)	0.806
≥ 41	81 (85.2)	127 (84.1)		
Gender				
Male	24 (25.3)	50 (33.1)	0.68 (0.38-1.21)	0.191
Female	71 (74.7)	101 (66.9)		
Skin color				
White	16 (22.2)	27 (21.3)	1.05 (0.52-2.13)	0.874
Non-White	56 (77.8)	100 (78.7)		
Income				
≥ R$ 1,875.00	64 (78.1)	106 (78.5)	0.97 (0.50-1.89)	0.935
< R$ 1,875.00	18 (21.9)	29 (21.5)		
Years of schooling				
< 9	64 (69.6)	103 (69.1)	1.02 (0.58-1.79)	0.942
≥ 9	28 (30.4)	46 (30.9)		
Alcoholism				
Yes	60 (64.5)	121 (82.3)	2.55 (1.40-4.66)	0.001
No	33 (35.5)	26 (17.7)		
Smoking status				
Smoker or former smoker	32 (35.6)	45 (35.1)	0.93 (0.50-1.73)	0.833
Never smoker	58 (64.4)	81 (64.3)		
Marital status				
Single/separated/widowed	45 (51.7)	70 (51.1)	1.02(0.59-1.75)	0.926
Married/living as married	42 (48.3)	67 (48.9)		
Homeless				
No	87 (91.6)	146 (96.7)	0.37 (0.11-1.17)	0.081
Yes	8 (8.4)	5 (3.3)		
Dyspnea				
No	41 (45.1)	43 (28.7)	2.04 (1.18-3.51)	0.009
Yes	50 (54.9)	107 (71.3)		
Fever				
Yes	44 (50.0)	94 (64.8)	0.54 (0.31-0.93)	0.025
No	44 (50.0)	51 (35.2)		
Cough				
Yes	13 (14.0)	15 (9.9)	1.47 (0.66-3.25)	0.335
No	80 (86.0)	136 (90.1)		
Hemoptysis				
Yes	59 (72.0)	102 (68.9)	1.15 (0.63-2.09)	0.630
No	23 (28.0)	46 (31.1)		
Previous treatment for tuberculosis				
Yes	58 (61.7)	78 (60.9)	1.03 (0.59-1.78)	0.908
No	36 (38.3)	50 (39.1)		
Presence of comorbidities				
Yes	32 (33.7)	31 (21.0)	0.52 (0.29-0.93)	0.027
No	63 (66.3)	117 (79.0)		
Type 2 diabetes mellitus				
Yes	87 (91.6)	131 (88.5)	0.70 (0.29-1.71)	0.442
No	8 (8.4)	17 (11.5)		
HIV/AIDS				
No	94 (99.0)	141 (93.4)	0.15(0.01-1.19)	0.054
Yes	1 (1.0)	10 (6.6)		
COPD				
No	90 (94.7)	118 (79.7)	0.22 (0.08-0.60)	0.001
Yes	5 (5.3)	30 (20.3)		
Cavitation pulmonary				
Yes	30 (49.2)	76 (86.4)	6.54 (2.97-14.40)	< 0.001
No	31 (50.8)	12 (13.6)		
Chest X-ray pattern				
Suggestive/typical	9 (13.4)	62 (67.4)	13.31 (5.82-30.43)	< 0.001
Atypical	58 (86.6)	30 (32.6)		

R$: Brazilian reals. [a]Values expressed in n (%). [b]Missing data in some cases.

Table 3. Multivariate analysis of factors associated with tuberculosis.

Factor	OR	p	95% CI
Alcoholism	3.70	0.012	1.33-10.98
Presence of comorbidities	0.24	0.004	0.09-0.64
Pulmonary cavitation	2.88	0.032	1.09-7.62
Chest X-ray pattern	7.43	< 0.001	2.82-19.58

Table 4. Factors associated with multidrug-resistant tuberculosis using univariate analysis.

Factors	Groups[a,b]		ORs	p
	DS-TB (n = 84)	MDR-TB (n = 11)		
Skin color				
White	15 (24.2)	1 (10.0)	2.87 (0.33-24.56)	0.439
Non-White	47 (75.8)	9 (90.0)		
Income				
≥ R$ 1,875.00	54 (76.1)	10 (90.9)	0.31 (0.03-2.66)	0.441
< R$ 1,875.00	17 (23.9)	1 (9.1)		
Years of schooling				
< 9	57 (71.2)	7 (58.3)	1.77 (0.50-6.15)	0.501
≥ 9	23 (28.7)	5 (41.7)		
Alcoholism				
Yes	51 (63.0)	9 (75.0)	0.56 (0.14-2.25)	0.528
No	30 (37.0)	3 (25.0)		
Smoking status				
Smoker or former smoker	58 (73.4)	8 (72.7)	1.03 (0.25-4.27)	1.000
Never smoker	21 (26.6)	3 (27.2)		
Homeless				
No	75(90.4)	12(13.8)	Undefined	0.590
Yes	8(9.6)	0 (0.0)		
Previous treatment for tuberculosis				
Yes	55 (67.1)	3 (25.0)	6.11 (1.52-24.42)	0.008
No	27 (32.9)	9 (75.0)		
Presence of comorbidities				
Yes	28 (33.7)	4 (33.3)	1.01 (0.28-3.67)	1.000
No	55 (66.3)	8 (66.7)		
Type 2 diabetes mellitus				
Yes	76 (91.6)	11 (91.7)	0.98 (0.11-8.80)	1.000
No	7 (8.4)	1 (8.3)		
HIV/AIDS				
Yes	82 (98.9)	12 (100)	Undefined	1.000
No	1 (1.2)	0		
Pulmonary cavitation				
Yes	29 (55.8)	1 (11.1)	10.08 (1.17-86.57)	0.026
No	23 (44.2)	8 (88.9)		
Chest X-ray pattern				
Suggestive/typical	9 (15.8)	0 (0.0)	Undefined	0.335
Atypical	48 (84.2)	10 (100.0)		

DS-TB: drug-susceptible TB; and MDR-TB: multidrug-resistant tuberculosis. [a]Values expressed in n (%). [b]Incomplete data in some cases.

was not associated with MDR-TB, unlike what has been reported in other studies.[17,18] The in vivo and in vitro association between alcohol consumption and tuberculosis has long been known. Alcohol use significantly alters the immune response, thereby increasing susceptibility to tuberculosis. [5] In addition, alcohol abuse influences not only the incidence of tuberculosis but also its clinical course and outcomes, with higher rates of treatment noncompliance and relapse due to precarious living conditions and the increased risk of hepatotoxicity.[5] This association underscores the need to try to achieve the goals proposed by the WHO described in Pillar 1 (prevention and integrated patient-centered care) of the National Plan, likewise supported by the Brazilian National Ministry of Health, to eliminate tuberculosis as a health problem.[1,3]

Although we found no association between smoking and tuberculosis, some authors, through observational

analyses, have shown an unfavorable association between smoking and the global tuberculosis epidemic, as well as having pointed out the psychosocial aspects of smoking that are related to lower treatment compliance rates.[5]

Despite the low socioeconomic conditions in Belo Horizonte, homelessness was not associated with tuberculosis, although such an association has been reported by other authors.[6] That can probably be explained by the small number of homeless individuals among the patients selected in the present study.

The classic symptomatology of pulmonary tuberculosis is characterized by cough, sputum (sometimes bloody), chest pain, weakness, weight loss, fever, and night sweats.[19] Of those symptoms, only fever and dyspnea were associated with tuberculosis. For the other three symptoms (sputum, cough, and hemoptysis) there was no association with tuberculosis, probably because they are also associated with other diseases found in patients treated at the JKH, which, in addition to being a tertiary referral hospital for tuberculosis, is a comprehensive regional general hospital. Therefore, those data constitute not only a warning but also an indication of the diagnostic confusion and difficulties that occur when differentiating tuberculosis from other important diseases.

The presence of comorbidities (alcoholism, DM, pulmonary silicosis, chronic kidney disease, HIV/AIDS, etc.) is associated with tuberculosis. In fact, some studies[5,20] show that tuberculosis is approximately three times more prevalent in patients with DM. However, comorbidities, except for alcoholism when analyzed separately, were not found to be risk factors for tuberculosis in our sample. That may be related to the high prevalence of comorbidities among our patients, which acts as a protective factor. The JKH treats patients with highly complex diseases, which shows the need for patient follow-up with a multidisciplinary team, because comorbidities may increase costs, alter the course of the disease, and modify its outcome.[1]

In 2016, the WHO estimated that 10% of the 10.4 million patients with tuberculosis are also infected with HIV.[2] In the present study, only 1 patient presented tuberculosis-HIV coinfection, possibly because coinfected patients are typically referred to the Minas Gerais State Referral Hospital for Infectious Diseases, located in the same city.

Atypical or typical chest X-ray findings presented a strong association in the final model (OR = 7.43; 95% CI: 2.82-19.58), as did the presence of pulmonary cavities (OR = 2.88; 95% CI: 1.09-7.62). That demonstrates that chest X-ray, which is still the most widely used imaging examination, is an important tool for diagnostic investigation and enables the differentiation of images suggestive of tuberculosis,[15,19] thereby underscoring the relevance of radiological data.

In recent years, the Brazilian National Ministry of Health has used the targets proposed by the WHO in tuberculosis control: diagnose at least 70% of the expected cases; properly treat 100% of the diagnosed cases; cure at least 85% of those cases; and maintain treatment noncompliance at levels considered acceptable (below 5%). Among the patients who were started on tuberculosis treatment in the present study, the cure rate was 74.7%, below the level recommended by the WHO and close to the 67.2% and 76.2% reported for the state of Minas Gerais in 2013[15] and 2014,[16] respectively. In addition, the treatment noncompliance rate was 11.6%, close to the 9.0% and 11.5% reported for Brazil as a whole in 2013 and 2014, respectively, and more than double the target recommended by the WHO.[1]

In recent years, there has been a reduction in tuberculosis mortality in Brazil.[3] Although not the object of the present study, the outcomes were analyzed due to the importance of those data. There were 3 deaths due to tuberculosis in our study, which demonstrates the need for continued efforts to improve the quality of care given to people with the disease. The rates of cure and treatment noncompliance among the patients with MDR-TB were comparable to those reported by the Brazilian National Ministry of Health in 2017 (66.5% and 16.7%, respectively).[3]

In the univariate analysis, an association between pulmonary cavitation and the occurrence of MDR-TB was observed, because primary and acquired resistance are phenomena that are dependent on bacillary load and active multiplication, which are higher in the presence of cavitary disease.[17,18] In addition, MDR-TB was associated with previous treatment for tuberculosis, as has previously been reported.[18,21] That finding is of concern, because there were 13,347 cases of retreatment in the country, corresponding to 16.1% of all cases reported, during the study period.[1] Those data underscore the importance of giving special attention to patients who have previously been treated for disease, because they are at a higher risk of being infected with a drug-resistant strain of *M. tuberculosis*.

One of the main limitations of our study was that the small number of MDR-TB cases included limited the statistical power, precluding a multivariate analysis of resistance exposure factors. Another limitation was the fact that the study was conducted at only one center.

In conclusion, alcoholism, a chest X-ray with a pattern suggestive of tuberculosis, the presence of comorbidities, and the presence of pulmonary cavitations were factors associated with tuberculosis. MDR-TB was associated with previous treatment for tuberculosis and cavitation. Despite the significant progress made in the fight against tuberculosis, there is a need for coordinated actions that include social protection measures and patient support.

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ORIGINAL ARTICLE

Brain death effects on lung microvasculature in an experimental model of lung donor

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Study carried out in the Laboratório Cirúrgico de Pesquisa Cardiovascular, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.

ABSTRACT

Objective: Brain death (BD) triggers important hemodynamic and inflammatory alterations, compromising the viability of organs suitable for transplantation. To better understand the microcirculatory alterations in donor lungs caused by BD. The present study investigated the pulmonary microcirculation in a rodent model of BD via intravital microscopy. **Methods:** Male Wistar rats were anaesthetized and mechanically ventilated. They were trepanned and BD was induced through the increase in intracranial pressure. As control group, sham-operated (SH) rats were trepanned only. In both groups, expiratory O_2 and CO_2 were monitored and after three hours, a thoracotomy was performed, and a window was created to observe the lung surface using an epi-fluorescence intravital microscopy. Lung expression of intercellular adhesion molecule (ICAM)-1 and endothelial nitric oxide synthase (eNOS) was evaluated by immunohistochemistry, and cytokines were measured in lung samples. **Results:** Three hours after the surgical procedures, pulmonary perfusion was 73% in the SH group. On the other hand, BD animals showed an important decrease in organ perfusion to 28% ($p = 0.036$). Lung microcirculatory compromise after BD induction was associated with an augmentation of the number of leukocytes recruited to lung tissue, and with a reduction in eNOS expression and an increase in ICAM-1 expression on lung endothelial cells. BD rats showed higher values of expiratory O_2 and lower values of CO_2 in comparison with SH animals after three hours of monitoring. **Conclusion:** Data presented showed that BD triggers an important hypoperfusion and inflammation in the lungs, compromising the donor pulmonary microcirculation.

Keywords: Brain death; Inflammation; Intravital microscopy; Lung microcirculation.

INTRODUCTION

Lung transplantation continues to be restricted by limited long-term survival and high incidence of lung donor impairment. Several studies have shown that microvascular damage seems to be an important cause of early and long-term lung graft failure,[1,2] like other solid organs,[3,4] thus stressing the importance of preserving a functional microvasculature as a therapeutic strategy for preventing chronic fibrotic remodeling.

Besides alloimmune inflammation and ischemia-reperfusion injury, which are considered as the causes of microvascular injury in the solid organs used for transplantation,[1-4] pathophysiological changes triggered by brain death (BD) also can adversely affect the microcirculatory system. In a previous study,[5] observation of rat mesenteric microcirculation *in situ* and *in vivo* by intravital microscopy showed that BD triggers a persistent mesenteric hypoperfusion, local inflammation, and organ dysfunction. To better understand the microcirculatory alterations in the BD donor lungs, the present study aimed to investigate pulmonary microcirculation in a rodent model of BD via intravital microscopy.

METHODS

Experimental groups and surgical procedures

We used male Wistar rats (300±30 g) divided in two groups: BD group (n=10), rats in which BD was induced; sham-operated group (n=10), animals subjected to the same surgical process without BD induction. Each group included five rats for intravital microscopy analysis and other five per group were used for lung tissue analysis to avoid any bias related to the invasive lung study. All rats received humane care in compliance with the ethical principles adopted by the Brazilian College of Animal Experimentation.

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The protocol was approved by the Faculdade de Medicina of the Universidade de Sao Paulo Ethics Committee for Research Projects Analysis.

The anesthesia was induced in a chamber with 5% of isoflurane, followed by intubation and mechanical ventilation (Harvard Apparatus, USA) with tidal volume of 10 mL/kg, frequency of 70 breaths/min and FiO_2 of 100%. The ventilator was connected to a gas analyzer (AD Instruments, Australia) and the percentage of exhaled O_2 and CO_2 was measured in all animals during the experiment.

Brain death model

Surgical procedures to BD induction were performed as previously described.[5] BD was induced by a Fogarty-4F catheter placed into intracranial cavity and suddenly inflated with 0.5 mL of saline solution; then, anesthesia was immediately interrupted. The sudden increase in intracranial pressure was confirmed by hypertensive episode, maximal pupil dilatation and absence of reflex, according to a previously described protocol.[5] Sham-operated rats, submitted to trepanation only, were used as control group.

Before trepanation, the left carotid artery was cannulated, and a catheter was placed into the vessel for continuous monitoring of mean arterial blood pressure in rats of both groups and draw blood samples. As previously observed in other experimental models, this procedure does not lead to important cerebral hemispheric ischemia.[6] The jugular vein was cannulated for continuous infusion of 0.9% saline solution (2 mL/h) to minimize dehydration.

Intravital microscopy study

Three hours after BD induction, a right thoracotomy was performed in five animals per group, creating a window for in vivo microscopic examination of the lung tissue. Pulmonary microcirculation was evaluated using an epifluorescence intravital microscopy system (Carl Zeiss, Germany). To evaluate lung perfusion, fluorescein isothiocyanate (FITC) conjugated to albumin was administered to the rats by a catheter placed into the jugular vein. The number of microvessels with a diameter of less than 30 µm with or without blood flow were determined in an area of 0.2 mm^2, which were calculated using the AxioVision LE software. After intravenous injection with rhodamine 6G, the number of adhered or migrated leukocytes was determined in the same area of the lung tissue. For better resolution, the ventilation was performed with positive end-expiratory pressure (PEEP) of 5 cm H_2O during the intravital microscopy analyses. During video recording the animals were maintained in apnea at the end of the maximal inspiration. All images were recorded using 20 x objective lens.

Immunohistochemistry analyses

After the euthanasia, the lungs of the other five animals per group were removed, insufflated with OCT solution (Tissue Teck, USA), immersed in hexane and frozen with liquid nitrogen. The expression levels of intercellular adhesion molecule (ICAM)-1 and endothelial nitric oxide synthase (eNOS) on the endothelium of pulmonary microvessels were determined by immunohistochemistry. Cryosections of lungs (8 µm) were fixed in cold acetone for 10 min. The slides were washed with TRIS buffer-saline tween-20 (TBS-T) solution and the nonspecific binding sites were blocked using TBS-T containing 1% bovine serum albumin (BSA), and the endogenous peroxidase was blocked using 2% H_2O_2 solution. The sections were incubated for 1 hour at 37 °C with an anti-rat ICAM-1 antibody (Santa Cruz, USA) or with an anti-eNOS antibody (Abcam, USA). After incubation, the slide glasses were washed with TBS-T and incubated with horseradish peroxidase (HRP) secondary antibodies (Millipore, USA). Horseradish peroxidase substrate (3-amino-9-ethylcarbazole; Vector Laboratories, USA) was used for staining, and 3-amino-9-ethylcarbazole-stained objects in vessels walls were identified after threshold determination. The staining area fractions were quantified using an image analyzer (NIS-elements; Nikon, Japan). The background reaction was determined in lung sections incubated in the absence of primary antibody (negative control)

Tissue concentrations of cytokines

The lung tissue levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 were quantified by enzyme-linked immunosorbent assay (ELISA; R&D Systems, USAR&D Systems Inc). A fragment of the frozen harvested lungs was sectioned, immersed in saline solution and milled in a tissue mixer (Miltenyi Biotec, USA). The supernatant was analyzed by ELISA as recommended by the manufacturer. The results were expressed as ng/g or pg/gtissue.

Statistical analyses

The sample size was based on similar studies previously performed by our group.[5,7] All data are presented as mean±SEM (standard error of the mean). The results between groups were compared by a non-parametric Mann-Whitney test using Graph Pad Prism software version 6.1. A p value less than 0.05 was considered significant.

RESULTS

There were no deaths and the overall volume of fluid administered during the experiments was similar between the groups. As expected, BD induced a hypertensive episode sudden after the increase in intracranial pressure followed by hypotension, whereas in SH rats the mean arterial blood pressure did not change over time. The hypertensive episode is a peculiar feature in BD and confirms that the model is well established.

Microvascular pulmonary perfusion was evaluated after 3 hours of surgical procedures by epifluorescence

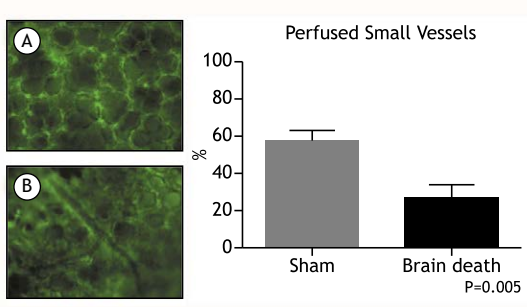

Figure 1. Percentage of perfused small vessels in pulmonary microcirculation. In vivo photomicrographs obtained by intravital microscopy in sham-operated (A) and in brain-dead (B) rats. Data are presented as mean±standard error mean; 5 rats per group.

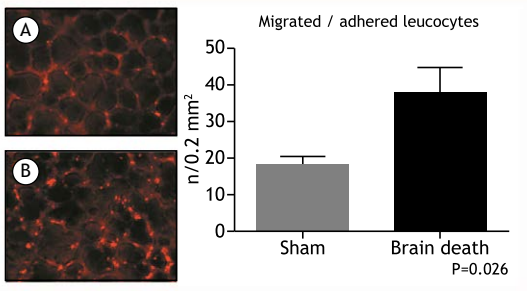

Figure 2. Number of migrated and adhered leukocytes in lung tissue. In vivo fluorescent photomicrographs obtained by intravital microscopy in sham-operated (A) and in brain-dead (B) rats. Data are presented as mean ± standard error mean; 5 rats per group.

microscopy. As illustrated in Figure 1, BD rats showed a significant decrease in the percentage of perfused microvessels compared with SH rats in an area of 0.2 μm². The pulmonary hypoperfusion was associated with increased leukocyte recruitment to the lung tissue as illustrated in Figure 2. We observed a higher number of recruited leukocytes to the lungs in BD rats when compared with SH group.

The endothelial cells of pulmonary microvessels in BD rats showed augmentation in the expression of ICAM-1 in comparison with SH group. In contrast, BD presented lower levels of eNOS expression on the endothelium (Figure 3A and B). Table 1 shows the values of tissue cytokines levels in the pulmonary tissue of both groups. We observed no differences in pulmonary IL-1β, IL-6 and IL-10 concentrations between groups, but TNF-α concentration increased significantly in BD group in comparison with SH animals.

Before BD induction, partial pressure of arterial oxygen and carbon dioxide were similar in both groups. At the end of the experiment, PaO_2 in the BD group (204.5 ± 35.07 mmHg) was lower than in SH group (303.4 ± 26.47 mmHg, p=0.039). There were no significant differences in $PaCO_2$ values between the groups (SH, 31.66 ± 3.81; BD, 37.68 ± 2.64 mmHg, p=0.235). The expiratory gases were measured throughout the experiment in animals of both groups. After three hours of monitoring, BD rats showed higher values of expiratory O_2 and lower values of CO_2 in comparison with SH animals at the same time point (Figure 4A and B).

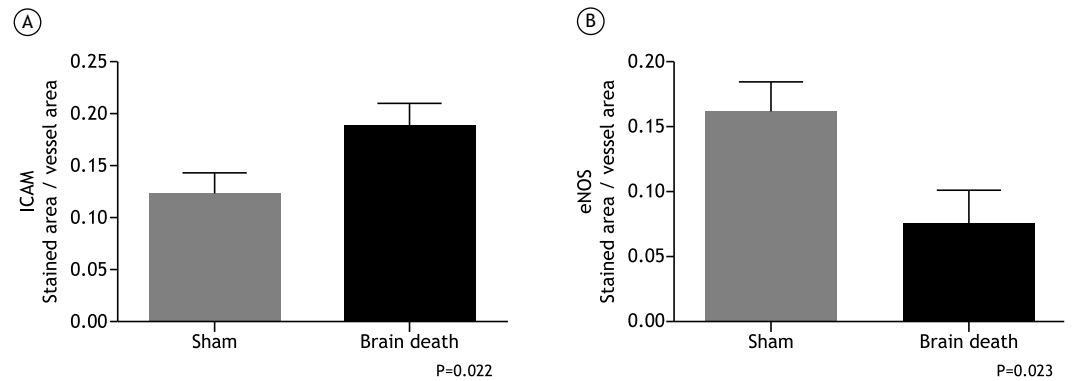

Figure 3. (A) Expression of intercellular adhesion molecule (ICAM)-1 in lug vessels; (B) Intraluminal expression of endothelial nitric oxide synthase (eNOS) in lung tissue. Data are presented as mean ± standard error mean; 5 rats per group.

Table 1. Levels of cytokines in the lung tissue.

	Sham	Brain Death	P value
TNF-α	1.63 ± 0.35	18.22 ± 7.24	0.008
Interleukin 1ß	67.4 ± 20.4	74.6 ± 31.3	0.854
Interleukin 6	74.4 ± 15.1	92.5 ± 25.4	0.772
Interleukin 10	36.8 ± 16.6	28 ± 25.1	0.672

Data (pg/g tissue) are presented as mean ± standard error mean; 5 rats per group.

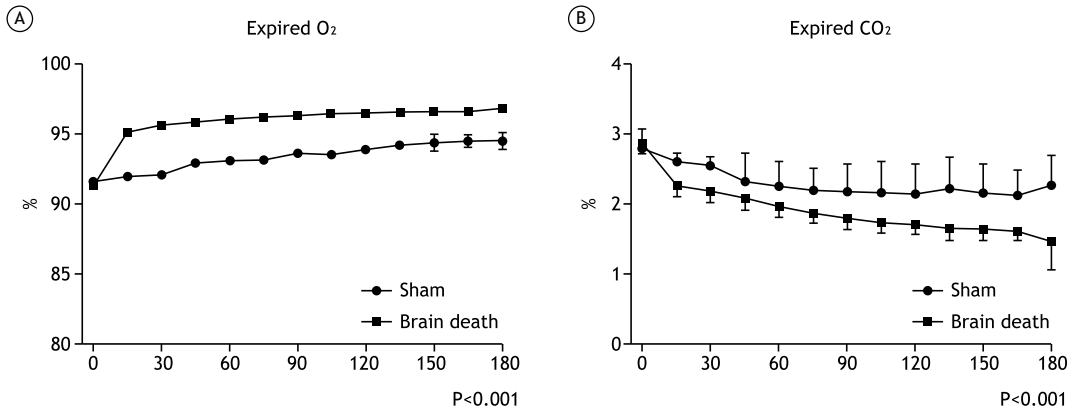

Figure 4. Analyses of (A) Expired O_2 and (B) expired CO_2 in sham-operated and brain-dead rats along 3 hours of monitoring. Data are presented as mean ± standard error mean of the percentage of expiratory volume; 10 rats per group.

DISCUSSION

The elucidation of the pathophysiological events triggered by BD may lead to a better treatment of organ donors and, consequently, a better outcome after transplantation. Several studies have been performed to clarify these events, either in clinical or experimental models. Notwithstanding, the observation of pulmonary microcirculation in vivo and in situ was not performed in brain dead lung donor before this study and, herein, we demonstrated that BD was responsible for a pulmonary hypoperfusion with an increase in the leukocyte recruitment to the lungs. These microcirculatory changes were associated with endothelial alterations such as augmentation in the expression of ICAM-1 and decrease in eNOS expression. Finally, these observations were correlated with alterations on the percentage of exhaled O_2 and CO_2, indicating that BD was responsible for an impairment on donor lung function, despite the absence of blood gases compromise due to the short period of observation.

Regarding lung intravital microscopic data, Ivanov and co-workers describe that the alveolus is a spherical body surrounded by a microvascular network.[7] On the other hand, Sack et al.[2] showed that pulmonary microcirculation exhibits a significant number of no-reflow areas with extremely reduced red blood cell velocities after lung transplantation in pigs, situation that was probably triggered by the ischemia-reperfusion injury. The analogous observation regarding the lung microcirculation documented in the current study after BD induction indicates another cause to this phenomenon and reinforces the relevance of microvascular injury since the donor management.

The correlation between BD and microcirculation compromise was previously verified in abdominal organs. It was shown that BD leads to a decreased organ perfusion in mesenteric, pancreatic and hepatic microcirculation and that these changes were associated with increased local inflammation.[5,8-10] The described events started early after BD induction and may be clearly observed three hours after brain damage.[5,8] Similarly, the increase of leukocyte recruitment to the lungs was also observed in the current study after BD induction, as a cascade of events that includes margination, rolling, adhesion, crawling and transmigration to perivascular tissue.[11]

The microvasculature has an important role in the long-term health of transplanted organs, and the mechanisms of microvascular damage after transplantation are mainly related to the endothelial cells injury by oxidative stress and immune response associated factors.[1-4,12] Oxidative stress is normally perceived in solid organ transplants and has been attributable to several factors including ischemia-reperfusion injury, post-transplant graft dysfunction, immunosuppressive drugs, as well as primary illness of the transplanted organ that has BD as its most important cause.

During oxidative stress augmentation, alloantibodies may induce endothelium cells death by complement-dependent mechanisms, which stimulate endothelium cells expression of adhesion molecules. Endothelial cells apoptosis via suppression of eNOS can be also observed.[12] The current documentation that these mechanisms are also directly involved in the lung microvascular compromise induced by BD, clearly demonstrated that microvascular damage initiates before the transplantation process, adding a worse substrate for the subsequent changes due to ischemia-reperfusion injury and immunological alterations.

The inflammation observed in BD rats was also characterized by an increase in ICAM-1 expression on lungs, demonstrating the interaction between leukocytes and endothelial cells.[11] In a previous study, the augmentation in the expression of the adhesion molecules was associated with an important reduction in the serum corticosterone levels.[5] It is well known that BD interrupts the hormonal axis leading to a reduction in the release of hormones, including glucocorticoids, such as corticosterone, which act controlling the inflammation and avoiding the overexpression of adhesion molecules on the endothelial cells.[5]

Other studies demonstrated that BD leads to an augmentation in the serum level of cytokines, adhesion molecules expression, and leukocyte recruitment to

perivascular tissue,[10,13-17] suggesting a connection among these features. This study showed that rats with BD exhibited increased serum levels of TNF-α in comparison to SH animals in the lung tissue, despite the absence of differences between the other investigated cytokines. This observation was similarly observed in a previous study with the same protocol, suggesting that some of the observed inflammatory events are triggered by BD-associated trauma.[5]

From these findings, we can conclude that BD triggers an important hypoperfusion in the lungs, compromising the pulmonary microcirculation and function, once leukocyte recruitment associated with endothelial activation is observed. During the different phases of the lung transplantation process, including the management of BD organ donors, extensive microvascular injury with insufficient repair leads to pathogenic angiogenesis and subsequent fibrosis. Therefore, the preservation of a healthy microvasculature by inhibiting pathways that lead to microvascular injury is a fundamental strategy to improve both lung donor availability and graft survival extension.

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ORIGINAL ARTICLE

The role of endobronchial ultrasound-guided transbronchial needle aspiration in isolated intrathoracic lymphadenopathy in non-neoplastic patients: a common dilemma in clinical practice

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Study carried out in the Serviço de Endoscopia Respiratória, Disciplina de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.

ABSTRACT

Objective: To determine the diagnostic yield of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in non-neoplastic patients with isolated intrathoracic lymphadenopathy (IL). **Methods:** This was a retrospective study of patients with isolated IL referred for EBUS-TBNA. We calculated the sensitivity, specificity, accuracy, positive predictive value (PPV), and negative predictive value (NPV) of EBUS-TBNA in the diagnosis of granulomatous, reactive, and neoplastic lymphadenopathy. In cases of nonspecific granulomas, reactive lymphadenopathy, or inconclusive results, a definitive diagnosis was established by other diagnostic procedures or during a follow-up period of at least 18 months. **Results:** Among the 58 patients included in the study, EBUS-TBNA established a diagnosis of granulomatous disease in 22 (38%), reactive lymphadenopathy in 15 (26%), cancer in 8 (14%), and other diseases in 3 (5%). Results were inconclusive in 10 (17%), the diagnosis being established by other bronchoscopic procedures in 2 (20%) and by surgical procedures in 8 (80%). A final diagnosis of reactive lymphadenopathy was established in 12. Of those, 11 (92%) had their diagnosis confirmed during follow-up and 1 (8%) had their diagnosis confirmed by mediastinoscopy. In another 3, a final diagnosis of sarcoidosis or neoplasm was established. For the diagnosis of granulomatous disease, neoplasms, and reactive lymphadenopathy, EBUS-TBNA was found to have a sensitivity of 73%, 68%, and 92%, respectively; a specificity of 100%, 100%, and 93%, respectively; an accuracy of 86%, 93%, and 93%, respectively; a PPV of 100%, 100%, and 80%, respectively; and an NPV of 78%, 92%, and 98%, respectively. **Conclusions:** In non-neoplastic patients, granulomatous disease and reactive lymphadenopathy appear to be common causes of isolated IL. EBUS-TBNA shows promising results as a first-line minimally invasive diagnostic procedure. The results obtained by EBUS-TBNA can be optimized by examining clinical and radiological findings during follow-up or by comparison with the results obtained with other bronchoscopic methods.

Keywords: Endoscopic ultrasound-guided fine needle aspiration; Lymphadenopathy/diagnosis; Neoplasms.

INTRODUCTION

Isolated intrathoracic lymphadenopathy (IL) can be caused by malignant or benign diseases, and it is often difficult to establish a minimally invasive definitive diagnosis.[1] Tuberculosis, sarcoidosis, and other inflammatory diseases are the most common benign conditions and have a substantial clinical and diagnostic overlap; because they demand completely different therapeutic regimens, this creates a dilemma for pathologists and clinicians.[1-3] Therefore, histopathological/microbiological confirmation is essential for the differential diagnosis.[2]

Lymph nodes (LNs) may become enlarged as a reaction to underlying pulmonary or cardiovascular comorbidities; in such cases, lymph node enlargement is referred to as reactive lymphadenopathy.[4] Reactive lymphadenopathy has been reported to be present in almost 50% of COPD patients and in 35-66% of the patients with chronic heart failure.[4] Other chronic conditions, such as bronchiectasis, pulmonary arterial hypertension, and connective tissue disease, have also been associated with reactive lymphadenopathy.[4]

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a useful and minimally invasive diagnostic procedure that provides cytological sampling under real-time ultrasound viewing, resulting in improved accuracy and safety during LN sampling.[1-3,5] Nowadays, EBUS-TBNA has an established

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role for IL evaluation, particularly for the diagnosis and staging of cancer patients and for the diagnosis of granulomatous disease, with an accuracy comparable to mediastinoscopy.[4,5] However, less is known about the role of EBUS-TBNA in nonspecific IL.

The objective of the present study was to evaluate the diagnostic yield of EBUS-TBNA in non-neoplastic patients with isolated IL.

METHODS

This was a retrospective longitudinal study of patients with isolated IL undergoing EBUS-TBNA who were seen in the *Serviço de Endoscopia Respiratória, Disciplina de Pneumologia, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo*, located in the city of São Paulo, Brazil, between August of 2011 and April of 2017. The data were obtained from the institutional database. The study was approved by the Research Ethics Committee of *Hospital das Clínicas* (Protocol no. 1,630,604).

Patients referred for isolated IL evaluation were included in the study. We defined IL as the presence of at least one mediastinal or hilar LN > 1 cm in short-axis diameter based on CT or with a standardized uptake value > 2.5 on positron emission tomography (PET)/CT. The exclusion criteria were as follows: presence of an endobronchial lesion during conventional video-assisted bronchoscopy performed immediately prior to EBUS-TBNA, history or suspicion of cancer, and loss to follow-up.

To localize enlarged LNs, CT or PET/CT scans of the chest were assessed before the procedure. The LN map recommended by the International Association for the Study of Lung Cancer was used in order to standardize the LN station nomenclature in all procedures and to facilitate communication.[6]

All procedures were performed with topical anesthesia—1% lidocaine using "spray-as-you-go" delivery—and moderate sedation with midazolam (5 mg), fentanyl (100 µg), and slow infusion of propofol (approximately 200 mg). EBUS-TBNA was preceded by conventional video-assisted bronchoscopy (BF-Q180; Olympus Medical Systems Corp., Tokyo, Japan) in order to access the airways and identify any endobronchial lesions to be biopsied. All linear-probe EBUS procedures were performed through natural orifices—nose or mouth—by an experienced bronchoscopist who had been trained in standard and interventional bronchoscopy. In all instances, EBUS sectorial scope (BF-UC180F; Olympus Medical Systems, Tokyo, Japan) and a disposable 22-gauge needle compatible with the ultrasound scope were used: NA-201SX-4022 (Olympus Medical Systems), ECHO-HD-22-EBUS-O (Cook Medical, Winston-Salem, NC, USA), or GUS-45-18-022 (Medi-Globe, Achenmühle, Germany).

TBNA was performed using a 22-gauge needle and negative pressure, and sample collection followed standardized routine protocols. TBNA aspirates were immediately smeared on glass slides and fixed in 95% ethanol for cytology; the remaining aspirates were fixed in 10% formaldehyde and embedded in paraffin for cell-block analysis. When there was suspicion of granulomatous disease, the TBNA aspirate was also flushed into a sterile container with normal saline and sent for fungal and mycobacterial culture; transbronchial biopsy (TBB) was taken, fixed in 10% formaldehyde and embedded in paraffin for histological analysis; and BAL fluid was collected and sent for microbiological and cytological analysis. When there was suspicion of tuberculosis, the specimens obtained were smeared for Ziehl-Neelsen staining and sent for mycobacterial culture on Löwenstein-Jensen medium. Rapid on-site evaluation (ROSE) was not carried out.

When EBUS-TBNA indicated nonspecific granulomas, reactive LNs, or inconclusive results, a definitive diagnosis was established by other endoscopic procedures, by surgical procedures, or by clinical and radiological follow-up for at least 18 months. The diagnostic criteria were as follows: presence of caseating or noncaseating granulomas consistent with tuberculosis, fungal disease, sarcoidosis, or other granulomatous diseases; positive culture for a specific microorganism in the LN sample; and presence of neoplastic cells in the aspirate.

The EBUS-TBNA procedure was considered diagnostic if it resulted in the specific diagnosis of malignancy or inflammatory disease. An LN was considered reactive if further investigation showed it to be reactive or if it remained stable on CT scans and clinical evaluation for at least 18 months of follow-up.

Statistical methods

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy for each condition were calculated based on the number of successful diagnoses made by EBUS-TBNA and the number of EBUS-TBNA procedures. We considered true positive cases to be those in which EBUS-TBNA established a correct diagnosis. False positive cases were considered to be those in which a diagnosis was established and then changed due to other procedures or during follow-up. True negative cases were those when the patient did not have the disease according to EBUS-TBNA, other procedures, or follow-up. False negative cases were considered to be those in which EBUS-TBNA could not provide a diagnosis, other procedures or follow-up therefore being required. The variables were described as absolute and relative frequencies. All of the analyses were performed with the IBM SPSS Statistics software package, version 19.0 for Windows (IBM Corporation, Armonk, NY, USA).

RESULTS

During the study period, a total of 196 patients underwent EBUS-TBNA for the diagnosis of isolated IL. Of those, 138 were excluded: 132 due to a history or suspicion of cancer; 3 because they had

an endobronchial lesion detected during conventional bronchoscopy; and 3 who were lost to follow-up (Figure 1). Therefore, 58 patients were included, 33 (56.9%) of whom were female. The mean age of the patients was 53 ± 15 years. The most common comorbidities were HIV and/or HCV infection and autoimmune disease, followed by COPD. All CT scans revealed IL. The most common CT findings were pulmonary nodules, pulmonary masses, pleural effusion, and pulmonary infiltrates. A total of 159 LNs were assessed by EBUS; of those, 79 (49.7%) were sampled by EBUS-TBNA. The sampled LNs had a mean short-axis diameter of 17.8 ± 6.6 mm, and most were subcarinal or paratracheal LNs. The mean number of punctures made in each LN was 3.5 ± 1.5. These characteristics are summarized in Table 1.

EBUS-TBNA diagnosed granulomatous disease in 22 patients (38%), reactive LNs in 15 (26%), neoplasms in 8 (14%), and other diseases in 3 (5%). In 10 cases (17%), EBUS-TBNA samples were not suitable for histopathology. Of those, 8 were also sent for culture and microbiological analysis, the results of which were negative and therefore inconclusive (Figure 1). No major complications were recorded.

With regard to granulomatous diseases, EBUS-TBNA identified mycobacteriosis in 5 patients (22.7%); histoplasmosis, in 1 (4.5%); sarcoidosis, in 1 (4.5%); and silicosis, in 1 (4.5%; Figure 1). Tuberculosis was diagnosed in 4 patients (caseating granulomas identified by cell-block analysis in 2 and positive AFB in aspirate smear in 2). All 4 patients responded to tuberculosis treatment. *Mycobacterium kansasii* was isolated in the aspirate culture in 1 patient. In the remaining 14 patients with nonspecific granulomas by EBUS-TBNA (63.6%), the definitive diagnosis was made by association with other bronchoscopic methods, in 6 (42.9%); surgical biopsy, in 3 (21.4%); and during follow-up, in 5 (35.7%; Table 2).

Of the 15 patients diagnosed with reactive lymphadenopathy, 12 (80%) had their diagnosis subsequently confirmed. Of those, 11 (92%) had their diagnosis confirmed during the follow-up period and 1 (8%) had their diagnosis confirmed by mediastinoscopy. The remaining 3 patients (20%) were diagnosed with sarcoidosis (during the follow-up period), epithelioid hemangioendothelioma (by TBB), or lymphoma (by cerebrospinal fluid analysis; Table 2). Underlying comorbidities were present in 83% of the 12 patients with a definitive diagnosis of reactive lymphadenopathy: infectious/inflammatory disease (pleural tuberculosis under treatment, organizing pneumonia, or myocarditis), in 25%; and chronic diseases (HIV infection, hepatitis C, autoimmune disease, COPD, severe mitral regurgitation, or hypothyroidism), in 58%.

Of the 8 neoplastic results, EBUS-TBNA made a definitive diagnosis in 4: adenocarcinoma, in 2, and non-small cell lung cancer, in 2. Histopathology raised the suspicion of non-Hodgkin lymphoma in 3 cases, all of which were confirmed by surgical biopsy of the LNs. In 1 case, the diagnosis of undifferentiated carcinoma remained the same after surgical biopsy of LNs. In addition, EBUS-TBNA established the diagnosis of substernal goiter in 2 patients and of bronchogenic cyst in 1.

Table 1. General characteristics of the patients, radiological findings, and lymph nodes sampled by endobronchial ultrasound-guided transbronchial needle aspiration (N = 58).[a]

Characteristic	Result
Gender	
Male	25 (43.1)
Female	33 (56.9)
Age, years	53 ± 15
Comorbidities	
HCV/HIV	3 (5.2)
Autoimmune disease	3 (5.2)
COPD	2 (3.4)
Pleural tuberculosis	1 (1.7)
Pulmonary tuberculosis scars	1 (1.7)
Pericardial effusion	1 (1.7)
Heart transplant	1 (1.7)
Diabetes	1 (1.7)
Arterial hypertension	1 (1.7)
CT findings	
Lymph nodes	58 (100)
Pulmonary nodules	12 (20.7)
Bilateral	9 (15.5)
RUL	2 (3.4)
RUL + LUL	1 (1.7)
Size, mm	14 ± 8
Masses	4 (6.9)
Mediastinal	2 (3.4)
Paratracheal	1 (1.7)
Hilar	1 (1.7)
Size, mm	64 ± 14
Pulmonary micronodules	1 (1.7)
Pleural effusion	4 (6.9)
Pulmonary infiltrates	3 (5.2)
Emphysema	1 (1.7)
Pulmonary atelectasis	1 (1.7)
Ground-glass opacities	1 (1.7)
Lymph nodes sampled	79 (49.7)
Location	
Paratracheal region	27 (34.2)
Subcarinal region	41 (51.9)
Hilar region	2 (2.5)
Interlobar region	9 (11.4)
Size, mm	17.8 ± 6.6
Number of punctures	3.5 ± 1.5

RUL: right upper lobe; and LUL: left upper lobe. [a]Values expressed as n (%) or mean ± SD.

On cytology, the presence of red blood cells (n = 9) and few lymphoid cells (n = 1) rendered the samples inadequate for diagnosis, and this could explain the inconclusive results. In 8 of those cases, the samples were also sent for microbiological analysis, but the results were negative. The definitive diagnosis of those inconclusive results by EBUS-TBNA was established by other bronchoscopic methods, in 2 cases (20%); and by surgical biopsy, in 8 cases (80%; Table 2).

Performing BAL or TBB together with EBUS-TBNA helped achieve a definitive diagnosis of nonspecific

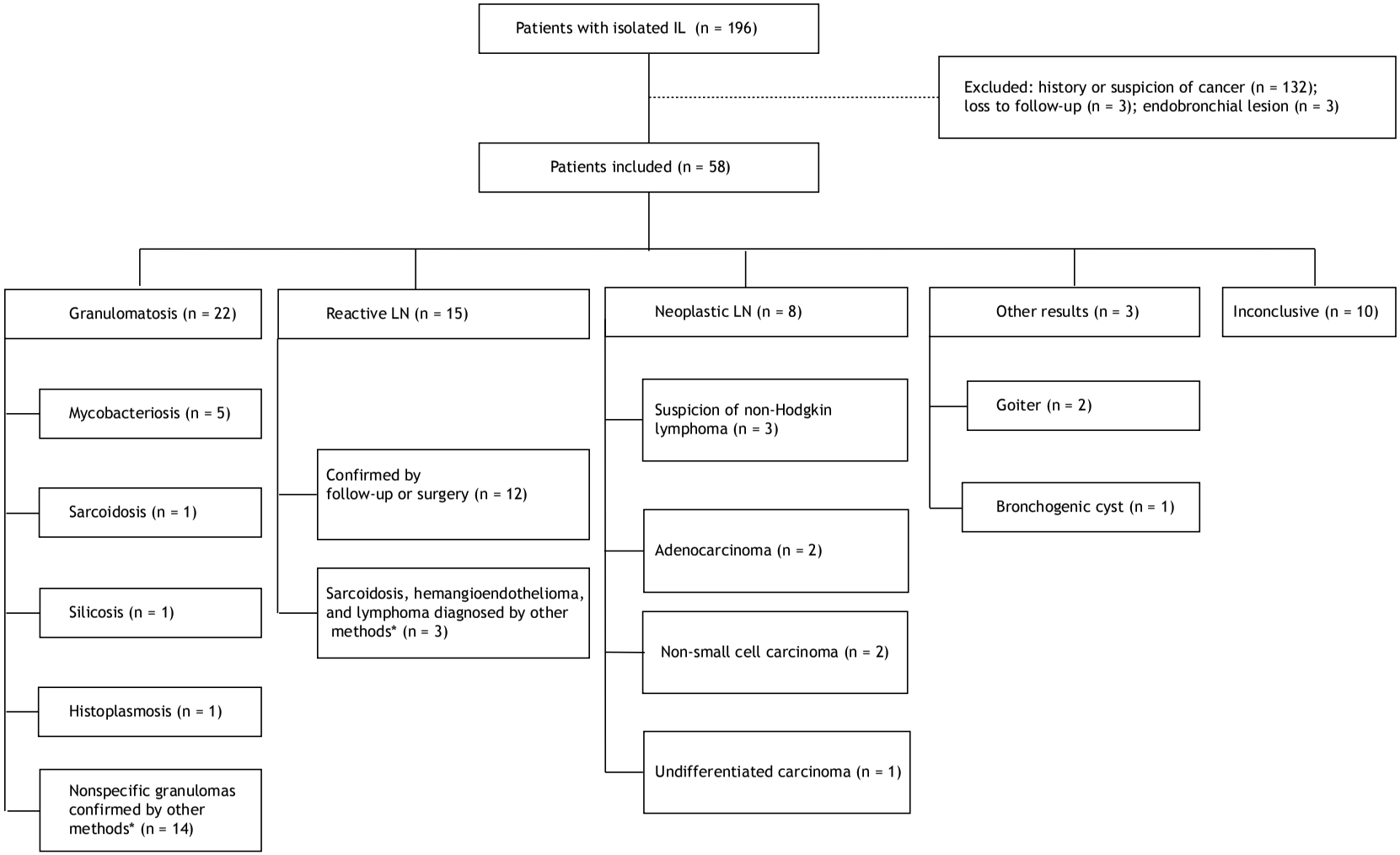

Figure 1. Flow chart of patients who underwent endobronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of isolated intrathoracic lymphadenopathy. IL: intrathoracic lymphadenopathy; and LN: lymph node. *Other methods (bronchoscopy, surgery, or cerebrospinal fluid analysis).

Table 2. Diagnostic methods other than endobronchial ultrasound-guided transbronchial needle aspiration that established the diagnosis in cases of nonspecific granulomas, reactive lymph nodes, and inconclusive results.

Diagnostic method	Nonspecific granulomas	Inconclusive results	Reactive lymph nodes
BAL	Sarcoidosis (n = 1) (plus clinical and radiological findings)	Aspergillosis (n = 1)	
Transbronchial biopsy	Sarcoidosis (n = 5) (lymphocytosis in BAL fluid and/or elevated CD4/CD8)	Sarcoidosis (n = 1) (lymphocytosis in BAL fluid and/or elevated CD4/CD8)	Epithelioid hemangioma (n = 1)
Mediastinoscopy	*M. avium* (n = 1)	*M. tuberculosis* (n = 1) Sarcoidosis (n = 1) Histoplasmosis (n = 1) Fibrosing mediastinitis (n = 1)	
Surgical biopsy of extrathoracic LN	*M. tuberculosis* (n = 1) Granulomatous slack skin (n = 1)	Reactive lymphadenopathy (n = 1) Sarcoidosis (n = 2)	
Transthoracic biopsy		Lymphoma (n = 1)	
Cerebrospinal fluid analysis			Lymphoma (n = 1)
Follow-up	Sarcoidosis (n = 5)		Sarcoidosis (n = 1)

granuloma, reactive lymphadenopathy, or "inconclusive" results by EBUS-TBNA in 33.3% of the cases.

The sensitivity, specificity, PPV, NPV, and accuracy of EBUS-TBNA for the diagnosis of granulomatosis, neoplasms, and reactive lymphadenopathy are specified in Table 3. The global diagnostic yield was 77.6%, and possible false results were 5%.

DISCUSSION

In our sample of patients with no history or suspicion of cancer, isolated IL mostly revealed benign diseases, which is in accordance with the literature.[1,7] EBUS-TBNA has shown high sensitivity and cost-effectiveness for first-line investigation, rendering mediastinoscopy unnecessary in 87% of the patients with IL.[4] Our study showed that EBUS-TBNA is a useful tool in the differential diagnosis of isolated IL, an important step that informs the utility and applicability of different therapeutic options.

Granulomatous diseases—tuberculosis, fungal infections, and sarcoidosis—are the most common benign causes of IL and, likewise, were the diseases that were most commonly diagnosed by EBUS-TBNA in our study, corresponding to 38% of the cases.[1,7] Granulomatous diseases share various clinical, radiological, and pathological features, which makes their differentiation occasionally arduous, increasing the importance of tissue diagnosis.[8] Morphological characteristics of granulomas—noncaseating in sarcoidosis and caseating in tuberculosis and fungal infections—associated with clinical and radiological findings can help differentiate such disorders. Aspirate smears and cultures can confirm infectious causes and exclude sarcoidosis.[8-11]

The sensitivity of EBUS-TBNA for the diagnosis of granulomatous disease has been reported to range from 64.0% to 80.9%, with an accuracy between 70.0% and 83.3%, and an NPV of 33.0-42.8%,[7,12] the highest results having been shown in a prospective study performed by Çağlayan et al.,[7] in which 72 patients with suspected granulomatous disease were enrolled. In our study, we found sensitivity values within the range reported in the current literature, excellent specificity and PPV, as well as high NPV and accuracy. These results were achieved despite the retrospective nature of our study and a lower suspicion of granulomatous disease than that reported in the aforementioned study.[7]

Sarcoidosis is a multisystem disease that affects the lungs and LNs in almost all patients.[13,14] Its diagnosis is based on clinical and radiological findings, CD4/CD8 ratio (> 3.5) on BAL fluid, tissue confirmation of noncaseating epithelioid cell granulomas, and the exclusion of infectious and malignant conditions.[13-15] Flexible bronchoscopy has a diagnostic yield of approximately 70% for pulmonary sarcoidosis, and EBUS-TBNA can reach a diagnostic yield of 86%.[15] A randomized multicenter clinical trial that included 304 patients with sarcoidosis showed that the overall diagnostic yield of TBB was 53%, with better results in patients with stage II sarcoidosis than in those with stage I sarcoidosis (66% vs. 38%).[13] However, the overall diagnostic yield of EBUS-TBNA was higher (80%), with better results in patients with stage I sarcoidosis than in those with stage II sarcoidosis (84% vs. 77%).[13] Similarly, a systematic review and meta-analysis on the efficacy and safety of EBUS-TBNA in sarcoidosis revealed an overall diagnostic yield of 79%.[14] The sensitivity of EBUS-TBNA for the diagnosis of sarcoidosis has been reported to be between 64% and 84%.[7,12] These excellent results and the knowledge that the combination of EBUS-TBNA with TBB and endobronchial biopsies can

Table 3. Sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of endobronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of granulomatous disease, neoplasms, and reactive lymph nodes.

Diagnosis	Sensitivity	Specificity	PPV	NPV	Accuracy
Granulomatosis	73.3	100	100	77.8	86.2
Neoplasms	66.7	100	100	92.0	93.1
Reactive LNs	92.3	93.3	80.0	97.7	93.1

LN: lymph node; PPV: positive predictive value; and NPV: negative predictive value.

significantly add to the diagnostic yield suggest that EBUS-TBNA should be routinely employed for the diagnosis of sarcoidosis whenever available. In our study, a definitive diagnosis was obtained by EBUS-TBNA in only 1 patient, the remaining diagnoses being confirmed by other bronchoscopic methods, such as lymphocytosis in BAL fluid, elevated CD4/CD8, and/or noncaseating granuloma in TBB; in other cases, there were strong clinical and radiological findings suggesting a diagnosis of sarcoidosis, which was later confirmed during patient follow-up. Although EBUS-TBNA alone was unable to establish the differential diagnosis in 14 cases of granulomatous disease in our study, it was able to detect granulomas in 11 cases diagnosed as sarcoidosis, and its results were inconclusive or indicated reactive LNs in only 5 cases.

IL is the most common form of extrapulmonary tuberculosis, accounting for 30-40% of cases.[9] Unfortunately, its diagnosis is challenging because of the lack of specific clinical and radiological features, as well as common negative sputum smear and culture results due to the absence of parenchymal involvement.[9-11] EBUS-TBNA has been shown to be an effective diagnostic tool in extrapulmonary tuberculosis, with a high sensitivity and a diagnostic yield of 80-94%, because it can reach mediastinal and hilar LNs that are commonly involved, so that the samples can be sent to cytological and microbiological analysis.[10,11,16-18] However, the positive culture rate for tuberculosis in this type of sample is reported to be 14-62%, which might be due to the scarcity of AFB in the LNs or lack of suitable cellular material in samples obtained from necrotic tissue.[9,16,18,19] In our study, EBUS-TBNA diagnosed tuberculosis in 4 cases, half of which were diagnosed by means of aspirate smears together with necrotizing granulomas or reactive LNs. Unfortunately, due to the small number of diagnoses of tuberculosis in our study, we cannot compare our results with those of other studies.

We had a considerable rate of patients diagnosed with reactive lymphadenopathy by EBUS-TBNA (26%), reactive LNs being our second most common cause of isolated IL. The diagnostic yield of EBUS-TBNA was good; 80% of the diagnoses of reactive lymphadenopathy were confirmed, the majority of which (92%) during the follow-up period. Only 1 case was confirmed by mediastinoscopy. Only 2 patients with reactive LNs had no associated comorbidities. The sensitivity and NPV for that diagnosis by EBUS-TBNA were 92.3% and 91.1%, respectively. These results allow us to rely on EBUS-TBNA when it indicates reactive LNs, especially in patients with other inflammatory or chronic diseases and in the absence of a high degree of suspicion of malignancy or alternative diagnoses. Some studies have reported an IL incidence of approximately 35% in HIV patients on the basis of CT findings, and this can be a challenge for clinicians due to a variety of differential diagnoses.[20] Although EBUS-TBNA can help patients avoid surgical procedures in such cases, few studies have evaluated its utility in such patients. According to Han et al.,[20] EBUS-TBNA can prevent mediastinoscopy in 89% of the cases; dispenses with general anesthesia and hospitalization in most centers; and has a low rate of major complications and mortality. In our study, there were only 2 HIV patients: 1 was diagnosed with reactive lymphadenopathy that was confirmed during the follow-up period, and 1 was diagnosed with nonspecific granuloma revealed to be due to *Mycobacterium avium* by mediastinoscopy.

In our study, EBUS-TBNA showed very good specificity in benign diseases, almost all cases being confirmed with no need for more invasive procedures. Furthermore, the accuracy of EBUS-TBNA can be improved with other bronchoscopic procedures, such as BAL, TBB, and endobronchial biopsy, performed during the same procedure when pulmonary infiltrates or secretions are present and especially when nonspecific granulomas, reactive LNs, or inconclusive results are found. In our study, EBUS-TBNA provided a definitive diagnosis in one third of such cases when associated with other bronchoscopic procedures.

Lymphoma is a common cause of mediastinal tumors; however, only 10% are primary mediastinal lymphomas in adults.[21] Despite the relatively lower sensitivity of EBUS-TBNA for lymphoma—because large samples are often required to achieve adequate cellularity and evaluate tissue architecture—EBUS-TBNA has been reported to have a sensitivity ranging from 76.0% to 90.9% and a specificity of 100% when combined with flow cytometry and immunohistochemical analysis.[1,22] In a study by Nunez et al.,[22] 89% of patients with deep-seated lymphadenopathy were diagnosed by endoscopic ultrasound or EBUS-guided fine-needle aspiration. The authors also observed that at least two additional passes can provide an adequate number of cells for flow cytometric analysis, and most of the patients who underwent this diagnostic modality had no need for subsequent surgical LN excision.[22] In our study, EBUS-TBNA failed to yield a definitive diagnosis

of lymphoma; it raised the suspicion of lymphoma in 2 cases, which were subsequently confirmed by surgical biopsy of the LNs. This finding might be due to a low level of suspicion at the time of the procedure and, consequently, the absence of flow cytometry analysis and immunohistochemistry.[1]

The limitations of our study are the typical limitations of any retrospective study. Furthermore, ours was a relatively small population, and some subgroups—particularly malignancy cases—were underrepresented. Finally, ROSE was not available in our routine. We understand the importance of ROSE to raise the suspicion of a particular diagnosis and direct the analysis of the collected material (culture, flow cytometry, or other).

In conclusion, inflammatory and infectious LNs were the most common findings in our non-neoplastic patients with isolated IL. EBUS-TBNA showed good sensitivity and high NPV and should therefore be considered a first-line minimally invasive diagnostic procedure in such cases. Accuracy can be optimized by using clinical and radiological findings appropriately in order to guide further evaluation, follow-up, and the use of bronchoscopic methods.

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ORIGINAL ARTICLE

Effects of varenicline on lung tissue in the animal model

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ABSTRACT

Objective: This study aimed to investigate acute and chronic effects of varenicline on lung tissue in an experimental study. **Methods:** A total of 34 rats were randomly allocated into study (varenicline) and control groups. The rats were divided into two groups (i) control group, (ii) varenicline group. Then, the rats in the each group were sub-divided equally in turn as acute (C1; V1) and chronic (C2; V2) ; all rats of acute and chronic groups were sacrificed under the anesthesia on the 45th day for acute group [C1 (n=5) and V1 (n=12)] and the 90th day for chronic group [C2 (n=5) and V2 (n=12)], respectively. Thus, biochemical and histopathological analysis were carried out. **Results:** Thirty four rats completed the study, 24 were in varenicline group and 10 were in control group. In chronic exposure to varenicline, oxidant levels comprising of malondialdehyde (MDA), and myeloperoxidase (MPO) increased and superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) levels, named as antioxidants, decreased significantly when compared to the control group. MDA and MPO levels were also significantly higher and SOD, CAT, GPx, GSH levels were also significantly lower in chronic varenicline group when compared to acute varenicline group. These findings were also supported by histopathological observations. **Conclusion:** This is the first study, which evaluated pulmonary effects of varenicline experimentally on an animal model. It was observed that chronic varenicline treatments cause inflammation and lung cell injury.

Keywords: Varenicline; Lung tissue; Oxidative stress; Pulmonary toxicity.

INTRODUCTION

Smoking tobacco is the most important and preventable cause of pulmonary diseases such as chronic obstructive pulmonary disease, lung cancer, and interstitial lung diseases. Furthermore, it has been associated with approximately 50% of deaths due to cardiovascular and pulmonary diseases. The most important pathological changes associated with smoking tobacco are inflammation and oxidative stress of the respiratory tract. Metaplastic and dysplastic changes in bronchial epithelium are accompanied by elevated expression of adhesion molecules and secretion of many cytokines and oxidative stress products.[1-3]

Quitting smoking is the most effective method of prophylaxis and treatment of pulmonary diseases. The effects of nicotine are mediated through a variety of mechanisms including actions at various nicotinic receptor subtypes (nAChR) and modulation of the neurotransmitters release such as dopamine, serotonin and glutamate.[2] Varenicline is the most widely used agent for quitting smoking and it is a highly selective partial agonist at the α4β2 nAChR and a full agonist at the α7 nicotinic acetylcholine receptor.[3,4]

In randomised clinical trials varenicline has been shown to increase the chances of successful long-term results in 2–3 times compared to pharmacologically unsupported attempts to quit smoking.[3] Varenicline has been reported to suppress the withdrawal symptoms and feeling of enjoyment derived from smoking.[5] Smokers treated with varenicline reported that cigarette smoking was less satisfying and rewarding than those given a placebo.[6] The most common side effects of varenicline include nausea, insomnia, abnormal dreams, headache, agitation, anxiety, tachycardia, dyspepsia, and constipation.[7]

Although there are lots of studies evaluating the non-smoking related effects of varenicline, but the number of studies evaluating the effects on tissues is extremely low, whether or not it has a negative effect on rats' lung tissue which has never been evaluated. To this end, it was aimed to explore the acute and chronic effects of varenicline using histopathological examinations to assess oxidative stress and apoptosis

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in the lung tissue of rats treated with varenicline. It was also detected biochemical parameters and weighed individual lungs.

METHODS

The current study protocol was approved by the Animal Ethics Committee (Number: 2012/A-61). In the present study, 34 male Wistar Albino rats, 10-12 weeks of age and weighing 250–300 g were supplied from the Inonu University Laboratory Animals Research Center. The rats were kept in a temperature (21±2°C) and in humidity (60±5%) controlled room in which a 12:12 h light:dark cycle was provided during the trial. The rats were randomly divided into varenicline (V: n=24) and control (C: n=10) groups and the rats in each group were sub-divided equally as either acute (C1; V1) or chronic (C2; V2). The rats in the control group were administered distilled water orally. Modified animal-to-human dosing tables were used to adjust the duration and dose scheme of varenicline (Champix 1 mg Tb®, Pfizer Corporation, Istanbul, Turkey) according to the human therapy protocol.[8,9] Accordingly, the dosage of oral varenicline was adjusted as 9 µg/kg daily on days 1–3, 9 µg/kg twice daily on days 4–7, and 18 µg/kg twice daily on days 8-90 (total of 83 days). The rats in the acute group were sacrificed on day 45 [C1 (n=5) and V1 (n=12)] and the rats in the chronic group [C2 (n=5) and V2 (n=12)] were sacrificed on day 90 by using ketamine and xylazine. The lung tissues were stored for biochemical and histological studies.

Spectrophotometric analyses of TBARS ingredient of the homogenates were performed by thiobarbituric acid reaction use.[10] Three millilitres of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid solution were added to 0.5 ml of plasma in a tube. The mixture was heated in boiling water for 45 minutes and after cooled, and extracted into 4 ml of n-butanol. The absorbance was measured spectrophotometrically (UV-1601; Shimadzu, Kyoto, Japan) at 532 nm. The amount of lipid peroxides was calculated as per TBARS of lipid peroxidation. A standard graph prepared for evaluation of standard fluids was used and the results were expressed in nanomoles per gram (nmol/g tissue) (1,1,3,3-tetramethoxypropane).

In this study, it was used the method developed by Sun et al.,[11] which involves inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide ($O2^{\bullet-}$) generator in order to evaluate total SOD (EC 1.15.1.1) activity (Cu-Zn and Mn). The enzyme amount that caused 50% inhibition in the NBT reduction ratio was described as one unit of SOD. SOD activity was expressed as units per gram protein (U/g protein).

It was used the method defined by Paglia and Valentine[12] in order to evaluate GPx activity (EC 1.6.4.2). It was initiated an enzymatic reaction by adding H_2O_2 to a tube, which contained nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH), sodium azide, and glutathione reductase. The change in absorbance at 340 nm was monitored by a spectrophotometer. The results were expressed as U/g protein.

It was used a 4-aminoantipyrine/phenol solution as the substrate for MPO-mediated oxidation by H_2O_2 in order to evaluate the MPO (EC 1.11.1.7) production and recorded the change in absorbance at 510 nm.[13] The amount causing degradation of 1 µmol H_2O_2/min at 25°C was described as one unit of MPO. The results were expressed as U/g protein.

It was used Aebi's method in order to assay catalase activity (CAT, EC 1.11.1.6).[14] This method is based on the determination of the rate constant (k, s−1) or the H_2O_2 decomposition rate at 240 nm. The activity was expressed as k per gram protein (k/g protein).

A previously defined method was used in order to analyse the GSH ingredient in the lung tissue as non-protein sulphydryls.[15] It was mixed aliquots of tissue homogenate with distilled water and 50% trichloroacetic acid in glass tubes and centrifuged at 3000 rpm for 15 min. The supernatants were mixed with Tris buffer (0.4 M, pH 8.9) and added 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB, 0.01 M). It was assayed absorbance of reaction content at 412 nm within 5 min of the addition of DTNB against a blank with no homogenate. It was extrapolated the absorbance levels from a glutathione standard curve and defined as GSH (µmol/g tissue).

At the end of the study, all animals were sacrificed under ketamine anaesthesia. The lung tissues were removed. Tissue samples were placed in 10% formalin and prepared for routine paraffin embedding. Paraffin blocks were cut into slices 5 µm thick, mounted on slides, and stained with hematoxylin-eosin (H-E). It was examined lung sections histopathologically for severity of alterations including haemorrhage, inflammatory cell infiltration, thickened alveolar wall, and congestion. Lung injury was classified semi-quantitatively for each criterion as (0) normal, (1) mild, (2) moderate, (3) or severe, with scores ranging between 0 and 12. Lung sections were observed by using a Leica DFC280 light microscope and a Leica Q Win and Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK).

Statistical power analysis was used in order to determine the necessary sample sizes required to detect even minor effects. It was employed the NCSS packet program in order to calculate the necessary of sample sizes for a power of 0.80 was. The analyses were performed by using SPSS software, 22.0 version (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov test was used in order to examine normality of distribution. And the Kruskal-Wallis H test was used in order to examine the data that were not distributed normally. After a significant Kruskal–Wallis H test, a Conover test was also utilized for biochemical and histopathological analyses. A value of $P<0.05$ was accepted as statistically significant. The results were presented as medians (min–max).

RESULTS

No mortality occurred due to varenicline exposure or anaesthesia, none of them caused any animal mortalities. All of the animals survived until the end of the study.

Body weight (BW), lung weight (LW), oxidant/antioxidant parameters (MDA, SOD, CAT, GPx, GSH, and MPO), and lung injury scores are presented in Tables 1, 2, and 3. Acute exposure to varenicline did not lead to a significant decrease in LW and BW in comparison to the control group, whereas, a numeric increase was observed in MDA and MPO levels and a significant decrease was observed in SOD, CAT, GSH, and GPx content. On the other hand, chronic exposure to varenicline significantly increased MDA and MPO and decreased SOD, CAT, GSH and GPx levels in comparison to the control group. In addition, significantly higher levels of MDA and MPO and CAT, SOD, GPx were observed in the lung tissues of the chronic varenicline group and GSH levels were significantly lower than the acute varenicline group.

The histological appearance of the lung tissue was normal in the acute and chronic control groups (Figures 1A and B). The lung sections from V1 and

Table 1. The histopathological damage score of all groups.

Groups	C1 (n=5)	V1 (n=12)	C2 (n=5)	V2 (n=12)
Lung injury score	0 (0-1)	6 (5-7)[a]	0 (0-1)	7 (6-8)[b,c]

Data are expressed median (min-max). [a]p =0.001 vs C1; [b]p =0.001 vs C2; [c]p =0.001 vs V1.

Table 2. Changes of SOD, CAT, GPx activities and GSH, MDA contents in the lung tissue of rats administered by acute and chronic varenicline [Median (min-max)].

		MDA nmol/g tissue	SOD U/g protein	CAT K/g protein	GPx U/mg protein	GSH µmol/g tissue	MPO U/g protein
Acute	Control Group (n=5)	7.37 (6.39-9.12)	46.99 (36.64-50.49)	14.17 (10.82-17.75)	4.28 (2.76-5.66)	0.47 (0.43-0.55)	31.32 (24.69-37.78)
	Varenicline Group (n=12)	7.09 (4.62-15.90)	44.40 (32.15-59.05)	13.34 (18.83-7.73)	3.93 (2.05-6.96)	0.46 (0.28-0.67)	31.65 (22.14-40.26)
Chronic	Control Group (n=5)	7.12 (6.44-8.02)	45.01 (34.12-54.29)	13.20 (8.14-21.81)	3.98 (2.90-4.65)	0.46 (0.41-0.50)	30.90 (20.71-35.43)
	Varenicline Group (n=12)	18.75 (11.19-21.60)[a,b]	14.89 (6.04-18.15)[a,b]	4.87 (3.15-5.12)[a,b]	1.11 (0.7-1.92)[a,b]	0.15 (0.10-0.20)[a,b]	90.78 (70.46-109.12)[a,b]

[a]Significantly different compared with both control groups (p≤0.05); [b]Significantly different compared with acute varenicline group (p≤0.05).

Table 3. Lung weight changes.

		Lung Weight (g)	Body Weight (g)
Acute	Control Group (n=5)	20.1±1.62	300±10
	Varenicline Group (n=12)	18.8±2.81	295±12
Chronic	Control Group (n=5)	19.0±1.47	310±8
	Varenicline Group (n=12)	19.4±2.90	318±12

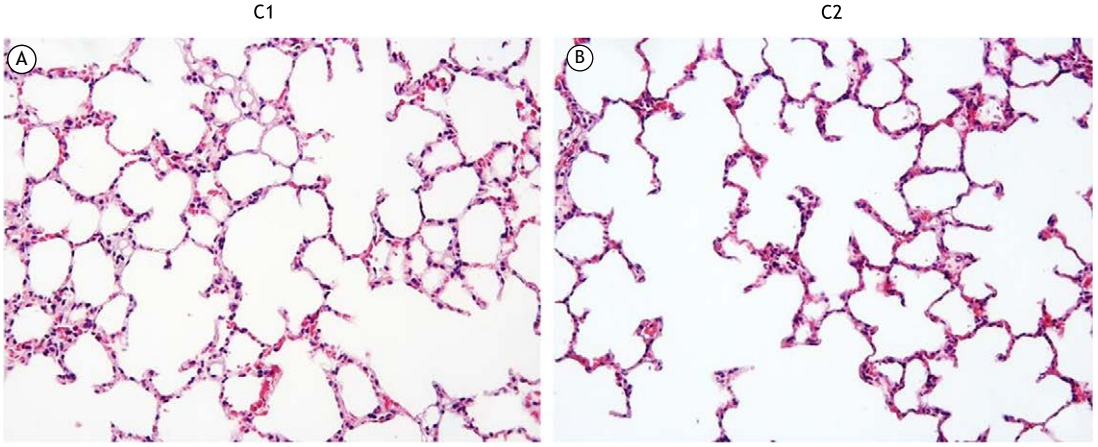

Figure 1. Normal appearance of lung histology in C1 **(A)** and C2 **(B)** groups, H-E; X20. GROUPS. C1: Acute Control (n=5); C2: Chronic Control (n=5).

V2 exhibited some histopathological changes including inflammatory cell infiltration, haemorrhage, thickened alveolar wall, and congestion (Figures 2A, B, C and D). In addition to these changes, it was also observed lipid-laden macrophages (Figures 3A and B) and intrabronchial macrophages in some areas (Figures 3C and D). When C1 and V1 as well as C2 and V2 were compared, significant differences were detected (p=0.001, for all). Significant differences were also noticed between V1 and V2 (p=0.001).

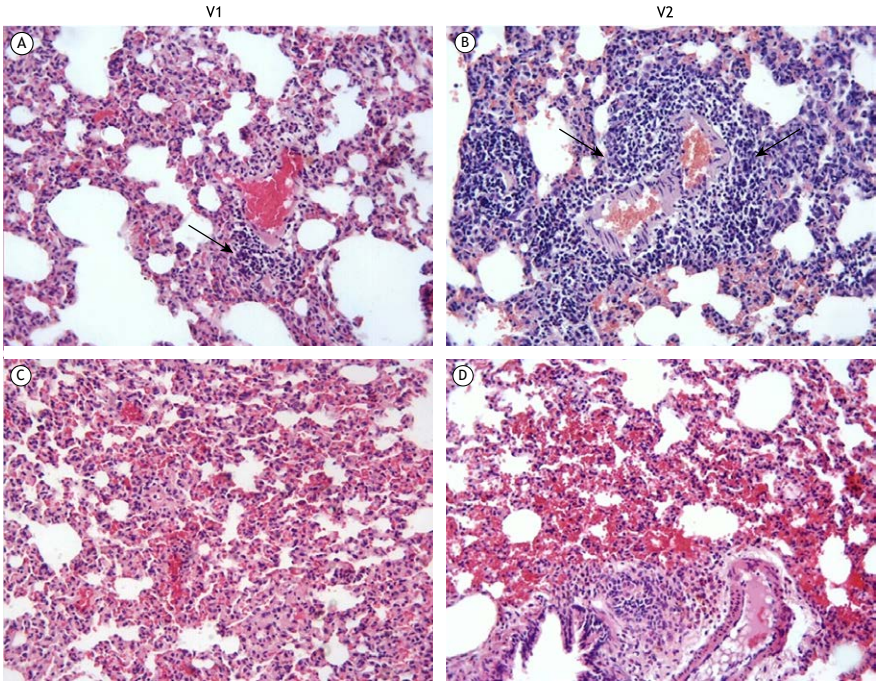

Figure 2. Visible lymphocytes accumulation around the blood vessels **(A** and **B)** (arrows) and congestion of the parenchyma **(C** and **D)** in V1 and V2 goups. It was noticed that inflammation and congestion are more prominent in V2 group than V1 group. H-E; X20. V1: Acute Varenicline (n=12); V2: Chronic Varenicline (n=12).

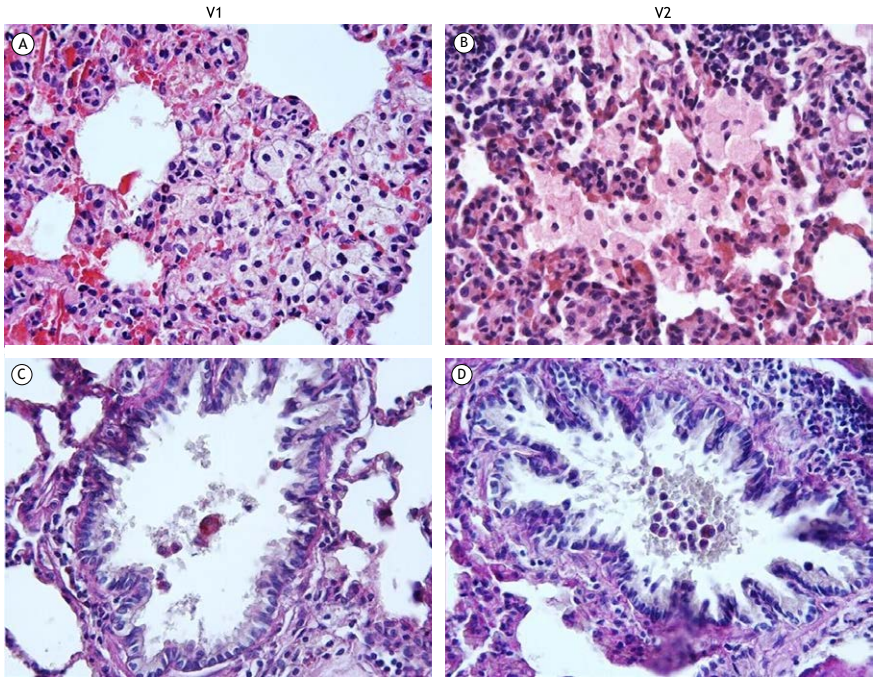

Figure 3. The appearance of lipid-laden macrophages accumulation **(A** and **B),** H-E; X40. Macrophages in bronchial lumen are observed **(C** and **D)** PAS; X40. V1: Acute Varenicline (n=12); V2: Chronic Varenicline (n=12).

DISCUSSION

The purpose of our study was to evaluate the effects of varenicline on lung tissue. In the authors' knowledge, this is the first study that demonstrates the toxic effects of varenicline on lung tissue based on biochemical and histopathological parameters.

Cigarette smoking is an important risk factor for the development of COPD and several other diseases. Additionally, tobacco smoke exposure is associated with exacerbations of pulmonary diseases.[16] Also, it is possible to notice that smoking cessation improves respiratory function and decreases pulmonary symptoms and airway inflammation; for this reason smokers are strongly advised to quit. Comprehensive programs are regarded as the most effective method of reducing tobacco-related mortality and morbidity. Without this type of support, only 6% of attempts to quit smoking are successful.[17,18]

A partial agonist for the nicotinic acetylcholine receptor α4β2 as Varenicline is the drug most widely used to quit smoking.[19] Its side effects include nausea, headache, insomnia, vivid dreams, and gastrointestinal and cardiovascular effects; however, its pulmonary side effects remain unknown.

In this study, it was demonstrated that chronic varenicline treatment increased the level of TBARS, which is an important sign of oxidative stress caused by increasing lipid peroxidation in lung tissue. However, chronic treatment with varenicline significantly reduced the levels of the antioxidants SOD, CAT, GPx, and GSH in lung tissue. MDA, which is a cursor of lipid peroxidation, was created by peroxidation by reactive oxygen species of fatty acids and leads to irreversible cell damage.[20,21] On the other hand, antioxidant defence systems protect the cell against oxidative damage under normal physiological conditions.[22] Oxidative stress results from an imbalance between TBARS and the antioxidant defence system. Membrane lipids, proteins, nucleic acids, and deoxyribonucleic acid molecules are the most sensitive cellular conformations to reactive oxygen species (ROS), which cause to cell injury, membrane damage, protease activation, DNA destruction and protein-lipid peroxidation.[23]

MPO is a highly sensitive index of tissue neutrophil sequestration included in the pathogenesis of various inflammatory diseases and is an inflammatory marker.[24,25] In this work, it was determined that chronic varenicline treatment caused a significant increase in the levels of MPO in the lung tissue leading to pulmonary inflammation.

It was also perceived that chronic varenicline exposure increased inflammatory cell infiltration and congestion histopathologically. The main role of alveolar macrophages is the phagocytosis and to eliminate the inhaled particulates, preventing damage to the delicate and highly functional alveolar epithelium. The word foamy is used generically to describe the vacuolated appearance of alveolar macrophage cytoplasm under light microscopy, which can be classified ultrastructurally based on the presence of lysosomal lamellar bodies, neutral lipid droplets, or drug particles in response to a variety of conditions.[26]

Inflammation is an essential component in the pathogenesis of lung damage that is orchestrated in part by endogenous and migrating leukocytes, which, along with pulmonary epithelial and endothelial cells, it create a feedback loop through which stimuli from cell damage activate alveolar and interstitial macrophages.[27] Activated leukocytes can deliver reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radical, hypochlorous acid, nitric oxide, and peroxynitrite) and proteases that maintain the injury/repair processes that are thought to contribute to fibrotic processes.[28] Data of this study indicate that acute administration of varenicline did not exert oxidative or inflammatory effects on the lung, in contrast to chronic administration.

Also, these results are consistent with another varenicline studies by Selcuk et al. regarding its detrimental cardiovascular effects,[9] which demonstrated adverse effects of chronic varenicline exposure on cardiovascular tissue via electrocardiographic, biochemical, and histopathological analyses. Recently, randomized controlled clinical trials and meta-analyzes of varenicline, that can be safely used in smoking cessation therapy without increased risk of cardiovascular disease have been published.[29,30] There are also clinical studies demonstrating the positive effects of varenicline on smoking cessation in patients with mild, moderate and severe COPD.[31,32] In these studies, no respiratory side effects of varenicline besides the expected side effects were found in COPD patients comparing placebo during a-year period. Tashkin et al.[33] showed that there was no difference between continuous abstainers and continuous smokers in terms of lung function and respiratory symptoms in COPD patients who quit smoking with varenicline following 1 year period, but also they showed improvement in lung function during first 12 weeks. Although it is not methodologically appropriate to compare these results with findings of this sutdy. Once, that the pulmonary function of patients using long-term varenicline for smoking cessation should be closely monitored, because it also was not detected any acute toxicity during the 45-day varenicline treatment period. Moreover, in these clinical studies, the positive effect of smoking cessation on the improvement of pulmonary function should not be ignored. There is only one study that evaluated the prolonged exposure to varenicline in rats.[34] Zaccarelli-Magalhães et al.[34] studied possible toxicity through haematological, biochemical and anatomopathological parameters of 30 days exposure to varenicline. Opposite to the present study, the authors showed no significant kidneys, heart, liver, adrenal glands, spleen and lung tissues alterations, which indicates that varenicline was not able to alter these organs either macroscopic or microscopic aspects. However, the inflammatory and oxidative stress parameters were not evaluated in this study.

It is also relevant to note that the doses and times applied to rats and the doses and duration applied to humans are different. Andreollo et al.[35] showed that rats rapidly develop during childhood and become sexually mature at about six weeks old, but reach social maturity five to six months later. In adulthood, every month of the animal is approximately equivalent to 2.5 human years. The rats of this study were 10-12 weeks of age. According to this, the use of varenicline for 3 months in rats corresponds to 7.5 years of use in humans.

Although, most recently cardiovascular safety of varenicline was reported by Benowitz et al.[29], Selçuk et al.[9] reported that in an animal study, chronic varenicline exposure caused heart weight loss and decreased mean blood pressure, induced lipid peroxidation, and reduced antioxidant activity. Both acute and chronic varenicline exposure caused impairment of mean oxygen saturation. QT interval was prolonged in the chronic varenicline group, while PR interval prolongation was statistically significant in both control and acute varenicline groups. And the authors also confirmed their results both biochemically and histopathologically.

This study had some limitations. The aim was especially on the toxic effects of chronic varenicline administration on lung tissue of rats while ignoring its exposure-dependent effects. If the antioxidant enzymes had been studied in one-week intervals in a study design with a larger sample size, high levels were detected, which would have clearly established that short-term use has no toxic effects. Additionally, the use of advanced analysis methods, molecular mechanisms, and histopathological staining could have helped to assess the effects of varenicline on lung tissue. Studies with different drug doses should be performed to determine the highest effective treatment dose with the least toxic effects. In patients using varenicline, toxic effects can be determined by assessing inflammatory markers in expired air.

In conclusion, it was demonstrated the pulmonary effects of varenicline for the first time in lung tissue of rats. Chronic (3 months in rats, equivalent to 7,5 years in human) varenicline treatment caused inflammation and injury in lung cells by changing the biochemical and histological parameters. However, there is no reference of varenicline use in humans such a long time period.

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ORIGINAL ARTICLE

Nontuberculous mycobacteria in patients with suspected tuberculosis and the genetic diversity of *Mycobacterium avium* in the extreme south of Brazil

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ABSTRACT

Objective: Nontuberculous mycobacteria (NTM) are a heterogeneous group of bacteria that are widely distributed in nature and associated with opportunistic infections in humans. The aims of this study were to identify NTM in patients with suspected tuberculosis who presented positive cultures and to evaluate the genetic diversity of strains identified as *Mycobacterium avium*. **Methods:** We studied pulmonary and extrapulmonary samples obtained from 1,248 patients. The samples that tested positive on culture and negative for the *M. tuberculosis* complex by molecular identification techniques were evaluated by detection of the *hsp65* and *rpoB* genes and sequencing of conserved fragments of these genes. All strains identified as *M. avium* were genotyped using the eight-locus mycobacterial interspersed repetitive unit–variable-number tandem-repeat method. **Results:** We found that NTM accounted for 25 (7.5%) of the 332 mycobacteria isolated. Of those 25, 18 (72%) were *M. avium*, 5 (20%) were *M. abscessus*, 1 (4%) was *M. gastri*, and 1 (4%) was *M. kansasii*. The 18 *M. avium* strains showed high diversity, only two strains being genetically related. **Conclusions:** These results highlight the need to consider the investigation of NTM in patients with suspected active tuberculosis who present with positive cultures, as well as to evaluate the genetic diversity of *M. avium* strains.

Keywords: Mycobacterium avium; Nontuberculous mycobacteria; HIV; Genotyping techniques.

INTRODUCTION

The incidence of nontuberculous mycobacterial (NTM) infections has increased worldwide, attracting attention in routine diagnostic settings, particularly among patients with suspected tuberculosis. Recognized as true pathogens in humans, NTM are major causes of opportunistic infections in people living with HIV.[1] Infections with NTM often occur in the respiratory tract, and such infections can progress to severe lung disease, thus increasing morbidity and mortality.[2]

Among the most important and frequently isolated NTM species are members of the *Mycobacterium avium* complex (MAC), particularly *M. avium* and *M. intracellulare*, followed by *M. abscessus*.[3] Given the ubiquitous presence of MAC in the environment, it has been assumed that exposure to environmental conditions would be the most common form of transmission of these NTM to the host, although it is a huge challenge to prove transmission by an environmental source or directly from patient to patient. However, it has been suggested that there is transmission of *M. abscessus* from patient to patient,[4] and a recent study used a nematode model to determine whether *M. avium* can be transmitted from host to host.[5] The results of that study suggested that *M. avium* may be acquired from a living source, such as an infected patient with a chronic pulmonary disease, as well as from the outside environment.

The distribution of NTM species that cause infections differs by geographic region. Therefore, defining the epidemiology of infections caused by NTM in developing countries is more challenging than delineating that of tuberculosis, because, unlike what is the case for tuberculosis, there is no compulsory reporting of cases of NTM infection.[3,6] To obtain reliable epidemiological data and prescribe the appropriate therapy, it is important to accurately identify the NTM responsible. The identification of acid-fast bacilli or a positive culture does not allow mycobacterial species to be differentiated.[7,8]

The American Thoracic Society issued diagnostic criteria to assist in diagnosis of NTM disease cases.[7] Clinical, radiographic, and (mainly) microbiological data are required; three or more sputum specimens should be collected for microscopy and culture or specimens should be collected by bronchoscopy. Although the diagnosis of NTM disease is based on isolation of the organisms from the culture of diagnostic specimens, simply isolating an

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NTM does not mean that disease is present. There are at least three factors that can help clinicians differentiate between disease and colonization[9]: the bacterial load; the species isolated; and whether or not there is clinical or radiographic progression of disease.

Although species identification can be carried out by biochemical methods, that approach is cumbersome and poorly reproducible. Molecular techniques, such as whole genome sequencing, restriction fragment analysis by PCR, line probe hybridization, and sequencing of *hsp65* and *rpoB* fragments, have been widely used and have a clear advantage over phenotypic methods.[10,11]

In addition to the identification of the species involved in the infectious process, molecular biology tools have allowed the genotyping and differentiation of isolates of the same species, thus allowing epidemiological links to be established. The use of RFLP with IS*1245* as the target, which is considered the gold-standard method for the genotyping of *M. avium* strains, has been changed by the introduction of the mycobacterial interspersed repetitive unit–variable-number tandem-repeat (MIRU-VNTR) method, which presents similar IS*1245* RFLP discriminatory power. The main advantages of the MIRU-VNTR method are its simplicity, the fact that it produces rapid results, and its reproducibility.[12,13]

The main objectives of this study were to determine the prevalence of NTM in patients with suspected tuberculosis who present with positive cultures and to evaluate the clonal diversity of *M. avium*.

METHODS

Study design

This was a retrospective cross-sectional study of pulmonary and extrapulmonary samples collected from 1,248 patients suspected of tuberculosis who were seen at the Dr. Miguel Riet Corrêa Junior University Hospital, in the city of Rio Grande, located in the southern Brazilian state of Rio Grande do Sul. The samples were received at the Mycobacteria Laboratory of the Federal University of Rio Grande between January of 2014 and December of 2016. The characteristics of the patients were collected from medical records and from the Mycobacteria Laboratory database. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande (Reference no. 47/2017).

Experimental procedures

For PCR, sequencing, and genotyping experiments, we used DNA extracted from samples testing positive in liquid culture in an automated culture system (BACTEC Mycobacteria Growth Indicator Tube; Becton Dickinson, Sparks, MD, USA). Samples that were not identified as *M. tuberculosis* by IS*6110* PCR were submitted to PCR for the detection of fragments of the *hsp65* and *rpoB* genes. Samples testing positive for both genes were sequenced for identification of the mycobacterial species. Subsequently, all strains identified as *M. avium* were genotyped by the eight-locus MIRU-VNTR method (Figure 1).

DNA extraction

For DNA extraction, colonies of mycobacteria grown in liquid media were resuspended in 1× Tris-EDTA and incubated for 30 min at 80°C for inactivation of the bacteria. Subsequently, DNA was extracted by the cetyltrimethylammonium bromide/NaCl method, as described by van Soolingen et al.[14]

PCR for hsp65 and rpoB

A fragment of the *hsp65* gene was detected by using the primers TB11 (5′-ACCAACGATGGTGTGTCAT-3′) and TB12 (5′-CTTGTCGAACCGCATACCCT-3′), which amplify a 441-bp fragment. For detection of the *hsp65* gene fragment, PCR was performed as described by Telenti et al.[15] In addition, a fragment of the *rpoB* gene was detected by using the primers MycoF (5′-GGCAAGGTCACCCCGAAGGG-3′) and MycoR (5′-AGCGGCTGCTGGGTGATCATC-3′), which amplify a 764-bp fragment. For detection of the *rpoB* gene fragment, PCR was performed as described by Adekambi et al.[16]

Sequencing

Sequencing was performed in an automated sequencer (ABI 3500 Genetic Analyzer; Applied Biosystems, Foster City, CA, USA). The PCR products were labeled with 5 pmol of TB11 primer (5′-ACCAACGATGGTGTGTCAT-3′, for the *hsp65* gene) or with 5 pmol of MycoF primer (5′-GGCAAGGTCACCCCGAAGGG0-3′, for the *rpoB* gene), together with 1 µL of reagent (BigDye Terminator v3.1 Cycle Sequencing Kit; Applied Biosystems), for 4.5 µL of purified PCR product in a final volume of 10 µL. The labeling reactions were performed in a 96-well thermocycler (Veriti; Applied Biosystems) with denaturation at 96°C for 1 min, followed by 35 cycles at 96°C for 15 s, 50°C for 15 s, and 60°C for 4 min. After labeling, the samples were purified by ethanol/EDTA precipitation and analyzed in the automated sequencer. The sequences obtained were analyzed with the software Chromas, version 2.6 (Technelysium, Southport, Australia), and sequence alignment was performed on the National Center for Biotechnology Information Basic Local Alignment Search Tool site (http://blast.ncbi.nlm.nih.gov).

Genotyping of M. avium

The MIRU-VNTR method was performed with the primers described by Thibault et al.[12] and using eight loci. The PCR was performed as described in the MAC-INMV database (http://mac-inmv.tours.inra.fr). The fragment sizes were determined by the number of tandem repeats at each locus. The PCR products were visualized by 3% agarose gel electrophoresis, involving staining with 0.001 mg/mL ethidium bromide and fluorescence visualization under a UV light source. A 50-bp DNA ladder and a 100-bp DNA ladder (Ludwig

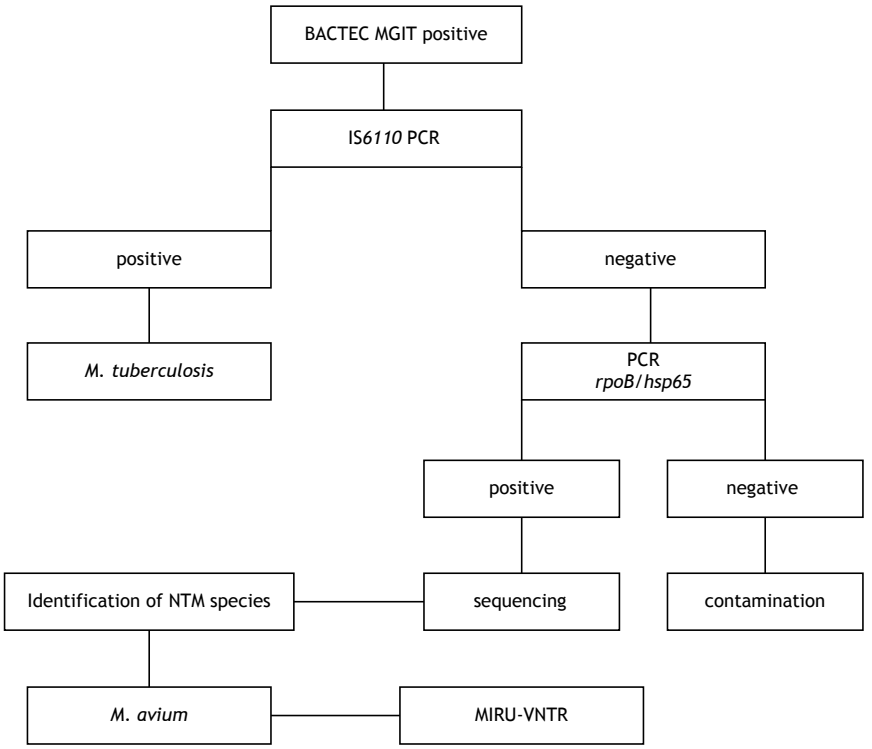

Figure 1. Flow chart of the experimental procedures. MGIT: Mycobacteria Growth Indicator Tube; *M.*: *Mycobacterium*; NTM: nontuberculous mycobacteria; and MIRU-VNTR: mycobacterial interspersed repetitive unit–variable-number tandem-repeat (method).

Biotec, Alvorada, Brazil) were used in order to define the size of the PCR products.

The allelic diversity of each MIRU-VNTR locus was calculated by the following equation:

$$h = 1 - \sum xi^2 [n / (n - 1)]$$

where h is the heterozygosity at the locus, xi is the allele frequency at the locus, and n is the number of strains. According to the h, the discriminatory power of the loci was classified as high (h > 0.6), moderate (h ≤ 0.6), or low (h < 0.3).[17]

RESULTS

Identification of NTM

Of the 1,248 patients suspected of having tuberculosis, 332 had positive mycobacterial cultures, 25 (7.5%) of whom were found to be infected with NTM. Of those 25 patients, 20 (80%) had undergone HIV testing and 13 (52%) were HIV positive. In addition, 18 (72%) were men, whereas only 7 (28%) were women, and the median age was 46 years (range, 26-78 years).

The NTM species were identified as *M. avium* in 18 (72%) of the 25 patients, as *M. abscessus* in 5 (20%), as *M. gastri* in 1 (4%), and as *M. kansasii* in 1 (4%). Of the 18 patients infected with *M. avium*, 10 (55.5%) were HIV positive, 5 (20.0%) were HIV negative, and 3 (16.7%) were of unknown HIV status. As can be seen in Table 1, 23 (92%) of the samples in which NTM species were identified were of pulmonary origin (sputum, bronchoalveolar lavage fluid, or tracheal aspirate).

Genotyping of M. avium

Eighteen strains of *M. avium* were analyzed by eight-locus MIRU-VNTR, resulting in 16 (88.9%) being classified as orphan strains and 2 being grouped to form the only cluster (Figure 2). As detailed in Table 2, we identified 17 previously unknown INMV patterns and one known pattern (INMV 78).

The values of allelic diversity in the samples analyzed were calculated for each locus and are presented in Table 3. Loci X3, 25, 10, and 32 were highly discriminatory (h ≥ 0.6); X3 and 10 were the most polymorphic, with eight different alleles each.

DISCUSSION

The increase in the incidence of NTM infections in cases of suspected tuberculosis is a huge challenge in clinical practice. The possible explanations for the increase in the number of such cases include the improvement in the diagnostic capabilities of laboratories and greater awareness of such infections in clinical settings.[18] In the present study, NTM were identified in 7.5% of the positive cultures in patients with suspected tuberculosis. This result is consistent with those of other studies, in which NTM were identified in 4-10% of positive cultures in such patients.[19-21]

Table 1. Characteristics of patients infected with nontuberculous mycobacteria.

Patient	Age	Gender	HIV status	CD4 count (cells/mm³)	Clinical specimen	Sequencing result
1676	71	F	Positive	12	Sputum	*M. abscessus*
1871	67	M	Negative		Sputum	*M. avium*
1895	41	M	Positive	56	BALF	*M. avium*
1896	58	M	ND		Sputum	*M. avium*
1901	69	M	ND		Sputum	*M. abscessus*
2006	46	F	Negative		Sputum	*M. abscessus*
2091	26	F	Positive	54	LB	*M. avium*
3036	37	F	Positive	183	BALF	*M. avium*
3145	28	F	Positive	544	Sputum	*M. avium*
3168	46	M	Positive	152	TA	*M. avium*
3366	49	M	Negative		Sputum	*M. avium*
3390	30	F	Positive	ND	Sputum	*M. avium*
3491	54	M	Positive	22	Sputum	*M. gastri*
3471	38	M	Positive	266	Sputum	*M. avium*
3594	58	M	ND		Sputum	*M. avium*
3717	55	M	ND		Sputum	*M. avium*
3811	32	M	Positive	290	Sputum	*M. avium*
3870	42	M	Positive	4	Sputum	*M. avium*
3913	36	F	Negative		Sputum	*M. avium*
4111	78	M	Negative		Sputum	*M. avium*
4127	59	M	ND		Sputum	*M. kansasii*
4161	40	M	Positive	55	Sputum	*M. abscessus*
4307	45	M	Negative		BALF	*M. abscessus*
4425	46	M	Positive	153	CG	*M. avium*
4695	37	M	Negative		Sputum	*M. avium*

F: female; M: male; *M.*: *Mycobacterium*; BALF: BAL fluid; ND: no data; LB: liver biopsy; TA: tracheal aspirate; and CG: cervical ganglion.

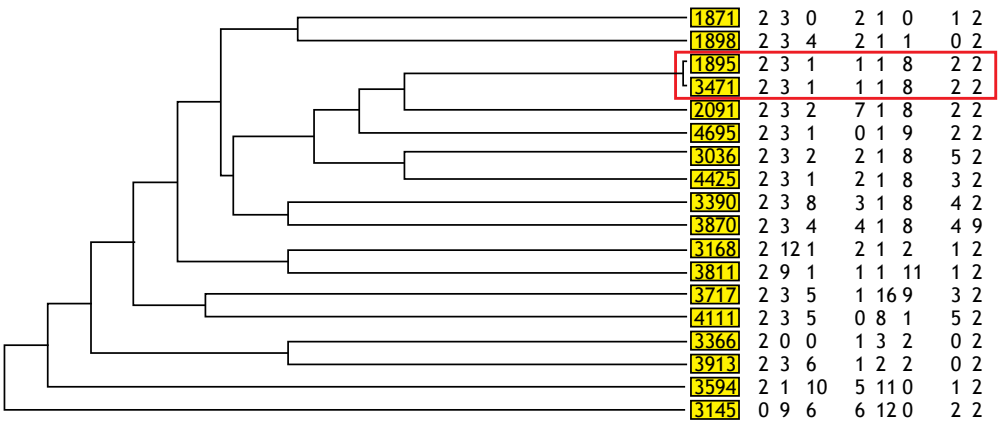

Figure 2. Epidemiological links between patients.

Other studies have shown that the prevalence of NTM is higher in men and in individuals over 40 years of age (72% and 68%, respectively).[22,23] In addition, 92% of NTM are isolated from respiratory specimens and individuals of advanced age could therefore be more susceptible to respiratory infections caused by NTM.[19]

It should be noted that the incidence of NTM infection can be 9.7-fold higher in patients with HIV infection, especially those with CD4 cell counts < 100 cells/mm³.[24] Recent studies conducted in Brazil have reported that *M. avium* is the NTM species most often isolated from respiratory specimens in HIV-infected patients.[25,26]

The present study was carried out at a referral hospital for HIV-infected patients, located in the Brazilian state of Rio Grande do Sul, where the prevalence of HIV infection is 38.3 cases/100,000 population, the second highest among all of the states in the country.[27] Among the patients infected with NTM, most were infected with *M. avium* (72.0%), and 55.5% of those patients were coinfected with HIV. Such infections

Table 2. Patterns of 18 *Mycobacterium avium* strains evaluated with the mycobacterial interspersed repetitive unit–variable-number tandem-repeat method.

Patient	Loci of interest in the MIRU-VNTR method								INMV pattern
	292	X3	25	47	3	7	10	32	
	Number of tandem repeats								
1871	2	2	1	3	1	2	0	0	Not yet listed
1895	2	1	2	3	1	2	1	8	Not yet listed
1896	2	2	0	3	1	2	4	1	Not yet listed
2091	2	7	2	3	1	2	2	8	Not yet listed
3036	2	2	5	3	1	2	2	8	Not yet listed
3366	2	1	0	0	3	2	0	2	Not yet listed
3390	2	3	4	3	1	2	8	8	Not yet listed
3471	2	1	2	3	1	2	1	8	Not yet listed
3594	2	5	1	1	11	2	10	0	Not yet listed
3717	2	1	3	3	16	2	5	9	Not yet listed
3811	2	1	1	9	1	2	1	11	Not yet listed
3870	2	4	4	3	1	9	4	8	Not yet listed
3913	2	1	0	3	2	2	6	2	Not yet listed
4695	2	0	2	3	1	2	1	9	Not yet listed
4425	2	2	3	3	1	2	1	8	78*
3145	0	6	2	9	12	2	6	0	Not yet listed
3168	2	2	1	12	1	2	1	2	Not yet listed
4111	2	0	5	3	8	2	5	1	Not yet listed

MIRU-VNTR: mycobacterial interspersed repetitive unit–variable-number tandem-repeat; INMV: MAC-*Institut National de la Recherche Agronomique* (French National Institute for Agricultural Research) Nouzilly MIRU-VNTR; and *M.*: *Mycobacterium*. **M. avium* subsp. *paratuberculosis*.

Table 3. Allelic diversity using the eight-locus mycobacterial interspersed repetitive unit–variable-number tandem-repeat method.

N of alleles	MIRU 292	MIRU X3	MIRU 25	MIRU 47	MIRU 3	MIRU 7	MIRU 10	MIRU 32
0	1	2	3	1			2	3
1		6	4	1	12		6	2
2	17	5	5		1	17	2	3
3		1	2	13	1			
4		1	2				2	
5		1	2				2	
6		1					2	
7		1						
8					1		1	7
9				2		1		2
10							1	
11					1			1
>12				1	2			
Measures of diversity								
h	0.104	0.783	0.808	0.456	0.540	0.104	0.820	0.765
DP*	Low	High	High	Mod	Mod	Low	High	High

MIRU: mycobacterial interspersed repetitive unit; h: heterozygosity; DP: discriminatory power; and Mod: moderate. *Discriminatory power is defined, according to the allelic diversity (heterozygosity), as high (h > 0.6), moderate (h ≤ 0.6), or low (h < 0.3).

generate high morbidity and economic costs, because the current treatments have multiple side effects and an intention-to-treat cure rate < 50%.[28]

In Brazil, the available data suggest regional differences in the distribution of NTM species, especially in the relative proportions of MAC and *M. kansasii*. Carneiro et al.[25] also found MAC species to be the most common NTM species causing respiratory infection in the state of Rio Grande do Sul. However, at a referral center in the state of Rio de Janeiro, *M. kansasii* was found to account for a third of all NTM infections.[29] The fact that there are a greater number of HIV-infected patients in the state of Rio Grande do Sul than in the state of Rio de Janeiro is the most likely reason for

the higher prevalence of respiratory infection caused by MAC in the former.[29] It has been suggested that, in addition to the presence of cofactors such as HIV infection, host and environmental factors interact to influence the risk of disease and the geographic distribution of NTM infection.

Infection with *M. avium* can have clinical and radiological presentations indistinguishable from those of tuberculosis, making its differentiation and diagnosis difficult. The accurate identification of NTM species is critical because the management and treatment of infected patients, as well as the epidemiological control tools implemented, should reflect the specific mycobacterial species isolated and its sources.[30]

Studies involving the epidemiology of *M. avium* have been based on typing methods such as RFLP analysis using IS*1245* as a probe and are now based on typing methods such as MIRU-VNTR.[13] Thibault et al.[12] standardized the MIRU-VNTR method using eight loci to study variability in *M. avium* strains obtained from different hosts and from different geographic regions.

In the last five years, several genotypes of *M. avium* from diverse hosts (humans and animals) have been identified and registered in a web application known as the MAC-INMV database (http://mac-inmv.tours.inra.fr).[12] In the present study, we have described, for the first time, seventeen patterns that will later be included in the database. The only pattern that had previously been described was INMV 78 (*M. avium* subsp. *paratuberculosis*), which was previously isolated from a goat.[31] That pattern differs from those previously reported to be the most prevalent in different parts of the world (INMV 1 and 2).[12,32] However, caution is needed when using VNTR subtyping, given that it may overestimate or underestimate the relationship between strains because of the instability of some repetitive elements in the genome.[33]

Despite the wide acceptance of the MIRU-VNTR method, it is often difficult to make comparisons across studies reporting the results obtained with the method, because of the lack of standardization. Such studies have involved various hosts (such as cattle, goats, and sheep), loci (such as 7, 8, 16, and 20), and methodologies.[32,34,35] However, eight specific loci (292, X3, 25, 47, 3, 7, 10, and 32) are the most commonly used in the MIRU-VNTR method and have shown high discriminatory power.[12,36]

In the present study, we found only one strain cluster, comprising two strains of *M. avium*. We find it interesting that patients 1895 and 3471 occupied the same bed in the hospital within a short time period (30 days). Both were HIV positive and were immunocompromised, according to their CD4 count. The first patient to occupy the bed (patient 1895) reported having worked in fields and having had contact with birds. Although there are reports that humans and animals acquire *M. avium* infection from environmental sources,[37] direct transmission between animals and humans cannot be excluded, because the genetic profiles of strains isolated from both hosts are similar. In addition, soil, water, and biofilms can be important sources of transmission of *M. avium* because of its ability to survive for a long time (200-600 days) in those environments.[36-38]

In relation to allelic diversity, some loci were highly discriminatory and should be prioritized for rapid differentiation of *M. avium* strains. According to one previous study,[39] X3 is one of the most discriminatory loci, as are loci 3 and 10, although the last two were described as being less suitable for typing. The seven loci presented low allelic diversity (0.104), which is consistent with the findings of another study.[40]

In the present study, *M. avium* was the NTM most frequently identified among positive cultures in cases of suspected tuberculosis. In addition, HIV infection was the main condition predisposing patients to the development of NTM infectious diseases.

To our knowledge, this was the first study using the eight-locus MIRU-VNTR method as a tool to evaluate the clonal diversity of *M. avium* strains isolated from humans in the extreme south of Brazil. We observed high clonal diversity, with only one cluster (comprising two strains). It is noteworthy that the two strains in the cluster were obtained from patients who had an epidemiological link. Although we cannot affirm that there was a connection between those two cases, we also cannot rule it out.

Our study was limited by the small number of *M. avium* strains studied. However, our findings highlight the need to implement the rapid, accurate identification of NTM in positive cultures in patients with suspected tuberculosis, as well as to use molecular tools to monitor the clonal diversity of *M. avium* strains and establish possible epidemiologic links.

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Presentation of pulmonary infection on CT in COVID-19: initial experience in Brazil

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ABSTRACT

The disease caused by the new coronavirus (SARS-CoV-2), designated COVID-19, emerged in late 2019 in China, in the city of Wuhan (Hubei province), and showed exponential growth in that country. It subsequently spread to all continents, and infection with SARS-CoV-2 is now classified as a pandemic. Given the magnitude achieved, scientific interest in COVID-19 has also grown in the international literature, including its manifestations on imaging studies, particularly on CT. To date, no case series have been published in Brazil. Therefore, our objective was to describe the CT findings in an initial series of 12 patients.

Keywords: Coronavirus infections; Coronavirus; Multidetector computed tomography.

In late 2019, a new coronavirus—severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—was identified as the causative agent of an outbreak of pneumonia in China, in the city of Wuhan (Hubei province). An association was found between the first cases and a local food market that sold live animals, where most of such patients had been on business or visiting.

The spread of the new coronavirus was rapid, resulting in an epidemic, with the main form of transmission being person-to-person, either via the airways or by touching contaminated surfaces and then the eyes, nose, or mouth. The epidemic hit other continents, and the disease was called coronavirus disease 2019 (COVID-19). New cases started being reported in other countries, initially in individuals who had travelled to China and in people who had had contact with them, and outbreaks related to local transmission were subsequently documented.[1-4] COVID-19 is currently considered a pandemic.

The spectrum of clinical presentation of COVID-19 is wide, ranging from absence of symptoms to critical disease. Most respiratory infections are mild, but severe or critical forms have been also described, especially in the elderly and individuals with comorbidities, who can present with dyspnea, hypoxemia, extensive pulmonary involvement on imaging studies, respiratory failure, shock, and multiple organ failure. The estimated COVID-19 mortality in China was 2.3%, most cases having occurred in elderly patients or patients with comorbidities (cardiovascular disease, diabetes mellitus, chronic lung disease, hypertension, and cancer).[5] However, the reported mortality rates vary in different populations; in Italy, for example, where the mean age of the affected population is higher, the currently reported mortality rate is approximately 5.8%.[6]

The possibility of COVID-19 should be considered in symptomatic patients who, in the past 14 days, have had close contact with a suspected or confirmed case of COVID-19, have been in areas where widespread transmission has been documented, or have had potential exposure due to participation in events or have been in places where cases of COVID-19 have been reported. Among the most commonly reported clinical manifestations of COVID-19 are fever, fatigue, dry cough, anorexia, myalgia, dyspnea, and sputum production.[7]

The diagnosis of COVID-19 is confirmed through detection of SARS-CoV-2 RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) from nasopharyngeal or oropharyngeal swab samples. However, if RT-PCR is negative and clinical suspicion is high, the recommendation is that RT-PCR testing be performed again, including on swabs from other respiratory sites. It is also worth highlighting the value of testing for other viruses that can clinically present in a similar way.

Multiple articles have been published reporting CT findings in COVID-19, even in patients with negative RT-PCR results, arousing interest in the role of CT in the current clinical scenario. The American College of Radiology and the Brazilian College of Radiology recommend that chest CT be used in hospitalized patients with pneumonia symptoms and with specific clinical indications for CT; it is important to point out that neither of them recommend using CT for screening of COVID-19 or as the test of choice for diagnosing COVID-19.[8,9]

Chest CT can help in the diagnosis of COVID-19, but it cannot confirm or exclude it alone. When RT-PCR is used as a reference, chest CT has high sensitivity (97%) but low specificity (25%), given the overlap of findings with those of respiratory infections of different etiologies.[10]

The most commonly observed CT findings in cases of COVID-19 are pulmonary ground-glass opacities and, occasionally, consolidations, with a predominantly peripheral distribution, sometimes associated with fine reticulation (forming the crazy-paving pattern), vascular

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thickening, and the reversed halo sign. Central parenchymal involvement or the presence of nodules, cavitation, lymph node enlargement, or pleural effusion are less common.[11-13] It has been suggested that the reversed halo sign, described by some authors, indicates that organizing pneumonia might be one of the mechanisms of lung injury.[14,15]

The objective of the present study was to describe the major CT findings in the first case series of COVID-19 in Brazil. In this initial experience in Brazil, involving the first 12 patients with a confirmed diagnosis of COVID-19 (based on RT-PCR results) who underwent chest CT at our facility, the following CT features were observed: ground-glass opacities, in 12 patients (100%); crazy-paving pattern, in 7 (58%); alveolar consolidation, in 4 (33%); reversed halo sign, in 1 (8%); and pleural effusion, in 1 (8%). Nodules, cavitation, and lymph node enlargement were not identified in our sample.

We observed involvement of both lungs in 11 patients (92%) and a peripheral predominance in 9 patients (75%); the changes affected mainly the lower lobes in 8 patients (67%), were multilobar and relatively diffuse in 3 patients (25%), and predominated in the left lower lobe in 1 patient (8%).

Figure 1 illustrates a typical case and demonstrates the evolution of the findings, whereas Figure 2 demonstrates not only the major changes caused by the disease but also another possible but relatively uncommon finding (small pleural effusion).

Because the major imaging finding in COVID-19 is ground-glass opacities, chest X-ray plays a less important role in the imaging evaluation of patients, given that the sensitivity of chest X-rays for detecting this type of opacity is low. However, of course, chest X-rays can be useful in monitoring inpatients, including those who are in the ICU, because it is a widely available, rapid, and inexpensive test that enables more frequent (often daily) monitoring of the extent of the pulmonary involvement in the disease.

It has been demonstrated that asymptomatic individuals can present with pulmonary findings (clinical-radiological dissociation), but less frequently than do symptomatic patients and, in general, with less extensive involvement and predominant ground-glass opacities. In contrast, symptomatic patients more commonly present with pulmonary findings, with predominant consolidations and more extensive parenchymal involvement.[16]

Despite the variety of presentations, it has generally been reported that, in the first 4 days after symptom onset, the ground-glass opacity pattern predominates. Between days 5 and 8 after symptom onset, there is an increase in the extent of the pulmonary involvement, with the presence of crazy-paving pattern and consolidations. Between days 9 and 13 after symptom onset, when CT findings are usually at their peak, consolidations are noted to predominate; after day 14, the process of consolidation resorption begins and the crazy-paving pattern tends to resolve, although ground-glass opacities may persist.[17] The resolution of the findings is usually relatively slow, taking approximately 30 days, and scarring changes in the lung parenchyma have been described.

The major CT features identified in the first 12 Brazilian patients evaluated at our facility are very similar to those being described in the literature and fundamentally include bilateral multifocal ground-glass

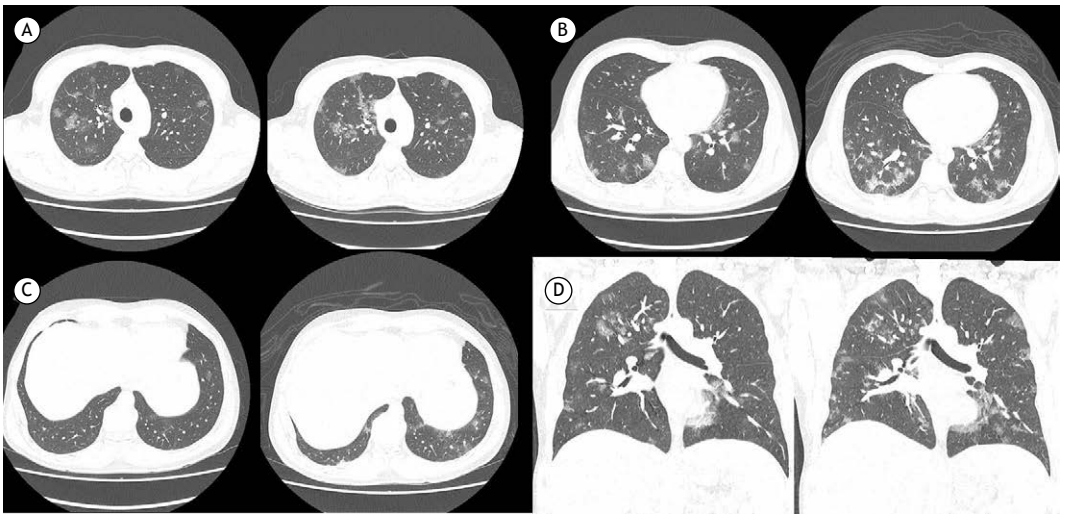

Figure 1. Axial images (in A to C) and coronal reconstructions (in D) of chest CT scans of a 44-year-old man with clinical findings suggestive of COVID-19 (fever, sore throat, and frequent dry cough), demonstrating the most commonly described pattern: numerous bilateral multifocal ground-glass opacities, associated with fine reticulation and interlobular septal thickening (crazy-paving pattern), involving various lung lobes and being predominantly peripheral in distribution in the parenchyma and a little more extensive in the posterior regions of the lower lobes. The patient had a positive RT-PCR result for COVID-19 on the day he underwent the first CT scan (images on the left in each pair) and was hospitalized. A second CT scan, which was performed three days later (images on the right in each pair) because he continued to have fever spikes and dry cough, demonstrated an increase in the number and extent of pulmonary opacities.

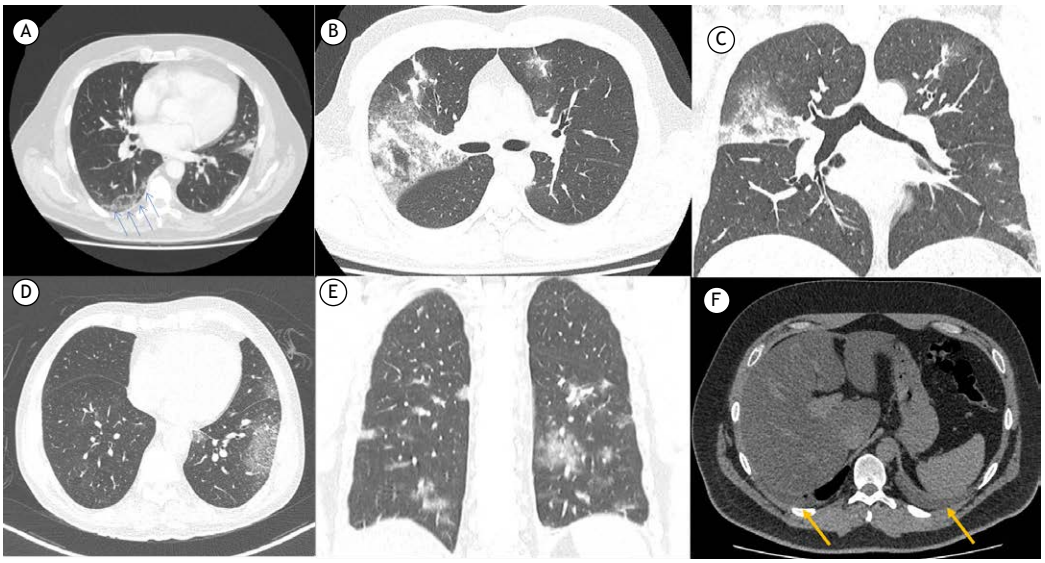

Figure 2. Chest CT scans of different patients illustrating the spectrum of findings of COVID-19 in our sample. In A, a 61-year-old male patient with peripheral and posterior ground-glass opacities in the lower lobes (blue arrows in the right lower lobe), as well as a focus of parenchymal opacification in the lingula. In B and C, a 41-year-old male patient with extensive ground-glass opacities associated with septal thickening and fine reticulation (crazy-paving) in the right upper lobe, in addition to other small scattered foci in the upper and lower left lobes. In D, an 85-year-old male patient with ground-glass opacities, associated with fine reticulation and thickening of some interlobular septa, extending mostly into the periphery of the left lower lobe, but also present in the lingula and in the right lower lobe. In E and F, a 42-year-old male patient with ground-glass opacities and bilateral foci of consolidation, predominantly in the most posterior regions of the lower lobes. The patient also had bilateral minimal pleural effusion (arrows), a relatively uncommon finding in patients with COVID-19. In addition, signs suggestive of hepatic steatosis were identified.

opacities, sometimes associated with fine reticulation and septal thickening (crazy-paving pattern), in general involving several lung lobes and being predominantly peripheral in distribution in the parenchyma. The low incidence of pleural effusion and the absence of other findings, such as lymph node enlargement, nodules, and cavitary lesions, are in line with recent international experiences.[10-17]

The most common CT findings in COVID-19 overlap with those observed in pulmonary infections caused by other agents (particularly other viruses), and, ideally, the definitive diagnosis of COVID-19 should be based on RT-PCR results. Nevertheless, given the magnitude achieved by COVID-19 in recent months, with the disease being classified as a pandemic by the World Health Organization, it is essential that clinicians and radiologists be familiar with the most common imaging presentations of COVID-19, as well as with the expected evolution of the findings, so that they can contribute to earlier identification of cases and thus to reducing the consequences and mortality of the disease.

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SPECIAL ARTICLE

Brazilian guidelines for the pharmacological treatment of idiopathic pulmonary fibrosis. Official document of the Brazilian Thoracic Association based on the GRADE methodology

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ABSTRACT

Idiopathic pulmonary fibrosis (IPF) is a form of chronic interstitial lung disease of unknown cause, which predominantly affects elderly men who are current or former smokers. Even though it is an uncommon disease, it is of great importance because of its severity and poor prognosis. In recent decades, several pharmacological treatment modalities have been investigated for the treatment of this disease, and the classic concepts have therefore been revised. The purpose of these guidelines was to define evidence-based recommendations regarding the use of pharmacological agents in the treatment of IPF in Brazil. We sought to provide guidance on the practical issues faced by clinicians in their daily lives. Patients of interest, Intervention to be studied, Comparison of intervention and Outcome of interest (PICO)-style questions were formulated to address aspects related to the use of corticosteroids, N-acetylcysteine, gastroesophageal reflux medications, endothelin-receptor antagonists, phosphodiesterase-5 inhibitors, pirfenidone, and nintedanib. To formulate the PICO questions, a group of Brazilian specialists working in the area was assembled and an extensive review of the literature on the subject was carried out. Previously published systematic reviews with meta-analyses were analyzed for the strength of the compiled evidence, and, on that basis, recommendations were developed by employing the Grading of Recommendations Assessment, Development and Evaluation approach. The authors believe that the present document represents an important advance to be incorporated in the approach to patients with IPF, aiming mainly to improve its management, and can become an auxiliary tool for defining public policies related to IPF.

Keywords: Idiopathic pulmonary fibrosis; GRADE approach; Pulmonary fibrosis/drug therapy; Practice guideline.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a form of chronic interstitial lung disease of unknown cause, limited to the lungs, which predominantly affects elderly men who are current or former smokers.[1-5] From a histological standpoint, IPF is characterized by the usual interstitial pneumonia pattern that can currently be inferred with a reasonable degree of certainty in cases of typical radiological findings on HRCT.[1-5] Even though it is an uncommon disease, IPF is of great clinical importance because of its severity. Although the natural history of the disease may vary and it is difficult to make accurate prognostic predictions for a given patient, the median survival for untreated patients with IPF is only 2.9 years.[6]

In recent decades, several pharmacological treatment modalities, with varied mechanisms of action, have been investigated for the treatment of IPF, and a substantial number of studies have reported negative outcomes.[7-36] Nevertheless, new drugs have shown benefits for the

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treatment of this disease, and some of them are already commercially available for this indication.

The purpose of these guidelines was to define evidence-based recommendations regarding the use of pharmacological agents in the treatment of IPF. We sought to provide guidance on the practical issues faced by clinicians in their daily routine. To that end, we carried out an extensive review of the literature on the subject, employing the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach.[37] It should be noted that, to date, there have been no studies using a methodology similar to that employed here to address the topic in Brazil.

METHODOLOGY

The development of the guidelines began with the formation of a group of coordinators, which included two recognized specialists in the subject area and two specialists in methodology. Pulmonologists familiar with the care of patients with IPF, working in different regions of Brazil, were invited to join a specialist committee. Everyone involved in the process provided signed conflict-of-interest forms (Chart S1, supplementary material: http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=73).

In a face-to-face meeting, held in September of 2017 in the city of São Paulo, Brazil, the objectives of the project were defined. It was decided that the GRADE approach would be used and that priority would be given to questions related only to the pharmacological treatment of IPF. The specialists then received written materials and training videos related to each step of the GRADE approach.[38-40]

The specialists formulated Patients of interest, Intervention to be studied, Comparison of intervention and Outcome of interest (PICO)-style questions related to the pharmacological treatment of patients with IPF. Through an online voting process, seven PICO questions and their corresponding highest-scored outcomes were selected on the basis of degree of importance. The outcomes were classified as unimportant, important, or critical, taking into account the IPF patient perspective, in accordance with the GRADE approach (Chart 1).

A librarian searched for articles published in English in PubMed and EMBASE, following a standardized methodology, under the supervision of the methodologists (Chart S2). In our search strategy, we focused on systematic reviews with meta-analyses, using pre-established keywords and covering a period of 10 years or less, with an inclusion data limit of November 2018. A decision was made to employ a pragmatic strategy of searching for completed meta-analyses, rather than searching for clinical trials and subsequently performing meta-analyses.

After the preliminary selection of articles, the methodologists separately evaluated the articles by reviewing their titles and abstracts to decide which ones would be included in the guidelines. Disagreements were resolved by consensus. The next step involved qualitative analysis of the full texts of the selected articles, which was carried out by the two methodologists, working independently. Again, disagreements about inclusion or exclusion of articles were resolved by consensus. The selected articles were then evaluated by the specialist coordinators, each working separately, and agreement among the analyses regarding the inclusion or exclusion of articles was assessed. The reasons for excluding articles, as presented in Figures S1 through S7 of the supplementary material, were documented in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations.[41]

Tables summarizing the evidence for each question (Tables S1 through S7) were prepared following the GRADE approach, using the GRADEpro Guideline

Chart 1. Questions and corresponding outcomes selected for the development of these guidelines.

Question	Critical outcome	Important outcome	Unimportant outcome
1. Should we recommend the use of nintedanib for patients with IPF?	Mortality Decline in FVC Number of exacerbations	Quality of life	Adverse events
2. Should we recommend the use of pirfenidone for patients with IPF?	Mortality Decline in FVC Number of exacerbations	Quality of life	Adverse events
3. Should we recommend the use of phosphodiesterase-5 inhibitors for patients with IPF?	Mortality	Quality life Dyspnea	–
4. Should we recommend the use of endothelin-receptor antagonists for patients with IPF?	Mortality Decline in FVC	–	Adverse events
5. Should we recommend pharmacological treatment of gastroesophageal reflux for patients with IPF?	Mortality Decline in FVC Number of exacerbations Number of hospitalizations	–	–
6. Should we recommend the use of N-acetylcysteine for patients with IPF?	Mortality Decline in FVC	–	–
7. Should we recommend the use of corticosteroids for patients with IPF?	Mortality Decline in FVC	–	–

IPF: idiopathic pulmonary fibrosis.

Development Tool (GDT; McMaster University, HamiltonON, Canada).[42] The quality of the evidence for each meta-analysis included, as a function of each analyzed outcome, was classified as high, moderate, low, or very low (Chart 2).

The quality of evidence was reduced by one or two grades if a risk of bias, indirect evidence, inconsistency, imprecision, or publication bias was identified. In contrast, the quality of evidence was upgraded if there was a strong association, no plausible confounders, or a dose response relationship, or if all plausible confounders would have reduced the effect (Chart 3). In the GRADE approach, the quality of the studies determines the confidence level and degree of certainty of the estimated effect of the intervention on each of the outcomes selected.[40]

In September of 2019, the Coordinating Committee met face to face with the specialists, in the city of São Paulo, to review the results and all tables summarizing the evidence. The attending members reviewed the tables, and corrections were made as appropriate.

Recommendations based on critical outcomes were made for each question, following the GRADE approach (Chart 4). When there was no consensus, votes were taken, the results of which were documented (Chart S3). The recommendations could be either strong or conditional.[40] The term "we recommend" was used for strong recommendations, and the term "we suggest" was used for conditional recommendations. Factors influencing the strength of recommendation included the balance between benefits and undesirable consequences, the overall quality of evidence, patient values/preferences, costs, and resource allocation.

For the preparation of the manuscript, the text was divided among the participants, each of whom returned their sections to the specialist coordinators within certain deadlines. A preliminary version of the guidelines was then edited and sent to all of the participants for corrections and suggestions, in an interactive process. All of the individuals listed as authors take responsibility for the final text, in its entirety. The seven PICO questions, as well as the evidence, recommendations, and comments related to them, are described below.

QUESTION 1: SHOULD WE RECOMMEND THE USE OF NINTEDANIB FOR PATIENTS WITH IPF?

Nintedanib was initially developed as an inhibitor of VEGF and FGF receptors, being intended for use in the treatment of cancer.[43] However, since nintedanib inhibits PDGF receptors, it has also been investigated as a therapy for IPF.[43] Nintedanib competitively inhibits tyrosine kinases, which explains its many potential actions, such as impeding the migration and proliferation of myofibroblasts and fibroblasts, as well as the deposition of extracellular matrix. In addition, more recent evidence indicates that nintedanib can reduce TGF-β production, inhibit the formation of the collagen fibrin network, and stimulate surfactant protein D production.[44-46]

Chart 2. Quality of evidence interpretation following the Grading of Recommendations Assessment, Development and Evaluation system.[a]

Quality of evidence	Implication	Example
High ⊕⊕⊕⊕	Further research is very unlikely to change our confidence in the estimate of effect; we are confident that we can expect a very similar effect in the population for which the recommendation is intended	Randomized trials without serious limitations Well-performed observational studies with very large effects
Moderate ⊕⊕⊕◯	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate	Randomized trials with serious limitations Well-performed observational studies yielding large effects
Low ⊕⊕◯◯	Further research is very likely to have a major impact on our confidence in the estimate of effect and is likely to change the estimate	Randomized trials with very serious limitations Observational studies without special strengths or important limitations
Very low ⊕◯◯◯	Any estimate of effect is very uncertain	Randomized trials with very serious limitations and inconsistent results Observational studies with serious limitations Unsystematic clinical observations (e.g., case series or case reports)

[a]Adapted from the Brazilian National Ministry of Health.[37]

Chart 3. Factors that can affect the quality of evidence.[a]

Quality of evidence	Reasons for downgrading	Reasons for upgrading
• High • Moderate • Low • Very low	• Risk of bias • Indirect evidence • Inconsistency • Imprecision • Publication bias	• A strong association, with no plausible confounders • Evidence of dose response • All plausible confounders would have reduced the effect

[a]Adapted from Guyatt et al.[38]

One phase II clinical trial and two phase III clinical trials, all observing subjects over a one-year period, evaluated the effects that nintedanib at a target dose of 150 mg twice daily had on the rate of decline in FVC.[27,28] On the basis of the results observed for this primary outcome, the US Food and Drug Administration approved nintedanib for use in patients with IPF.[47] The most common adverse effects of nintedanib, when it is used at the doses recommended for the treatment of IPF, are related to the gastrointestinal tract, especially diarrhea, of varying intensity, which, in the original clinical trials, affected approximately 62% of the participants who used the drug.[28]

Evidence

Using the methodology described above, we selected six systematic reviews with meta-analyses (Figure S1). [48-55] Even though not all of them analyzed the exact same sets of outcomes, they all indicate a beneficial therapeutic effect of nintedanib versus placebo in patients with IPF (Table S1).

With regard to mortality (a critical outcome), there was no statistically significant effect (OR = 0.70; 95% CI: 0.45-1.09) and the quality of evidence was moderate.[48]

With regard to a decline in FVC (a critical outcome), nintedanib was found to be beneficial. For a > 10% decline in FVC, the estimated OR was 0.61 (95% CI: 0.48-0.78), indicating a high quality of evidence.[48]

Finally, with regard to number of exacerbations (a critical outcome), nintedanib was found to be effective in reducing the number of acute exacerbations (OR = 0.50; 95% CI: 0.31-0.79), indicating a moderate quality of evidence.[54]

Recommendation

For patients with IPF, we suggest using nintedanib (conditional recommendation; moderate quality of evidence).

Comments

The available meta-analyses included only randomized, double-blind, controlled clinical trials. Therefore, the results obtained apply primarily to patients who meet the same selection criteria as those used for the participants in those trials. Those trials did not include patients with very early-stage IPF (DLCO ≥ 80%) or very advanced IPF (FVC < 50% of predicted or DLCO < 30%).

Therefore, the effects of nintedanib in these two groups of patients have yet to be well characterized. In addition, it is not possible to determine, on the basis of the selected articles, the long-term efficacy and safety of nintedanib, because the maximum duration of the trials included here was 52 weeks.

The recommendation made implies that using nintedanib is the right course of action to be taken in 50-95% of cases.[42] Clinicians should acknowledge that different choices may be appropriate for individual patients and that they are responsible for helping patients and families make decisions consistent with their values and preferences (Chart 4).[37-42] This recommendation does not take into account cost analyses or aspects of drug economics.

QUESTION 2: SHOULD WE RECOMMEND THE USE OF PIRFENIDONE FOR PATIENTS WITH IPF?

Pirfenidone is a drug with anti-inflammatory and antifibrotic properties that acts through regulation of TNF-α and TGF-β pathways, as well as through modulation of cellular oxidation.[56] Ultimately, pirfenidone inhibits fibroblast proliferation, consequently decreasing collagen synthesis and deposition.[56-59]

The therapeutic potential of pirfenidone was initially demonstrated in two small clinical trials, which compared it with placebo.[16,20] Subsequently, three other clinical trials stood out for greater homogeneity of inclusion

Chart 4. Implications of the recommendations of the Grading of Recommendations Assessment, Development and Evaluation system.[a]

Target audience	Strong recommendation		Conditional recommendation	
	We recommend	We do not recommend	We suggest	We do not suggest
Patients	Most individuals would want the intervention to be recommended, and only a small number would not accept this recommendation	Most individuals would not want the intervention to be recommended, and only a small number would accept this recommendation	Most individuals would want the intervention to be recommended, although a considerable number would not accept this recommendation	Most individuals would not want the intervention to be recommended, although a considerable number would accept this recommendation
Health professionals	Most patients should receive the recommended intervention		The health professional should acknowledge that different choices may be appropriate for individual patients and should help them make a decision consistent with their values and preferences	
Administrators	The recommendation can be adopted as a health policy in most situations		Substantial debate and involvement of all stakeholders are required	

[a]Adapted from Guyatt et al.[39] and Andrews et al.[40]

criteria and outcomes, including assessment of > 10% decline in FVC at week 52.[25,26] Those three clinical trials, in a combined analysis, demonstrated a reduced decline in percentage predicted FVC, as well as a reduced risk of disease progression, with the use of a target dose of 2,403 mg/day. In the five clinical trials, adverse events were more common in the group receiving pirfenidone, being mainly related to the skin (rash and photosensitivity) and the gastrointestinal tract (nausea, dyspepsia, and loss of appetite).

Evidence

Nine systematic reviews with meta-analyses comparing pirfenidone with placebo were selected (Figure S2).[55,60-65] The meta-analyses evaluated several outcomes, including mortality, progression-free survival, acute exacerbation, functional decline, change in six-minute walk distance (6MWD), and adverse events, indicating that pirfenidone has a favorable therapeutic effect and an acceptable safety profile.

Table S2 summarizes the quality of evidence of the selected articles for the question related to pirfenidone. Pirfenidone treatment was shown to reduce mortality—relative risk (RR) = 0.53; 95% CI: 0.32-0.88, indicating a moderate quality of evidence. [61,62] Likewise, pirfenidone was found to be effective in reducing the occurrence of a > 10% decline in FVC (RR = 0.64; 95% CI: 0.50-0.83).[61,62] With regard to the reduction in the number of acute exacerbations, there was no statistically significant effect (RR = 0.59; 95% CI: 0.19-1.84), indicating a low quality of evidence.[61,62]

Recommendation

For patients with IPF, we suggest using pirfenidone (conditional recommendation; low quality of evidence).

Comments

The available systematic reviews and meta-analyses evaluating the use of pirfenidone in patients with IPF included only randomized, double-blind, controlled clinical trials. The results obtained apply to patients who meet the same selection criteria as those used for the participants in those trials, that is, patients with mild to moderate disease. Those trials did not include IPF patients who had very early-stage functional changes (DLCO ≥ 90%), had very advanced functional changes (FVC < 50% of predicted or DLCO < 30%), or were over 80 years of age. Therefore, the effects of pirfenidone in those subgroups of patients have yet to be determined. On the basis of the results found, it is not possible to determine the impact of pirfenidone in terms of long-term efficacy and safety, because the maximum duration of the trials included here was 72 weeks.

The recommendation made implies that using pirfenidone is the right course of action in 50-95% of cases.[42] Clinicians should acknowledge that choices need to be individualized and that they should help patients and their families make decisions consistent with their values and preferences (Chart 4).[37-42] This recommendation does not take into account cost analyses or aspects of drug economics.

QUESTION 3: SHOULD WE RECOMMEND THE USE OF PHOSPHODIESTERASE-5 INHIBITORS FOR PATIENTS WITH IPF?

Phosphodiesterase-5 (PDE5) inhibitors stabilize cyclic guanosine monophosphate, the second messenger of nitric oxide, leading to pulmonary vasodilation.[66] The vasodilation produced by PDE5 inhibitors appears to have a preference for well-ventilated lung tissue, which could improve the ventilation-perfusion ratio and gas exchange in patients with IPF.[66] In addition, pulmonary hypertension is a common finding in patients with IPF, being associated with higher rates of morbidity and mortality.[67] The commercially available PDE5 inhibitors are sildenafil, tadalafil, and vardenafil; however, only sildenafil has been tested in patients with IPF.

Three clinical trials, of which only two were randomized, have evaluated the effects of sildenafil in patients with advanced IPF.[68-70] The primary outcome used was always a change in the 6MWD, which was not achieved in any of the investigations. Sildenafil treatment also had no impact on exacerbation or mortality rates.

Evidence

As a result of the methodology employed for these guidelines, two systematic reviews with meta-analyses were selected (Figure S3 and Table S3). One of those reviews found no evidence that sildenafil can provide relief of dyspnea in patients with IPF (relative risk not estimable; very low level of evidence) or improve quality of life in patients with IPF (relative risk not estimable; very low level of evidence).[71] The other meta-analysis, which used a network methodology, evaluated the effects of sildenafil treatment on mortality in patients with IPF, thus finding that the death rates in the treatment group and placebo group were 2.9% and 8.5%, respectively.[55] However, the OR was 0.84 and did not reach statistical significance (95% CI: 0.31-2.41).

Recommendation

For patients with IPF, we suggest not using a PDE5 inhibitor (conditional recommendation; moderate quality of evidence).

Comments

The critical outcome selected for this question by the specialists working on these guidelines was mortality. With regard to this parameter, the selected articles did not show significant benefits of PDE5 inhibitors. The same was true for two outcomes classified as important: dyspnea and quality of life. It is of note that other guidelines for the treatment of IPF, developed by other medical societies, also do not recommend the use of a PDE5 inhibitor (sildenafil) for this group of patients.[4,72,73] In addition, a randomized clinical trial

comparing the use of nintedanib plus sildenafil with that of nintedanib alone in patients with IPF and a DLCO < 35% of the predicted value, which was published after the analysis of the articles selected for these guidelines had been completed, found no significant differences regarding quality of life or dyspnea.[34] A pre-specified analysis of the subgroup of patients with right ventricular dysfunction, published separately, also showed no impact on the relevant variables.[74]

QUESTION 4: SHOULD WE RECOMMEND THE USE OF ENDOTHELIN-RECEPTOR ANTAGONISTS FOR PATIENTS WITH IPF?

Although the pathogenesis of IPF has yet to be fully elucidated, endothelin-1, a potent vasoconstrictor and growth factor, has been related to the fibroproliferative process of IPF.[75] Endothelins have a potent proliferative effect on mesenchymal cells and can induce cell differentiation, increasing the synthesis and deposition of extracellular matrix components, as well as their contractility.[75] On the basis of these elements, clinical trials of endothelin-receptor antagonists have been conducted with the aim of reducing the fibrotic process.

Two clinical trials have evaluated the efficacy of bosentan versus that of placebo in patients with IPF. No significant effects of bosentan were detected, either on the primary outcome (6MWD) or on the rate of disease progression, quality of life, or dyspnea intensity.[18,22,76]

The effect of macitentan on the rate of decline in FVC in patients with IPF was also not significant in comparison with that of a placebo.[24] Finally, one study investigating the effects of ambrisentan versus those of placebo in patients with IPF was terminated early because an interim analysis showed that ambrisentan-treated patients were more likely to meet the criteria for disease progression and had a greater number of respiratory hospitalizations.[23]

Evidence

Using the methodology proposed above, we selected two review articles with meta-analyses (Figure S4). Both evaluated individual results for bosentan, macitentan, and ambrisentan.[48,55] None of the drugs investigated showed significant effects with regard to mortality (critical outcome) or adverse effects (unimportant outcome; Table S4).

Recommendation

For patients with IPF, we recommend not using endothelin-receptor antagonists (strong recommendation; low quality of evidence).

Comments

In addition to the fact that the available evidence indicates no significant effects of endothelin-receptor antagonists on relevant clinical outcomes, it has been suggested that the use of ambrisentan, in particular, is associated with deleterious effects. It is unlikely that further studies of this class of drugs in patients with IPF will be conducted.

QUESTION 5: SHOULD WE RECOMMEND PHARMACOLOGICAL TREATMENT OF GASTROESOPHAGEAL REFLUX FOR PATIENTS WITH IPF?

Gastroesophageal reflux (GER) is highly prevalent in patients with IPF and, although there is as yet no evidence of a causal relationship, chronic aspiration is considered a risk factor for disease progression and exacerbation.[77-79] Although the importance of microaspiration of gastric content in the pathogenesis of IPF has yet to be established, there have been anecdotal reports of stabilization and (clinical and functional) improvement in patients with IPF after pharmacological or surgical treatment of GER.[80,81] Recent international guidelines suggest regular antacid use for all patients with IPF, although the quality of evidence is very low.[3,4]

All of the studies evaluating the possible effects of pharmacological treatment of GER in patients with IPF have been observational, and most have used data derived from the placebo arms of clinical trials aimed at investigating other drugs.[82-86] To date, there have been no clinical trials with an appropriate design and an adequate number of volunteers that have evaluated the routine use of GER medications in symptomatic or asymptomatic patients with IPF.[87]

Evidence

The methodology employed in developing these guidelines did not allow us to select articles suitable for developing recommendations regarding pharmacological treatment of GER in patients with IPF (Figure S5 and Table S5).

Recommendation

For patients with IPF, there is insufficient evidence to make a recommendation for or against the use of pharmacological treatment of GER.

Comments

Studies evaluating the possible effects of pharmacological treatment of GER in patients with IPF have involved patients who had been randomized to the placebo arms of clinical trials of other drugs for IPF.[82-86] This strategy introduces a very large selection bias, because it is not possible to know why pharmacological treatment of GER was prescribed, and these patients may therefore not be representative of all patients with IPF. Further controlled randomized clinical trials of pharmacological treatment of GER are needed in order to properly elucidate its effects in patients with IPF. Given that pharmacological treatment of GER may be associated with potential adverse effects, its use should be evaluated on a case-by-case basis and should be considered in patients with symptoms suggestive of GER or with GER symptoms confirmed by ancillary tests.

QUESTION 6: SHOULD WE RECOMMEND THE USE OF N-ACETYLCYSTEINE FOR PATIENTS WITH IPF?

N-acetylcysteine (NAC) is a precursor drug to glutathione, a water-soluble antioxidant present in most cells in the body.[88] A potential contribution of oxidative stress to the progression of IPF has led to studies being conducted to determine whether the use of NAC could restore lung glutathione levels.[88,89] Thus, NAC could slow the progression of the disease.

A multicenter double-blind, placebo-controlled study evaluated the use of oral NAC plus a corticosteroid and azathioprine versus placebo.[11] Participants who received NAC plus a corticosteroid and azathioprine had a significant reduction in the rate of decline in FVC and in DLCO.[11] In contrast, studies of inhaled or oral NAC monotherapy versus placebo demonstrated no significant differences in the evolution of lung function or in other clinical outcomes.[90-92]

Evidence

We selected five systematic reviews with meta-analyses comparing NAC and placebo with regard to mortality, as well as one evaluating reductions in the rate of decline in FVC in the treatment of IPF (Figure S6 and Table S6).[48,55]

In comparison with placebo, NAC did not reduce mortality in patients with IPF (OR = 0.84; 95% CI: 0.20-4.50).[54] With regard to the reduction in the rate of decline in FVC at 12 months, it was not possible to estimate the effect of NAC versus that of placebo because of the heterogeneity of and high risk of bias within the studies selected for the meta-analysis.[48]

Recommendation

For patients with IPF, we suggest not using NAC (conditional recommendation; low quality of evidence).

Comments

The lack of effect of NAC on reducing mortality and the difficulty in estimating positive effects of NAC on reducing the rate of decline in FVC make it unlikely that the drug has any beneficial effects on the course of IPF. It has been suggested that IPF patient responses to the use of NAC may be influenced by *TOLLIP* gene polymorphisms.[93] However, these aspects still require further clarification so that robust conclusions can be drawn.

QUESTION 7: SHOULD WE RECOMMEND THE USE OF CORTICOSTEROIDS FOR PATIENTS WITH IPF?

Classical hypotheses regarding the pathogenesis of IPF suggest that the onset of the disease is due to an inflammatory process, in which proinflammatory cytokines released by alveolar macrophages play a decisive role.[94,95] In this context, the use of corticosteroids would potentially be beneficial to the clinical course of patients with IPF. However, the most widely accepted hypothesis for the pathogenesis of IPF is alveolar epithelial aggression and damage followed by release of profibrotic mediators, with abnormal repair, myofibroblastic proliferation, and collagen deposition, without evident inflammation.[96]

The current concept of IPF was formulated in 2000; since then, there have been no controlled clinical trials of corticosteroid monotherapy in patients with IPF.[1]

Evidence

Within the literature review date range pre-established for these guidelines, it was not possible to find any systematic reviews or meta-analyses evaluating the effects of corticosteroid monotherapy versus those of placebo in the treatment of IPF (Figure S7 and Table S7).

Recommendations

For patients with IPF, there is insufficient evidence to make a recommendation for or against the use of corticosteroids.

Comments

Although there is no evidence regarding the use of corticosteroids in IPF, it is unlikely that studies of these drugs in patients with IPF will be conducted, because, given the noninflammatory pathogenesis of the disease, there appears to be little chance of therapeutic success. This recommendation is intended for patients with stable IPF. The potential use of corticosteroids in patients with acute IPF exacerbations was not analyzed in these guidelines.

FINAL CONSIDERATIONS

A summary of the recommendations for the pharmacological treatment of IPF is shown in Chart 5.

Although there is currently no drug that can cure IPF, these guidelines suggest that nintedanib and pirfenidone be considered for the treatment of the disease (conditional recommendation). The evidence indicates that these antifibrotic agents are, in fact, the only pharmacological treatment options that can lead to a reduction in functional decline in IPF. Both reduce the rate of decline in FVC, which is a strong independent predictor of IPF mortality. However, when considering whether or not to use either of these drugs, it is essential to evaluate the specifics of each case, including the severity of the functional impairment, the presence of comorbidities, the concomitant use of other drugs (i.e., potential drug interactions), potential adverse events, and costs, as well as, in particular, the concerns of patients and their families. It should also be emphasized that nintedanib and pirfenidone were not compared with each other in our guidelines, and determining the superiority of one over the other is therefore not possible. In addition, the combined use of these drugs was not evaluated.

Although the prevalence of GER is high in IPF, we found insufficient evidence to define the role of the routine use of GER medications in patients with IPF.

Chart 5. Summary of the recommendations for the pharmacological treatment of idiopathic pulmonary fibrosis.

Question	Recommendation	Grade of recommendation	Quality of evidence
1. Should we recommend the use of nintedanib for patients with IPF?	Yes (suggestion)	Conditional	Moderate
2. Should we recommend the use of pirfenidone for patients with IPF?	Yes (suggestion)	Conditional	Low
3. Should we recommend the use of phosphodiesterase-5 inhibitors for patients with IPF?	No (suggestion)	Conditional	Moderate
4. Should we recommend the use of endothelin-receptor antagonists for patients with IPF?	No (recommendation)	Strong	Low
5. Should we recommend pharmacological treatment of gastroesophageal reflux for patients with IPF?	Unknown Lack of eligible studies for selection		
6. Should we recommend the use of N-acetylcysteine for patients with IPF?	No (suggestion)	Conditional	Low
7. Should we recommend the use of corticosteroids for patients with IPF?	Unknown Lack of eligible studies for selection		

IPF: idiopathic pulmonary fibrosis.

Again, the decision should be made on the basis of the clinical characteristics of each case.

The lack of reliable data does not allow recommendations to be made regarding the use of corticosteroids in IPF. That does not imply that this category of drugs might not be used in other forms of interstitial lung disease, such as sarcoidosis and proliferative bronchiolitis.

With regard to the other drugs investigated, it is suggested that neither a PDE5 inhibitor nor NAC be used (conditional recommendation), and it is strongly recommended that endothelin-receptor antagonists not be prescribed for patients with IPF.

It should be emphasized that non-pharmacological approaches to IPF, including oxygen supplementation, pulmonary rehabilitation, immunizations, and lung transplantation, were not considered in the development of these guidelines. It should also be emphasized that the guidelines in question apply only to patients with IPF, which means that the results cannot be extrapolated to patients with fibrotic lung diseases from other causes.

We believe that the present document represents an important tool to be incorporated in the approach to patients with IPF, aiming mainly to improve its management, as well as aiding in the development of public policies related to the disease.

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REVIEW ARTICLE

Management of multidrug-resistant tuberculosis: main recommendations of the Brazilian guidelines

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ABSTRACT

Over the years, various recommendations have been made in pursuit of controlling resistance to antituberculosis drugs, especially multidrug resistance, in Brazil. Given the importance of standardizing those recommendations, the aim of this study was to describe the main recommendations of the Brazilian guidelines, primarily those related to the treatment and follow-up of cases of tuberculosis. From August through October of 2018, a document search was conducted via the websites of the Brazilian National Ministry of Health, the Brazilian National Tuberculosis Control Program, the JBP, and the Official Gazette of the Federal Republic of Brazil. Data were collected systematically by using a protocol designed specifically for this study. Documents published between 2004 and 2018 were selected. It was possible to understand and trace the history of the measures for the control of multidrug-resistant tuberculosis in Brazil from 2004, when the first documents related to the disease were published, up to 2018, when the second edition of the Brazilian National Guidelines for the Control of Tuberculosis was published. The contents of the documents were analyzed and grouped by case definition, diagnostic criteria, treatment, use of directly observed treatment; mechanisms of social protection for patients; data tools; and organization of care. This analysis allowed us to understand the efforts towards standardizing some measures in Brazil, not only identifying advances in the alignment with international prerogatives (case definition, incorporation of diagnostic technology, and treatment regimens) but also underscoring the need for greater clarity regarding the mechanisms of social protection and the organization of the care provided via the Brazilian health care system.

Keywords: Tuberculosis, multidrug-resistant; Organization and administration; Practice patterns, physicians'; Practice patterns, nurses'.

INTRODUCTION

Drug-resistant tuberculosis (DR-TB)—tuberculosis that is resistant to drugs used in the treatment of tuberculosis—poses a serious threat to attempts to control tuberculosis worldwide.[1] Multidrug-resistant tuberculosis (MDR-TB) is defined as that which is resistant to both rifampin and isoniazid, whereas extensively drug-resistant tuberculosis (XDR-TB) is defined as that which, in addition to being resistant to rifampin and isoniazid, is resistant to a fluoroquinolone and a second-line injectable drug; these are the most worrying forms of tuberculosis.[2]

Another form of DR-TB that is a major concern is rifampin-resistant tuberculosis (RR-TB), given that approximately 82% of RR-TB cases are also resistant to isoniazid.[3,4] According to the World Health Organization (WHO), there were an estimated 558,000 new cases of MDR/RR-TB and an estimated 230,000 deaths from MDR/RR-TB worldwide in 2017.[1] In 2017 in Brazil, there were 1,119 laboratory-confirmed cases of MDR/RR-TB, 746 of which had started treatment, and 26 laboratory-confirmed cases of XDR-TB, all of which had started treatment.[1]

The WHO has been working to keep a systematic record of the occurrence of DR-TB worldwide,[1] as well as setting clear objectives to address this problem: prevent the emergence of drug-resistant forms through adequate treatment of drug-susceptible forms; expand the network for rapid testing for the timely identification of drug resistance; ensure immediate access of drug-resistant cases to treatment; prevent transmission; and ensure political and financial commitment to fight DR-TB.[1]

In Brazil over the years, some recommendations have been made on the basis of international documents

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and the experience of specialists working in the country.[5] The aim of this study was to describe the main recommendations of past and current Brazilian guidelines on DR-TB care, primarily those related to the treatment and follow-up of cases.

METHODS

A document search was conducted of official Brazilian documents that guide MDR-TB care in the country. The search was conducted via the websites of the Brazilian National Ministry of Health (NMH), the *Programa Nacional de Controle da Tuberculose* (PNCT, Brazilian National Tuberculosis Control Program), the JBP, and the Official Gazette of the Federal Republic of Brazil. The document selection process occurred between August and October of 2018.

For the document review, a protocol was designed based on the results of a recently published integrative review of the literature[6] and the WHO guidelines on MDR-TB care.[7-9] The aspects addressed were as follows: capability/need for communication between levels of care; standardization of practices related to referrals; outpatient care; return visit schedules; capability/need for provision of social, economic, and emotional support to patients undergoing treatment, as well as ways of effecting that; capabilities and involvement of various health care facilities, with a focus on patient and information flow via the Brazilian Unified Health Care System; and adoption of and recommendations on pharmacological treatment regimens.

Documents were read in full, and data related to the aspects addressed by the protocol were extracted by two researchers, working independently, and then compared. A researcher other than those two performed the role of third reviewer when there was divergence between the two sets of extracted information. In the subsequent phase of the study, the data were analyzed, grouped by key themes and concepts, and summarized.

A search of selected sources resulted in the analysis of the following documents: the Second Brazilian Consensus on Tuberculosis: 2004 Brazilian Guidelines on Tuberculosis[10]; the 2007 Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis[11]; the 2009 Technical Note on Changes in the Treatment of Tuberculosis in Brazil for Adults and Adolescents[12]; the (2009) Third Brazilian Thoracic Association Guidelines on Tuberculosis[13]; the 2011 Brazilian National Guidelines for the Control of Tuberculosis[14]; NMH Technical Memo no. 9, in 2014[15]; NMH Technical Memo no. 8, in 2016[16]; and the 2018 Brazilian National Guidelines for the Control of Tuberculosis.[17] A review of the bibliographic references of those eight documents led to the inclusion of a 2007 study on MDR-TB[18] in the analysis, resulting in a final sample of nine documents.

The analysis made it possible to understand and trace the history of the measures for the control of MDR-TB in Brazil from 2004, when the first documents related to the disease were published, up to 2018, when the second edition of the Brazilian National Guidelines for the Control of Tuberculosis was published.[17] The key milestones in this regard are depicted in Figure 1.

ANALYSIS OF DOCUMENTS

The analysis of documents resulted in the division of results into two categories, which are discussed below.

History of the measures for the control of multidrug-resistant tuberculosis in Brazil

The establishment of short-course (6-month) treatment regimens in 1979, standardized by the PNCT, is a major milestone in tuberculosis control in Brazil.[19] The first-line basic regimen, known as regimen I, included a 2-month intensive phase of rifampin, isoniazid, and pyrazinamide (2RHZ), followed by a 4-month continuation phase of rifampin and isoniazid (4RH). This regimen was recommended for treatment-naive patients and for patients who discontinued treatment for < 30 days. For patients who discontinued treatment for > 30 days and for patients who experienced recurrence, regimen I boosted with ethambutol (2RHZE/4RH) was recommended. For patients with meningoencephalitis, the duration of treatment was extended to 9 months: 2RHZ/7RH. For patients who experienced clinical and bacteriological treatment failure, regimen III, which consisted of 3 months of streptomycin, ethambutol, ethionamide, and pyrazinamide, followed by 9 months of ethambutol and ethionamide (3SEEtZ/9EEt), was recommended.[5,10,14,20] However, at health care facilities, there remained patients who did not achieve cure after the standard treatments, cases known as cases of chronic tuberculosis, probably due to drug resistance.[19]

In the 1980s and early 1990s, several experiments with alternative regimens were conducted in Brazil in an attempt to treat patients who did not respond to the recommended drugs.[18] In 1992, the NMH recognized the lack of reliable data on cases of drug-resistance,[20] and, in 1994, the first two cases of MDR-TB were reported in Brazil.[11] Therefore, the need for conceptualization and classification of cases of drug-resistance in Brazil dates from the early 1990s, and for that purpose at that time, operational and bacteriological aspects related to care in the Brazilian context were considered, resulting in a different definition from the one that was then adopted internationally.[21] That definition was seen as a breakthrough in terms of delineating the problem in Brazil, since it was related to the reality experienced by patients and health care professionals. On the basis of that definition, a nationwide survey conducted in 1995 revealed that there were approximately 1,500 cumulative cases of MDR-TB.[20]

With regard to the broader picture of the efforts against tuberculosis in Brazil, the 1990s were marked by severe setbacks in tuberculosis control policies, with the dismantling of the PNCT at the federal level and the weakening of the coordination of local programs.[22] All of those events impacted negatively on the results

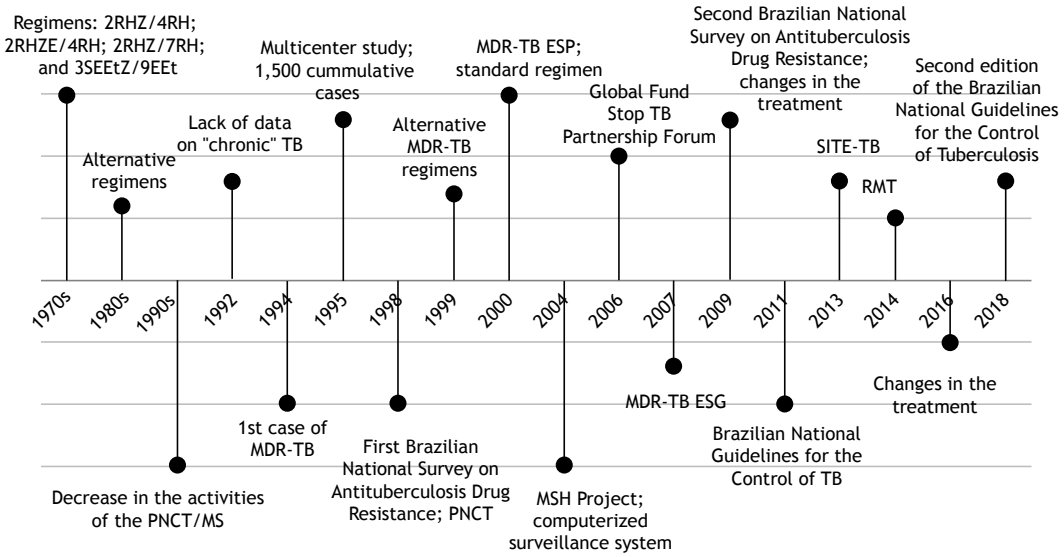

Figure 1. Timeline of milestones in the standardization of practices related to multidrug-resistant tuberculosis in Brazil. Ribeirão Preto, 2019. PNCT/MS: *Plano Nacional de Controle da Tuberculose/Ministério da Saúde* (Brazilian National Tuberculosis Control Program/Brazilian National Ministry of Health); TB: tuberculosis; MDR-TB: multidrug-resistant tuberculosis; ESP: epidemiological surveillance program; MSH: Management Sciences for Health; ESG: epidemiological surveillance guide; SITE-TB: *Sistema de Informação de Tratamentos Especiais da Tuberculose* (Brazilian Database of Special Treatments for Tuberculosis); and RMT: rapid molecular test(ing).

and indicators, which added up to the spreading AIDS epidemic.[23]

Worldwide at that time, there was also a lack of commitment to the problem of tuberculosis, reflected internationally as the occurrence of MDR-TB outbreaks in the United States and the reemergence of the disease as a matter of concern in developed countries in North America and Europe,[20] which was called the "resurgence of tuberculosis" in the northern hemisphere. In developing countries, such as Brazil, the disease has always been "the neglected calamity" that was "ever present and enduring".[23] However, increasing social inequalities, poverty, and a lack of access to goods and services, as well as the growth of the population, an increasing number of people living in urban areas, and the AIDS pandemic, exacerbated the problem of tuberculosis, resulting in increased resistance of the disease to drugs and treatment regimens.[24]

In view of this challenge, in 1995, the NMH *Centro de Referência Professor Hélio Fraga* (CRPHF, Professor Hélio Fraga Referral Center), located in the city of Rio de Janeiro, Brazil, developed a national treatment protocol that was validated through a multicenter study involving the Clemente Ferreira Institute, located in the city of São Paulo, Brazil, the *Hospital Sanatório Partenon*, located in the city of Porto Alegre, Brazil, and the Raphael de Paula Souza Hospital, also located in the city of Rio de Janeiro; this multicenter study was conducted between 1995 and 1998.[11]

The First Brazilian National Survey on Antituberculosis Drug Resistance, conducted between 1996 and 1997, was published in 1998.[20] Concomitantly, all such drugs were registered with the Brazilian National Health Oversight Agency, the purchase of such medications was regulated, and most of such drugs began to be produced by Brazilian public companies.[24] In addition, in a broader context related to disease control measures, a tuberculosis control program was launched; the program addressed the need to provide, free of charge, antituberculosis drugs, including those in special regimens such as regimen III, to patients, as well as to ensure supervised treatment and drug resistance surveillance.[23]

In 1999, the results of a study[20] on the effectiveness of alternative MDR-TB regimens were published in the JBP, providing guidance in the search for standardization of practices, regimens, treatment duration, and the management of cases and outbreaks, as well as of tests and operational aspects that are required for a broad and multidisciplinary approach. In 2000, the proposed regimen was validated by the NMH and an epidemiological surveillance program for MDR-TB was initiated, including case reporting and the consolidation of a database dedicated to this health problem.[11] From then on, the CRPHF began to provide, free of charge, medications for the treatment of all cases of MDR-TB reported in Brazil.[11]

In 2004, a partnership was established with Management Sciences for Health, enabling the continued implementation of epidemiological surveillance systems. The objective of this collaboration between Management Sciences for Health and CRPHF was to improve the reporting system for case follow-up, allowing assessments and searches in the dedicated database, which was then up to date and computerized. In addition, drug shipments and drug stocks were controlled via this database, and the epidemiological

surveillance program for MDR-TB, which has since been computerized, was strengthened and decentralized.[11] The CRPHF became a national referral center, being responsible for drug dispensing, case validation, and the management of the database.[11]

The partnership with Management Sciences for Health also led to the publication of the Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis.[11] This guide allowed the dissemination of standardized recommendations, including diagnosis, pharmacological treatment, case follow-up, prevention, biosafety, database, and human resources, throughout Brazil. The professional and managerial responsibilities and duties of the various actors involved in health care were defined.[5,11,19] In addition, the content of the guide was widely disseminated through refresher courses on MDR-TB, which were offered in all Brazilian states and the Federal District of Brasília.[11]

In 2006, financing was granted by the Global Fund against AIDS, Tuberculosis and Malaria, and this resulted in the creation by the NMH of the "Stop TB Partnership" Forum, which represented an achievement in disease control by officially acknowledging the role of social participation/mobilization.[11,24]

The Second Brazilian National Survey on Antituberculosis Drug Resistance occurred between 2006 and 2008[12]; the survey involved a representative Brazilian sample and sought to determine patterns of resistance to first- and second-line drugs in both outpatient and inpatient follow-up cases.[11,25] Its preliminary results indicated an increase in primary resistance to both isoniazid (from 4.4% to 6.0%) and rifampin (from 0.2% to 1.5%) and led the PNCT to change in 2009 several aspects related to the treatment of tuberculosis in Brazil.[5,14,19]

Also in 2009, the Third Brazilian Thoracic Association Guidelines on Tuberculosis were published.[13] From then on, a "basic regimen", which included the use of ethambutol as a fourth drug in the intensive phase of treatment (for patients aged 10 years and older), was adopted, as were fixed-dose combination tablets.[5,14,19]

All tuberculosis-related guidelines were revised and published in the 2011 Brazilian National Guidelines for the Control of Tuberculosis.[14] There were specific recommendations for confirmed cases of drug-resistance and for early detection of cases of DR-TB.[5,14] However, unlike the Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis,[11] the 2011 guidelines[14] provided general guidance on all forms of tuberculosis, defining drug-resistance and offering the related recommendations in one of the chapters. In addition, the 2011 guidelines provided the definition of cases of and special regimens for DR-TB and nontuberculous mycobacteria, as well as of patient flow through health care facilities and levels of care, establishing secondary and tertiary referral centers.[5,14] In 2013, the database was changed and renamed *Sistema de Informação de Tratamentos Especiais da Tuberculose* (SITE-TB, Brazilian Database of Special Treatments for Tuberculosis).[26]

In July of 2014, the use of a rapid molecular test (RMT)—Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA)—performed on the GeneXpert platform,[26] began to be implemented throughout Brazil. Xpert MTB/RIF is an automated, real-time polymerase chain reaction-based assay that, in approximately 2 h, detects bacillus DNA and mutations that can cause resistance to rifampin.[27,28] The intent of the implementation was to reduce the time to diagnosis of tuberculosis and to optimize early identification of resistance to rifampin, which is in line with the WHO recommendations and the goals of the End TB Strategy.[26,29]

The General Council of the PNCT prepared, discussed, and validated new recommendations in consultation with national and international specialists, on the basis of the WHO recommendations and literature review. A technical memo in which the standard MDR-TB regimen was changed was published in 2016.[7,16] The technical memo also emphasized the need to carry out directly observed treatment (DOT) in partnership with primary health care teams to ensure appropriate follow-up, and the importance of reporting cases to the Brazilian Case Registry Database and SITE-TB.[16]

The last change during the study period is related to the Brazilian National Guidelines for the Control of Tuberculosis, which were disclosed to the members of the Brazilian Tuberculosis Research Network in September of 2018 and published in March of the following year.[17] The document included recommendations for the diagnosis, management, and follow-up of drug-susceptible and drug-resistant cases, pointing out innovations in case management within the *Redes de Atenção à Saúde* (RAS, Health Care Networks).

Measures taken to control multidrug-resistant tuberculosis

Chart 1 presents the documents that were analyzed and that guide MDR-TB care in Brazil by title, year of publication, institution responsible for its development and/or dissemination, and major contributions. The data from the documents were selected and grouped by category as follows: MDR-TB case definition, diagnostic criteria, treatment, use of DOT; mechanisms of social protection for patients; data tools; and organization of care.

Up until 2004 in Brazil, MDR-TB cases were defined as those in which patients experienced bacteriological treatment failure, as mentioned above. In 2007, with the publication of the Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis,[11] MDR-TB case definition was qualified by the requirement of drug susceptibility test results showing resistance to rifampin and isoniazid and to at least one more component of regimens I or III, regardless of the treatment regimen being used. Another possible definition for MDR-TB in the guide was the occurrence of resistance to rifampin and isoniazid accompanied by confirmed bacteriological

Chart 1. Identification of the publications that guide multidrug-resistant tuberculosis care in Brazil by title, year of publication, institution responsible for its development and/or dissemination, and major contributions. Ribeirão Preto, Brazil, 2019.

Document (year of publication)	Institution responsible for its development/ dissemination	Major contributions
Second Brazilian Consensus on Tuberculosis: Brazilian Guidelines on Tuberculosis (2004)[10]	Brazilian Thoracic Association JBP	Regulates the measures proposed by the First Brazilian Consensus on TB: DOT as a recommended strategy to improve treatment adherence, reduce treatment abandonment rates, increase cure rates, and intervene in disease transmission and in the risk of developing drug resistance. The components of the DOTS strategy are perceived by the Brazilian National Ministry of Health as viable for implementation in the different regions of Brazil, as long as local characteristics are taken into account.
Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis (2007)[11]	Brazilian National Ministry of Health. Professor Hélio Fraga Referral Center	Provides specific information about MDR-TB, such as concepts, diagnosis management, treatment, and case follow-up, as well as prevention and required human resources. Of note are the specificities of the professional categories and the management of the databases, which are reported in an organized and clear manner in the document.
Technical Note on Changes in the Treatment of Tuberculosis in Brazil for Adults and Adolescents (2009)[12]	Brazilian National Ministry of Health. Brazilian National Tuberculosis Control Program	Disseminates changes in the TB treatment system. The change in the treatment regimens creates a "basic regimen" for drug-susceptible cases and alters the definition of MDR-TB in Brazil from then on, in line with the WHO recommendations.
Third Brazilian Thoracic Association Guidelines on Tuberculosis (2009)[13]	Brazilian Thoracic Association JBP	Clearly show the need for health care administrators, health care workers, members of society, and organized segments of society to work together. Address MDR-TB, describing a standard treatment regimen, new drugs, surgical indications, and case reporting.
Brazilian National Guidelines for the Control of Tuberculosis (2011)[14]	Brazilian National Ministry of Health. Brazilian National Tuberculosis Control Program	Address MDR-TB in one of their chapters, offering recommendations regarding practices for case management and reporting and the duties of various levels of MDR-TB care.
Technical Memo no. 9 (2014)[15]	Brazilian National Ministry of Health. Brazilian National Tuberculosis Control Program	Reports the news of RMTs for TB and the identification of resistance to rifampin, as well as addresses the status of the implementation of the RMT network in Brazil.
Technical Memo no. 8 (2016)[16]	Brazilian National Ministry of Health. Brazilian National Tuberculosis Control Program	Disseminates new recommendations for the treatment of MDR-TB and RR-TB. Recommends that RR-TB be treated as MDR-TB until AST results become available. Reports changes in the drugs used and in the duration of injectable drug therapy.
Brazilian National Guidelines for the Control of Tuberculosis (2018)[17]	Brazilian National Ministry of Health. Brazilian National Tuberculosis Control Program	Address MDR-TB in one of their chapters, offering recommendations regarding practices for case management and reporting and regarding the duties of the various levels of MDR-TB care. In other chapters, these guidelines address contexts within which multidrug-resistance is embedded, such as within the Health Care Networks.

TB: tuberculosis; DOT: directly observed treatment; DOTS: directly observed treatment, short-course; MDR-TB: multidrug-resistant tuberculosis; WHO: World Health Organization; RMT: rapid molecular test(ing); RR-TB: rifampin-resistant tuberculosis; and AST: antimicrobial susceptibility testing.

treatment failure with regimen III. Already at that time, the WHO defined MDR-TB as a disease caused by a bacillus that was resistant only to rifampin and isoniazid. This criterion was adopted in Brazil in 2011 and is still used today.[14,17]

More recently, with the advent of RMTs, especially Xpert MTB/RIF, another definition has been gaining increasing prominence in the international literature: that of RR-TB cases. The use of RMTs was incorporated into Technical Memo no. 9,[15] and its importance for the diagnosis of RR-TB cases and for control of the disease in Brazil was reaffirmed in the 2018 Brazilian National Guidelines for the Control of Tuberculosis.[17] The 2018 guidelines also incorporated the concept of "pre-XDR-TB", which is used to describe cases in which, in addition to resistance to rifampin and isoniazid, there is resistance to a fluoroquinolone or a second-line injectable drug.[17]

The diagnostic criterion is closely associated with case definition and with laboratory confirmation of

drug resistance via sputum culture and antimicrobial susceptibility testing (AST). The first important recommendation in this regard was found in the 2009 Technical Note,[12] in which it was recommended that culture and AST be performed in all cases of retreatment; up until then, the use of these tests depended on the clinical and epidemiological status of the patient under investigation and the available laboratory resources.[13]

In 2014, Technical Memo no. 9[15] recommended the use of an RMT in the following cases: screening for rifampin resistance; early identification of treatment failure in patients using the basic regimen; and diagnosis of new cases in the general population and in vulnerable populations. However, the use of RMTs was not recommended for treatment follow-up or diagnosis in cases of retreatment, because an RMT detects live and dead bacilli.

In the 2018 Brazilian National Guidelines for the Control of Tuberculosis,[17] an algorithm for the diagnosis of cases in settings where RMTs are available and in settings where RMTs are unavailable was provided, as were the steps for the diagnostic investigation and management of cases with conflicting test results. The need for performing sputum culture and AST was reaffirmed, and the need for assessment of social and clinical risks (clinical course of the case, previous treatment, and bacteriological curve) by specialists at tertiary referral centers in order for a treatment regimen to be selected was also highlighted. In addition, the process of validating cases that are reported to SITE-TB, through consideration of outside specialist opinion, was described.[17]

With regard to the treatment of MDR-TB, Brazil chose to standardize the drug regimens because of laboratory difficulties, although the evidence points to better results when the treatment regimen is individualized on the basis of AST results,[30] and has exercised this choice since then.[5,11] The first standard regimens were the result of a multicenter study conducted in the 1990s and validated nationwide in 2000.[20]

From then on, some changes, mainly related to the use of injectable drugs of choice and their frequency of administration, were made to the regimens. Up until 2016, the recommended frequency of administration of injectable drugs was five times per week for 2 months, then three times per week until treatment month 12.[10] In 2016, this frequency was changed to three times per week for 8 months. However, optimal treatment prescription was dependent on the clinical and bacteriological status of each patient receiving pharmacological therapy.

With regard to injectable drugs, the Second Brazilian Consensus on Tuberculosis and the Guide for Epidemiological Surveillance of Multidrug-Resistant Tuberculosis recommended a regimen containing amikacin as the regimen of choice.[10,11] However, in 2009, when the treatment regimens as a whole were changed, the use of injectable streptomycin began to be recommended, and amikacin was reserved for use in cases in which it was not possible to use streptomycin.[12-14] In 2016,[16] the use of injectable capreomycin was standardized for the first time, on the basis of the WHO recommendations, literature review, and approval by the PNCT. In addition, the use of ethionamide began to be recommended in cases of confirmed resistance to ethambutol and adjustment in the dose of levofloxacin, as recommended by the WHO.[16] These changes were reaffirmed in 2018[18] and included the addition of clofazimine to the treatment regimens, which is supported by recent studies.[25,31]

It is of note that the 2007 Guide for Epidemiological Surveillance of MDR-TB[11] presented the treatment options in a much more complete way than the documents that had been published up until then, providing a classification and division of antituberculosis drugs into pharmacological groups and a system for choosing the amount and diversity of drugs, as well as information about the properties of each drug and its major side effects. These aspects were maintained in the 2011 and 2018 Brazilian National Guidelines for the Control of Tuberculosis,[14,17] and the latter expanded the treatment options for RR-TB cases in settings where RMTs are available.[17]

It is recommended that tuberculosis therapy be administered as an outpatient regimen under supervision via DOT. All documents, to a greater or lesser extent, refer to the need to ensure treatment adherence; however, up until 2018,[17] the characteristics of patients with drug resistance had not been addressed. In 2004, the Second Brazilian Consensus on Tuberculosis[10] already acknowledges the need for treatment supervision, at that time named supervised treatment, priority being given to patients with MDR-TB. In 2011, the chapter on DOT had five pages, with no specific recommendations for cases of MDR-TB.[14] Up until then, there had been a significant gap in the organization of care because of the profile of the patients with greater clinical severity and greater social deprivation, whose management required greater efforts on the part of the teams.[32]

In this regard, the recommendations provided by the 2018 Brazilian National Guidelines for the Control of Tuberculosis[17] made a great leap, approaching those provided by the End TB strategy, namely: DOT throughout treatment, ideally five times per week or at least three times per week; possibility of carrying out DOT in various places; and the need to make choices in partnership with patients and their families "in a welcoming and supportive way".[17] In addition, the need was highlighted for DOT to be carried out in partnership with primary care, which, in turn, would have the responsibility of monitoring treatment adherence, adverse effects, and complications on a permanent basis. The possibility of referring patients and patient information to a tertiary referral center was acknowledged, although the forms of referral were not regulated.[17]

Another important aspect that has been discussed in other contexts in order to improve treatment

adherence is family involvement and support, which was recommended in 2018, but no great details about how to put that into practice were provided.[17] Studies conducted in Peru and Africa have demonstrated the importance of a community supporter, who may be a family member or someone who has been cured of MDR-TB, for strengthening treatment adherence and fighting the social stigma of the disease.[33,34]

With regard to mechanisms of social protection for patients, three of the documents analyzed indicated the need for provision of incentives, such as public transportation passes and baskets of food items, in order to increase treatment adherence.[11,14,17] In none of the documents analyzed was it possible to find the regulation of financial resources specifically intended for the provision of incentives at health care facilities.

Advances toward providing a holistic approach to patients can be observed in the 2018 Brazilian National Guidelines for the Control of Tuberculosis,[17] which address actions and partnerships to fight the social determinants of health that affect the various stages of disease pathogenesis. The challenges of overcoming poverty and promoting social development are presented, and intersectoral action is reported as an overcoming strategy.[17] The document addresses the continuing care benefit and the special social protection mechanisms to which patients with tuberculosis may resort to depending on their needs, as well as the possibilities of access to Social Security, sickness benefit, and disability retirement, all of which are conditional on previous contributions by the beneficiary. In addition, the document presents the main goals of the joint efforts of the NMH and the Brazilian Ministry of Social Development regarding intersectoral actions, including the establishment of flows between health care facilities and social care centers; assurance of food and nutritional security; integrated care plans (health and social care) for patients and their families; search for institutional care alternatives during the treatment of homeless patients; "promotion of health education activities at social care centers and dissemination of social care mechanisms at health care facilities"; and assurance of care for people who do not have an identification document, at health care facilities.[17]

With regard to information flow, it was possible to observe the evolution of the dedicated database over time through the analysis of the documents mentioned above. In the 2007 document,[18] it is possible to have a preliminary idea of the structure of the database that had been developed through a partnership between the CRPHF and Management Sciences for Health. The document presented the Internet computerized MDR-TB surveillance database, access to which was restricted to registered professionals with diverse profiles; the use of the database allowed one to follow patients undergoing treatment, seek information, and rationalize the use of medications. In addition, the document provided a flowchart of the information flow within the database and addressed the need for case validation by a referral center.[18]

Since 2013, the database called SITE-TB, which is a modified version of the MDR-TB surveillance database, has allowed case reporting, case follow-up, and case outcome reporting, classifying the different types of DR-TB.[8] In addition to being used for the reporting of cases of drug resistance, the database has been used for managing information about special treatments and nontuberculous mycobacteria, as well as for medication management, which allows dispensing, ordering, receipt, transfers, and inventory control.[35]

SITE-TB shows significant similarities to other databases used in the follow-up of cases of MDR-TB, because it allows communication among the various professionals involved in care. However, in parallel to its use, there is a need to report cases to other databases, none of which share data, and this results in data redundancy and rework for the professionals involved.[36] Another important limitation of SITE-TB is that it does not allow access by primary health care professionals; its information is intended solely for secondary and tertiary levels of health care.

Technical Memo no. 9[15] offers recommendations on the timing of case reporting to SITE-TB—when drug resistance is confirmed—which should occur concomitantly with case outcome reporting to the Brazilian Case Registry Database. It highlights the importance of updating the database to prevent drug shortages.[15] Technical Memo no. 8 also supports these recommendations.[16] However, it is in the 2018 Brazilian National Guidelines for the Control of Tuberculosis[17] that SITE-TB gains prominence with a detailed description of its functionality; case reporting and validation flow; definition of admission types and predicted outcomes; classification of cases entered in the database by drug resistance pattern; treatment follow-up and post-cure follow up; and medication management and planning.[17]

Given that patients with MDR-TB move through various levels of care, regulating how care should be organized, acknowledging its flows and counterflows, is extremely important for case management and for obtaining favorable results. The role of health care facilities was first defined in 2007.[11] Up until then, more complex cases were referred to referral centers, usually state ones.[19]

The 2007 Guide for Epidemiological Surveillance of MDR-TB[11] describes in detail the activities that should be carried out at referral health care centers, the role and duties of the various occupational categories (both at the primary and specialist care level), and the role of the PNCT's national, state, and municipal councils. It is recommended that cases be followed by a multidisciplinary team, and tertiary referral centers are responsible for the therapeutic management of all cases of drug resistance. Although emphasis is placed on primary care as the initial support network and on the importance of a structured referral and counter-referral network, there are no details regarding the duties at this level of care.[11]

The 2011 Brazilian National Guidelines for the Control of Tuberculosis[14] made it clearer which cases should be followed at each specific level of care in the chapter "Structure of Care for the Person with Tuberculosis", in which cases of drug resistance are included. Among the duties in primary care was patient referral to referral centers when there was drug resistance, treatment failure, comorbidities, important adverse effects, or difficulty in making a diagnosis: "receive and follow patients treated at referral centers and referred back to primary care, carrying out supervised treatment and investigating contacts (counter-referral)". As for communication among the various facilities, the need to record DOT on a "supervised treatment card" was mentioned; however, no model or definition of such a card was provided. The 2011 guidelines[14] also addressed hospital units and the laboratory network.

The 2018 Brazilian National Guidelines for the Control of Tuberculosis[17] went a step further regarding the organization of care, because they incorporated the 2011 RAS, contextualized tuberculosis care, and defined the role of long-stay hospitals.[37] However, in a practical sense, they did not present significant changes in the definition of flows and counterflows in the Brazilian Unified Health Care System or provide mechanisms for promoting the integration of the health care system in terms of MDR-TB care. In addition, they did not clearly identify the tertiary referral centers, where they were located, or what was their catchment area.[17] That information was not found in any of the documents analyzed.

Another significant gap in MDR-TB care in Brazil is the lack of formal mechanisms of communication among levels of care, given that the aforementioned "supervised treatment card" was not found and SITE-TB does not allow access by primary health care professionals. Therefore, although the 2018 Brazilian National Guidelines for the Control of Tuberculosis[17] went a step further in that they incorporated the RAS principles, in practice, the tools for care coordination among the various health care facilities involved were not well established, leading to fragmentation of care.

FINAL CONSIDERATIONS

The present study summarized the recommended actions and the efforts made in recent decades in Brazil for the effective control of MDR-TB. We noted a closer approximation to the WHO recommendations and definitions, which is a reflection of the change in case definition and adjustments in the standard regimens, as well as of the incorporation of new diagnostic technologies that allowed the identification of RR-TB.

The documents went a step further in that they acknowledge the need for social protection for patients with MDR-TB. However, they still lack a concrete proposal for the operationalization of social protection within the Social Security System in Brazil, with equitable measures to treat such a socially diverse population. From the same perspective, the fact that providing incentives has the potential to improve treatment adherence is acknowledged; however, the financial resources to be allocated for that purpose are not defined.

The necessary skills for the various professionals that are charged with treating MDR-TB at the various levels of care were defined, as were the measures that should be taken at each such level. Such organization needs to be presented in official publications. We believe that this is well established in the work routine of health care teams, given that MDR-TB patients are treated at referral centers. In this regard, the organization of care is moving toward the establishment of tools for communication among the various health care facilities involved in the care of these patients.

AUTHOR CONTRIBUTIONS

JGAB conceived the proposal, guided data collection, and assisted in the organization of results, discussion of findings, and writing of the manuscript. JMG contributed to the writing of the research proposal, data collection, organization of results, and writing of the manuscript. VRB, ARN, and MPD contributed to the improvement of the methodology, discussion of results, and writing and revision of the final version of the manuscript. ACSM contributed to the organization and discussion of results, as well as to the writing of the manuscript. IZR and NSM assisted in data collection, literature review, updating of reverences, and writing of the manuscript. PFP guided the proposal, obtained institutional funding, and contributed to data collection, organization of results, and discussion and revision of the final version of the manuscript.

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REVIEW ARTICLE

Shortened tuberculosis treatment regimens: what is new?

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ABSTRACT

Given the global burden of tuberculosis, shortened treatment regimens with existing or repurposed drugs are needed to contribute to tuberculosis control. The long duration of treatment of drug-susceptible tuberculosis (DS-TB) is associated with nonadherence and loss to follow up, and the treatment success rate of multidrug-resistant tuberculosis (MDR-TB) is low (approximately 50%) with longer regimens. In this review article, we report recent advances and ongoing clinical trials aimed at shortening regimens for DS-TB and MDR-TB. We discuss the role of high-dose rifampin, as well as that of clofazimine and linezolid in regimens for DS-TB. There are at least 5 ongoing clinical trials and 17 observational studies and clinical trials evaluating shorter regimens for DS-TB and MDR-TB, respectively. We also report the results of observational studies and clinical trials evaluating a standardized nine-month moxifloxacin-based regimen for MDR-TB. Further studies, especially randomized clinical trials, are needed to evaluate regimens including newer drugs, drugs proven to be or highly likely to be efficacious, and all-oral drugs in an effort to eliminate the need for injectable drugs.

Keywords: Tuberculosis/drug therapy; Tuberculosis, multidrug-resistant/drug therapy; Drug resistance, bacterial.

INTRODUCTION

Given the global burden of tuberculosis, shortened regimens with existing or repurposed drugs are needed to contribute to tuberculosis control. The current standard antituberculosis chemotherapy treatment regimen currently recommended by the World Health Organization (WHO) consists of a 2-month intensive phase with isoniazid, rifampin, pyrazinamide, and ethambutol, followed by a 4-month continuation phase with isoniazid and rifampin. Isoniazid and rifampin are the drugs with the greatest early bactericidal activity, and rifampin and pyrazinamide are the drugs with the greatest sterilizing power. Ethambutol is bacteriostatic and is strategically associated with the more potent drugs to prevent the emergence of resistant bacilli. The major justification for using this longer treatment regimen is to reduce recurrence.[1] In addition, previously published data do not support the use of shortened treatment regimens in adults with newly diagnosed pulmonary drug-susceptible tuberculosis (DS-TB). However, the long duration of DS-TB treatment is associated with nonadherence and loss to follow-up. Four-month treatment regimens that replace ethambutol with moxifloxacin or gatifloxacin, or those that replace isoniazid with moxifloxacin, increase relapse substantially when compared with standard 6-month treatment regimens.[2] However, the treatment success rate of multidrug-resistant tuberculosis (MDR-TB) is low (approximately 50%) with longer regimens, although recent studies involving new drugs have suggested that better results are possible also at the programmatic level.[3] The development of efficacious, safe, and shorter treatment regimens for both DS-TB and MDR-TB could significantly improve tuberculosis management and treatment success rates.[4]

In the present review article, we report recent advances and ongoing clinical trials aimed at shortening regimens for DS-TB and MDR-TB.

METHODS

In this nonsystematic review we searched PubMed, Google, Google Scholar, and ClinicalTrials.gov for studies evaluating short regimens for DS-TB and MDR-TB and published in English, Spanish, Portuguese, Italian, or French, published between January 1, 2014 and December 20, 2019. The following search terms were used: "treatment" AND "tuberculosis" OR "drug-susceptible tuberculosis" OR "MDR-TB".

SHORTENED REGIMENS FOR DS-TB

The treatment currently recommended by the WHO for patients with DS-TB lasts at least 6 months: a 2-month intensive phase (isoniazid, rifampin, pyrazinamide, and ethambutol) followed by a continuation phase of 4 months with isoniazid and rifampin. The long duration of this treatment regimen is a major barrier to adherence and has a significant negative impact on tuberculosis control.[5]

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Current evidence from in vitro, animal, and human studies suggests that high-dose rifampin can shorten the duration of tuberculosis treatment.[6] In a multicenter, randomized, controlled, triple-blinded clinical trial[7,8] involving 180 treatment-naïve adults diagnosed with pulmonary tuberculosis (positive sputum smear) were allocated to receiving rifampin at 10 mg/kg per day (control group), 15 mg/kg per day, or 20 mg/kg per day during the intensive phase. Higher rifampin doses were associated with more rapid sputum sterilization, and toxicity was similar to that of the standard dose.

Finding the optimal higher-than-standard dose of rifampin and avoiding toxicity is a challenge. Svensson et al.[9] evaluated 336 patients with newly diagnosed pulmonary tuberculosis from seven sites in Tanzania and South Africa who were treated with rifampin at 10, 20, or 35 mg/kg. Higher rifampin doses increased the probability of a reduction in time to culture conversion, with no maximal limit of the effect, suggesting that doses > 35 mg/kg could be more effective.[9]

Clofazimine, an antileprosy agent, showed significant bactericidal and sterilizing activity in a mouse model of MDR-TB treatment[10] and enabled significant shortening of treatment duration in MDR-TB patients.[11-14] Recently, clofazimine was repurposed in the new short-course MDR-TB regimen.[11] When added to the first-line regimen for DS-TB in a mouse model of tuberculosis, clofazimine demonstrated greatest activity when used continuously throughout the treatment together with the first-line drugs, doses at 12.5 mg/kg and 25 mg/kg being equivalent.[15] However, the optimal dose of clofazimine in the first-line regimen is uncertain, especially as it may cause dose-dependent skin discoloration.[16,17] Also, adding clofazimine and replacing rifampin with high-dose rifapentine in the first-line regimen was associated with greater bactericidal and sterilizing activity when compared with either modification alone in another mouse model.[18] In a large program-based Brazilian study,[19] clofazimine was well tolerated, with a low proportion of adverse events, such as gastrointestinal complaints (10.5%) and neurological disturbances (9-13%); however, hyperpigmentation was present in 50.2%.

Linezolid, an oxazolidinone currently recommended for MDR-TB treatment,[11] could have a potential role in shortening DS-TB treatment. Previous studies[20,21] suggested that a reduction in the dose from 1,200 to 600 mg/day was able to reduce the proportion of serious adverse events.

In a recent phase 2, multicenter, randomized, open-label trial[22] for patients with pulmonary tuberculosis at three hospitals in South Korea, the authors evaluated the substitution of linezolid for ethambutol during the intensive phase of treatment. The use of linezolid at 600 mg once daily for 2 weeks was associated with a higher proportion of 8-week culture negativity when compared with control arms.[22] Nevertheless, linezolid has a narrow therapeutic window, and its prolonged use could result in peripheral/optic neuropathy and bone marrow suppression.[23] In a recent active drug-safety monitoring and management global study by the Global Tuberculosis Network, the proportion of serious adverse events attributed to linezolid, such as peripheral neuropathy, optic neuritis, severe urticaria, anemia, and bone marrow depression, was 2.8% (15/536).[24,25]

Table 1 shows details of ongoing clinical trials evaluating shortened regimens for DS-TB. A trial designated S31/A5349 (NCT02410772)[26] is currently underway to determine whether one or two 4-month regimens for tuberculosis treatment are as effective as the standard 6-month regimen. The first short regimen is a single substitution of rifapentine for rifampin: isoniazid, rifapentine, ethambutol, and pyrazinamide for 2 months, followed by isoniazid and rifapentine for 2 months. The second short regimen is a double substitution of rifapentine for rifampin and of moxifloxacin for ethambutol: isoniazid, rifapentine, moxifloxacin, and pyrazinamide for 2 months, followed by isoniazid, rifapentine, and moxifloxacin for 2 months.[26]

The trial designated TRUNCATE-TB (NCT03474198)[27] is a randomized, open-label, multi-arm, multistage trial to test the hypothesis that treatment for 2 months (8 weeks, extended to 12 weeks if there is inadequate clinical response) with four potentially boosted regimens is non-inferior to the standard management strategy.[27]

The designated RIFASHORT trial (NCT02581527)[28] is an open-label three-arm trial in order to compare a standard 6-month control regimen with two 4-month treatment regimens (with increased doses of rifampin—1,200 mg or 1,800 mg) for the treatment of tuberculosis. The objective is to assess whether high doses of rifampin for 4 months will result in greater and faster killing of tuberculous bacilli in the lungs, as well as in relapse rates similar to those obtained with the standard 6-month regimen.

SHORTENED REGIMENS FOR MDR-TB

From 2005 to 2011, 515 patients were enrolled in a prospective, observational study from the Damien Foundation in Bangladesh[29] in order to evaluate the first treatment for MDR-TB using a standardized regimen, consisting of high-dose gatifloxacin, ethambutol, pyrazinamide, and clofazimine for at least 9 months, supplemented during the intensive phase (for 4 months at least) with kanamycin, prothionamide, and isoniazid. The 4-month intensive phase was extended until sputum smear conversion. Due to extensive disease with delayed sputum conversion, only half of the patients completed treatment within 9 months; however, 95% were able to complete

Table 1. Ongoing clinical trials evaluating shortened regimens for drug-susceptible tuberculosis.

ClinicalTrials.gov Identifier (study name)	Phase	Study population	Study groups	Status	Results expected in (year)
NCT02410772 (TBTC 31/A5349)	3	2,500 adults and children (≥ 12 years), HIV+ and HIV-	2 months of isoniazid, rifapentine, ethambutol, and pyrazinamide, followed by 2 months of isoniazid and rifapentine, or 2 months of isoniazid, rifapentine, moxifloxacin, and pyrazinamide, followed by 2 months of isoniazid, rifapentine, and moxifloxacin vs. standard 6-month therapy	Active, not recruiting	2020
NCT03474198 (TRUNCATE-TB)	2/3	900 adults, HIV+ and HIV-	Standard 6-month therapy vs. Regimen B: rifampin (35 mg/kg), isoniazid, pyrazinamide, ethambutol, and linezolid; or Regimen C: rifampin (35 mg/kg), isoniazid, pyrazinamide, ethambutol, and clofazimine; or Regimen D: rifapentine, isoniazid, pyrazinamide, linezolid, and levofloxacin; or Regimen E: isoniazid, pyrazinamide, ethambutol, linezolid, and bedaquiline	Recruiting	2022
NCT02581527 (RIFASHORT)	3	654 adults, HIV-	2 months of ethambutol, isoniazid, rifampin (1,200 mg or 1,800 mg), and pyrazinamide daily, followed by 2 months of isoniazid and rifampin (1,200 mg or 1,800 mg) daily vs. standard 6-month therapy	Recruiting	2021
NCT03338621	2c/3	450 adults, HIV+ and HIV-	BPaMZ regimen: bedaquiline 200 mg daily for 8 weeks then 100 mg daily for 9 weeks, together with pretomanid 200 mg + moxifloxacin 400 mg + pyrazinamide 1,500 mg daily for 17 weeks (total treatment duration: 4 months) vs. standard 6-month therapy	Recruiting	2022
NCT03561753	2b	300 adults, HIV-	PRS regimen: 4 months of daily clofazimine, ethambutol, prothionamide, and high dose pyrazinamide vs. standard 6-month therapy	Enrolling by invitation	2021

treatment within 12 months, and 84.4% had a bacteriologically favorable outcome. An external review of that project[29] conducted in 2007 by the WHO concluded that additional data from a clinical trial were needed.[30] The 2011 WHO guidelines recommended an intensive treatment phase of 8 months and a total treatment duration of 20 months.[31]

A clinical trial initiated in 2012 (designated STREAM) compared a 9-month moxifloxacin-based regimen with the WHO-recommended 20-24 month regimen.[32]

In 2016, the WHO introduced new guidelines for the treatment of MDR-TB, including recommendations for isolated use of bedaquiline or delamanid and a shorter MDR-TB treatment regimen.[11] They recommended that in patients with rifampin-resistant tuberculosis or MDR-TB who had not previously been treated with second-line drugs and in whom resistance to fluoroquinolones and second-line injectable drugs was excluded or considered as highly unlikely, a shorter (9-12 months) MDR-TB regimen might be used instead of longer regimens.[11]

In 2018, Trébucq et al.[12] reported the results of a prospective observational study in nine African countries that evaluated a standardized 9-month moxifloxacin-based regimen in 1,006 MDR-TB patients. The treatment success rate was 81.6% and did not differ by HIV status. Despite being an observational study, its results support the efficacy and good tolerability of the regimen. However, hearing loss was reported in 7.1% of the patients.

In March of 2019, the initial results of the abovementioned trial[32] were published.[33] The authors found that, in patients with rifampin-resistant tuberculosis that was susceptible to fluoroquinolones and aminoglycosides, a shorter regimen (9-11 months, including high-dose moxifloxacin) was non-inferior to the longer regimen (20 months, following the 2011 WHO guidelines) with respect to the primary efficacy outcome (negative cultures at week 132) and was similar to the longer regimen in terms of safety. However, the participants in the short-regimen group had more adverse events (grade 3 or more), prolongation of either the QT interval or the QTc to 500 milliseconds, acquired resistance to fluoroquinolones or aminoglycosides, and death; nevertheless, the differences were not significant. A development of that clinical trial,[32] also designated STREAM (NCT02409290),[34] is currently evaluating the efficacy of a short, fully oral regimen containing bedaquiline; the results are expected in 2022.[35] Recently, a study on individual patient data meta-analysis[36] compared longer and shorter regimens in

terms of safety and efficacy, substantially confirming the results of that trial.[33]

A regimen approved by the U.S. Food and Drug Administration in mid-2019 (bedaquiline, pretomanid, and linezolid for 6-9 months) was recommended by the WHO in a rapid communication published in December of 2019.[37] The regimen improved treatment outcomes in patients with extensively drug-resistant tuberculosis and can be used under operational research conditions in those patients for whom design of an effective regimen based on existing recommendations is not possible, as well as in those who have not had previous exposure to bedaquiline and linezolid (defined as < 2 weeks). However, further evidence on efficacy and safety is needed to consider its programmatic use worldwide.[37]

Table 2 shows details of ongoing clinical trials evaluating shortened regimens for MDR-TB. A phase 2/3, multicenter, randomized, open-label clinical trial of non-inferiority design designated MDR-END (NCT02619994)[38] aims at comparing a new shorter regimen including delamanid, linezolid, levofloxacin, and pyrazinamide for 9 or 12 months (depending on the time to sputum culture conversion) with a conventional treatment regimen with second-line drugs, including injected drugs, for 20-24 months. The primary outcome is treatment success rate at 24 months after treatment initiation, and the results are expected by 2021.[38]

The designated endTB clinical trial (NCT02754765)[39] is a phase 3, randomized, controlled, open-label, non-inferiority, multi-country trial evaluating the efficacy and safety of five new, all-oral, shortened regimens for MDR-TB. The regimens examined combine the newly approved drugs bedaquiline and/or delamanid with existing drugs known to be active against *Mycobacterium tuberculosis* (linezolid, clofazimine, moxifloxacin or levofloxacin, and pyrazinamide). Results are expected by 2021.

The designated TB-PRACTECAL trial (NCT02589782)[40] is a multicenter, open-label, multi-arm, randomized, controlled phase 2/3 trial, aimed at evaluating short treatment regimens containing bedaquiline and pretomanid in combination with existing and repurposed antituberculosis drugs for MDR-TB treatment. The study will be divided into two stages: the primary outcome measures in stage 1 are the proportion of patients with culture conversion in liquid media and the proportion of patients who discontinue treatment for any reason or die at 8 weeks after randomization; the primary outcome measure in stage 2 is the proportion of patients with an unfavorable outcome at week 72.

The designated GRACE-TB trial (NCT03604848)[41] is a multicenter, open-label, randomized, controlled clinical trial involving patients with MDR-TB. The objective is to assess the feasibility and effects of individualized regimens for MDR-TB based on rapid molecular drug susceptibility tests of key second-line drugs by means of next-generation sequencing. The trial will evaluate a shorter course regimen (pyrazinamide, amikacin, moxifloxacin, prothionamide, and cycloserine for 9 or 12 months) among patients whose MDR-TB is proven to be susceptible to fluoroquinolones, second-line injectable drugs, or pyrazinamide by next-generation sequencing.

The designated TB-TRUST trial (NCT03867136)[42] is a phase 3, multicenter, open-label, randomized controlled trial aiming at assessing the efficacy, safety and tolerability of an ultra-short treatment regimen of all-oral antituberculosis drugs (levofloxacin, linezolid, cycloserine, and pyrazinamide [or clofazimine if pyrazinamide-resistant]) compared with the WHO standardized shorter regimen of 9-11 months. The primary outcome measure is the treatment success rate without relapse in 24 months. The results are expected by 2022.

FINAL CONSIDERATIONS

The present review shows that many observational studies and clinical trials have demonstrated the potential of shortened regimens for the treatment of both DS-TB and MDR-TB. In addition to reducing costs, the use of shorter regimens can improve adherence and, consequently, treatment completion. However, further studies, especially randomized clinical trials[32] and pragmatic clinical trials, are needed to evaluate regimens including newer drugs, drugs drugs proven to be or highly likely to be efficacious, and all-oral drugs in an effort to eliminate the need for injectable drugs.[43,44] The potential of using programmatic data and individual patient data meta-analysis has recently been highlighted.[43]

New approaches need to be identified to shorten treatment duration, including the possibility of administering new drugs (e.g., bedaquiline and delamanid) together, as recent evidence suggests that it can be safer than it was initially considered.[45]

Importantly, active drug-safety monitoring and management, which is recommended by the WHO for new drugs and regimens for drug-resistant tuberculosis, should be integrated into tuberculosis programs.[24,25,46] In addition, drug susceptibility testing should be available where new regimens are to be implemented in order to allow the identification of resistance patterns and to avoid selecting strains resistant to promising new regimens.[37,44]

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Table 2. Ongoing clinical trials evaluating shortened regimens for multidrug-resistant tuberculosis.

ClinicalTrials.gov Identifier (study name)	Phase	Study population	Study groups	Status	Results expected in (year)
NCT02619994 (MDR-END)	2/3	238 adults, HIV+ and HIV-	Delamanid, linezolid, levofloxacin, and pyrazinamide for 9 or 12 months (depending on time to sputum culture conversion) vs. locally-used WHO-approved MDR-TB regimen (intensive phase regimen consists of four effective second-line antituberculosis drugs, including injectable second-line drugs, and pyrazinamide for at least 20 months)	Recruiting	2021
NCT02409290 (STREAM stage 2)	3	530 adults and children (≥ 15 years), HIV+ and HIV-	Moxifloxacin, clofazimine, ethambutol, and pyrazinamide daily for 9 months, with initial isoniazid, kanamycin, and prothionamide daily for 2 months; or bedaquiline, clofazimine, ethambutol, levofloxacin, and pyrazinamide daily for 9 months, with initial isoniazid (high dose) and prothionamide daily for 2 months (all oral); or bedaquiline, clofazimine, levofloxacin, and pyrazinamide daily for 6 months, with initial isoniazid (high dose) and kanamycin for 2 months vs. 20-24-month local regimen	Recruiting	2022
NCT02754765 (endTB)	3	750 adults and children (≥ 15 years), HIV+ and HIV-	Bedaquiline, linezolid, moxifloxacin, and pyrazinamide daily for 9 months; or bedaquiline, linezolid, clofazimine, levofloxacin, and pyrazinamide daily for 9 months; or bedaquiline, linezolid, delamanid, levofloxacin, and pyrazinamide daily for 9 months, or delamanid, linezolid, clofazimine, levofloxacin, and pyrazinamide daily for 9 months; or delamanid, clofazimine, moxifloxacin, and pyrazinamide daily for 9 months vs. local regimen	Recruiting	2021
NCT02589782 (TB PRACTECAL)	2/3	630 adults and children (≥ 15 years), HIV+ and HIV-	Bedaquiline, pretomanid, moxifloxacin, and linezolid daily for 6 months; or bedaquiline, pretomanid, linezolid, and clofazimine daily for 6 months; or bedaquiline, pretomanid, and linezolid daily for 6 months (all oral) vs. local regimen	Recruiting	2021
NCT01918397 (Opti-Q)	2	111 adults (> 18 years), HIV+ and HIV-	Levofloxacin (14, 17, or 20 mg/kg daily) plus optimized background regimen for 6 months vs. levofloxacin (11 mg/kg daily) plus optimized background regimen for 6 months	Active, not recruiting	2020
NCT02333799	3	109 adults and children (≥ 14 years), HIV+ and HIV-	Single arm study: bedaquiline (200 mg daily for 2 weeks, then 200 mg three times weekly), pretomanid (200 mg daily), and linezolid (600 mg twice daily) for 6 months	Active, not recruiting	2021
NCT02454205 (NEXT)	2/3	154 adults (> 18 years), HIV+ and HIV-	Bedaquiline, linezolid, levofloxacin, pyrazinamide, and either high-dose isoniazid or ethionamide or terizidone daily (all oral) for 6-9 months vs. conventional treatment (kanamycin, moxifloxacin, pyrazinamide, ethionamide, and terizidone for 21-24 months)	Active, not recruiting	2020
NCT03141060	1/2	48 children (<18 years), HIV+ and HIV-	Single arm study: bedaquiline (100 mg twice daily) plus optimized background regimen for 6 months	Recruiting	2021
NCT03338621	2c/3	450 adults, HIV+ and HIV-	Single arm study: bedaquiline, pretomanid, moxifloxacin, and pyrazinamide for 6 months	Recruiting	2022

WHO: World Health Organization; and MDR-TB: multidrug-resistant tuberculosis.

Table 2. Continued...

ClinicalTrials.gov Identifier (study name)	Phase	Study population	Study groups	Status	Results expected in (year)
NCT03086486 (ZeNix)	3	180 adults and children (≥ 14 years), HIV+ and HIV-	Linezolid (600 or 1,200 mg daily, double-blinded), bedaquiline (200 mg daily for 2 weeks, then 100 mg daily), and pretomanid (200 mg daily) for 2 or 6 months	Recruiting	2021
NCT02354014	2	60 children (< 18 years), HIV-	Single arm study: bedaquiline (daily for 2 weeks, then 3 times a week) plus optimized background regimen for 6 months	Recruiting	2025
NCT02583048	2	84 adults (> 18 years), HIV+ and HIV-	Bedaquiline plus optimized background regimen for 6 months; or delamanid plus optimized background regimen for 6 months, or bedaquiline and delamanid plus optimized background regimen for 6 months	Active, not recruiting	2021
NCT03604848 (GRACE-TB)	3	488 adults (≥ 18 years), HIV-	Pyrazinamide, amikacin, moxifloxacin, prothionamide, and cycloserine for 9 or 12 months vs. WHO-approved MDR-TB regimen for 24 months	Not yet recruiting	2024
NCT03867136 (TB-TRUST)	3	354 adults (≥ 18 years), HIV-	Levofloxacin, linezolid, cycloserine and pyrazinamide(or clofazimine if resistant to pyrazinamide) for 6 to 8 months vs. WHO standardized shorter regimen of 9-11 months	Not yet recruiting	2022
NCT01859923	2	37 children (<18 years), HIV+ and HIV-	Delamanid plus optimized background regimen for 6 months	Active, not recruiting	2020
NCT03896685 (endTB-Q)	3	324 adults and children (≥ 15 years), HIV+ and HIV-	BeDeCLi regimen: bedaquiline-delamanid-linezolid-clofazimine for 24 or 39 weeks vs. local regimen according to WHO guidelines	Not yet recruiting	2022
NCT04062201 (BEAT)	3	400 adults and children (≥ 12 years), HIV+ and HIV-	Bedaquiline, delamanid, and linezolid plus levofloxacin and clofazimine for 6 months vs. local regimen for 9 months	Recruiting	2023

WHO: World Health Organization; and MDR-TB: multidrug-resistant tuberculosis.

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Cost of precision medicine at a referral center for cystic fibrosis

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TO THE EDITOR:

We are currently experiencing an exciting period of scientific advances in patient care. Precision medicine and personalized medicine have allowed us to dream of the ability to treat numerous diseases based on their root causes. In cystic fibrosis (CF), the recent integration of precision medicine into routine patient care has enabled the management of CFTR protein expression and has brought hope for the treatment of the disease, with improved quality of life and increased life expectancy.

In CF, precision medicine uses three US Food and Drug Administration-approved drugs, namely ORKAMBI® (lumacaftor/ivacaftor), SYMDEKO® (tezacaftor/ivacaftor and ivacaftor), and KALYDECO® (ivacaftor), all of which are manufactured by Vertex Pharmaceuticals, Inc. (Boston, MA, USA). Substantial clinical benefits have been obtained with a novel combination therapy (VX-659–tezacaftor-ivacaftor) in comparison with placebo, with a change of 14 percentage points in percent predicted FEV_1 (FEV_1%) in individuals with one F508del mutation and one minimal function mutation, as well as a change of 10 percentage points in FEV_1% in individuals with two F508del mutations initially treated with tezacaftor-ivacaftor and subsequently treated with tezacaftor-ivacaftor plus VX-659. In addition, treatment with the triple combination therapy of VX-455–tezacaftor-ivacaftor has been tested in phase I and II clinical trials, with significant improvement in FEV_1%.[1-5]

The outcomes of CF clinical trials have been remarkable. Although the initial results obtained from precision medicine clinical trials showed only a slight improvement in FEV_1% (of < 2-4 percentage points), recent studies have shown a significant improvement in the quality of life and life expectancy of CF patients. However, the economic dimension of precision medicine, including the high cost of developing drugs and running trials, is a barrier to the use and implementation of new therapies. From the discovery of a new molecule to the clinical application of a new drug, high costs are involved. In CF, the final cost of new precision medicine drugs depends on the following: the high cost of clinical trials; lengthy timelines; difficulties in recruiting participants (because the *CFTR* genotype needs to be identified); limited clinical research capacity; strict regulations; administrative barriers; data collection and interpretation; and difficulties in maintaining and monitoring safety. In addition, health care costs rise exponentially when precision medicine is used.

Although the Brazilian *Agência Nacional de Vigilância Sanitária* (ANVISA, National Health Surveillance Agency) is charged with the approval and regulation of pharmaceutical drugs, public health care facilities need further authorization to dispense drugs free of charge to the population. In 2018, the Brazilian government approved the first precision medicine drug for use in CF patients in Brazil. However, the costs must be borne by the patient. The next step should be the support from the public health care system to provide the drug free of charge to all CF patients on the basis of their *CFTR* genotype. However, this raises a controversial question: How much can we afford?

In Brazil, approximately 140 patients at a referral center for CF are eligible for treatment with a precision medicine drug, with total treatment costs estimated at US$ 40,308,420 per year. The classification of *CFTR* mutations was not taken into account because the US Food and Drug Administration did not approve the use of precision medicine drugs for all *CFTR* mutations (Table 1),[6] and the costs were calculated on the basis of the US market in order to provide an international overview of the drug price. Neither our institution nor the public health care system can afford to spend that much on (treating) a single disease. A total financial support of US$ 123,710,785.70 should cover all hospital procedures, including all routine medical consultations. In addition, the cost of treating CF patients amounts to approximately one third of the total cost of maintaining hospital activities.

Some insights can help resolve the controversy over the (estimated) cost of treating a disease and pricing the priceless, i.e., the improvement of health. First, a new drug should be prescribed only for patients who will truly benefit from it, primarily on the basis of the individual response to CF drugs in nasal cell cultures (from patients with *CFTR* and modifier gene variants).[7-10] Second, all *CFTR* genotypes should be identified in order to determine whether precision medicine is feasible. Third, the government and the pharmaceutical industry should discuss costs, benefits, and a partnership for mutual benefit. Fourth, medical societies, as well as patients and their families, together with nongovernmental organizations and researchers, should discuss the possibilities of precision medicine, implementing medication adherence policies and reducing the costs of long-term therapies. Finally, precision medicine should be used in the treatment of other diseases. For example, ataluren

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Table 1. Precision medicine drugs approved for use in the treatment of cystic fibrosis at a referral center in Brazil, as well as an overview of the hospital where the referral center is located.[a]

Drug[b]	n	Monthly cost/ patient	Annual cost/ patient	Total annual cost
ORKAMBI® or SYMDEKO®[c]	57	US$ 26,880	US$ 322,560	US$ 18,385,920
ORKAMBI®	76	US$ 21,583	US$ 259,000	US$ 19,884,000
KALYDECO®	5	US$ 28,675	US$ 344,100	US$ 1,720,500
ORKAMBI® or KALYDECO®[d]	2	US$ 21,583	US$ 259,000	US$ 518,000
Total				US$ 40,308,420
Overview of the hospital where the referral center is located				
Financial support (university and health care system)		US$ 123,710,785.70 (R$ 460,000,000.00)[e]		
Number of hospital beds		419		
Number of beds in the adult ICU		409		
Number of beds in the pediatric ICU		56		
Bed occupancy rate		85%		
Number of hospitalizations		14,442 per year		
Number of medical specialties		47 (580 subspecialties)		
Number of patients receiving emergency room treatment		69,573 per year		
Number of patients receiving outpatient treatment		373,574 per year		
Geographic area of coverage		~100 cities (~5,000,000 inhabitants)		
Number of visitors		~10,000 per day		
Number of operating rooms		16		
Number of surgical procedures		15,509 per year		
Number of transplants		485 per year[f]		
Number of medical records since the first year of the hospital's inception		1,000,000		
Number of new records		~150 per day		
Number of laboratory tests		2,529,209 per year (more than 300 different types of tests)		
Number of radiological examinations		146,375 per year		
Number of nuclear medicine examinations		9,532 per year		
Number of radiotherapy cycles		47,906 per year		
Hospital pharmacy		2,313,771 units of medication + 843,265 saline bottles		
Number of blood bags used		6,730 per month		
Number of surgical gloves used		2,500,000 per year		
Number of thermometers used		60 per month		
Amount of water consumed		9,227 m^3 per month		
Amount of oxygen consumed		35,715 m^3 per month		
Number of hospital sheets used		2,000 per day		

[a]Adapted from Pereira.[6] [b]ORKAMBI®: lumacaftor/ivacaftor as 100 mg/125 mg and 150 mg/188 mg granule packets for children ≥ 2 years of age or 100 mg/125 mg and 200 mg/125 mg tablets for children ≥ 6 years of age; SYMDEKO®: tezacaftor 50 mg/ivacaftor 75 mg and ivacaftor 75 mg for patients ≥ 12 years of age with the F508del/F508del genotype; KALYDECO®: ivacaftor 150 mg, approved for use in individuals ≥ 2 years of age with at least one copy of a class III variant (E56K, G178R, S549R, K1060T, G1244E, P67L, E193K, G551D, A1067T, S1251N, R74W, L206W, G551S, G1069R, S1255P, D110E, R347H, D579G, R1070Q, D1270N, D110H, R352Q, S945L, R1070W, G1349D, R117C, A455E, S977F, F1074L, R117H, S549N, F1052V, or D1152H). The prices of all three medications have been established by the manufacturer (Vertex Pharmaceuticals, Inc., Boston, MA, USA). [c]Values calculated for SYMDEKO®. [d]Values calculated for ORKAMBI®. [e]Calculated on the basis of the exchange rate on July 27, 2018 (US$ 1.00 = R$ 3.718). [f]No lung transplants were performed at our facility during that period. Note: At this writing, the Brazilian *Agência Nacional de Vigilância Sanitária* (ANVISA, National Health Surveillance Agency) has yet to approve the use of SYMDEKO® in the country. Because of that, we used the drugs approved by the US Food and Drug Administration protocol and their age recommendation for drug use in order to facilitate the comparison of our findings with those of other studies.

has been discontinued for the treatment of CF, but it is still prescribed for the treatment of Duchenne muscular dystrophy and Becker muscular dystrophy caused by nonsense mutations in the *DMD* gene.

Precision medicine gives us hope, and genome editing tools are being investigated for the treatment of CF. In the long run, gene therapy will be used as a treatment model for CF.

Is precision medicine cost-effective? Is the heavy upfront investment legitimate? What is the total cost of innovation: developing and releasing a new drug and the moral issue of pricing and profit? This letter is a reflection on the application of new therapies (using CF as model) and their financial impact on health care systems. In addition, this letter invites patients, civil society, governmental officials, and the pharmaceutical

industry to discuss the major outcomes of new therapies and markers, including quality-adjusted life years.

The *CFTR* gene was described as the cause of CF in 1989. Since then, we have dreamed of treating the root cause of the disease. We have made remarkable progress with research on phenotype variability, *CFTR* variants, and modifier genes, and we should continue to research and translate these findings into new diagnostic methods and therapies. Although high costs can be a barrier, they can be overcome through the collaborative input of all involved parties. We believe in the promise of precision medicine to improve quality of life and life expectancy, and our efforts should be geared toward allowing precision medicine to reach its full expectations.

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COVID-19 pneumonia: what is the role of imaging in diagnosis?

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TO THE EDITOR:

The current global COVID-19 pandemic is related to an acute respiratory disease caused by a new coronavirus (SARS-CoV-2), which is highly contagious and whose evolution is still little known. Considering the current case definition, based on the diagnosis of pneumonia, more than 100,000 cases of COVID-19 infection have been confirmed worldwide, and the associated mortality rate has fluctuated around 2%.[1] However, recent changes in the diagnostic criteria of the disease have led to an increase in the rate of new cases and, on a daily basis, increasing numbers and challenges have been the subject of intense debate on the topic by the scientific community.

To date, reverse-transcriptase polymerase chain reaction (RT-PCR) remains the gold standard for the definitive diagnosis of COVID-19 infection, despite reports of false-negative results (due to insufficient cellular material or inadequate detection and extraction techniques) in face of positive radiological findings.[2] Because currently available laboratory tests might not be widely accessible to a growing infected population, new screening strategies are necessary. In this context, chest X-rays have not been recommended as a first-line imaging modality for the diagnosis of COVID-19 due to its limited sensitivity in the detection of ground-glass opacities and other incipient pulmonary findings of the infection.[3,4] However, although the use of chest CT as a screening tool has yet to be determined, recent studies have demonstrated a central role of CT in the early detection and management of COVID-19 pulmonary manifestations. It has shown high sensitivity but limited specificity.[3,5]

To date, most of the cases reported have presented similar tomography findings (Figure 1), with alveolar changes predominating, such as ground-glass opacities, focal consolidations, and mixed opacities (including reversed halo sign), usually with bilateral and multifocal involvement, peripheral distribution, and predominance in the middle, lower, and posterior lung fields.[6-8] Septal thickening and reticular changes superimposed on alveolar changes have also been described and reflect concomitant interstitial involvement, especially in patients in advanced stage (8-14 days after the appearance of symptoms).[9] Incipient lung scarring (fibrotic bands) and pleural effusion have also been more common in the advanced stage of the disease when compared with the early stages, when alveolar changes, especially ground-glass opacities, predominated.[9]

The dissociation between clinical, laboratory, and imaging findings has been demonstrated in some cases. It is estimated that up to 50% of the patients infected with COVID-19 might have normal chest CT scans within the first two days after the onset of the symptoms.[5] In addition, the existence of patients infected with COVID-19 confirmed by positive RT-PCR and with normal chest CT at admission and during a follow-up period after 2-3 weeks[10] reinforces the current understanding that normal chest CT results should not be considered for the exclusion of the diagnosis, especially in patients with recent onset of the disease.[11] Patients with a high degree of clinical suspicion, typical tomographic findings, and negative RT-PCR results have also been found; in such cases, it is recommended that laboratory tests be repeated and contact isolation should be considered.[2] Given these possibilities, chest CT findings were excluded from the

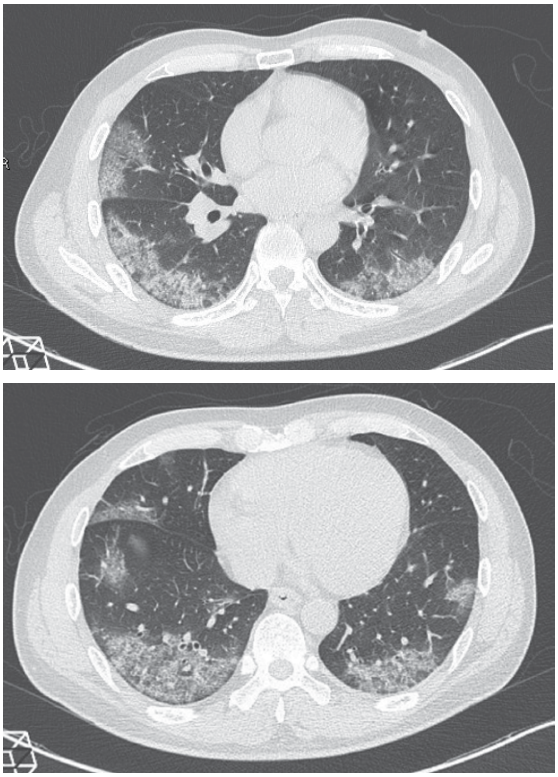

Figure 1. Axial chest HRCT scans showing multifocal and bilateral ground-glass opacities, with peripheral and posterior predominance, which are typical pulmonary findings of COVID-19 infection (confirmed by RT-PCR).

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diagnostic criteria of COVID-19 in the sixth edition of the Coronavirus Pneumonia Diagnosis and Treatment Program guidelines proposed by General Office of China Health Commission.[12] However, until further ongoing studies on the role of CT in COVID-19 pneumonia are published, an integrated analysis of clinical, laboratory, and radiological aspects should be recommended, aiming at the early diagnosis of the disease.

In conclusion, CT should not be used for COVID-19 screening in asymptomatic patients, but may be considered in hospitalized patients, symptomatic cases, or in specific clinical situations. Tomography findings of COVID-19 pneumonia are nonspecific and similar to those of other pulmonary infections, and they vary according to the stage of the disease onset. They must be correlated with clinical and laboratory evidence of COVID-19 infection. To date, it is recommended that the final diagnosis of the disease be confirmed by a positive RT-PCR test or genetic sequencing. Clinicians and radiologists should be familiar with the spectrum of COVID-19 involvement and be vigilant to identify and treat the affected patients early. Those patients may have few clinical symptoms, normal findings on chest CT, and even negative laboratory tests at the beginning.

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Analysis of the incidence of latent *Mycobacterium tuberculosis* infection among primary health care professionals in two Brazilian capitals

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TO THE EDITOR:

One quarter of the world's population is estimated to have latent *Mycobacterium tuberculosis* infection (LTBI), which generates potential new cases of active tuberculosis.[1] Most cases progressing to active disease occur within the first 2-5 years after exposure to a tuberculosis index case.[2] Health care professionals in whom there has been conversion of an LTBI test—tuberculin skin test (TST) or interferon-gamma release assay (IGRA)—are at risk of developing active disease. Therefore, the Brazilian National Ministry of Health[3] recommends annual LTBI screening of this group and the prescription of prophylaxis for those cases in which any of the tests used in order to diagnose this infection have converted.

In the present study, QuantiFERON-TB Gold In-Tube test (QFT; Qiagen, Hilden, Germany), an IGRA, was used to determine the incidence of LTBI among primary health care professionals in two Brazilian capitals: Vitória (incidence rate = 40/100,000 population); and Manaus (incidence rate = 71/100,000 population).

This was a prospective cohort study, conducted between 2011 and 2013, involving primary health care professionals (physicians, nurses, nursing assistants or technicians, and community health agents) who received QFT at two different time points, between 18 and 24 months of follow-up.

All participating professionals gave written informed consent. Blood sample collection for the QFT and rapid HIV testing was carried out as described by Souza et al.,[4] as were other methodological details.

In 2013, the cohort was evaluated again. In addition to the previous inclusion criteria, having a negative result on the first QFT was considered an inclusion criterion for the second phase of the study. To minimize variability, similar protocols were used for the baseline and follow-up tests.

Since, at that time, QFT results were not standardized for diagnosing LTBI in Brazil,[5] participants with positive results in the first phase of the study were evaluated by an infectious disease specialist, who considered QFT and TST results. At that time, those with TST conversion were referred to the local tuberculosis control program.

The present study was approved by the Research Ethics Committee of the Federal University of Espírito Santo, located in the city of Vitória, Brazil (Protocol no. 007/10, March of 2010). All information was coded and stored anonymously in a database created with Microsoft Excel for Windows. For the descriptive analysis, we used STATA software, version 13.1 (StataCorp LP, College Station, TX, USA), and we included the estimate of the risk of QFT conversion, calculated using the actuarial method Cumulative Incidence Based on the Life-Table Interval Approach (Actuarial Life Table).[6,7] In this method, the cumulative probability of an event over a given time interval ($t_0 - t$) is the proportion of new events over that period, the denominator being the initial population (N_0) corrected for losses (W) that are assumed to be evenly distributed over the time interval of interest; therefore, on average, it is as if all losses occur in the middle of the observation period (W/2), according to the following equation[6,7]:

$$R_{(t_0 - t)} = \frac{1}{\left(N_0 - \left(\frac{W}{2}\right)\right)}$$

where $t0 - t$ is the time interval of interest (between 18 and 24 months); I is the number of new cases at $t0 - t$ (QFT conversion occurred in 12 primary health care professionals); $N0$ is the number of individuals at risk at the beginning of the follow-up period (339 primary health care professionals); and W is the number of losses at $t0 - t$ (276 primary health care professionals).

In the first phase of the study, 339 primary health care professionals were evaluated, 303 (89.4%) of whom were women, and the median age (interquartile range) was 41 (26-63) years.

The prevalence of LTBI, as determined by QFT, was 23.3% in the first phase of the study. Therefore, 260 primary health care professionals were eligible for follow-up, given that their first QFT result was negative. Because of the high turnover of these professionals in the public health care system, plus their absence from the health care facilities on the scheduled day, 63 primary health care professionals (24.2%) were available for the second evaluation.

The median age in the second phase of the study was 46 (39-53) years, 10 individuals (16.4%) reported morbidity, and only 7 (11.1%) reported having had close

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contact with someone with tuberculosis in the last 2 years. With regard to occupational and biosafety characteristics, 44 individuals (69.8%) reported having cared for tuberculosis patients in the last 2 years, and 40 (64.4%) reported that N95 masks were not always available for use.

Considering random losses and the ratio of the proposed calculation, the probability of QFT conversion between 18 and 24 months of follow-up was 7.4%.

Studies have shown that the rate of IGRA conversion among health care professionals in low- and middle-income countries ranges from 10-30%.[8] In studies conducted in primary and secondary care settings in countries with high tuberculosis incidence rates, the reported rate of IGRA conversion ranges from 14-22%.[9] In a study conducted in a tertiary care setting in India, the rate of IGRA conversion was 11.6%.[10] However, in none of those studies were losses to follow-up considered in the calculation of the incidence of IGRA conversion.

To our knowledge, this was the first study to provide estimates of the incidence of LTBI among primary health care professionals in Brazil and to use an IGRA as a diagnostic test to evaluate this measure of frequency. However, the present study has some limitations: loss to follow-up was high (approximately 75%); and, because there was no reference test for the diagnosis of LTBI, the estimated incidence of LTBI may have been influenced by the performance of the QFT.

Among the tuberculosis control strategies proposed by the World Health Organization and the End TB strategy, the diagnosis and treatment of LTBI is of note as one of the actions that can produce the necessary changes. Finally, proper assessment, screening, and treatment of people at risk for LTBI, such as health care professionals, are some of the actions that can contribute to ending the tuberculosis epidemic by 2035.

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Bacteriological diagnosis of tuberculosis in prison inmates: actions taken by the primary health care teams in prisons

Karine Zennatti Ely[1], Renata Maria Dotta[2], Carla Adriane Jarczewski[3], Andréia Rosane de Moura Valim[1], Lia Gonçalves Possuelo[1]

TO THE EDITOR:

Tuberculosis is a major public health problem in the penal institutions of Brazil, with a prevalence of 1,236 cases/100,000 population.[1] In a five-year period, the number of prison inmates in the Brazilian state of Rio Grande do Sul increased by 28%, reaching a total of 40,000 in 2018. In the same period, the prevalence of tuberculosis in prison inmates increased from 1,995 cases/100,000 population to 2,488 cases/100,000 population.[2,3]

The *Política Nacional de Atenção Integral à Saúde das Pessoas Privadas de Liberdade no Sistema Prisional* (PNAISP, National Policy for Comprehensive Health Care for Inmates of the Prison System) provides for the establishment of prison primary health care teams (pPHCTs) as a strategy to ensure the right of prison inmates to health. The pPHCTs are part of the Health Care Network, and their function is to improve the quality of primary health care in prisons and the quality of territorial coordination.[4] The duties of the pPHCTs include effective and timely surveillance of infectious diseases, such as tuberculosis.[5] In Rio Grande do Sul, the pPHCTs provide coverage for approximately 70% of prison inmates.[6] The expansion of coverage depends on municipal adherence to the PNAISP.

The bacteriological diagnosis of pulmonary tuberculosis (PTB) is established by the identification of individuals with respiratory symptoms and laboratory test results. Prison inmates are considered a specific, highly vulnerable population. Inmates who had had a cough for two weeks or more were classified as having respiratory symptoms. Bacteriological investigation includes sputum smear microscopy, mycobacterial culture, drug susceptibility testing, and rapid molecular testing.[7,8]

To collect data on actions for the bacteriological diagnosis of PTB in prison inmates treated by the 29 pPHCTs in Rio Grande do Sul, an e-mail invitation to complete a questionnaire, hosted on the LimeSurvey platform, was sent to all 29 teams. Data were requested for the period January to December 2017. Quantitative data were analyzed with the IBM SPSS Statistics software package, version 23.0 (IBM Corporation, Armonk, NY, USA). Values are expressed as absolute and relative frequencies.

The present survey is an arm of the project known as "Analysis of the dynamics of tuberculosis transmission and tuberculosis control strategies in prison settings in Rio Grande do Sul" and was approved by the Research Ethics Committee of the University of Santa Cruz do Sul, located in the city of Santa Cruz do Sul, Brazil (Reference no. 2.170.472), in accordance with Brazilian National Health Council Resolution no. 466/2012. All participants gave written informed consent.

Of the 29 pPHCTs, 22 (75.9%) completed the questionnaire: 14 (48.3%) within the stipulated time period and 8 (27.6%) after being contacted again by e-mail or telephone. A total of 15,529 inmates, 14,634 (94.2%) of whom were male, were under the responsibility of these pPHCTs during the study period, corresponding to 55% of all prison inmates with pPHCT coverage in Rio Grande do Sul. Sixteen pPHCTs (72.7%) reported that their staff was complete and included all professionals recommended by current legislation.

The actions for the bacteriological diagnosis of PTB taken by the pPHCTs in 2017 are summarized in Table 1. The number of individuals identified with respiratory symptoms (N = 3,516) could be higher, because tuberculosis screening at prison entry and identification of individuals with respiratory symptoms were not performed by all pPHCTs. A study conducted in Rio Grande do Sul in 2010 identified a prevalence of 20.6% of individuals with respiratory symptoms among prison inmates in the city of Santa Cruz do Sul.[9]

All cases newly diagnosed through sputum smear microscopy, sputum culture, or rapid molecular testing were added to those already under treatment, resulting in a total of 463 cases of PTB in 2017, which corresponds to a prevalence of 2,981/100,000 population. Previous studies have reported lower prevalence rates, ranging from 1,236 cases/100,000 population among prison inmates in Brazil as a whole to 1,898 cases/100,000 population among those in southern Brazil.[1,8,9]

Sputum smear microscopy is the main diagnostic test and can detect 60-80% of PTB cases.[7] Eighteen pPHCTs (81.1%) reported being prepared to collect sputum samples. Mycobacterial culture is recommended for prison inmates, regardless of sputum smear results, because it increases the bacteriological diagnostic yield by 30%.[7] In the present study, we found that 9 pPHCTs (40.9%) followed that recommendation. The main reasons for not following the recommendation included a lack of knowledge, the fact that there was no protocol in place, and the absence of a regional referral center to perform the tests. Culture results confirm mycobacterial infection, species identification characterizes tuberculosis, and drug susceptibility testing determines whether the strain is resistant to the first-line drugs used to treat tuberculosis.[7]

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Table 1. Actions for the bacteriological diagnosis of pulmonary tuberculosis taken by prison primary health care teams (N = 22). Rio Grande do Sul, Brazil, 2017.

Diagnostic actions	pPHCT[a]		Actions taken, n
	n	%	
Tuberculosis screening	14	48.3	6,379
Identification of individuals with respiratory symptoms	16	72.7	3,516
Sputum smear microscopy	18	81.8	3,637
Sputum culture	16	72.7	2,005
Drug susceptibility testing	10	45.5	551
Rapid molecular testing	3	13.6	2,239

pPHCT: prison primary health care team. [a]pPHCTs that took diagnostic actions.

Rapid molecular testing for tuberculosis was routinely available to 3 pPHCTs (13.6%), which diagnosed 309 (70.1%) of the cases of tuberculosis and were responsible for 8,040 (51.7%) of the inmates. Rapid molecular testing is of great relevance to the timely diagnosis, treatment initiation, detection of rifampin resistance, and increased detection of tuberculosis cases among smear-negative patients.[7] The pPHCTs to which rapid molecular testing was available to diagnose tuberculosis are those that had access to a laboratory and ordered a significant number of sputum smear microscopy examinations as recommended by the Brazilian National Ministry of Health.[10]

One pPHCT ordered no diagnostic laboratory tests for PTB. Four pPHCTs working in small prisons did not diagnose any cases of tuberculosis, and 3 of those collectively investigated 23 patients and were responsible for 250 inmates (1.6%). In general, 18 pPHCTs (81.8%) took actions for the bacteriological diagnosis of PTB in prison inmates.

In our sample, 4 pPHCTs (18.2%) reported asking inmates about the presence of cough at prison entry. Tuberculosis screening at prison entry and periodic active case finding among individuals with respiratory symptoms are relevant strategies for tuberculosis control, because they allow early detection of the disease and disruption of the chain of transmission.[7]

All pPHCTs reported difficulties completing the questionnaire because of the frequent change or lack of lead professionals, lack/inadequacy of records, lack of technical knowledge, and lack of protocols for taking actions for the bacteriological diagnosis of PTB, all of which can be considered limitations of the present study.

In addition, the health care professionals reported that putting protocols into practice depended largely on the prison staff (guards), that there was insufficient staff to meet the demand, and that the physical plant was not appropriate for health care.

In summary, we identified a set of effective strategies for the diagnosis of PTB in the penal institutions in Rio Grande do Sul that limit the burden of the disease and its economic and social costs, thereby reducing the transmission of the disease to the general population. However, there is a need for monitoring, organization of workflows, and continuing education and training of health care and health safety workers in order to improve the quality of the diagnostic actions taken by the pPHCTs.

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Thinking inside the box: nebulizer care, safe storage, and risk of infection in cystic fibrosis

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TO THE EDITOR:

Patients with cystic fibrosis (CF) are recommended to wash and disinfect their nebulizers on a regular basis, ideally after each use,[1] both to ensure that devices are maintained properly for optimal drug delivery and to minimize infection risks. In practice, approaches to nebulizer hygiene vary among pediatric[2,3] and adult patients, both in the home[2] and hospital environments.[4] Recently, Riquena et al.[5] demonstrated a contamination rate of 71.6% of the nebulizers used by CF patients who were chronically colonized with *Pseudomonas aeruginosa.* Nebulizers were contaminated with clinically significant organisms, including *Stenotrophomonas maltophilia* (11.9%), nonmucoid *P. aeruginosa* (4.8%), *Staphylococcus aureus* (4.8%), and *Burkholderia cepacia* complex (2.4%), as well as yeasts and filamentous fungi. Overall, such contamination was exacerbated by the use of tap water and outdoor drying of nebulizers, concurrent with poor nebulizer hygiene among patients.

Recently, CF centers in the United Kingdom highlighted a common practice to wash and store clean devices in sealed plastic boxes.[4] Given that there is no evidence in the published literature regarding microorganisms found in nebulizer storage boxes, we examined the microbiology of such boxes used during inpatient stays to help guide safe practice recommendations for the storage of nebulizers after cleaning/disinfection.

We collected 24 disposable plastic storage boxes (approximate dimensions: 152 mm in length × 98 mm in width × 68 mm in depth) used during inpatient stays from 15 pediatric patients and a new/unused control box. All microbiological analyses were performed blinded. Microbiology rinse cultures were performed aseptically on each box by adding 18 mL of 0.1% (w/v) peptone saline diluent (CM0733; Oxoid Ltd., Basingstoke, United Kingdom) into the box and agitating the diluent for 10 min. Resulting rinses were cultured aerobically on Columbia agar (CM0331; Oxoid Ltd.) supplemented with 5% (v/v) defibrinated horse blood (SR0050; Oxoid Ltd.) at 37°C/48 h, as well as in nonselective enrichment broth (Mueller-Hinton Broth; CM0405; Oxoid Ltd.) at 37°C/48 h and on Sabouraud dextrose agar with chloramphenicol (PO0161; Oxoid Ltd.) at 25°C/5 days, for the detection of yeasts/fungi. Resulting bacterial colonies were identified using matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry, and fungal colonies were identified using internal transcribed spacer/PCR/DNA sequencing. Microbiological analysis of boxes was subsequently compared with contemporary sputum microbiology from respective patients.

Eighty percent of the patients had at least one of their storage boxes positive for bacteria (Table 1). Overall, 20 boxes (83%) were positive for bacteria; however the majority of these (65%) had a contamination rate of $< 10^3$ CFU/box, whereas 15% of positive boxes were contaminated between 10^3–10^4 CFU/box, with the remainder (20%) contaminated between 10^4–10^5 CFU/box. The most highly contaminated box harbored 5.4×10^4 CFU/box. Bacterial diversity demonstrated a predominately gram-positive flora, representing 15 genera and 22 species. *Micrococcus luteus* and *Dermacoccus nishinomiyaensis* were the most commonly isolated species, with coagulase-negative staphylococci and the viridans group (oral) streptococci having the greatest species diversity within their respective genera. Gram-negative bacteria were in the minority, representing 8.3% of bacterial species isolated, namely *Stenotrophomonas maltophilia* and *Neisseria flava/perflava/subflava.* Fungi were isolated from 4 (26.7%) of 15 boxes and included *Penicillium* sp., *Penicillium expansum*, *Cladosporium* sp. and *Candida albicans*.

With the exception of *Stenotrophomonas maltophilia*, none of the organisms identified are considered major pathogens of CF. None of the boxes grew organisms which were contemporary to the organisms found in patients' sputum (Table 1). Most of the organisms identified were of skin, mouth, or throat/oropharyngeal origin. In contrast to Riquena et al.,[5] a recent study in the USA[3] found contamination of nebulizers used by pediatric CF patients, the most frequently observed microbial contaminants being viridans streptococci, *Micrococcus* sp., coagulase-negative staphylococci, and *Candida albicans.* Our findings in relation to storage boxes largely concur with those of the US report[3] in terms of bacterial contamination. Our study demonstrated the presence of yeast and fungal contaminants, similar to the Brazilian report.[5] The occurrence of fungi may be due to inadequate drying of nebulizer parts prior to storage, which emphasizes the importance of thorough drying prior to storage.

Therefore, what is the significance of the storage boxes being largely contaminated with oral and environmental organisms? Although the organisms detected are not believed to be clinically significant, such organisms

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Table 1. Comparison between microbial contaminants found in plastic storage boxes used to store nebulizers and current sputum microbiology in patients with cystic fibrosis.

Patient	Box	Sputum
1	*Staphylococcus epidermidis*	*Pseudomonas aeruginosa*
2	*Micrococcus luteus*, *Dietzia cinnamea*	*MRSA*
3	*Staphylococcus capitis*, *Dermacoccus nishinomiyaensis*, *Kocuria rhizophila*, *Corynebacterium afermentans*, *Paenibacillus macerans*, *Bacillus licheniformis*	*Pseudomonas aeruginosa*
4	*Micrococcus luteus*, *Gemella haemolysans*, *Streptococcus sanguinis*, *Rothia aeria*, *Rothia dentocariosa*, *Dermacoccus nishinomiyaensis*, *Bacillus licheniformis*, *Kocuria rhizophila*, *Streptococcus parasanguinis*, *Rothia mucilaginosa*	*Stenotrophomonas maltophilia*
5	*Streptococcus parasanguinis*, *S. mitis*, *S. oralis*, *Streptococcus* sp., *Neisseria flava*, *N. perflava*, *N. subflava*	Yeasts
6	*Staphylococcus warneri*, *Stenotrophomonas maltophilia*, *Microbacterium paraoxydans*, *Bacillus licheniformis*, *Penicillium expansum*, *Penicillium* spp., *Candida albicans*	*Pseudomonas aeruginosa*
7	No growth	*MRSA*
8	*Micrococcus luteus*	*Long-standing* ABPA
9	*Streptococcus mitis*, *S. oralis*, *S. sanguinis*, *S. parasanguinis*	*Pseudomonas aeruginosa*
10	*Micrococcus luteus*, *Bacillus* sp., unidentified fungus	*Staphylococcus aureus*
11	*Micrococcus luteus*	*Staphylococcus aureus*
12	*Staphylococcus saprophyticus*, *Dermacoccus nishinomiyaensis*,*Cladosporium* spp.	*Staphylococcus aureus*, *Haemophilus influenzae*, *Stenotrophomonas maltophilia*
13	*Brevibacillus* sp., *Dermacoccus nishinomiyaensis*, unidentified fungus	*Pseudomonas aeruginosa*
14	No growth	*Pseudomonas aeruginosa*, *Staphylococcus aureus*, ABPA
15	No growth	*Staphylococcus aureus*, *Haemophilus influenzae*
Control	*Staphylococcus epidermidis*	New control box

MRSA: methicillin-resistant *Staphylococcus aureus*; and ABPA: allergic bronchopulmonary aspergillosis.

may harbor antibiotic resistance gene determinants and, if nebulized, could provide a reservoir for such determinants to be horizontally transferred to established CF pathogens in the lung, thereby potentially increasing the antimicrobial resistance burden. Studies are therefore required to elucidate the potential for such horizontal gene transfer events from nonpathogenic to pathogenic organisms.

The efficiency of nebulizer cleaning and disinfection will directly affect the hygienic status of boxes, used subsequently for the storage of nebulizers. Therefore, in alignment with current evidence, patients should wash and disinfect their nebulizers after each use with steam disinfection in a baby bottle disinfector and leave their nebulizers in such disinfector units until next required.[6] Where storage in the steam disinfector is not practical, then, after disinfection, nebulizers should be air dried fully and stored on absorbent tissue in dedicated clean storage boxes, separate from those used to wash nebulizers.

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All authors contributed to the design, execution, analysis, and writing of this letter.

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COVID-19 pneumonia and the reversed halo sign

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A 56-year-old male patient with chronic bronchitis reported worsening of his usual dyspnea for one week prior to hospital admission, evolving to fever, productive cough, and non-massive hemoptysis, as well as a progressive need for oxygen supplementation, in the last 24 h.

Multidetector CT scans of the chest showed diffuse, extensive ground-glass opacities, together with foci of consolidation, many of them representing reversed halo signs, in both lungs. Screening for multiple respiratory pathogens was positive for SARS-CoV-2, indicating that the patient had COVID-19.

The reversed halo sign, defined as an area with ground-glass attenuation surrounded by partial or complete rings of consolidation, is a radiological finding present in patients with pneumonia caused by the new coronavirus. The reversed halo sign has been reported in other forms of viral pneumonia.[1,2] When identified, the reversed halo sign typically occurs longer after symptom onset, suggesting that this CT finding correlates with the underlying pathophysiology of the disease process as it organizes.[2] Such findings indicate that organizing pneumonia is one of the mechanisms of lung injury.[3]

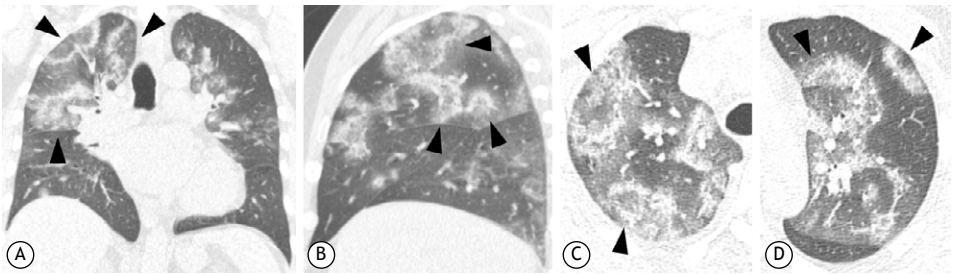

Figure 1. Images in coronal (A), sagittal (B), and axial (C and D) reconstructions from multidetector CT, showing multiple diffuse ground glass areas in both lungs, surrounded by partial or complete rings of consolidation, known as the reversed halo sign (arrows), in a 56-year-old male patient with COVID-19 pneumonia.

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Endobronchial aspergilloma

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A 68-year-old man, during the postoperative period after video-assisted thoracoscopic esophagectomy for esophageal adenocarcinoma, experienced an air leak via the chest drainage system (Thopaz+; Medela, Baar, Switzerland), at an approximate flow rate of 3.0-3.5 L/min, as a complication of the surgical procedure. Physical examination showed normal breath sounds bilaterally and usual fluid drainage through the chest tube (150-200 mL/day). In addition, a sagittal CT image of the chest did not reveal clear changes in the bronchial anatomy (Figure 1). Bronchoscopic examination of the bronchial tree was ordered for suspected air leak in the upper bronchial tree.[1] A blackened, villous vegetative lesion, located in the right main bronchus (Figure 2), was submitted to an incisional biopsy. Direct microbiological assay and cultures revealed *Aspergillus niger* (Figure 2), with no vascular involvement. A diagnosis of endobronchial aspergilloma (EA) was made.

EA is a rare entity, characterized by *Aspergillus* sp. growth within the bronchial lumen. It is a noninvasive form of aspergillosis, and most cases are diagnosed incidentally during bronchoscopy for reasons other than the initial diagnostic suspicion.[2] The patient was treated for two weeks with voriconazole and micafungin.[3] One month later, there were no clinical signs of invasive aspergillosis or treatment complications.

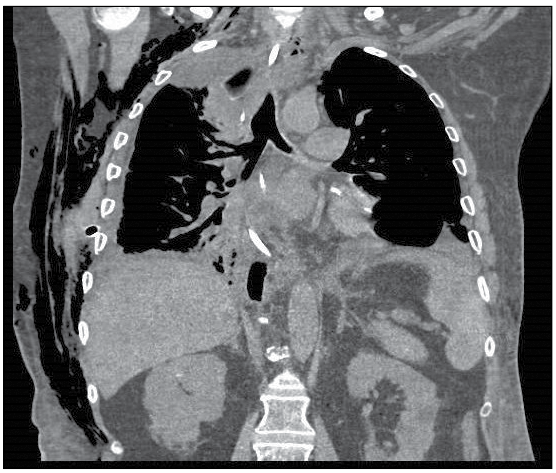

Figure 1. Sagittal slice from a CT scan of the chest. It was not possible to clearly identify the vegetative lesion in the right main bronchus and lobar bronchi (including the right upper lobar bronchus) with this imaging modality.

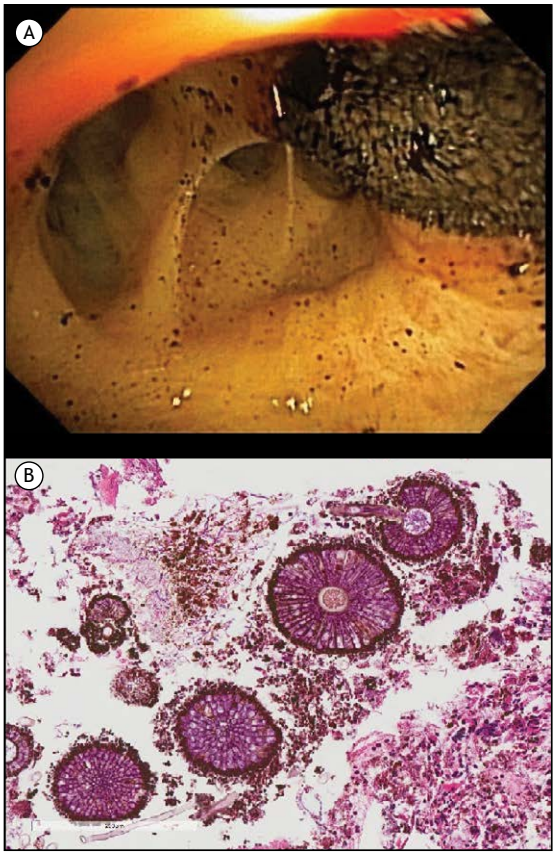

Figure 2. In A, image demonstrating a macroscopic lesion, caused by *Aspergillus niger*, in the right upper lobar bronchus. In B, photomicrograph of a direct microbiological assay (H&E; magnification, ×150).

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Difference between slow vital capacity and forced vital capacity in the diagnosis of airflow limitation

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In an article published in the JBP, Fernandez et al.[1] evaluated 187 patients referred for pulmonary function testing and concluded that a difference between slow VC (SVC) and FVC (ΔSVC−FVC) > 0.20 L is useful for defining airflow limitation (AFL) in patients with normal test results and for reducing the number of cases designated as nonspecific (i.e., cases in which there is a proportional reduction in FVC and FEV_1). In 82 of those patients, AFL had already been characterized by forced spirometry.[1]

The value of 0.20 L was suggested in the Brazilian Thoracic Association 2002 Guidelines for Pulmonary Function Testing.[2] In 2019, Saint-Pierre et al.[3] evaluated functional test results of 13,893 individuals and reported that a preserved FEV_1/FVC ratio with a reduced FEV_1/VC ratio was observed in 20.4% of cases. The low predicted value used in that study to characterize the lower limit for the FEV_1/FVC ratio greatly decreases the sensitivity of this parameter for detecting AFL.

Several considerations should be made regarding ΔSVC−FVC. The higher degree of alveolar gas compression during a forced maneuver results from several factors, including obstructive disease, greater muscle effort, and higher thoracic gas volume to be compressed. Since gas compression is based on muscle effort during a maximal expiratory maneuver, some degree of compression can be found in all normal individuals. Soares et al.[4] measured lung volumes in a sample of 244 normal individuals in Brazil. In that sample, 10% had a ΔSVC−FVC > 0.20 L. In comparison with the remaining individuals in the sample, those 10% were found to be more frequently male, to be taller, and to have higher FVC, a characteristic profile of what is called the normal variant, given the increased expiratory effort generated in men with larger lungs.

Similarly to Fernandez et al.,[1] most authors use percentages of the predicted values for SVC, assuming that these values are equal to those derived for FVC. They are not. Kubota et al.[5] evaluated pulmonary function in 20,341 normal individuals in Japan and found that, due to the expected loss of elastic recoil, the ΔSVC−FVC increases with age, although a greater difference was also found in certain younger individuals. It is not surprising that Saint-Pierre et al.[3] acknowledge that, in the elderly, in whom the prevalence of COPD is higher, the FEV_1/SVC ratio should not be considered.

In the study by Fernandez et al.,[1] half of the sample consisted of obese individuals. A ΔSVC−FVC > 0.20 L was significantly associated with a body mass index > 30 kg/m². Likewise, Saint-Pierre et al.[3] observed lower values for the FEV_1/SVC ratio versus the FEV_1/FVC ratio in obese individuals. Reference values do not generally include obese individuals, and, therefore, ΔSVC−FVC data in obese individuals without cardiopulmonary disease are not available for large samples. Campos et al.[6] evaluated 24 individuals before and after bariatric surgery and showed that ΔSVC−FVC dropped from 0.21 L to 0.080 L after the intervention. Obese individuals have lower FVC relative to SVC. Many obese individuals have the so-called nonspecific pattern, and caution should be exercised in assuming that the measurement of SVC alone solves the problem, without those of TLC and RV. An SVC within the predicted range does not exclude the possibility of a reduced TLC if an appropriate percent prediction equation is used.

In the study by Fernandez et al.,[1] the values for specific airway conductance and for RV did not differ between the groups with and without a ΔSVC−FVC > 0.20 L. These data are surprising, since consistency with other data indicative of obstruction should have been observed. Finally, mid and end-expiratory flows, which could detect obstruction in the presence of an FEV_1/FVC ratio within the predicted range, were not reported.

Patients with obstructive disease more frequently have a ΔSVC−FVC > 0.20 L when compared with normal individuals—20% of 190 cases evaluated at the *Centro Diagnóstico Brasil* compared with 10% of normal individuals (unpublished data)—however, that difference in patients with mild airflow limitation was similar to that observed in normal individuals.

We consider it unwise, in patients whose forced spirometry parameters, including end-expiratory flows, are within the normal range, to characterize the presence of AFL solely on the basis of a ΔSVC−FVC > 0.20 L, or even > 0.25 L, as suggested by Saint-Pierre et al.[3] Obtaining acceptable and reproducible values for measurement of SVC is time consuming. This time-consuming factor does not justify the routine use of measuring SVC in high-volume laboratories, given the uncertain meaning of this measurement compared with that of forced spirometry.

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Authors' reply

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In the article by Fernandez et al., a diagnosis of obstructive lung disease (OLD) was made if there was a reduced FEV_1/slow VC (SVC) ratio and/or a reduced FEV_1/FVC ratio.[1] The finding of a ΔSVC−FVC ≥ 200 mL alone did not define OLD. As the correspondence points out, in 82 of 187 cases, the FEV_1/FVC ratio had already revealed OLD; however, in 46 (25%) of those 187 cases, there was disagreement between diagnoses, a finding that is similar to that of Saint-Pierre et al.[1,2] In 21 of 73 cases with normal spirometry and in 15 of 32 cases considered nonspecific based on the analysis of forced expiratory maneuver parameters, obstruction was revealed only by a reduced FEV_1/SVC ratio.[1]

Kubota et al.[3] evaluated normal individuals and found a greater ΔSVC−FVC in the elderly, probably because of air trapping or heterogeneous lung emptying in the forced expiratory maneuver because of the loss of elastic recoil; that difference was less pronounced in young individuals. Therefore, the authors suggested that "reference values for SVC would be preferable for the interpretation of pulmonary function in the elderly".[3] Pistelli et al.[4] also calculated predicted values for SVC, finding a difference of only 50 mL between the mean values for SVC and FVC; however, the age group studied (8-64) was younger than that in the study by Kubota et al. (17-95 years).[3,4] There are no spirometry predicted values for SVC in Brazilians.

In our study, the values for specific airway conductance did not differ between those with and those without a ΔSVC−FVC ≥ 200 mL, a finding that may be attributable to the characteristics of the sample, which also included individuals with interstitial lung diseases (such as sarcoidosis, hypersensitivity pneumonitis, and fibrosis with emphysema), in whom changes in volume and flow can be masked by the balance of interstitial and airway involvement. In addition, reductions in FEV_1% and FEV_1/(F)VC, OLD, increased functional residual capacity, and reduced inspiratory capacity/TLC (i.e., findings of airflow limitation and air trapping) were predictors of a ΔSVC−FVC ≥ 200 mL.

ΔSVC−FVC correlates positively with body mass index, and analysis of FEV_1/SVC may increase the prevalence of the diagnosis of OLD. In general, functional residual capacity and expiratory reserve volume are the volumes most affected in obese individuals, and impairment of TLC is less pronounced. In individual cases, especially if there is dissonance with the clinical findings, plethysmography is essential for assessing the mechanisms underlying the reduction in (F)VC and FEV_1.

The finding of reduced end-expiratory flows alone (similarly to that of ΔSVC−FVC ≥ 200 mL alone) should be supported by other functional test results in order to confirm OLD. In the study by Saint-Pierre et al.,[2] discordant cases (i.e., normal FEV_1/FVC, but reduced FEV_1/SVC) had lower $FEF_{25-75\%}$ values.

Determination of the ΔSVC−FVC provides an additional piece of information, since, although that difference can occur in healthy individuals due to dynamic compression of the airways (young individuals) or loss of elastic recoil (elderly individuals), it can also be due to airflow limitation. Recommendations by the American Thoracic Society continue to support the use of the highest VC value as the denominator of the FEV_1/(F)VC ratio.[5]

In the Pulmonary Function Laboratory of the *Instituto de Assistência ao Servidor Público Estadual de São Paulo* (São Paulo Institute for the Medical Care of State Civil Servants), we perform approximately 800 tests/month. We serve a wide age group with a wide variety and great complexity of diseases. The SVC maneuver is performed without disrupting the laboratory's routine, and the analysis of its parameters additionally provides information about bronchodilator response.

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