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HIGHLIGHT

Prevalence of alpha-1 antitrypsin deficiency in COPD patients

Pulmonary rehabilitation in tuberculosis patients

Applications for a hybrid operating room in thoracic surgery

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Diagnosing alpha-1 antitrypsin deficiency: does it prevent or improve the course of COPD?

Irma Godoy[1]

Alpha-1 antitrypsin (AAT) is a protein whose main function is the inhibition of neutrophil elastase. The gene that encodes AAT is transmitted by simple autosomal codominant Mendelian inheritance through two alleles, one from each parent. The normal allele is designated Pi*M, and the most common deficient alleles are the Pi*S and Pi*Z alleles, which encode abnormal proteins that undergo polymerization in the liver. Therefore, 80-90% of the Z proteins and 40-50% of the S proteins are retained within the hepatocytes, grouped into polymers. The loss of the anti-inflammatory and antiproteolytic activity, together with the pro-inflammatory effects of polymers, contribute to protein degradation and the exacerbation of inflammation, resulting in an increased risk of developing COPD, with a predominance of emphysema, especially in smokers.

AAT deficiency (AATD) is a rare disease and, like most such diseases, is underdiagnosed. The diagnosis is usually made late (at an average patient age of 45 years), and estimates suggest that 85% of patients with AATD have gone un diagnosed.[1] These findings indicate the low adherence to the recommendations of the World Health Organization, as well as to the guidelines of the American Thoracic Society and the European Respiratory Society, suggesting that patients with COPD or persistent airway obstruction should be tested for AATD.[2,3] The potential reasons for misdiagnosis include a lack of knowledge about the disease, about the tests necessary for the diagnosis, the lack of availability of such tests, and about the algorithm required in order to confirm the diagnosis.

The prevalence of AATD varies depending on the population studied. Attempts to determine the prevalence of genetic predisposition in patient populations will inevitably overestimate the prevalence in the general population, whereas limiting screening to the healthy portion of the general population can underestimate the prevalence. In Europe, the prevalence of the Pi*Z mutation is highest in the northeastern region, where it ranges from 0.029 to 0.049.[4-6] In the United States, the prevalence is similar (0.019-0.030).[7,8] In Asia, the prevalence is extremely low (0.006).[1]

The data presented in the study conducted by Russo et al.,[9] published in the current issue of the JBP, are unprecedented in that they show the prevalence of AATD in 926 COPD patients in five Brazilian states. The diagnosis of AATD was based on an AAT cut-off point of < 2.64 mg/dL in dried blood samples on filter paper. For patients in whom the AAT value was below the cut-off point, the serum concentration of AAT was determined. For those with a serum AAT concentration < 113 mg/dL, genetic sequencing was performed. In inconclusive cases, *SERPINA* gene sequencing was performed. Among the patients evaluated by Russo et al.,[9] the AAT values indicated the need for additional investigation in 9.2%, the serum AAT concentration was < 113 mg/dL in 2.6%, and the prevalence of the PI*Z allele was 0.8%, similar to that described in other countries.[9]

The study underscores the need to investigate AATD in patients with COPD, in accordance with the recommendations and guidelines mentioned above.[2,3] The alternative strategy is to prioritize investigation for specific risk groups, including patients with early-onset emphysema or emphysema that is predominantly in the lower lobes, as well as family clusters of COPD patients or first-degree relatives of individuals diagnosed with moderate or severe AATD.[10] It is also important to screen for AATD in individuals with unexplained liver disease, including newborns, children, and adults, as well as in adults with necrotizing panniculitis. Screening for AATD is recommended in adults with bronchiectasis of unclear etiology, adolescents with a persistent obstructive pattern, and adolescents with cytoplasmic-antineutrophil cytoplasmic antibody-positive vasculitis.[11]

Given that the main risk factor in individuals with AATD is smoking, which promotes the earlier emergence of COPD in smokers than in non-smokers,[12] early identification of the disease allows preventive measures to be taken, the most important of which are avoiding smoking (active and passive) and exposure to environmental pollutants, both of which are determinants of the prognosis of COPD. Early identification of COPD allows lung function to be monitored and the therapeutic decision to administer supplemental therapy while pulmonary function is still relatively preserved. The treatment of patients with COPD and AATD includes the usual therapy for the disease (smoking cessation, vaccination, use of bronchodilators, rehabilitation, and long-term home oxygen therapy when indicated).[10] The specific treatment of AAT replacement through the administration of concentrated purified AAT from human plasma is now available in Brazil.[11] However, that treatment is extremely expensive (approximately US$100,000 per year); the indications for its use and its efficacy are not well defined; and it is not recommended by the US National Institute for Health and Care Excellence,[13] although its use can be considered in young patients with COPD, according to the Global Initiative for Chronic Obstructive Lung Disease (grade C recommendation).[14,15]

In brief, despite the recommendations of the World Health Organization and the American Thoracic Society/European Respiratory Society, many physicians and COPD patients do not fully comprehend the risk of a rapid increase in airway obstruction associated with AATD.

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With the availability of effective smoking cessation interventions, testing patients with COPD, especially those most at risk, to identify carriers of AATD is important and justifiable. During the treatment of COPD, efforts should be directed toward early detection of airway obstruction and avoiding exposure to risk factors, especially smoking, the most important intervention to reduce the progression of the disease.

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Intracavitary nodule

Edson Marchiori[1,2], Bruno Hochhegger[3,4], Gláucia Zanetti[2,5]

A 41-year-old male presented with complaints of anorexia and weight loss followed by cough with hemoptysis. The final diagnosis was aspergilloma in previous tuberculous lesions.

The finding of a nodule in a lung cavity has important diagnostic and therapeutic implications. Although aspergilloma is the most common cause of intracavitary nodules, a number of other conditions should be included in the differential diagnosis. These conditions primarily include neoplasms (particularly bronchial carcinoma), angioinvasive aspergillosis during the recovery phase, Rasmussen aneurysm, and clots. Other, rarer, causes include foreign bodies, thick pus, dehydrated caseous material, teratoma, and hydatidosis. The air crescent sign is commonly seen in patients with intracavitary nodules, regardless of the etiology. It corresponds to a collection of air in the form of a crescent or half moon, located in the periphery of a nodule or mass of soft tissue density, separating the nodule from the cavity wall.

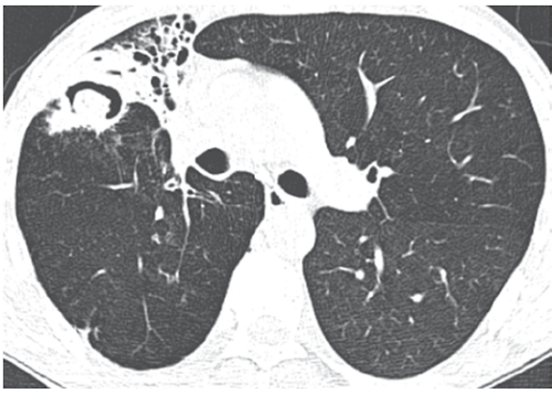

Figure 1. CT scan of the chest with lung window settings at the level of the main pulmonary artery showing volume loss in the right upper lobe, as well as bronchiectasis and a cavitary lesion containing a nodular density. Note air interposed between the nodule and the cavity wall (the air crescent sign)

A useful imaging criterion for the differential diagnosis is a change in the position of the nodule in the cavity when patient position is changed, especially during examination in the supine and prone positions. It is extremely important to determine whether the central mass is free or attached to the cavity wall because, unlike a fungus ball or a clot, cavitary lung cancer and Rasmussen aneurysm present as masses that are fixed to the cavity wall; that is, they do not move when patient position is changed. Contrast enhancement of the nodule on CT scans can aid in differentiating between aspergilloma and Rasmussen aneurysm. In cases of Rasmussen aneurysm, which is a pulmonary artery pseudoaneurysm secondary to pulmonary tuberculosis, hemoptysis is a common initial manifestation and can be fatal when it is massive. However, hemoptysis is also a common finding in patients with aspergilloma.

A fungus ball or aspergilloma is the most common cause of intracavitary nodules, generally resulting from fungal colonization of pre-existing lung cavities. Although such cavities are most commonly due to tuberculosis, fungus balls can develop in cysts, bullae, and bronchiectasis. Colonization with *Aspergillus* spp. occurs in most cases, which is why the term "aspergilloma" is commonly used. However, the air crescent sign has been reported in association with other fungal infections and bacterial infections, including coccidioidomycosis, actinomycosis, nocardiosis, and candidiasis. In conclusion, although aspergilloma is the most common cause of intracavitary nodules, other conditions should be considered in the differential diagnosis, including intracavitary tumors and Rasmussen aneurysm.

RECOMMENDED READING

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Randomization: beyond tossing a coin

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

INTRODUCTION

Randomization is a research strategy used in order to increase the validity of clinical trials evaluating the effect of interventions (e.g., drugs or exercise). It involves the random allocation of participants to either intervention or control groups and requires that participants have an equal chance of being allocated to either group. When properly implemented, randomization prevents selection bias and produces comparable study groups in terms of known and unknown baseline risk factors. For randomization to work, investigators and participants must be unable to predict to which group each of the participants will be allocated—this is called allocation concealment; in addition, investigators must be unable to change the allocation of any participant after randomization.

COMMONLY USED RANDOMIZATION STRATEGIES

Simple randomization is equivalent to tossing a coin: a new participant has an equal chance of being assigned to intervention or control groups, independently of previous assignments. Instead of tossing a coin, however, a randomization list is generated by a computer and used to prepare sequentially numbered, sealed envelopes, or, preferably, that list is administered by a central telephone service or website. The advantages of simple randomization are that it is inexpensive and easy to implement. The disadvantages include the risk of producing imbalances in the number of participants in the groups, as well as in the distribution of baseline risk factors, in studies with small sample sizes (N < 100; Figure 1).

In block randomization, the randomization list is a random sequence of blocks of participants instead of individual participants. The blocks have a pre-determined size; for example, four participants in one block, with six possible intervention and control sequences. This strategy ensures that intervention and control groups are balanced in terms of the number of participants (Figure 1). To ensure allocation concealment using this method, random variation of block sizes should be used (four to eight participants per block).

Stratified randomization is an alternative when balance for key baseline risk factors is desired. Each new participant is first classified into strata according to baseline characteristics (e.g., age or disease severity), and each stratum has a separate randomization list. Thereafter, once the participants are categorized into their stratum, they are randomized to either the intervention or the control groups. Stratification should be carried out using few relevant strata in order to work well. Stratified and block randomization strategies can be combined so that patients are first categorized into a stratum and then randomized in blocks.

Adaptive randomization uses computer algorithms that take into consideration baseline risk factors and the allocation of previous participants to allocate the next participant. The advantage of this method is that it accommodates more baseline risk factors than stratification and produces optimized group balance at the same time. However, it is more complex and requires a web-based randomization center available 24 h a day.

HOW TO CHOOSE

Simple randomization is easy to implement, is inexpensive, and can be a good option for large trials (N > 200). Block randomization is a good option when balance in the number of participants in each group is desired. Stratification is a good option to provide balance for important covariates. Adaptive randomization methods may be a good option when the trial structure includes statisticians and information technology support. For all methods, adequate implementation is paramount to ensure allocation concealment and to prevent manipulation and selection bias.

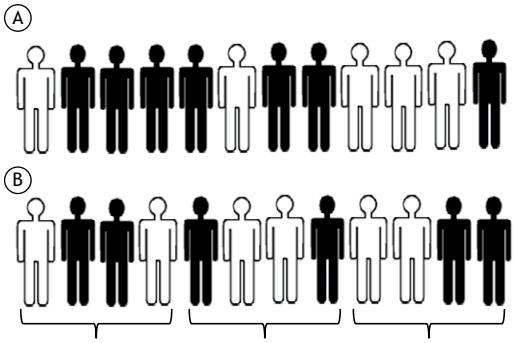

Figure 1. A) Simple randomization of 12 participants (black for intervention, white for control). This random sequence resulted in 7 subjects assigned to intervention and 5 to the control group; B) Block randomization of 12 participants with blocks of 4, resulting in 6 participants in each group.

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Prevalence of alpha-1 antitrypsin deficiency and allele frequency in patients with COPD in Brazil

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Study carried out at the Centro de Reabilitação Pulmonar, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo (SP); in the Divisão de Pneumologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (RS); in the Divisão de Pneumologia, Hospital Otávio de Freitas, Recife (PE); in the Departamento de Pneumologia, Hospital Geral de Goiânia Alberto Rassi, Goiânia (GO); in the Divisão de Pneumologia, Hospital do Servidor Público Estadual de São Paulo, São Paulo (SP); and in the Departamento de Pneumologia, Hospital de Messejana, Fortaleza (CE) Brasil.

ABSTRACT

Objective: To determine the prevalence of alpha 1-antitrypsin (AAT) deficiency (AATD), as well as allele frequency, in COPD patients in Brazil. **Methods:** This was a cross-sectional study involving 926 COPD patients 40 years of age or older, from five Brazilian states. All patients underwent determination of AAT levels in dried blood spot (DBS) samples by nephelometry. Those with DBS AAT levels ≤ 2.64 mg/dL underwent determination of serum AAT levels. Those with serum AAT levels of < 113 mg/dL underwent genotyping. In case of conflicting results, *SERPINA1* gene sequencing was performed. **Results:** Of the 926 COPD patients studied, 85 had DBS AAT levels ≤ 2.64 mg/dL, and 24 (2.6% of the study sample) had serum AAT levels of < 113 mg/dL. Genotype distribution in this subset of 24 patients was as follows: PI*MS, in 3 (12.5%); PI*MZ, in 13 (54.2%); PI*SZ, in 1 (4.2%); PI*SS, in 1 (4.2%); and PI*ZZ, in 6 (25.0%). In the sample as a whole, the overall prevalence of AATD was 2.8% and the prevalence of the PI*ZZ genotype (severe AATD) was 0.8% **Conclusions:** The prevalence of AATD in COPD patients in Brazil is similar to that found in most countries and reinforces the recommendation that AAT levels be measured in all COPD patients.

Keywords: alpha 1-antitrypsin deficiency/epidemiology; pulmonary disease, chronic obstructive/epidemiology; Alleles; alpha 1-antitrypsin.

INTRODUCTION

Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal codominant disorder primarily affecting the lungs and the liver.[1,2] The incidence of AATD is 1 per 2,000-5,000 live births; analysis of a database of 4.4 billion people from 58 countries estimated that 116 million individuals have the MS or MZ phenotype and that 3.4 million have the SS, SZ, or ZZ phenotype.[3,4]

AAT is a glycoprotein consisting of a chain of 394 amino acids and three carbohydrate side chains, being considered the prototype of a superfamily of proteins known as serpins (serine protease inhibitors). Also known as protease inhibitor (PI), AAT is encoded by the *SERPINA1* gene, located on the long arm of chromosome 14 (14q32.1), and inhibits neutrophil elastase, trypsin, and protease-3.[3,5,6]

Although smoking is a major cause of airflow obstruction, it is estimated that only 15-30% of smokers develop COPD.[7-9] Despite the clear association between smoking and COPD, the effects of smoking vary across individuals.[10] Studies have shown that AATD can increase the impact of smoking on the lungs, resulting in an increased rate of decline in lung function and early emphysema in smokers. Mutant S and Z alleles are the most commonly involved in severe AATD.[11,12]

The fact that the Brazilian population is racially diverse and includes immigrants from European countries where the frequency of alleles involved in early lung changes is high suggests that AATD is underdiagnosed in the country. Despite the estimated 5-7 million COPD patients in Brazil,[13] the prevalence of AATD in this population remains unknown, as does allele frequency. Therefore, the objective of the present study was to assess the prevalence of AATD, as well as allele frequency, in COPD patients from five Brazilian states.

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METHODS

Study design

The present study was approved by the Research Ethics Committee of the Federal University of São Paulo *Hospital São Paulo* (Protocol no. 0633/10), located in the city of São Paulo, Brazil, as well as by the research ethics committees of all participating centers. Between July of 2011 and August of 2012, 1,073 COPD patients followed at any of the six participating centers (two in northeastern Brazil, two in southeastern Brazil, one in southern Brazil, and one in central-western Brazil) were evaluated.

Patients

The inclusion criteria were as follows: being 40 years of age or older; having been diagnosed with COPD (on the basis of clinical history and spirometry results, including a post-bronchodilator percent predicted FEV_1/FVC ratio—FEV_1/FVC%—below the lower limit of normal); and having been stable for at least four weeks.[14] The exclusion criteria were as follows: having been diagnosed with any other lung disease or systemic disease that can increase serum AAT levels (including infections and inflammatory processes); having previously been diagnosed with AATD; being a relative of an index case of AATD; and having asthma (Figure 1).

The goal was to include 200 COPD patients from each participating center. At the end of the study period, no more patients were added to the study, regardless of whether or not the desired number of patients had been attained for each center.

Spirometry

The reference values for calculating percent predicted FVC, percent predicted FEV_1, and FEV_1/FVC% were based on the National Health and Nutrition Examination Survey equations.[15] Spirometry was performed with a portable spirometer (Easy One®; ndd Medical Technologies, Inc., Andover, MA, USA). At all participating centers, the American Thoracic Society acceptability and reproducibility criteria were used.[16]

Quantification of AAT

The study was divided into three phases. In the first phase, all patients underwent determination of AAT levels in dried blood spot (DBS) samples in order to identify those with a possible diagnosis of AATD. In the second phase, patients with DBS AAT levels ≤ 2.64 mg/dL (suspected AATD) underwent determination of serum AAT levels.[17] Finally, in the third phase, patients with serum AAT levels of < 113 mg/dL underwent genotyping. In case of conflicting results between serum AAT measurements and genotyping, genetic sequencing was performed (Figure 2). To determine the sensitivity and specificity of the eluate method, Zillmer et al. used the bootstrap resampling method, comparing the AAT values measured in serum with those measured in eluates from DBS samples in order to determine a cut-off point for AAT values in eluates; the value obtained was 2.02 mg/dL (97% CI: 1.45-2.64).[17] All patients whose DBS AAT levels were below 2.64 mg/dL underwent measurement of serum AAT levels in order to prevent AATD from going undiagnosed.

Genotyping

Blood samples were collected with the use of filter paper cards (Whatman 903, lot W101; Whatman/GE Healthcare, Florham Park, NJ, USA). They were transported to the Federal University of São Paulo *Hospital São Paulo* Central Laboratory, located in the city of São Paulo, Brazil, under temperature-controlled conditions (i.e., at a constant temperature of −20°C), in accordance with applicable International Air Transport Association regulations. All cards were stored at −20°C for subsequent analysis (determination of DBS AAT levels, genotyping, and *SERPINA1* gene sequencing). Serum and eluate samples were analyzed on a Siemens BNII system (Siemens Healthcare, Indianapolis, IN, USA) in July of 2012.

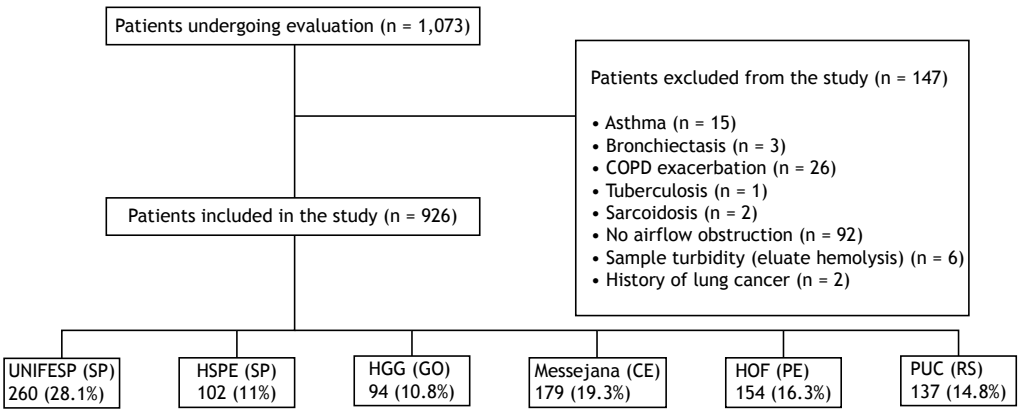

Figure 1. Flowchart of the patients included in the study and their distribution, by participating center. UNIFESP: *Universidade Federal de São Paulo*; HSPE-SP: *Hospital do Servidor Público Estadual de São Paulo*; HGG: *Hospital Geral de Goiânia Alberto Rassi*; Messejana: *Hospital de Messejana*; HOF: *Hospital Otávio de Freitas*; PUC: *Pontifícia Universidade Católica*; SP: Brazilian state of São Paulo; GO: Brazilian state of Goiás; CE: Brazilian state of Ceará; PE: Brazilian state of Pernambuco; and RS: Brazilian state of Rio Grande do Sul.

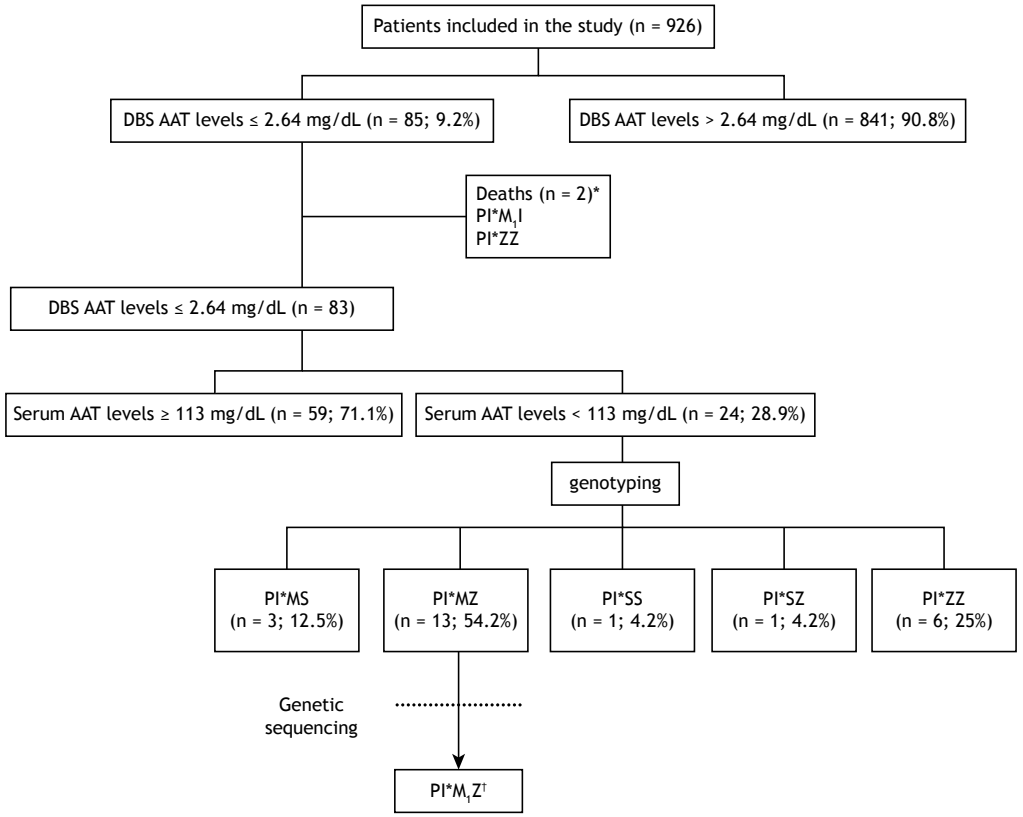

Figure 2. Flowchart of alpha 1-antitrypsin (AAT) deficiency screening and genotype distribution. PI: protease inhibitor; and DBS: dried blood spot. *Although 2 patients died before undergoing determination of serum AAT levels, *SERPINA1* gene sequencing was performed with previously collected DBS samples. †Only 1 PI*MZ patient underwent gene sequencing, because of discrepant results between determination of serum AAT levels and genotyping.

For DNA extraction, DBS samples were removed from the cards with a 6-mm paper punch, DNA being extracted with the QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany), in accordance with the manufacturer instructions. For identification of S and Z alleles in exons 3 and 5, respectively, real-time PCR was used with TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific Inc., Waltham, MA, USA). All patients with AATD but without S and Z alleles underwent *SERPINA1* gene sequencing (exons 2-5) in order to identify other polymorphisms described in the literature.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were expressed as absolute numbers and proportions. Data were entered into an Oracle database and analyzed with the Statistical Package for the Social Sciences, version 18.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 1,073 patients who were being followed at any of the six participating centers during the study period, 926 met the eligibility criteria and were therefore included in the study (Figure 1). The demographic characteristics of the patients included in the present study are shown in Table 1. There was no statistically significant difference between males and females regarding the prevalence of AATD, and two thirds of the participants were White. Although former smokers predominated (having accounted for 83.9% of the study sample), 36 (3.7%) of the patients had never smoked; 410 patients (44.3% of the sample) had a body mass index within normal limits, and 56 (6%) were underweight. As expected for patients with COPD, FEV_1/FVC% and percent predicted FEV_1 were low, characterizing obstructive lung disease.

Of the 926 COPD patients included in the present study, 85 had DBS AAT levels ≤ 2.64 mg/dL, therefore being suspected of having AATD. Of those 85 patients, 2 died. Therefore, 83 patients underwent determination of serum AAT levels. Of those 83 patients, 24 had serum AAT levels < 113 mg/dL and therefore underwent genotyping. Genotype distribution was as follows: PI*MS, in 3 (12.5%); PI*MZ, in 13 (54.2%); PI*SZ, in 1 (4.2%); PI*SS, in 1 (4.2%); and PI*ZZ, in 6 (25%). Although determination of serum AAT levels was not performed in the 2 patients who had DBS AAT levels ≤ 2.64 mg/dL and died, genotyping and *SERPINA1* gene sequencing were performed from the previously collected DBS samples, which had been stored at −20°C for subsequent analysis. Genotype distribution was as follows: PI*M_1I, in 1; and PI*ZZ,

in 1 (Figure 2). Given the discrepant results and the deaths, genetic sequencing was performed, and the following genotypes were found: PI*M_1Z; PI*M_1I; and PI*ZZ. These genotypes were included in a second analysis of allele frequency, and the results were as follows: PI*M, 28.8%; PI*M_1, 3.8%; PI*S, 11.5%; PI*Z, 53.8%; and PI*I, 1.9%.

Table 2 shows the demographic characteristics of a subset of 24 patients with DBS AAT levels ≤ 2.64 mg/dL and serum AAT levels < 113 mg/dL. As expected, the patients with the PI*ZZ genotype were younger and had lower serum AAT levels than those with other genotypes ($p < 0.001$). However, there were no differences in gender, smoking history, spirometric values, Medical Research Council scale scores, or COPD Assessment Test scores across patient genotypes.

Among the 926 COPD patients included in the present study, the overall prevalence of AATD was 2.8% and the prevalence of the PI*ZZ genotype (severe AATD) was 0.8%. Analysis of allele frequency in the subgroup of patients with serum AAT levels < 113 mg/dL (including the alleles found in the 2 patients who died, whose DBS AAT levels were ≤ 2.64 mg/dL) revealed frequencies of 53.8%, 11.5%, and 1.9% for the Z, S, and I alleles, respectively (Table 3). Table 4 shows the genotype prevalence among the five regions of Brazil.

Table 1. Demographic characteristics of the 926 COPD patients included in the present study.

Characteristic	COPD patients (N = 926)
Gender, n (%)	
Male	522 (56.4)
Female	404 (43.6)
Age, mean ± SD	
Age, years	67.3 ± 10.5
Race/ethnicity, n (%)	
White	612 (66.1)
Non-White	314 (33.9)
Smoking status, n (%)	
Smokers	113 (12.2)
Former smokers	777 (83.9)
Never smokers	36 (3.9)
Lung function, mean ± SD	
FEV_1/FVC	0.45 ± 0.10
FEV_1/FVC, % of predicted	61.0 ± 13.8
FVC, L	2.43 ± 0.76
FVC, % of predicted	75.6 ± 20.2
FEV_1, L	1.12 ± 0.45
FEV_1, % of predicted	42.9 ± 17.0

DISCUSSION

This is the first study to show the prevalence of AATD, as well as allele frequency, in a population of COPD patients in Brazil. The prevalence of AATD (i.e., 2.8%) and the Z allele (i.e., 0.8%) was found to be similar to that found in other countries.[18,19]

The decision to use the maximum confidence interval of the cut-off point for AAT values in eluates[17] was due to the need to identify all individuals who were suspected of having AATD and should therefore undergo genotyping, thus minimizing the chances of not identifying patients with AATD. Although AATD is one of the most common genetic diseases, the prevalence of AATD is low. However, failure to identify any patient with AATD would have had an impact on the final results of the present study. Nevertheless, the use of the maximum confidence interval width resulted in more patients being re-evaluated.[17] The cut-off point of 113 mg/dL was used in an attempt to

Table 3. Allele frequency in a subset of 24 COPD patients with serum alpha 1-antitrypsin levels < 113 mg/dL, including the alleles found in 2 patients with dried blood spot alpha 1-antitrypsin levels ≤ 2.64 mg/dL, both of whom died.

Allele	n	%
M	15	28.8
M_1	2	3.8
S	6	11.5
Z	28	53.8
I	1	1.9
Total	52	100

Genotypes include those in 2 patients who died (PI*M_1I and PI*ZZ) before undergoing determination of serum alpha 1-antitrypsin levels. PI: protease inhibitor.

Table 2. Demographic characteristics of a subset of 24 COPD patients with serum alpha 1-antitrypsin levels < 113 mg/dL, by genotype.[a]

Characteristic	Genotype					p*
	PI*MS	PI*MZ	PI*SS	PI*SZ	PI*ZZ	
Male gender, n (%)	2 (16.7)	7 (58.3)	1 (8.3)	1 (8.3)	1 (8.3)	0.07
Age, years	69.3 ± 9.4	69.0 ± 10.1	59.0	74.0	47.0 ± 2.3	< 0.001
Smoking history, pack-years	55.0	53.5 ± 41.1	40.0	12.6	19.1 ± 16.7	0.07
Post-BD FEV_1, % of predicted	33.8 ± 8.3	41.1 ± 14.0	54.7	45.8	37.5 ± 19.9	0.63
FEV_1/FVC	49.4 ± 5.8	57.4 ± 9.0	59.0	56.5	55.7 ± 12.6	0.92
Serum AAT, mg/dL	100 ± 13.5	93.7 ± 14.0	93.8	66.0	27.1 ± 4.8	< 0.001
MRC scale score	2.6 ± 1.1	2.7 ± 1.0	3.0	2.0	3.3 ± 1.6	0.27
CAT score, total	20.3 ± 6.4	16.6 ± 7.3	30	18	17.8 ± 6.3	0.42
Patients, n	3	13	1	1	6	N/A

PI: protease inhibitor; AAT: alpha 1-antitrypsin; MRC: Medical Research Council; BD: bronchodilator; and CAT: COPD Assessment Test. [a]Values expressed as mean ± SD, except where otherwise indicated. *PI*ZZ vs. the remaining genotypes.

Table 4. Genotypes involved in alpha 1-antitrypsin deficiency, distributed by mutation of the *SERPINA1* gene (genotype) and by participating center.

Brazilian region/state	Genotype					
	PI*MZ	PI*ZZ	PI*MS	PI*SS	PI*SZ	PI*M$_1$I
The northeast/Ceará	3	3	1	-	-	-
The northeast/Recife	-	-	1	1		
The central-west/Goiás	-	5	-	-	-	-
The southeast/São Paulo	3	3	-			1
The south/Rio Grande do Sul	2	1	1	-	1	-

PI: protease inhibitor.

identify not only patients with severe AATD but also those with moderate AATD.[20-22]

Despite methodological differences and the fact that not all participants underwent genotyping, our results regarding the PI*Z allele are similar to those found in the literature.[11,18,23] The frequency of the mutant PI*Z allele and other AATD-associated alleles in the present study can be explained by the large number of immigrants from Europe, primarily from countries where the prevalence of AATD is high, such as Portugal and Italy.[23-26]

Although DBS analysis is particularly useful as an initial screening test for AATD, it is not sufficient for a definitive diagnosis. Clinical history, physical examination findings, and family history should be taken into account when interpreting the results, which should be confirmed by measuring serum AAT levels in patients suspected of having AATD. If the results of DBS analysis are confirmed by serum ATT measurements, genotyping or phenotyping are necessary for a definitive diagnosis.

Health professionals providing care to patients with COPD should bear in mind that 2.8% of all COPD patients have some degree of AATD. Our study reinforces the knowledge that AATD is one of the most prevalent genetic diseases. Further studies are warranted, given that an AATD diagnosis can have a major impact on COPD prevention, especially in young smokers. In addition, our finding that the prevalence of the PI*ZZ genotype in the study population is 0.8% shows that severe AATD is present in patients with COPD in Brazil and reinforces the 1999 World Health Organization recommendation that "all COPD patients should be screened once for AATD using a quantitative test. Those with abnormal results on screening should undergo PI typing".[27] Determination of AAT levels in COPD patients has also been recommended by the American Thoracic Society/European Respiratory Society[11] and, more recently, the Canadian Thoracic Society.[28] However, these patients typically present at a younger age (< 45 years) with lower lobe emphysema. Family screening is useful for appropriate counseling. Few countries are as racially diverse as is Brazil, which is populated by a large number of immigrants, including Asians, Africans, Arabs, and, in particular, Europeans. The Portuguese brought centuries of genetic admixture among Europeans, including Celts, Romans, Germans, and Lusitanians. The differences in genotype prevalence among the five regions of Brazil might be due to the different immigrant origins.

One limitation of the present study is that not all participants underwent genotyping. However, serum AAT levels were measured in all patients with the use of the maximum confidence interval width, thus preventing AATD from going undiagnosed.

The prevalence of AATD in COPD patients in Brazil was found to be similar to that found in most countries, despite the racial diversity of the Brazilian population. The actual prevalence of AATD in this population can be best determined by investigating neonates. Genetic studies aimed at determining the ancestry of this population are critical in order to establish a correlation between mutated alleles and the actual ancestry of the individuals.

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Factors associated with disease-specific survival of patients with non-small cell lung cancer

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Study carried out at the Instituto Nacional de Câncer José Alencar Gomes da Silva – INCA – and at the Escola Nacional de Saúde Pública Sérgio Arouca – ENSP – Fundação Oswaldo Cruz – Fiocruz – Rio de Janeiro (RJ) Brasil.

ABSTRACT

Objective: Lung cancer is a global public health problem and is associated with high mortality. Lung cancer could be largely avoided by reducing the prevalence of smoking. The objective of this study was to analyze the effects of social, behavioral, and clinical factors on the survival time of patients with non-small cell lung cancer treated at Cancer Hospital I of the José Alencar Gomes da Silva National Cancer Institute, located in the city of Rio de Janeiro, Brazil, between 2000 and 2003. **Methods:** This was a retrospective hospital cohort study involving 1,194 patients. The 60-month disease-specific survival probabilities were calculated with the Kaplan-Meier method for three stage groups. The importance of the studied factors was assessed with a hierarchical theoretical model after adjustment by Cox multiple regression. **Results:** The estimated 60-month specific-disease lethality rate was 86.0%. The 60-month disease-specific survival probability ranged from 25.0% (stages I/II) to 2.5% (stage IV). The performance status, the intention to treat, and the initial treatment modality were the major prognostic factors identified in the study population. **Conclusions:** In this cohort of patients, the disease-specific survival probabilities were extremely low. We identified no factors that could be modified after the diagnosis in order to improve survival. Primary prevention, such as reducing the prevalence of smoking, is still the best method to reduce the number of people who will suffer the consequences of lung cancer.

Keywords: Lung neoplasms/epidemiology; Carcinoma, non-small-cell lung; Survival analysis.

INTRODUCTION

Lung cancer is the most common type of cancer worldwide; it is estimated that, in 2012, there were 1.8 million new cases.[1] In Brazil in 2015, 27,000 new cases were estimated.[2]

Although lung cancer has various histological types, the most widely used classification system is that which divides tumors into small cell carcinomas (15%) and non-small cell carcinomas (85%).[3]

The 60-month survival probability of patients with non-small cell lung carcinoma is lower than 15% in Europe.[4] A study conducted in the United States obtained estimates ranging from 66% (stage Ia) to 4% (stage IV).[5] A study involving patients from a university hospital in the city of Rio de Janeiro, Brazil, found that the 60-month survival probability was 6%, with it being 14% for the early stages and 5% for the advanced stages.[6]

Among the prognostic factors studied for lung cancer patients[7] are stage, performance status,[8] weight loss, gender, age, smoking, smoking history, quality of life, marital status, depression, and genetic mutations.[6,9-11]

Epidemiological studies have indicated that the effects of socioeconomic factors on health outcomes are indirect, occurring through behavioral and clinical factors. In this context, it is important to establish the hierarchy of these factors in determining the occurrence of lung cancer and in the survival probability of patients with this type of cancer.[12,13]

The objective of the present article was to analyze the importance of social, behavioral, and clinical factors on the survival time of patients with non-small cell lung cancer treated at *Hospital do Câncer I do Instituto Nacional de Câncer José Alencar Gomes da Silva* (HCI/INCA, Cancer Hospital I of the José Alencar Gomes da Silva National Cancer Institute), located in the city of Rio de Janeiro, Brazil, between 2000 and 2003.

METHODS

This was a retrospective observational hospital cohort study in which the object of interest was the time from diagnosis to death from lung cancer or metastasis.

The target population consisted of patients diagnosed with primary non-small cell lung carcinoma, between 2000 and 2003, who were registered in the *Registro Hospitalar de Câncer* (RHC, Hospital Cancer Registry) of HCI/INCA, which is a tertiary referral hospital for the treatment of cancer in the state of Rio de Janeiro, Brazil.

Eligible patients were defined as those from the state of Rio de Janeiro, where HCI/INCA is located, in whom diagnosis was confirmed by either anatomic pathological

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or cytological examination of the tumor and who had not been previously treated. The list of patients who met the eligibility criteria was extracted from the RHC of HCI/INCA, with the primary source of cancer registry information being medical records. At HCI/INCA, medical records were not electronic. To update data on patient survival, we searched the Rio de Janeiro State Mortality Database, and, for patients for whom the information was missing, we conducted an active search according to the RHC routine.[14] In addition, medical records were abstracted for information about smoking history and performance status, which is measured with scales that are used to evaluate how the disease progresses and affects the daily living abilities of the patient, in order to determine appropriate treatment and prognosis.[8] Patients who had ever smoked were considered smokers.

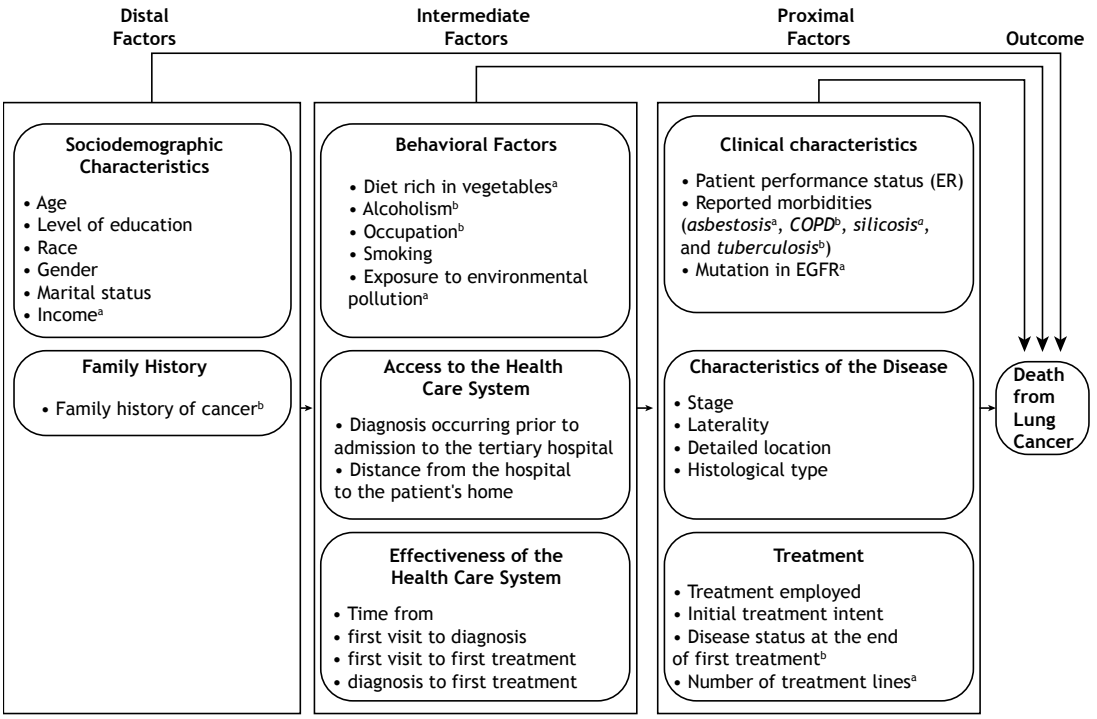
Of the 1,502 cases of non-small cell lung cancer registered in the RHC between 2000 and 2003, 1,394 lived in the state of Rio de Janeiro. Of those, 200 were excluded because it was impossible to determine disease stage by reviewing the medical records.

To reduce the influence of anticipation bias,[15] all analyses were stratified by clinical stage group, as determined by the tumor-node-metastasis classification system[16]: stages I/II (early stage); stage III; and stage IV. Stages I and II were gathered into one group in order to provide greater stability to the results of the models.

The factors identified in the review of the literature were organized into a hierarchical theoretical model (Figure 1).[1-6] Distal factors included sociodemographic characteristics and family history of cancer; intermediate factors included behavioral characteristics as well as access to and effectiveness of the health care system, all of which are generally influenced by sociodemographic characteristics; and proximal factors included patient clinical characteristics, disease characteristics, and treatment characteristics, all of which can be influenced by the previous level factors. Of the 28 factors identified, 10 were not analyzed because they were unavailable or because they were available in very few medical records. In the categorization of the studied factors, priority was given not only to coherence in the object of study but also to data stability as a function of the sample, especially in relation to stages I/II.

Age at diagnosis was included in all multiple regression models because it is directly related to death (from a biological standpoint), because it characterizes the birth cohort, and because it influences other factors (smoking, occupation, etc.)

The 60-month disease-specific survival probability in lung cancer was estimated with the Kaplan-Meier method, on the basis of the following criteria: i) initial event: diagnosis of lung cancer; ii) final event: death from lung cancer or metastasis; iii) survival time: time from initial to final event or time to loss to follow-up; and iv) censored cases: cases that were lost to follow-up over the 60-month period; cases in



Stage will be used as a stratification variable in order to reduce anticipation bias.
[a]Variables unavailable for analysis.
[b]Variables unavailable in a high proportion of cases.

Figure 1. Hierarchical theoretical model of prognostic factors and death from lung cancer.

which death from lung cancer or metastasis was not confirmed; and cases in which patients survived the 60-month follow-up.

Differences between the estimated probability curves were determined via log-rank test. Variables that had a value of $p < 0.20$ in the log-rank test were included in the Cox models.[17] The adjusted effects of factors on survival time were calculated, for each stage, by using the Cox model, on the basis of the hierarchical theoretical model proposed in Figure 1.

In the preparation of the models, variables were entered in blocks. First, distal factors were entered; those with a value of $p < 0.10$ were maintained in model 1. Subsequently, intermediate factors were added to model 1, and the same selection criterion was applied (model 2). The same procedure was repeated for proximal factors, and the final model was obtained. In the modeling process, the previous level factors that lost significance upon inclusion of more proximal-level factors were maintained. The goodness-of-fit of the models was determined by calculating the likelihood ratio, the probability of agreement, and the overall goodness-of-fit.[17]

The research project that generated the present article is registered with the research ethics committees of the INCA and the Sérgio Arouca National School of Public Health (Protocol nos. CAAE-012.0.007.031-11 and CAAE-0163.0.031.007-11).

RESULTS

A comparison of cases included and excluded as per the eligibility criteria indicated no statistically significant differences (chi-square test) in the distribution by gender, level of education, smoking, histological type, or treatment.

The mean age of the 1,194 patients included in the study was 62 years, and it decreased with the severity of tumor stage (stages I/II, 65 years; stage III, 62 years; and stage IV, 60 years). Most subjects were

Table 1. Distribution and 60-month disease-specific survival probability of the study cohort of patients with non-small cell lung cancer by clinical stage, as well as by distal factor and by intermediate factor of the proposed model. Cancer Hospital I, José Alencar Gomes da Silva National Cancer Institute, 2000-2003.

Distal and intermediate factors	Stage												Total	
	I/II				III				IV					
	n	%	SPr		n	%	SPr		n	%	SPr		n	%
			%	95% CI			%	95% CI			%	95% CI		
Total	207	17.3	25.0	19.1-32.8	506	42.4	6.7	4.5-9.9	481	40.3	2.5	1.3-4.9	1,194	100.0
Distal factor														
Age group, years														
30-49	18	8.7	41.2	23.3-72.7	63	12.5	7.3	2.5-21.8	72	15.0	0.0	N/A	153	12.8
50-59	41	19.8	32.5	20.0-52.9	128	25.3	8.7	4.5-16.8	144	29.9	0.8	0.1-5.9	313	26.2
60-69	67	32.4	22.4	13.0-38.4	189	37.4	8.6	4.9-14.8	179	37.2	4.9	2.3-10.6	435	36.4
70-99	81	39.1	19.0	11.1-32.4	126	24.9	1.2	0.2-8.3	86	17.9	2.5	0.4-15.2	293	24.5
Gender														
Female	68	32.9	29.3	19.2-44.8	134	26.5	5.9	2.7-12.8	138	28.7	2.9	1.0-8.8	340	28.5
Male	139	67.1	23.1	16.3-32.7	372	73.5	7.0	4.5-11.0	343	71.3	2.4	1.1-5.5	854	71.5
Intermediate factor														
Smoking[a]														
Never smoking	20	9.7	28.7	13.8-59.7	23	4.5	5.3	0.8-35.9	52	10.8	9.6	3.8-24.3	95	8.0
Current or former smoking[b]	185	89.4	24.6	18.4-33.0	480	94.9	6.8	4.6-10.1	422	87.7	1.5	0.6-3.9	1,087	92.0
Smoking history, pack-years[c]														
Up to 51	86	46.5	28.0	19.2-40.7	251	52.3	6.6	3.7-11.6	225	53.3	1.4	0.4-5.5	562	55.3
52-103	59	31.9	20.7	11.6-37.0	143	29.8	5.4	2.5-11.8	128	30.3	1.4	0.2-8.2	330	32.4
104-360	25	13.5	21.7	9.2-50.9	59	12.3	13.2	6.2-27.9	41	9.7	3.0	0.4-20.5	125	12.3
Diagnosis occurring prior to admission to HCI/INCA														
Yes	44	21.3	35.3	22.8-54.6	158	31.2	11.3	6.7-18.9	151	31.4	2.7	0.9-8.2	353	29.6
No	163	78.7	22.5	16.2-31.2	348	68.8	4.5	2.5-8.0	330	68.6	2.5	1.1-5.7	841	70.4
Distance from home to HCI/INCA, km														
≤10.00	54	26.1	26.9	16.5-43.9	131	25.9	4.7	1.8-12.2	119	24.7	1.2	0.2-8.4	304	25.5
10.01-20.00	55	26.6	31.2	19.7-49.3	134	26.5	4.5	1.7-11.6	144	29.9	4.2	1.6-10.8	333	27.9
20.01-30.00	31	15.0	9.6	2.7-33.9	88	17.4	11.1	5.8-21.4	76	15.8	3.2	0.8-12.4	195	16.3
30.01-40.00	25	12.1	10.8	2.9-39.8	61	12.1	2.3	0.3-16.1	60	12.5	0.0	N/A	146	12.2
> 40.00	42	20.3	36.1	23.2-56.2	92	18.2	10.5	5.2-21.1	82	17.0	3.3	0.9-12.9	216	18.1

SPr: survival probability; and HCI/INCA: *Hospital do Câncer I, Instituto Nacional de Câncer José Alencar Gomes da Silva*. [a]Data unavailable in 12 cases. [b]Category corresponding to ever smokers. [c]Data unavailable in 82 cases.

male and smokers; in stage III, the smoker/nonsmoker ratio reached its maximum value (20.9:1.0). The mean smoking history was 60 pack-years, and less than one third of the patients had a confirmed diagnosis prior to admission to HCI/INCA (Table 1).

At diagnosis, more than half of the patients in stages I/II and III presented with restrictions for performing vigorous physical activities. The most common first treatment was radiotherapy, and, in stages I/II, surgery was the first treatment in only one fourth of the cases. Adenocarcinoma predominated in stages I/II and stage IV, followed by squamous carcinoma, chiefly in stage III (Table 2).

By the end of the 60-month follow-up, 1,027 patients (86.0%) had died from lung cancer, 66 (5.5%) had died from other causes, 70 (5.9%) had survived, and 31 (2.6%) had been lost. The estimated 12-month and 60-month disease-specific survival probabilities were 32.7% (95% CI: 30.0-35.5%) and 7.9% (95% CI: 6.3-9.7%), respectively. The median survival time was estimated to be 17.7 months for stages I/II, 8.0 months for stage III, and 5.5 months for stage IV.

Patients with stage IV disease who were nonsmokers had a better prognosis than did those who were smokers/former smokers. Being admitted to HCI/INCA with a confirmed diagnosis doubled the survival probability of stage III patients (Table 1). Survival decreased with increasing limitation as assessed by the performance status scale, regardless of stage. Among the patients for whom information on tumor location was available, stage I/II and stage III patients had a better prognosis (Table 2).

In the modeling process, the cases with missing values for the variables included in the final models were excluded to allow comparability between the models of the different levels. We excluded 20 stage

Table 2. Distribution and 60-month disease-specific survival probability of the study cohort of patients with non-small cell lung cancer by clinical stage and by proximal factor of the proposed model. Cancer Hospital I, José Alencar Gomes da Silva National Cancer Institute, 2000-2003.

Proximal factor	Stage												Total	
	I/II				III				IV					
	n	%	SPr		n	%	SPr		n	%	SPr		n	%
			%	95% CI			%	95% CI			%	95% CI		
Performance status[a]														
Fully active	34	16.4	53.5	38.2-74.9	39	7.7	10.7	3.7-30.7	48	10.0	6.3	1.8-22.2	121	11.6
Limited in vigorous activities	114	55.1	24.7	17.3-35.2	308	60.9	8.0	5.2-12.2	233	48.4	2.5	1.1-5.9	655	62.8
Able of self-care but unable to work	39	18.8	0.0	N/A	93	18.4	1.3	0.2-9.0	105	21.8	1.3	0.2-8.8	237	22.7
Bedridden at least 50% of the day	0	0.0	N/A	N/A	8	1.6	0.0	N/A	22	4.6	0.0	N/A	30	2.9
Tumor laterality[b]														
Unilateral	192	92.8	26.2	20.0-34.3	467	92.3	6.5	4.4-9.6	423	87.9	2.9	1.5-5.5	1,082	98.5
Bilateral	1	0.5	0.0	N/A	4	0.8	25.0	4.6-100.0	11	2.3	0.0	N/A	16	1.5
Availability of detailed information on tumor location														
Yes	165	79.7	28.2	21.4-37.2	305	60.3	7.9	5.1-12.4	236	49.1	3.0	1.2-7.2	706	59.1
No	42	20.3	10.7	3.7-30.7	201	39.7	4.6	2.1-9.9	245	50.9	2.3	0.9-5.9	488	40.9
Initial treatment intent[c]														
Curative	132	78.6	33.8	25.9-44.3	231	55.8	9.7	6.2-15.3	82	22.9	4.8	1.4-16.5	445	47.3
Palliative	22	13.1	0.0	N/A	149	36.0	2.7	0.9-8.1	275	76.8	2.4	1.0-5.7	446	47.4
Neoadjuvant	14	8.3	33.8	13.5-84.5	34	8.2	13.6	5.5-33.8	1	0.3	0.0	N/A	49	5.2
Initial treatment modality														
Surgery	52	25.1	63.5	50.3-80.3	9	1.8	28.6	8.9-92.2	9	1.9	42.9	18.2-100.0	70	5.9
Radiotherapy	89	43.0	6.7	2.2-20.2	247	48.8	5.7	3.1-10.5	233	48.4	1.1	0.3-4.5	569	47.7
Chemotherapy	38	18.4	30.0	17.2-52.5	181	35.8	9.1	5.3-15.5	158	32.8	2.6	0.9-7.8	377	31.6
No treatment	28	13.5	0.0	N/A	69	13.6	0.0	N/A	81	16.8	0.0	N/A	178	14.9
Histological type														
Adenocarcinoma	87	42.0	30.6	21.3-43.9	198	39.1	6.8	3.7-12.6	237	49.3	2.9	1.3-6.9	522	43.7
Squamous carcinoma	81	39.1	17.3	9.9-30.2	207	40.9	6.7	3.7-12.2	130	27.0	1.8	0.3-10.3	418	35.0
Other carcinomas	39	18.8	28.2	15.9-50.2	101	20.0	6.6	2.6-16.6	114	23.7	2.3	0.6-9.1	254	21.3

SPr: survival probability. [a]Data unavailable in 151 cases. [b]Data unavailable in 96 cases. [c]Data unavailable in 76 cases.

I/II cases, 108 stage III cases, and 75 stage IV cases. Excluded and analyzed cases were compared, and no statistically significant differences were observed for the variables gender, age, level of education, race, marital status, smoking, histological type, treatment, vital status, or follow-up period.

In stages I/II, age, gender, performance status, detailed tumor location, histological type, initial treatment intent, and initial treatment modality proved to be important prognostic factors in the crude analysis of the Cox models and were used in the hierarchical modeling.

None of the intermediate factors were included in the multiple regression model of stages I/II ($p > 0.20$ in the log-rank test). When the proximal factors were added to model 1, the risk estimates decreased. The risk of death, adjusted for the other variables of the final model, was 2.34 times higher among patients who were unable to perform work activities than among those who were active, was twice as high among those for whom information on tumor location was unavailable as among those for whom this information was available, and was thirteen times higher among untreated patients than among operated patients. The risk of death associated with the use of radiotherapy or chemotherapy was high when compared with that related to surgery (Table 3).

In stage III, the prognostic factors used in the hierarchical modeling were age, diagnosis occurring prior to admission, distance from home to HCI/INCA, performance status, tumor laterality, tumor location, initial treatment intent, and initial treatment modality.

In the adjusted model for stage III, there were no differences in the estimates when the intermediate factors were added to model 1; however, when the proximal factors were included, the age-related risk estimate decreased. The effect of diagnosis occurring prior to admission increased when the proximal factors were included. The final model showed an excess risk of death of 70.0%, adjusted for the other factors, among the patients who had not been given a diagnosis prior to admission (Table 4).

Performance status maintained a strong association with outcome in the final model and showed an increasing gradient of risk of death/worsening of the patient's physical state. Patients who underwent palliative treatments had a risk of death, adjusted for the other factors in the final model, 2.48 times that of those who underwent curative treatments. Among the untreated patients, this estimate was even higher (Table 4).

In stage IV, the study characteristics gender, smoking, smoking history, diagnosis occurring prior to admission, distance from home to HCI/INCA, performance status, tumor laterality, histological type, initial treatment intent, and initial treatment modality were used in the hierarchical modeling. The age-related risk estimate decreased and gained statistical significance when the proximal factors were included in the final model. The effect associated with smoking decreased and lost statistical significance when the proximal factors were entered. This behavior probably occurs because the effect of smoking is mediated by proximal factors. Adjusted for the other factors, the risk of death by

Table 3. Results of the hierarchical Cox model of patients with stage I/II non-small cell lung cancer. Cancer Hospital I, José Alencar Gomes da Silva National Cancer Institute, 2000-2003.

Characteristic studied	Model 1 HR (95% CI)	Final model HR (95% CI)
Age	1.03[a] (1.01-1.05)	1.01 (0.99-1.03)
Gender		
Female	1.00	1.00
Male	1.43[b] (0.98-2.09)	1.28 (0.87-1.90)
Performance status		
Fully active, able to perform all activities		1.00
Limited in vigorous activities		1.23 (0.66-2.32)
Able of self-care but unable to work		2.34[a] (1.14-4.81)
Availability of detailed information on tumor location		
Yes		1.00
No		2.03[a] (1.29-3.20)
Initial treatment modality		
Surgery		1.00
Radiotherapy		5.13[a] (2.59-10.18)
Chemotherapy		2.70[a] (1.30-5.57)
No treatment		13.15[a] (6.39-27.06)
% of the explained variability (R^2)	8.22	44.42
Concordance probability, %	0.62	0.76
P-value of the deviance test (ANOVA)		< 0.001

HR: hazard ratio. [a]Estimate (Wald): $p < 0.05$. [b]Estimate (Wald): $0.05 < p < 0.10$.

Table 4. Results of the hierarchical Cox model of patients with stage III non-small cell lung cancer. Cancer Hospital I, José Alencar Gomes da Silva National Cancer Institute, 2000-2003.

Characteristic studied	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Final model HR (95% CI)
Age	1.02[a] (1.01-1.03)	1.02[a] (1.01-1.03)	1.01 (1.00-1.02)
Diagnosis occurring prior to admission to HCI/INCA			
Yes		1.00	1.00
No		1.63[a] (1.30-2.06)	1.70[a] (1.34-2.15)
Distance from HCI/INCA to home, km			
≤ 10.00		1.00	1.00
10.01-20.00		0.86 (0.64-1.16)	0.88 (0.65-1.19)
20.01-30.00		0.75[b] (0.54-1.04)	0.71[a] (0.43-0.85)
30.01-40.00		1.10 (0.77-1.58)	0.95 (0.65-1.37)
> 40.00		0.71[a] (0.52-0.99)	0.64[a] (0.46-0.90)
Performance status			
Fully active, able to perform all activities			1.00
Limited in vigorous activities			1.33 (0.89-2.00)
Able of self-care but unable to work			2.70[a] (1.73-4.21)
Bedridden at least 50% of awake hours			4.56[a] (1.93-10.75)
Tumor laterality			
Unilateral			1.00
Bilateral			0.32[b] (0.10-1.02)
Initial treatment intent			
Curative			1.00
Palliative			2.48[a] (1.93-3.21)
Neoadjuvant			0.97 (0.63-1.48)
No treatment			3.67[a] (2.56-5.25)
% of the explained variability (R^2)	2.62	8.37	32.86
Concordance probability, %	0.56	0.60	0.71
P-value of the deviance test (ANOVA)		< 0.001	< 0.001

HR: hazard ratio; and HCI/INCA: *Hospital do Câncer I, Instituto Nacional de Câncer José Alencar Gomes da Silva*. [a]Estimate (Wald): $p < 0.05$. [b]Estimate (Wald): $0.05 < p < 0.10$.

the end of the 60-month follow-up was found to be 50.0% higher among those who had not been given a diagnosis prior to admission. Those who lived within a radius of 20-30 km of HCI/INCA had an adjusted risk of death that was 28.0% lower than that for those who lived closer to HCI/INCA (Table 5).

Performance status maintained a strong association with outcome after adjustment and showed an increasing gradient of risk of death/worsening of the patient's physical state. The risks associated with nonsurgical treatments, as compared with surgical treatments, are extremely high in that stage. In addition, the risk of death by the end of the 60-month follow-up is estimated to be eight times higher among untreated patients than among operated patients (Table 5).

The inclusion of the proximal factors in the more distal-level models significantly increased the likelihood ratio of the final models, regardless of stage. In addition, the probability of agreement of these models can be classified as very good in stages I/II and stage III and as coherent in stage IV. The all-level adjusted models were significantly different from the null model ($p < 0.001$) in the three stages analyzed (Tables 2, 3, and 4).

DISCUSSION

Among the factors evaluated, performance status, initial treatment intent, and initial treatment modality stood out for influencing the survival time of patients with non-small cell lung cancer treated at HCI/INCA between 2000 and 2003, in all stage groups. The magnitudes and directions of the estimated effects related to these factors in the present study are consistent with those reported in other studies and will be addressed below.[6,18,19]

Survival studies of lung cancer patients commonly involve clinical trial patients. A review of the literature revealed two survival studies of patients with non-small cell lung cancer, both of which were conducted in the city of Rio de Janeiro, Brazil: one in a public hospital, in which 60-month survival probabilities were estimated[6]; and one in a private clinic,[19] in which 24-month survival probabilities were estimated.

The predominance of advanced stage disease (stage IV, 40.3%), males (71.5%), and smokers (92.0%) observed in the present study is consistent with the characteristics of other study populations.[1,6,18-20]

Results that are consistent and in agreement with findings of previous studies were observed for the

Table 5. Results of the hierarchical Cox model of patients with stage IV non-small cell lung cancer. Cancer Hospital I, José Alencar Gomes da Silva National Cancer Institute, 2000-2003.

Characteristic studied	Model 1 HR (95% CI)	Model 2 HR (95% CI)	Final model HR (95% CI)
Age	0.99 (0.98-1.00)	0.99 (0.98-1.00)	0.98[a] (0.97-0.99)
Gender			
Female	1.00	1.00	1.00
Male	1.21[c] (0.97-1.52)	1.13 (0.89-1.43)	1.11 (0.87-1.41)
Smoking			
Never smoking		1.00	1.00
Current or former smoking[b]		1.46[a] (1.03-2.07)	1.23 (0.86-1.77)
Diagnosis occurring prior to admission to HCI/INCA			
Yes		1.00	1.00
No		1.39[a] (1.12-1.73)	1.50[a] (1.19-1.88)
Distance from HCI/INCA to home, km			
≤ 10.00		1.00	1.00
10.01 -20.00		0.79 (0.60-1.05)	0.83 (0.63-1.10)
20.01 -30.00		0.79 (0.57-1.08)	0.72[a] (0.52-0.99)
30.01-40.00		1.18 (0.82-1.69)	1.00 (0.69-1.45)
> 40.00		0.76[c] (0.55-1.04)	0.85 (0.62-1.18)
Performance status			
Fully active, able to perform all activities			1.00
Limited in vigorous activities			1.46[a] (1.04-2.06)
Able of self-care but unable to work			2.58[a] (1.72-3.85)
Bedridden at least 50% of awake hours			3.87[a] (2.24-6.68)
Initial treatment modality			
Surgery			1.00
Radiotherapy			4.70[a] (1.70-12.97)
Chemotherapy			3.28[a] (1.20-9.03)
No treatment			8.03[a] (2.85-22.66)
% of the explained variability (R^2)	1.21	6.14	25.68
Concordance probability, %	0.53	0.58	0.69
P-value of the deviance test (ANOVA)		< 0.001	< 0.001

HR: hazard ratio; and HCI/INCA: *Hospital do Câncer I, Instituto Nacional de Câncer José Alencar Gomes da Silva*. [a]Estimate (Wald): $p < 0.05$. [b]Category corresponding to ever smokers. [c]Estimate (Wald): $0.05 < p < 0.10$.

distribution of the study population by smoking history (mean, 60 pack-years),[6] performance status (approximately 90.0% were limited),[6,18,19] and histological type (more than 40.0% had adenocarcinoma).[5,18,20]

The median estimated survival time was slightly higher than that found in the study conducted in a public hospital in Rio de Janeiro, for all stages.[6] It is likely that, because HCI/INCA is an oncology referral center, it has a health care infrastructure that favors a better prognosis, in comparison with the other public hospital not specializing in oncology. In contrast, the low proportion of operated patients, especially stage I/II patients, indicates limited access to this treatment modality.

The 60-month survival probabilities below 25.0% found in most of the analysis categories in stages I/II illustrate how devastating cancer is, regardless of what factors are evaluated, even when the disease is diagnosed in early stages. This finding underscores the importance of primary prevention with two major strategies: encouraging smoking cessation and increasing young people's awareness regarding the dangers of smoking in order to prevent them from acquiring this behavior.

In the evaluation of prognostic factors through the use of the Cox models, results that are consistent with those of other studies were observed for performance status,[18] that is, patients who are more limited have a lower survival probability.

Regarding initial treatment intent and initial treatment modality, the adjusted results of the final models of the present study are consistent with what is expected in oncology,[21,22] that is, survival probabilities are higher among those initially treated with curative intent and among those treated with surgery, which, for lung cancer, is the treatment modality that is most likely to result in a cure.

In stage IV, a mediation effect of smoking was observed for the proximal factors. Considering that variables are entered in blocks, it is impossible to determine which factor is responsible for this effect.[13]

The results associated with the distance from the patient's home to HCI/INCA can be explained on the basis of how the health care system in the state of Rio de Janeiro was organized in terms of lung cancer treatment during the study period. A medical referral, together with test results indicating the presence of a malignant tumor, was required for admission to HCI/INCA. Patients who lived outside the city of Rio de Janeiro and were referred to HCI/INCA usually benefited from a free shuttle service organized and provided by each municipal government. In general, this service facilitated continuation of treatment and follow-up. Perhaps other ways of evaluating access to the hospital in relation to place of residence, taking into account the route traveled and the transport used as reported by patients, can provide survival analysis results that are more consistent.

In the present study, the incompleteness of the medical records limited the use of some factors in the model proposed in Figure 1 and case inclusion in the analyses. Since the clinical stage was not recorded, we lost 14.3% of the eligible cases, which affected the stability of the estimates for some analysis categories. An evaluation of cases included and excluded on the basis of missing information revealed a statistically significant difference regarding treatment—approximately 50.0% of the patients excluded from the analysis had not been treated, whereas among the cases analyzed, the proportion was 14.9%. This loss could influence the results obtained, but it is impossible to determine the magnitude of this effect because we do not know the stage of the patients who were not analyzed. Progressive improvement in the quality of data entry into medical records should be encouraged, since these documents are often used as a source for database building. Despite the limitations inherent to retrospective studies that use medical records, such studies are of great value in increasing knowledge about disease involvement in populations that are treated at health care clinics.

In summary, it can be noted that the estimated 60-month disease-specific survival probabilities were very low, even in stages I/II. In addition, we identified no factors that could be modified after the diagnosis in order to improve survival. Lung cancer is a silent disease whose symptoms are associated with other less lethal diseases, which can lead to a delay in diagnosis in relation to the natural history of the disease. The best method to reduce the number of people who will suffer the consequences of lung cancer is primary prevention, reducing smoking.

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Bronchodilator response cut-off points and $FEV_{0.75}$ reference values for spirometry in preschoolers

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ABSTRACT

Objective: To determine the cut-off points for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ bronchodilator responses in healthy preschool children and to generate reference values for $FEV_{0.75}$. **Methods:** This was a cross-sectional community-based study involving children 3-5 years of age. Healthy preschool children were selected by a standardized questionnaire. Spirometry was performed before and after bronchodilator use. The cut-off point of the response was defined as the 95th percentile of the change in each parameter. **Results:** We recruited 266 children, 160 (60%) of whom were able to perform acceptable, reproducible expiratory maneuvers before and after bronchodilator use. The mean age and height were 57.78 ± 7.86 months and 106.56 ± 6.43 cm, respectively. The success rate for $FEV_{0.5}$ was 35%, 68%, and 70% in the 3-, 4-, and 5-year-olds, respectively. The 95th percentile of the change in the percentage of the predicted value in response to bronchodilator use was 11.6%, 16.0%, 8.5%, and 35.5% for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$, respectively. **Conclusions:** Our results provide cut-off points for bronchodilator responsiveness for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ in healthy preschool children. In addition, we proposed gender-specific reference equations for $FEV_{0.75}$. Our findings could improve the physiological assessment of respiratory function in preschool children.

Keywords: Spirometry; Bronchodilator agents; Reference values; Child, preschool.

INTRODUCTION

Although spirometry with bronchodilator testing is routinely used in order to investigate respiratory diseases in children and adults, it is rarely used in preschool children. In children, only a few studies have defined bronchodilator response cut-off points (for FEV_1), the established change in baseline FEV_1 and in percent predicted FEV_1 in response to bronchodilator use having varied across studies, from 9% to 14% and from 9% to 10%, respectively.[1-4] The American Thoracic Society (ATS) and the European Respiratory Society (ERS) have yet to determine the best cut-off points for children. In addition, given the lack of studies, the ATS and the ERS have not been able to determine cut-off points for preschool children.[5,6]

One obstacle is that only a low proportion (34-90%) of preschoolers are able to exhale for 1 s or more.[7-12] Therefore, measurements of FEV during the first 0.5 s of FVC ($FEV_{0.5}$) or during the first 0.75 s of FVC ($FEV_{0.75}$) can be used as surrogates for FEV_1. According to the ATS and the ERS, $FEV_{0.5}$ and $FEV_{0.75}$ should always be reported from spirometry maneuvers performed by preschool children.[6] Several studies have shown that $FEV_{0.5}$ and $FEV_{0.75}$ are reproducible.[7,8,10,12-14] Several reference equations for $FEV_{0.5}$ and $FEV_{0.75}$ have been established in various populations.[13-17]

In a case-control study,[18] bronchodilator response cut-off points of 14%, 14%, and 33% were found for baseline FEV_1, $FEV_{0.75}$, and $FEF_{25-75\%}$, respectively. In another study,[19] cut-off points of 10%, 11%, and 25% were found for baseline FEV_1, $FEV_{0.5}$, and $FEF_{25-75\%}$, respectively; however, their sensitivity for the diagnosis of asthma was found to be low (12%, 30%, and 41%, respectively), their specificity being 84%, 90%, and 80%, respectively.[9]

Given that spirometry is a low-cost and noninvasive test, and given that several studies[7,8,10,12-14] have demonstrated that preschool children can perform acceptable and reproducible $FEV_{0.5}$ and $FEV_{0.75}$ measurements, there is a need to determine bronchodilator response cut-off points for children in this age group so that spirometry can be used in daily clinical practice. Only two studies[18,19] have assessed bronchodilator response using spirometry exclusively in preschool children.

In the present study we sought to determine bronchodilator response cut-off points for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ using the 95th percentile of the change in each parameter and establish reference values for $FEV_{0.75}$ in healthy preschool children (i.e., preschoolers without respiratory symptoms).

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METHODS

This was a community-based study of preschool children 3-5 years of age selected from among those attending any one of 18 public day care centers and schools in the city of Recife, Brazil. Data were collected in the period between February and December of 2014.

We selected a convenience sample, giving priority to the schools and day care centers attended by the highest number of children and located in central, northern, and western Recife. We calculated the sample size required to achieve a mean increase in $FEV_{0.75}$, after bronchodilator use, of 4.5% and a standard deviation of 5.1%, a value found in a study by Borrego et al.,[18] with 95% confidence, assuming an estimation error of 1%, in accordance with the sample size calculation of Pardos et al.[20] The minimum sample size was calculated to be 100.

In order to characterize the study sample, we used the ATS and Division of Lung Diseases questionnaire for the diagnosis of asthma—designated ATS-DLD-78-C—previously adapted and validated for use in Brazil in children 4 months to 13 years of age.[21] The questionnaire was administered by two of the authors of the present study.

The inclusion criteria were as follows: being 3-5 years of age; having been a full-term infant; having had a birth weight ≥ 2,500 g; and having no respiratory symptoms, i.e., having no symptoms of asthma (dyspnea, wheezing, recurrent cough, or exertional dyspnea) or other respiratory diseases. The exclusion criteria were as follows: respiratory disease at birth requiring the use of oxygen for more than 24 h; chronic respiratory disease (including bronchopulmonary dysplasia, cystic fibrosis, and bronchiolitis obliterans); thoracic and pulmonary malformations; acute viral bronchiolitis in the last 6 months; acute nasopharyngitis; heart disease; and other severe diseases (including immunodeficiencies, neurological diseases, and genetic syndromes). A questionnaire administered up to one week before testing was used in order to determine whether prospective participants met any of the aforementioned criteria. Testing was not performed if there were signs of acute nasopharyngitis at the time of testing.

All tests were performed by the principal investigator, having been performed in the morning in all participating schools and day care centers. A back-extrapolated volume of < 80 mL or 12.5% of FVC was accepted, as recommended for preschool children.[6] The objective was to obtain two acceptable maximal expiratory curves, the variation between the two highest values of FVC, FEV_1, and $FEV_{0.75}$ being equal to or less than 10% and the variation between the two highest values of $FEV_{0.5}$ being equal to or less than 5%. Curves with a forced expiratory time (FET) of at least 0.5 s were accepted regardless of whether or not they ended abruptly. Each session of testing lasted a maximum of 25 min. Encouragement screens were used, and each session of testing was preceded by a brief (5-min) session of training. Spirometry was repeated 15 min after administration of 400 μg of albuterol delivered by a metered dose inhaler, as recommended by the ATS/ERS.[6] An aluminum spacer with a face mask was used (inAl-air; RSMed, Belo Horizonte, Brazil). Testing was performed with the children in the sitting position. No nose clips were used in the present study, because the use of nose clips in children undergoing spirometry has been shown to have no clear advantage.[22] Testing was performed with a portable spirometer validated by the ATS (Koko; Ferraris Respiratory, Louisville, CO, USA). Calibration was performed at the testing site, before each session of testing, with the use of a 3-L syringe, within the acceptable range of volume and flow.[23] Room temperature and humidity were measured, and the data collected were entered into the software. In order to obtain acceptable maneuvers, testing sessions were suspended after an average of eight attempts or, before that, if the child showed fatigue or disinterest in continuing.

The following spirometric parameters were assessed: FVC; FEV_1; $FEV_{0.75}$; $FEV_{0.5}$; and $FEF_{25-75\%}$. The values of the aforementioned parameters were obtained from the two best flow-volume curves, both of which were acceptable and reproducible.[6] The criteria for determining the values of $FEF_{25-75\%}$ were as follows: for curves with a maximum FET of < 0.75 s, $FEF_{25-75\%}$ was obtained from the curve with the highest $FEV_{0.5}$ value and the highest $FEV_{0.5}$ + FVC value; for curves with a maximum FET of < 1 s, $FEF_{25-75\%}$ was obtained from the curve with the highest $FEV_{0.75}$ value and the highest $FEV_{0.75}$ + FVC value; for curves with a maximum FET ≥ 1 s, $FEF_{25-75\%}$ was obtained from the curve with the highest FEV_1 value and the highest FEV_1 + FVC value. The variables used in order to determine bronchodilator response cut-off points for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ were the percent change regarding the predicted values, the percent change regarding the baseline values, and the change in absolute values.

Statistical analysis was performed with the IBM SPSS Statistics software package, version 21 (IBM Corporation, Armonk, NY, USA). Numerical variables are expressed as means, medians, and percentiles. Categorical variables are expressed as proportions. The reproducibility of the spirometric measurements was tested by the intraclass correlation coefficient (ICC). Weight, height, and BMI are expressed as Z scores.[24]

The Shapiro-Wilk test was used for testing data normality. The Student's t-test for paired samples was used in order to compare mean baseline and post-bronchodilator values of all spirometric parameters.

The changes in response to bronchodilator use were calculated by the following formulas:

$$(\text{post-bronchodilator value} - \text{baseline value}) \times 100/\text{baseline value}$$

$$(\text{post-bronchodilator value} - \text{baseline value}) \times 100/\text{predicted value}$$

The predicted values were derived from a reference equation for preschool children developed by our research group in a previous study.[17] Because the aforementioned equation[17] does not include reference values for $FEV_{0.75}$, they were calculated in the present study by linear regression.

In order to determine bronchodilator response cut-off points for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$, the 95th percentile of the change in each parameter was calculated for baseline, predicted, and absolute values.

Spearman's correlation coefficient was calculated in order to evaluate the correlation of the bronchodilator response indices tested with age, height, and baseline FEV_t (FEV_1, $FEV_{0.75}$, and $FEV_{0.5}$).

The study project was approved by the Research Ethics Committee of the Professor Fernando Figueira Institute of Integrative Medicine (Protocol no. 2616-11). The parents or legal guardians of all participating preschoolers gave written informed consent, and the researchers signed a statement of responsibility.

RESULTS

Of the 462 eligible children, 447 completed the questionnaires. Of those 447 children, 41 (9%) met the exclusion criteria and 34 (8%) constituted losses: 26 for missing school on the day of testing and 8 for declining to undergo testing. Of the remaining 372 preschoolers, 266 (71%) were classified as having no respiratory symptoms. Of those 266 children, 56 (21.0%) failed to perform spirometry correctly and 50 (19.0%) failed to perform bronchodilator testing correctly. The final sample consisted of 160 asymptomatic preschool children (60% of the initial sample of 266 asymptomatic children). A flowchart of the sample selection process is shown in Figure 1.

The demographic characteristics of the sample are presented in Table 1. Of the children who performed acceptable measurements, 19 (12%) were 3 years old, 74 (46%) were 4, and 67 (42%) were 5. Curves with a back-extrapolated volume ≤ 5% were obtained in 99% of the tests, and, in 95% of those, the difference between the two highest FVC, FEV_1, $FEV_{0.75}$, and $FEV_{0.5}$ values was < 5%, demonstrating a high reproducibility.

We calculated the ICCs for the two highest values of each of the spirometric variables tested. Mean ICCs (and their respective 95% CIs) for FVC and FEV_1 were 0.994 (0.990-0.996) and 0.993 (0.989-0.996), respectively. Mean ICCs (and their respective 95% CIs) for $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ were 0.993 (0.990-0.995), 0.992 (0.990-0.994), and 0.935 (0.913-0.951), respectively.

Among the 3-year-olds in the initial sample of 266 children, FVC, FEV_1, $FEV_{0.75}$, and $FEV_{0.5}$ measurements were considered acceptable and reproducible in 5%, 7%, 9%, and 37%, respectively; among the 4-year-olds, they were considered acceptable and reproducible in 23%, 29%, 39%, and 68%, respectively; and among the 5-year-olds, they were considered acceptable and reproducible in 23%, 26%, 44%, and 70%, respectively.

Spirometry was considered unacceptable in 63% of the 3-year-olds, in 32% of the 4-year-olds, and in 30% of the 5-year-olds. Bronchodilator response testing was considered inadequate in 19% of the

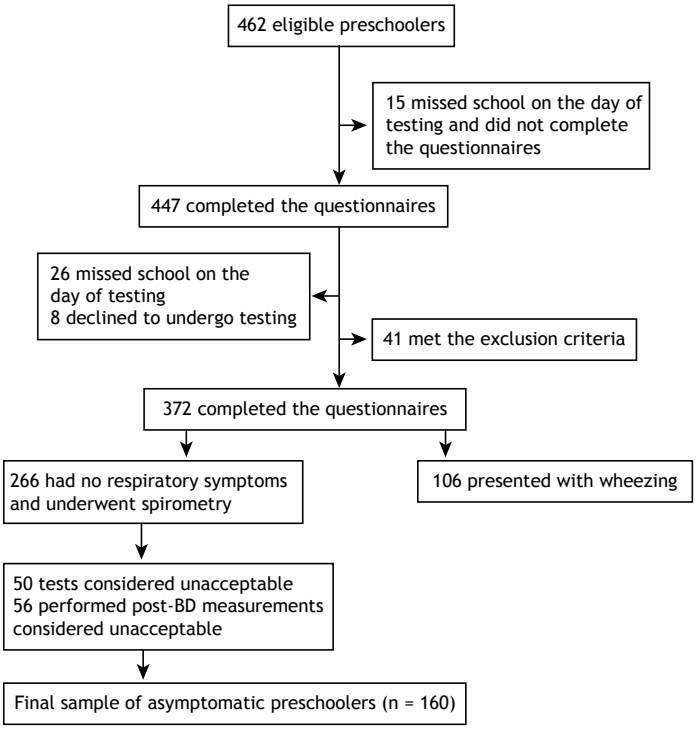

Figure 1. Flowchart of study sample selection. BD: bronchodilator.

3-year-olds, in 20% of the 4-year-olds, and in 20% of the 5-year-olds, proportions that were very similar.

Mean baseline and post-bronchodilator FVC, FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ values are shown in Table 2. There were significant differences between mean pre- and post-bronchodilator values of the aforementioned parameters ($p < 0.005$). Proportionally, mean and median pre- and post-bronchodilator changes in FVC, FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ with non-normal distribution can be seen in Table 2.

In order to calculate reference values for $FEV_{0.75}$, gender-specific reference equations were derived by linear regression. For males, the equation and the lower limit (LL) are as follows:

$FEV_{0.75}$ = height × 0.013 + weight × 0.010

LL = predicted value − 0.21(5th percentile of the residual)

For females, the equation and the LL are as follows:

$FEV_{0.75}$ = height × 0.008 + age × 0.008 + weight × 0.013

LL = predicted value − 0.19

The dispersion of $FEV_{0.75}$ values is shown in Figure 2.

Table 3 shows all cut-off points established by the 95th percentile of the change in FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$ in response to bronchodilator use.

Table 4 shows the Spearman's correlations of the bronchodilator response cut-off points for baseline, percent predicted, and absolute values of $FEV_{0.5}$ and $FEV_{0.75}$ with age, height, and baseline FEV_t ($FEV_{0.5}$ and $FEV_{0.75}$).

Table 1. Sociodemographic characteristics of the study population, Recife, Brazil, February-December of 2014.[a]

Variable	Result
Male gender	84 (52.5)
Age, months	57.8 ± 7.8
Race	
White	33 (20.6)
Black	11 (6.9)
Mulatto	116 (72.5)
Weight-for-age (Z score)[b]	0.20 ± 1.18
Height-for-age (Z score)[b]	−0.38 ± 1.03
BMI (Z score)[b]	0.65 ± 1.20

[a]Values expressed as n (%) or mean ± SD. [b]Based on data from the Brazilian National Ministry of Health.[24]

DISCUSSION

This is the first study to establish, by means of spirometry, bronchodilator response cut-off points in preschoolers, the cut-off points being expressed as the change in percent predicted FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$. Of the 4-year-olds in the study sample, 67% were able to perform $FEV_{0.5}$ measurements and 39% were able to perform $FEV_{0.75}$ measurements. Of the 5-year-olds in the study sample, 70% were able to perform $FEV_{0.5}$ measurements and 44% were able to perform $FEV_{0.75}$ measurements. Therefore, in preschoolers, $FEV_{0.5}$ measurements are more useful than $FEV_{0.75}$ measurements because the proportion of children who can perform the former is higher. In community-based samples, spirometry is not useful in 3-year-olds due to the high rate of unacceptable tests.

The low proportion of children who were able to perform acceptable and reproducible pre- and post-bronchodilator measurements of FVC, FEV_1, $FEV_{0.75}$, and $FEF_{25-75\%}$ can be explained by the fact that ours was a community-based sample, the children therefore being more inexperienced in performing such measurements; in general, children selected from among those treated at respiratory outpatient clinics have previously been evaluated by their physicians regarding their motor coordination to perform such tests. Given that it is difficult for children to perform pre- and post-bronchodilator spirometry, the proportion of preschoolers who can perform it is lower. The high ICCs for the spirometric parameters tested in the present study constitute evidence of the low variability and high reproducibility of the measurements performed, as well as of the technical skills of the professional who performed the tests.

We found no studies evaluating bronchodilator response exclusively in healthy preschool children. The studies that we found involved children with asthma. The mean changes in the percentage of the predicted values of FVC, FEV_1, and $FEV_{0.75}$ in response to bronchodilator use in the present study were 2.3%, 4.5%, and 5.6%, respectively, being similar to those found in another study (2.5%, 4.7%, and 4.5%, respectively).[18] For $FEF_{25-75\%}$, Borrego et al.[18] found a change of 11.7%, compared with 20.0% in the present study. This difference can be explained by the difference in study sample between the two studies: ours was a community-based sample, whereas that

Table 2. Means and dispersion of baseline and post-bronchodilator spirometric parameters in the preschoolers studied.

Variable	Baseline		Post-BD		Change (pre- and post-BD), %	p*
	n	Mean ± SD	n	Mean ± SD	Mean ± SD (median)[a]	
FVC, L	94	1.06 ± 0.21	52	1.09 ± 0.18	2.3 ± 4.3 (0.71)	< 0.001
FEV_1, L	93	1.00 ± 0.18	61	1.06 ± 0.17	4.5 ± 4.7 (2.67)	< 0.001
$FEV_{0.75}$, L	94	0.94 ± 0.17	92	1.00 ± 0.17	5.6 ± 5.6 (4.32)	< 0.001
$FEV_{0.5}$, L	160	0.80 ± 0.16	160	0.86 ± 0.16	6.8 ± 6.4 (5.47)	< 0.001
$FEF_{25-75\%}$, L/s	94	1.52 ± 0.40	92	1.79 ± 0.42	20.0 ± 20.2 (15.48)	< 0.001

BD: bronchodilator; $FEV_{0.5}$: FEV during the first 0.5 s of FVC; and $FEV_{0.75}$: FEV during the first 0.75 s of FVC. [a]Mean and median of the changes observed after bronchodilator use (variables with non-normal distribution). All parameters had a normal distribution. The greatest differences between mean and median values are due to a lower n of FVC and FEV_1 measurements after bronchodilator use. *Student's t-test for paired samples.

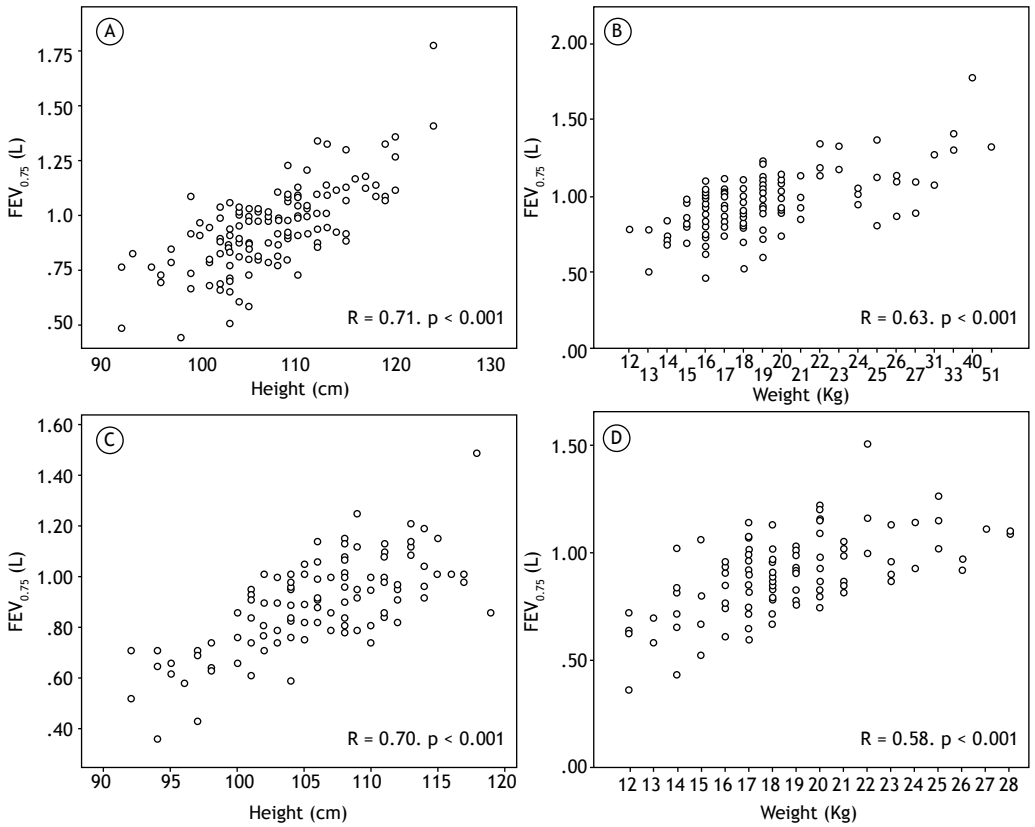

Figure 2. Scatter plots of FEV during the first 0.75 s of FVC ($FEV_{0.75}$) in relation to height and weight in male preschoolers (A and B) and in female preschoolers (C and D).

Table 3. Percentiles of the changes in FEV_1, $FEV_{0.5}$, $FEV_{0.75}$, and $FEF_{25-75\%}$ in response to bronchodilator use, the parameters being expressed as percentages of the predicted values, percent changes from baseline, and absolute changes in the preschoolers studied.

Variable		Percentile			
		5th	25th	75th	95th
FEV_1, L	% of predicted[a]	0	0	7.91	11.6
	% change from baseline	0	0	8.80	13.0
	Absolute change, L	0	0	0.09	0.13
$FEV_{0.5}$, L	% of predicted[a]	0.51	1.18	9.66	16.0
	% change from baseline	0	1.07	10.74	20.0
	Absolute change, L	0	0.01	0.08	0.15
$FEV_{0.75}$, L	% of predicted[b]	0	0.57	5.35	8.50
	% change from baseline	0	0.85	9.20	18.0
	Absolute change, L	0	0.01	0.08	0.14
$FEF_{25-75\%}$, L/s	% of predicted[a]	−2.88	3.87	22.02	35.5
	% change from baseline	−4.74	6.33	33.17	61.0
	Absolute change, L	−0.06	0.09	0.44	0.74

$FEV_{0.5}$: FEV during the first 0.5 s of FVC; and $FEV_{0.75}$: FEV during the first 0.75 s of FVC. [a]Values according to Piccioni et al.[13] [b]Values calculated on the basis of the data from the present study.

in the study by Borrego et al.[18] was a case-control sample. In addition, the high reproducibility and, consequently, low variability of $FEF_{25-75\%}$ measurements in the present study increase the power to detect differences between pre- and post-bronchodilator values. In the present study, the mean post-bronchodilator percentage changes of FEV_1, $FEV_{0.5}$, and $FEF_{25-75\%}$ were 4.5%, 6.8%, and 20.0%, respectively, whereas, in another study,[19] they were 8.9%, 2.9%, and 8.1%, respectively. The type of sample used in ours and in that study[19] (community-based and case-control samples, respectively), as well as the fact that dose of albuterol was lower in that study (200 µg),[19] might have contributed to those differences.

Table 4. Spearman's coefficients correlating the bronchodilator response indices with age, height, FEV during the first 0.5 s of FVC ($FEV_{0.5}$), and FEV during the first 0.75 s of FVC ($FEV_{0.75}$) in the study sample.

Variable	Absolute change from baseline $FEV_{0.5}$ after bronchodilator use, L	Percent change from baseline $FEV_{0.5}$ after bronchodilator use	Change in percent predicted $FEV_{0.5}$ after bronchodilator use
	SCC (p)	SCC (p)	SCC (p)
Age, months	0.04 (0.60)	−0.1 (0.28)	−0.05 (0.50)
Height, cm	0.11 (0.17)	−0.05 (0.50)	−0.03 (0.70)
Baseline $FEV_{0.5}$, L	−0.16 (0.47)	−3.27 (0.00)	−0.25 (0.02)
	Absolute change from baseline $FEV_{0.75}$ after bronchodilator use, L	**Percent change from baseline $FEV_{0.75}$ after bronchodilator use**	**Change in percent predicted $FEV_{0.5}$ after bronchodilator use**
	SCC (p)	SCC (p)	SCC (p)
Age, months	−0.17 (0.11)	−0.24 (0.02)	−0.23 (0.03)
Height, cm	0.53 (0.62)	−0.05 (0.66)	−0.01 (0.89)
Baseline $FEV_{0.75}$, L/s	−0.06 (0.60)	−0.18 (0.10)	−0.17 (0.11)

SCC: Spearman's correlation coefficient.

For FEV_1, the cut-off point found in the present study (a change of 13% from baseline in response to bronchodilator use) was similar to that found in another study (14%)[18] but different from that found by Linares et al. (10%, with a sensitivity of 12% and a specificity of 84%).[19] This low sensitivity suggests that there were no significant differences between the groups regarding bronchodilator response or that the administered dose of albuterol (200 µg) was insufficient to produce a bronchodilator effect. Because the aforementioned study was a case-control study involving patients with moderate to severe persistent asthma, it is more likely that the administered dose of albuterol was insufficient to provide effective bronchodilation. With regard to the change in the percentage of the predicted FEV_1 in response to bronchodilator use in the present study (i.e., 11.6%), we found no other studies evaluating this parameter in samples composed exclusively of preschool children.

For $FEV_{0.5}$, the cut-off point found in the present study (a change of 20% from baseline in response to bronchodilator use) differs from that found by other authors (11%, with a sensitivity of 30% and a specificity of 90%).[19] This low sensitivity suggests that is not the best cut-off point. The high ICCs for the measurements performed in the present study increase its power regarding the reliability of those measurements. It should also be taken into account that the cut-off points for a community-based study should be higher than those for studies comparing patients and healthy controls, as was the case of the two aforementioned studies.[18,19] With regard to the change in the percentage of the predicted $FEV_{0.5}$ in response to bronchodilator use in the present study (16%), the fact that there have been no studies evaluating this parameter makes it impossible to make comparisons.

For baseline $FEV_{0.75}$, the bronchodilator response cut-off point found in the present study (18%) was higher than that found in another study (14%).[18] This difference might be due to the type of study (a case-control study)[18] and how the cut-off point was calculated (mean + 2 standard deviations after bronchodilator use in healthy participants).[18] With regard to the change in the percentage of the predicted $FEV_{0.75}$ in response to bronchodilator use in the present study (8.5%), the lack of evidence in the literature makes it impossible to make comparisons.

Although some studies have included $FEF_{25-75\%}$ in the analysis of bronchodilator response,[1,19,20] $FEF_{25-75\%}$ is not given weight in studies evaluating bronchodilator response, because it varies widely.[6,20] For $FEF_{25-75\%}$, the cut-off point found in the present study (a change of 61% from baseline in response to bronchodilator use) is different from those found by Borrego et al. (33%) [18] and other authors (25%, with a sensitivity of 41% and a specificity of 80%).[1] Unlike the present study, the aforementioned studies were both case-control studies, and this might explain these discrepancies. The high ICC for $FEF_{25-75\%}$ in the present study indicates good reproducibility. With regard to the change in the percentage of the predicted $FEF_{25-75\%}$ in response to bronchodilator use in the present study (35.5%), the lack of studies on this topic makes it impossible to make comparisons. The use of this parameter in the evaluation of bronchodilator response in preschool children will require further studies.

Some studies have shown that, in children, it is best to express bronchodilator response as a percentage of the predicted values, because percent predicted values do not depend on age, height, or baseline FEV_1.[6,25] However, for preschool children, the present study showed correlations of baseline $FEV_{0.5}$ with the percent change from baseline after bronchodilator use and the percent change in the predicted value after bronchodilator use. With regard to $FEV_{0.75}$, regarding this age group, age correlates with the percent change from baseline after bronchodilator use and the percent change in the predicted value after bronchodilator use. Therefore, in preschool children, there is no difference between the percent change from baseline after bronchodilator use and the percent change in the predicted value after bronchodilator use for the two parameters.

One of the strengths of the present study is that it was a community-based study, the results of which are more generalizable than are those of studies conducted in secondary or tertiary care settings. Another strength of the present study is that we used predicted values that had been derived from preschoolers in the same region as those in the present study, thus increasing the reliability of the results obtained. The high reproducibility of the spirometric measurements in the present study shows that they are reliable.

To our knowledge, this is the first study to determine, in preschool children, bronchodilator response cut-off points for FEV_1, $FEV_{0.75}$, $FEV_{0.5}$, and $FEF_{25-75\%}$, expressed as percentages of the predicted values. We derived reference values for $FEV_{0.75}$. Given that $FEV_{0.5}$ is reproducible and that $FEV_{0.5}$ measurements can be performed by a higher proportion of preschoolers, it is the most useful of all of the parameters studied here. $FEV_{0.75}$ is useful in children ≥ 4 years of age. . In community-based samples, spirometry is not useful in 3-year-olds due to the high rate of unacceptable tests.

For clinical practice, the recommended bronchodilator response cut-off points for percent predicted FEV_1, $FEV_{0.75}$, and $FEV_{0.5}$ are ≥ 12%, ≥ 8%, and ≥ 16%, respectively; for baseline FEV_1, $FEV_{0.75}$, and $FEV_{0.5}$, the recommended cut-off points are ≥ 13%, ≥ 18%, and ≥ 20%, respectively. For percent predicted and baseline $FEF_{25-75\%}$, the recommended cut-off points are ≥ 35% and ≥ 61%, respectively. Given that $FEF_{25-75\%}$ showed good reproducibility, it might be useful in the evaluation of bronchodilator response. Further studies are needed in order to test the utility of these cut-off points in samples of patients with respiratory symptoms treated at respiratory outpatient clinics.

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Effects of passive inhalation of cigarette smoke on structural and functional parameters in the respiratory system of guinea pigs

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ABSTRACT

Objective: To evaluate the effects of passive inhalation of cigarette smoke on the respiratory system of guinea pigs. **Methods:** Male guinea pigs were divided into two **groups:** control and passive smoking, the latter being exposed to the smoke of ten cigarettes for 20 min in the morning, afternoon and evening (30 cigarettes/day) for five days. After that period, inflammatory parameters were studied by quantifying mesenteric mast cell degranulation, as well as oxidative stress, in BAL fluid. In addition, we determined MIP, MEP, and mucociliary transport (in vivo), as well as tracheal contractility response (in vitro). **Results:** In comparison with the control group, the passive smoking group showed a significant increase in mast cell degranulation (19.75 ± 3.77% vs. 42.53 ± 0.42%; $p < 0.001$) and in the levels of reduced glutathione (293.9 ± 19.21 vs. 723.7 ± 67.43 nM/g of tissue; $p < 0.05$); as well as a significant reduction in mucociliary clearance ($p < 0.05$), which caused significant changes in pulmonary function (in MIP and MEP; $p < 0.05$ for both) and airway hyperreactivity. **Conclusions:** Passive inhalation of cigarette smoke caused significant increases in mast cell degranulation and oxidative stress. This inflammatory process seems to influence the decrease in mucociliary transport and to cause changes in pulmonary function, leading to tracheal hyperreactivity.

Keywords: Inflammation; Inhalation exposure; Tobacco smoke pollution.

INTRODUCTION

Smoking is a risk factor for the leading causes of death worldwide, including cardiac and pulmonary diseases. [1,2] There are no safe levels of exposure to cigarettes. Passive or active smoking is directly related to irritation, inflammation, and changes in lung function within the first few hours of exposure.[3,4]

Airway contact with cigarette smoke induces changes in the respiratory system, such as mucus hypersecretion, deficit in mucociliary transport, tracheobronchial tree defects, small airway restriction accompanied by increased closing capacity, and a trend toward changes in the ventilation-perfusion ratio.[5]

A passive smoker is an individual who inhales environmental cigarette smoke. The immediate harmful effects of such exposure include irritation of the eyes, nose and throat, as well as increased respiratory and heart rates.[6] There is also an increase in inflammatory cytokine levels within the first few hours of inhalation, especially in men.[3]

The concern regarding passive inhalation of cigarette smoke is recent, having begun in the early 1980s. According to the Brazilian National Health Oversight Agency, approximately two thirds of the smoke produced by cigarettes (passive smoking, secondary exposure, passive inhalation, or involuntary smoking) is released into the ambient air through the lit end of the product. [7] In the USA, 3,000 lung cancer deaths per year are estimated to have been caused by passive inhalation of cigarette smoke.[8] In Brazil, the National Health Survey conducted in 2013 points out that the proportion of people aged 18 years or older who were exposed to passive smoking was 10.7% in the home and 13.5% in the indoor workplace. Regarding gender, that proportion is higher among women in the home (11.7%) and men in the workplace (16.9%).[9]

Some studies have pointed out that the harmful effects of passive inhalation of cigarette smoke may begin in childhood, causing cough,[10] endothelial dysfunction,[11] and prolonged expiratory apnea.[12]

Currently, the incidence of death and disease caused by cigarette smoking remains high. However, there have been few studies reporting the possible structural and functional changes in the respiratory system in passive smokers. This aroused our interest in developing this study. We hope to use the study findings to alert the population to and raise its awareness about the prevention of diseases caused by smoking, in order to contribute to improving public health in general.

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Therefore, the present study aimed at evaluating structural and functional aspects of the respiratory system of guinea pigs after passive inhalation of cigarette smoke.

METHODS

This was an experimental longitudinal exploratory study with quantitative analysis of results. We used male guinea pigs (*Cavia porcellus*; 5-8 per group) obtained from the animal facilities of the Estácio University Center of Ceará, located in the city of Fortaleza, Brazil. The mean body weight of the animals was 321.00 ± 6.72 g at the beginning of the experiments. All animals were handled in accordance with the Brazilian College for Animal Experimentation's guidelines for animal care and welfare. The study was approved by the Animal Research Ethics Committee of the Federal University of Ceará (Protocol no. 052/10).

The experiments were performed in the Laboratory of Biophysics, Physiology and Pharmacology of the *Centro Universitário Estácio do Ceará* (Estácio University Center of Ceará), in cooperation with the Department of Physiology and Pharmacology of the Federal University of Ceará, also located in the city of Fortaleza.

Cigarette smoke inhalation

The protocol consisted of inhalation of smoke from ten commercial filter cigarettes (Derby Autêntico; Souza Cruz S.A., Rio de Janeiro, Brazil), each containing 8 mg of tar, 0.7 mg of nicotine, and 7 mg of carbon monoxide. Those cigarettes were lit concomitantly for 20 min in the morning, afternoon, and evening, adding up to 30 cigarettes/day, for 5 days.[13,14] During inhalation, the animals were placed into acrylic boxes (whole body; n = 3 per box) measuring 30.0 × 16.6 × 19.8 cm.[15] Each box had a removable upper lid for introduction of the animals and two holes on its side walls: one for air drainage, allowing the smoke to escape; and one for placing the cigarettes (cigarette smoke was introduced into the acrylic box passively). Control animals did not inhale any type of toxic substance, and they were placed into control group acrylic boxes (whole body; n = 3 per box) to simulate the same conditions as those of the cigarette smoke inhalation groups.

For the purposes of this study, two groups were formed: control, consisting of guinea pigs that did not inhale any toxic substance and were killed after 25 days; and passive smoking, consisting of guinea pigs that were killed 25 days after completing the process of passive inhalation of cigarette smoke (Figure 1).

Analysis of the inflammatory process on the basis of mast cell degranulation counts

After anesthesia (urethane, 1.2g/kg, i.p.), the mesentery was removed and a wire was carefully inserted into the small intestine, which was then given a circular shape. Subsequently, the small intestine was transferred to a Petri dish containing a toluidine blue solution (toluidine blue dissolved in 70% alcohol at a concentration of 0.1g/L and diluted with 1% NaCl at a ratio of 1:10, resulting in a concentration of 0.01 g/L) for staining at room temperature. The preparation was then washed with Tyrode's solution (136 mM of NaCl; 5 mM of KCl; 0.98 mM of $MgCl_2$; 0.36 mM of NaH_2PO_4; 11.9 mM of $NaHCO_3$; 2 mM of $CaCl_2$; and 5.5 mM of glucose) and left to dry on a glass slide (for 10 min) at room temperature. After drying and excess tissue removal, 100 cells were counted in different fields by optical microscopy (magnification, ×200), and, on that basis, the percentage of mast cell degranulation was determined.

Assessment of oxidative stress

Oxidative stress was measured indirectly by quantification of nitrite and reduced glutathione (GSH) in BAL fluid. To that end, the lungs were initially filled with 5 mL of saline solution at 37ºC, which was instilled into the tracheal tube using a syringe. After a 3-min period, the instilled fluid was slowly recovered by aspiration. That procedure was repeated with another 5 mL of saline solution. The material was stored in a freezer (−70ºC).[16]

Determination of nitrite

Assays were prepared with 100 µL of Griess reagent—0.1% N-(1-naphthyl)ethylenediamine in water; and 1% sulfanilamide in 5% phosphoric acid—and 100 µL of the (centrifuged) supernatant of the 20% BAL fluid homogenate from the guinea pigs or 100 µl of various concentrations of the standards. Blanks were prepared with 100 µL of Griess reagent and 100 µL of saline solution (0.9% NaCl). Absorbance was measured at 560 nm using a plate reader.[17] Results are expressed as µM/g of tissue.

Figure 1. Model of passive inhalation of cigarette smoke. In the center, a photograph of the guinea pigs during the inhalation process. M: morning; A: afternoon; and E: evening.

Determination of GSH

The reagent was prepared using 0.02 M of EDTA and 50% trichloroacetic acid. After this process, centrifugation was performed (5,000 rpm for 15 min at 4°C). Subsequently, the supernatant was collected and homogenized. The samples were mixed with 0.4 M Tris-HCl buffer (pH = 8) and 0.01 M of 5.5-dithio-bis-2-nitrobenzoic acid. The material was kept cooled throughout the assay. The GSH activity was measured at 412 nm using a plate reader. Results are expressed as ng/g of tissue.

Measurement of mucociliary transport

Initially, the guinea pigs were anesthetized with urethane (1.2 g/kg, i.p.) and fixed horizontally in the supine position. Subsequently, 2 µL of a 0.3 g/mL gelatin solution containing 0.5% Evans blue dye was injected into their tracheas with a microsyringe. Two minutes later, the tracheas were opened, and mucociliary transport was measured, from the injection point, by using a caliper.[15,18]

Measurement of pulmonary pressures

The animals were anesthetized (urethane, 1.2 g/kg, i.p.). Subsequently, the trachea was cannulated and connected in a closed system to a pressure transducer, at the end of inhalation, not allowing airflow leaks. The connection was maintained until the animal had intercostal retraction (approximately 20 seconds). Three MIP and MEP measurements were taken in each animal, and the time interval between measurements depended on normalization of the respiratory pattern and rhythm.[19] The ventilatory parameters were recorded by PowerLab/8sp (ADInstruments Pty Ltd., Bella Vista, Australia) data acquisition system.

Isometric recordings of the tracheal rings

After anesthesia (urethane, 1.2 g/kg, i.p.) and subsequent euthanasia by exsanguination (through the left carotid artery), a ventral midline incision was made and the trachea was rapidly excised in one single segment of approximately 10-12 mm.[19] The excised segment was then rapidly transported to a Petri dish containing Krebs-Henseleit solution (in nmol/L: 118 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 1.2 $MgSO_4$; 25.0 $NaHCO_3$; 1.2 KH_2PO_4; and 10 glucose). After removal of adjacent tissues, the trachea was cut into four ring-shaped segments, which were transferred to individual organ bath chambers containing 5 mL of Krebs-Henseleit solution that was continuously aerated with carbogen ((95% O_2 and 5% CO_2); the pH of the solution was manually adjusted to 7.4 at a temperature of 37°C, which was kept constant through water circulation from a pumped water bath. The lumen of the tracheal rings was crossed with two pieces made of thin stainless steel, which were tied to two points: one fixed in the chamber; and one connected to a force transducer (ML870B60/C-V; ADInstruments) suitable for recording isometric contractions. The signals generated by the force transducer were recorded by a computerized data acquisition system (PowerLab™ 8/30; ADInstruments). The tension applied to each tracheal segment was set at 1 gf. The equilibration period was 1 h, and the incubation liquid was changed every 15 min.[20] After the stabilization period, concentration-effect curves were constructed for potassium (10-120 mM) and for carbachol (0.001-10 µM), with a 5 min-interval at each concentration. After completion of the protocol, the tracheas were removed from the incubation liquid and left to dry at room temperature (approximately 25°C) for 2 h. Subsequently, each tissue was weighed.

Data analysis

Results are expressed as mean ± standard error of the mean, and the number of experimental observations is noted in parentheses (n). For group comparison, we used one-way or two-way ANOVA, as well as the Student's t-test, Holm-Sidak's test, the Kruskal-Wallis test, and the Mann-Whitney test, according to the normality test. For all tests, values of $p < 0.05$ were considered statistically significant.

RESULTS

Mast cell degranulation

Mesenteric mast cell degranulation in the guinea pigs was 19.75 ± 3.77% (n = 5). However, in the guinea pigs that inhaled cigarette smoke over a short period of time (25 days), mast cell degranulation was 42.53 ± 0.42% (n = 5), being significantly higher than that observed in the control group ($p < 0.001$; Student's t-test; Figure 2).

Nitrite and GSH levels

Determination of nitrite (control group = 0.073 ± 0.007 µM/g of tissue vs. passive smoking group = 0.065 ± 0.004 µM/g of tissue) and GSH (control group = 293.9 ± 19.21 nM/g of tissue vs. passive smoking group = 723.7 ± 67.43 nM/g of tissue) in the BAL fluid from the guinea pigs revealed a significant difference ($p < 0.05$; Student's t-test) only in the GSH levels (Figure 3).

Mucociliary transport and pulmonary pressures

Analysis of mucociliary transport revealed that, in the control group, the distance traveled was 0.65 ± 0.08 cm; however, that distance was significantly reduced ($p < 0.05$; ANOVA followed by the Kruskal-Wallis post-test) to 0.30 ± 0.03 cm in the passive smoking group (n = 6; Figure 4A).

The mean MIP was −9.93 ± 0.94 cmH_2O in the control group (n = 5) and −40.44 ± 9.26 cmH_2O in the passive smoking group (n = 6); therefore, the latter group showed a significant decrease ($p < 0.05$; ANOVA followed by Holm-Sidak's post-test) as compared with the former (Figure 4B).

The mean MEP was 0.58 ± 0.05 cmH_2O in the control group (n = 6) and 3.60 ± 0.60 cmH_2O in the

passive smoking group (n = 6); therefore, the latter group showed a significant increase ($p < 0.05$; ANOVA followed by Holm-Sidak's post-test) as compared with the former (Figure 4C).

Tracheal contractility

Adding increasing cumulative concentrations of K^+ (10-120 mM) in the control group (n = 6) produced a contractile response with an amplitude of 0.05 ± 0.00 gf/mg of tissue. Comparatively, the passive smoking group (n = 8) showed a significantly increased ($p < 0.05$; two-way ANOVA followed by Holm-Sidak's post-test) contractile response, with a mean value of 0.10 ± 0.01 gf/mg of tissue (Figure 5A).

Concentration-effect curves were also constructed to determine the contractile response to carbachol (0.001-10 µM): the animals in the control group (n = 6) showed a mean value of 0.09 ± 0.02 gf/mg of tissue; comparatively, the animals in the passive smoking group (n = 8) showed a significantly increased ($p < 0.05$; two-way ANOVA followed by Holm-Sidak's post-test) contractile response (0.23 ± 0.03 gf/mg of tissue; Figure 5B).

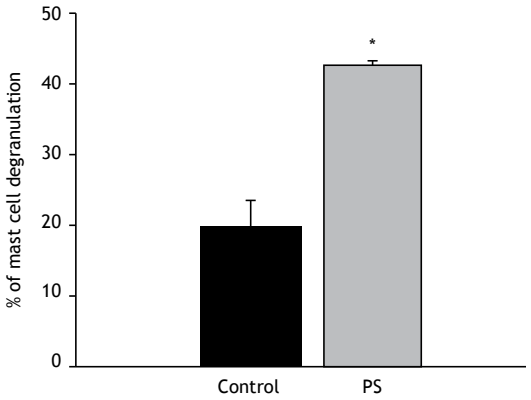

Figure 2. Comparison of the proportion of mast cell degranulation between the control group and the passive smoking (PS) group. Data expressed as mean ± standard error. *p < 0.001 in relation to the control group.

DISCUSSION

The findings of the present study reveal that passive inhalation of cigarette smoke over a short period of time produces structural and functional changes in the respiratory system. Such effects were proven by evidence of a deficit in lung tissue; an increase in inflammatory cells, leading to mucus accumulation; and pulmonary function impairment caused by tracheal hyperreactivity. Although some inhalation models[13,14,21,22] have reported the damages caused by constant and prolonged use of cigarettes, the relevance of the present study lies in reporting the changes caused by passive inhalation of cigarette smoke over a short period of time (5 days), with a change in the amount and duration of exposure to cigarette smoke as compared with previous studies.[23-25] In their study, Hernandez et al.[23] also opted to analyze the effects of passive inhalation of cigarette smoke over a short period of time. To that end, the animals were submitted to a protocol consisting of three 10-min exposures to smoke from one cigarette, separated by 30-min intervals, each day, for 4 days, and it was found that exposure of guinea pigs to cigarette smoke produced airway hyperreactivity to histamine and recruitment of inflammatory cells.

It is of note that, in recent years, the understanding of the pathophysiological mechanisms related to passive inhalation of cigarette smoke has enabled increasingly specific approaches, with the use of concise and reproducible methods to investigate the contractile and inflammatory repercussions of this inhalation process.

The animals that inhaled cigarette smoke exhibited destruction of lung tissue architecture, accompanied by migration of proinflammatory cells (data not shown), which corroborates other experimental models of cigarette smoke inhalation.[23-27] Such changes were pointed out by the study of Banerjee et al.,[28] in which the guinea pigs that inhaled cigarette smoke exhibited inflammation and apoptosis, which leads to destruction of alveolar membranes and septal cells, causing pulmonary airspace enlargement, and such enlargement may have influenced the changes in pulmonary pressures in the present study. Page

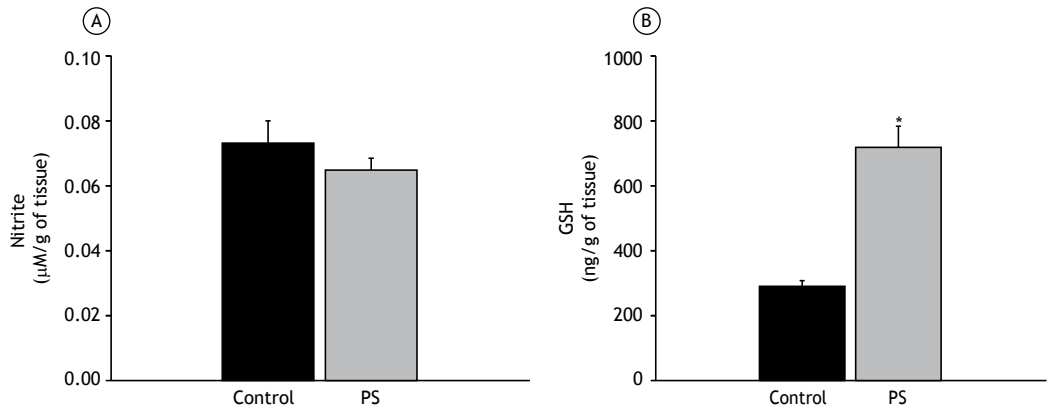

Figure 3. Comparison between the control group and the passive smoking (PS) group regarding tissue levels of nitrite (in A) and reduced glutathione (GSH; in B). Data expressed as mean ± standard error. *p < 0.05; Student's t-test.

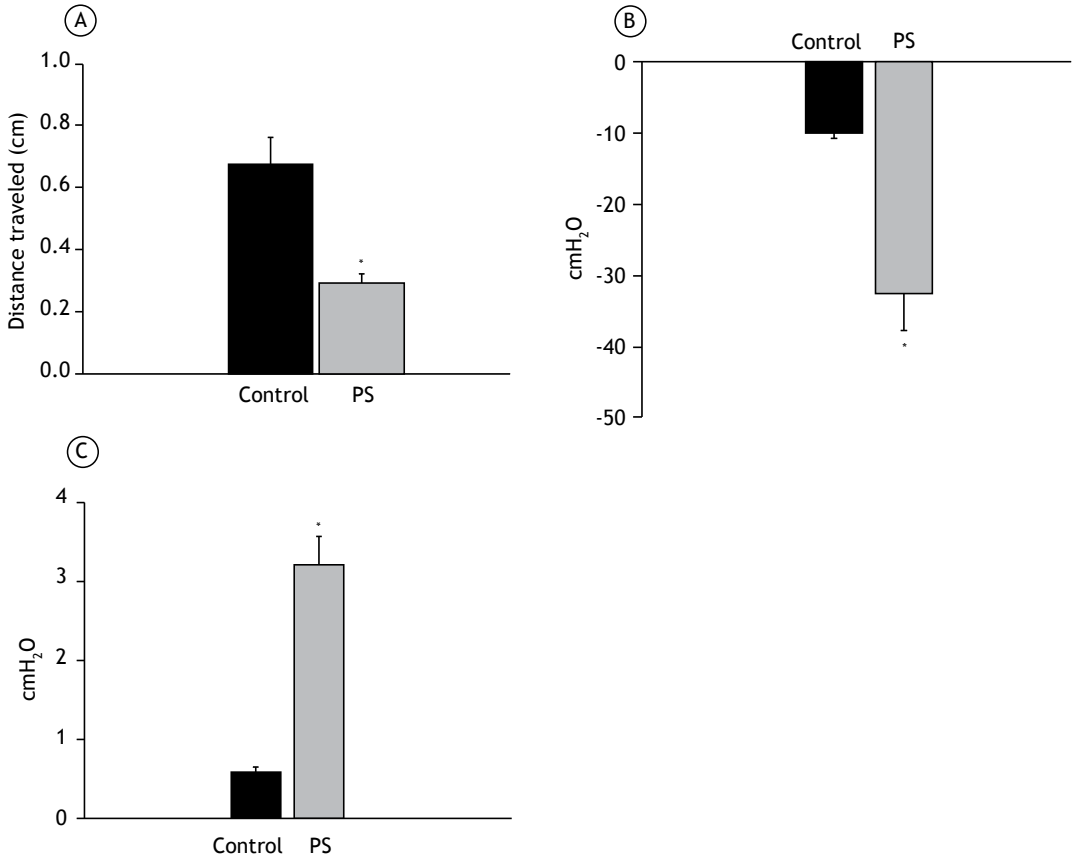

Figure 4. Comparison between the control group and the passive smoking (PS) group regarding mucociliary transport (in A), MIP (in B), and MEP (in C).Data presented as mean ± standard error. *p < 0.05 for all.

et al.[29] add that, after exposure of airway sensory nerves to cigarette smoke, there is damage caused by cytotoxic mediators to the ciliary epithelium layer, increased mucus secretion, hyperresponsiveness, and vasodilatation, findings that are in agreement with those of the present study.

In this sense, Valença & Porto[21] highlighted the participation of macrophages in the destruction of extracellular lung matrix in animals exposed to cigarette smoke over different periods of time; in contrast, in animals exposed to room air (control group), the alveoli were preserved and there were few alveolar macrophages. The study by Zhong et al.,[30] in which guinea pigs were exposed to inhalation of smoke from 10 cigarettes for 20 min, twice a day, for 14 days, reported that the animals exposed to cigarette smoke showed increased proinflammatory cytokine levels (TNF-α and IL-8), as well as an increase in inflammatory cells and tracheal thickness.

Another important harmful effect observed in the present study was the decrease in mucociliary transport in the guinea pigs submitted to passive inhalation of cigarette smoke, a finding confirmed by Furtado[5] when he states that smoking leads to a decrease in mucociliary transport because of the many toxic substances found in the cigarette that exhibit ciliostatic and ciliotoxic properties. Vasconcelos et al.[15] also observed a deficit in mucociliary transport in guinea pigs that mimicked asthma symptoms, corroborating the present study when they state that inflammatory diseases cause changes in ciliary function and in the amount of mucus secreted, which directly influences the protection of the respiratory system.

Therefore, to protect itself, the organism has an intracellular defense system that can act in two ways: either by reducing the toxic substances in the organism before they cause injury, through the activity of antioxidants, such as GSH, superoxide dismutase, catalase, and vitamin E; or by inhibiting the injury with ascorbic acid, glutathione reductase, glutathione peroxidase, etc.[31] Indirectly, measurement of antioxidant enzyme activity and determination of the concentration of tripeptides are the most widely used methods for the assessment of oxidative stress.[32] That same group of authors[32] points out the important antioxidant effect of GSH on the respiratory tract, an effect that can reduce oxidative stress.

The levels of GSH, one of the major antioxidant enzymes in the respiratory tract, were significantly higher in the passive smoking group than in the control group, demonstrating an adaptive response of the organism to an injury, but there were no changes in the nitrite

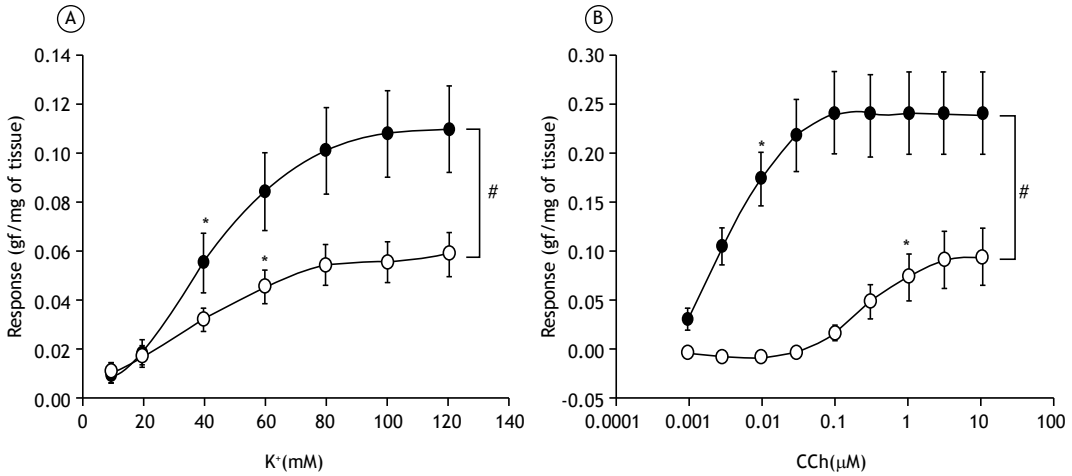

Figure 5. Tracheal hyperreactivity to contractile stimulation with K^+, in A, and with carbachol (CCH), in B, in the control group (white circles) and in the passive smoking group (black circles). Data expressed as mean ± standard error. *p < 0.05; first significant effect (one-way ANOVA followed by Holm-Sidak's post-test). #p < 0.05 (two-way ANOVA followed by Holm-Sidak's post-test).

levels. MacNee & Rahman[33] add that, initially, there is a decrease in GSH levels, but, over time, these levels are increased to protect against the toxic damage caused by cigarette smoke inhalation. However, this increase in GSH levels may not be sufficient to neutralize the excessive oxidant burden during acute smoking.

In this sense, several studies support the relationship between cigarette smoke inhalation and the production of oxidative stress,[33-35] because cigarettes are known to be a potential inducer of adverse effects in biological structures and in tissues, as well as influencing weight loss, inflammatory cell increase, and muscle fatigue.[26,35] Carlos et al.,[26] in a study in which male (C57BL/6) mice were exposed to smoke from 12 cigarettes a day for 7, 15, 30, 45, and 60 days, identified that the oxidative stress induced by cigarette smoke caused changes in the lungs and in the diaphragm. Those authors added that the oxidative stress caused by exposure to cigarette smoke occurs first in the skeletal muscle and subsequently in the lung tissue. Regarding deficits in muscle strength, Camargo Filho et al.[36] point out that cigarette smoke can lead to muscle atrophy, which is a way for the organism to adapt to oxygen limitation, causing loss of activity and disorganization of the muscle fibers.

The animals that inhaled cigarette smoke also exhibited tracheal hyperreactivity to electromechanical contractile stimulation (K^+) and to pharmacomechanical contractile stimulation (carcachol), possibly because of reduced epithelial protection after cigarette smoke inhalation.[37] The results presented here support the hypothesis that the process of cigarette smoke inhalation produces increased responses in voltage-operated Ca^{2+} channels and in receptor-operated Ca^{2+} channels.[38,39]

The hyperreactivity reported in the present model of passive inhalation of cigarette smoke is similar to that found in models of asthma and chronic inflammatory bronchoconstrictive disease.[22,23,37]

James et al.[40] pointed out that cigarette smoke inhalation produces harmful effects in the respiratory system of guinea pigs, such as increased airway resistance; however, such effects did not produce an increase in airway wall thickness. Omini et al.[41] add that passive exposure to cigarette smoke produces a significant increase in the number of macrophages and eosinophils in the BAL fluid, which further corroborates the tracheal hyperreactivity, but no mucus hypersecretion.

Hernandez et al.[23] conducted a study to investigate the ability of a mucoactive compound (CO 1408) to modulate the development of airway hyperreactivity induced by passive inhalation of cigarette smoke over a short period of time. Those authors found that passive inhalation of cigarette smoke causes airway hyperreactivity associated with an increase in inflammatory cells, and that the CO 1408 compound was able to reverse these harmful effects.

The limitations of the present study include the fact that the experimental model of passive inhalation of cigarette smoke was performed over a short period of time and the long-term effects of inhalation were not assessed. Therefore, new approaches that address these issues, as well as the insertion of forms of prevention and treatment, should be attempted.

In conclusion, the protocol of passive inhalation of cigarette smoke used in the present study was effective, because it mimicked structural and functional changes in the guinea pig respiratory system that characterize the damages caused by cigarette smoke.

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ORIGINAL ARTICLE

Lung volumes and airway resistance in patients with a possible restrictive pattern on spirometry

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Study carried out in the pulmonary function laboratories of Centro Diagnóstico Brasil and the Hospital do Servidor Público Estadual de São Paulo, São Paulo (SP) Brasil.

ABSTRACT

Objective: Many patients with proportional reductions in FVC and FEV_1 on spirometry show no reduction in TLC. The aim of this study was to evaluate the role that measuring lung volumes and airway resistance plays in the correct classification of patients with a possible restrictive pattern on spirometry. **Methods:** This was a prospective study involving adults with reduced FVC and FEV_1, as well as an FEV_1/FV(C) ratio within the predicted range. Restrictive lung disease (RLD) was characterized by TLC below the 5th percentile, as determined by plethysmography.

Obstructive lung disease (OLD) was characterized by high specific airway resistance, significant changes in post-bronchodilator FEV_1, or an $FEF_{25-75\%}$ < 50% of predicted, together with a high RV/TLC ratio. Nonspecific lung disease (NLD) was characterized by TLC within the predicted range and no obstruction. Combined lung disease (CLD) was characterized by reduced TLC and findings indicative of airflow obstruction. Clinical diagnoses were based on clinical suspicion, a respiratory questionnaire, and the review of tests of interest. **Results:** We included 300 patients in the study, of whom 108 (36%) were diagnosed with RLD. In addition, 120 (40%) and 72 (24%) were diagnosed with OLD/CLD and NLD, respectively. Among the latter, 24 (33%) were clinically diagnosed with OLD. In this sample, 151 patients (50.3%) were obese, and obesity was associated with all patterns of lung disease. **Conclusions:** Measuring lung volumes and airway resistance is often necessary in order to provide an appropriate characterization of the pattern of lung disease in patients presenting with a spirometry pattern suggestive of restriction. Airflow obstruction is common in such cases.

Keywords: Spirometry; Airway resistance; Lung volume measurements.

INTRODUCTION

The American Thoracic Society (ATS)/European Respiratory Society (ERS) task force proposed definitions for the various patterns of lung disease.[1] Restrictive lung disease (RLD) was defined as a reduction in TLC below the 5th percentile of the predicted value and a normal FEV_1/VC ratio. Obstructive lung disease (OLD) was defined as an FEV_1/VC ratio below the 5th percentile of the predicted value. Mixed or combined lung disease (CLD) was characterized by FEV_1/VC and TLC below the 5th percentile of the predicted values.

A combination of reduced VC and preserved FEV_1/(F)VC is used in order to infer the presence of RLD; however, in approximately 40% of such cases, TLC is not reduced.[2,3] According to the ATS/ERS task force, OLD is characterized by a combination of reduced (F)VC, FEV_1/(F)VC above the lower limit of normal, and TLC within the predicted range.[1] This functional abnormality was later designated nonspecific lung disease (NLD).[4] In a sample of 100 patients presenting with reduced (F)VC, FEV_1/(F)VC above the lower limit of normal, and TLC within the predicted range, 68 had evidence of airway disease, whereas the remaining 32 had signs of restriction.[4]

The proportional reduction in FVC and FEV_1 in patients with OLD can be explained by airway closure with air trapping.[5] Obesity reduces (F)VC more than it does FEV_1[6] and can therefore result in a preserved FEV_1/(F)VC ratio in the presence of OLD. As occurs with diseases affecting respiratory mechanics or respiratory muscle strength, obesity can, in and of itself, result in NLD.[4] Although COPD and asthma account for most OLDs, a wide range of other diseases, including bronchiolar diseases and some interstitial lung diseases, are associated with airflow obstruction and can result in proportional reductions in FVC and FEV_1.[7] In addition, smoking (either current or past) is associated with various lung diseases and can contribute to an obstructive component.

Spirometry is considered the method of choice for detecting airflow limitation caused by OLD. However, airflow limitation is multifactorial. One such factor is high airway resistance (Raw).[8] In many patients with spirometry results suggestive of RLD, Raw measurements can reveal airflow obstruction. It is commonly believed that Raw is a parameter that is not sufficiently sensitive in cases of peripheral airway disease; however, a classic study showed a close correlation between airway conductance

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(Gaw) and bronchiolar diameter.[9] It is possible that Gaw alone is abnormal in patients with bronchiolitis.[10] In 2012, reference values for specific Raw were derived from a large sample of healthy adults.[11]

The objective of the present study was to evaluate the role that measuring lung volumes and Raw plays in the final functional classification of patients with spirometry results suggestive of RLD.

METHODS

Data collection was performed in the pulmonary function laboratories of *Centro Diagnóstico Brasil* (n = 217) and the São Paulo Hospital for State Civil Servants (n = 83) in the period between December of 2011 and December of 2013. Pulmonologists certified in pulmonary function testing by the Brazilian Thoracic Association (BTA) and the lead author of the present study prospectively selected all spirometry results suggestive of RLD. Clinical diagnosis was established by the pulmonologist requesting the test, by administering a standardized respiratory questionnaire adapted from a previously published questionnaire (Appendix 1: http://www.jornaldepneumologia.com.br/detalhe_anexo.asp?id=46)[12] and by reviewing ancillary test results or analyzing the results of additional tests, including chest X-rays, chest CT scans, and echocardiograms, requested on the basis of clinical suspicion. All pulmonary function tests were performed in accordance with the BTA guidelines.[13] All patients gave written informed consent.

Inclusion criteria

The inclusion criteria were as follows: 1) being an adult whose age and height were within the reference range[14]; 2) having FVC below the lower limit of normal, i.e., below the 5th percentile of the reference population[14]; 3) having FEV_1/FVC and FEV_1/VC equal to or above the lower limit of normal, i.e., above the 5th percentile of the reference population[14]; 4) having a definitive clinical diagnosis (for asthma, physician-diagnosed asthma and a patient report of two or more episodes of wheezing, which were alleviated by bronchodilator use; for COPD, physician-diagnosed COPD, chronic cough/dyspnea—a Medical Research Council scale score ≥ 2—and past or current smoking; patients diagnosed with obesity were in most cases referred for preoperative evaluation for bariatric surgery, including those with a complaint of dyspnea without meeting criteria for diseases such as asthma); and 5) having performed pulmonary function tests in accordance with the BTA/ATS/ERS acceptability and reproducibility criteria.[13,15-17]

Patients whose tests were not in accordance with the aforementioned criteria were excluded, as were those without a definitive diagnosis by the end of the analysis period.

All pulmonary function tests were performed with a Sensor Medics 6200 Bodybox system and a Collins system (Ferraris Respiratory, Louisville, CO, USA). Lung volumes were determined by whole-body plethysmography. For lung volumes, the predicted values were those proposed by Crapo et al.[18] Reduced TLC was characterized by values below the 5th percentile. RV and the RV/TLC ratio were considered high when they were above the 95th percentile of the reference values.[18] Spirometry was repeated after administration of a bronchodilator (400 µg of albuterol aerosol). A significant bronchodilator response was characterized by FEV_1 ≥ 0.20 L and 7% of predicted, in accordance with Soares et al.[19]

Raw was measured by mean linear intercept values, as recommended by Matthys et al., after analysis of at least five pressure-flow loops.[20] Only satisfactory, reproducible loops were accepted. The predicted values used for calculation were those proposed by Piatti et al.[11] Values above 8.0 cmH_2O/s in females and 8.6 cmH_2O/s in males were considered high (mean ± 1.64 SD).

Satisfactory single-breath DLCO measurements were obtained in 260 patients. The reference values were based on those proposed by Miller et al.[21]

After data collection, the patterns of lung disease were divided into four groups:

RLD—characterized by TLC below the lower limit of normal and no obstruction[1]

OLD—characterized by one or more of the following: high specific Raw corrected for lung volume (Raw × Lv); a significant change in FEV_1 after bronchodilator administration (ΔFEV_1 > 0.20 L and 7% of predicted); and $FEF_{25-75\%}$ < 50% of predicted with a high RV/TLC ratio (see the Results section)

CLD—characterized by reduced TLC and findings indicative of airflow obstruction, including high Raw × Lv; $FEF_{25-75\%}$ < 50% with a high RV/TLC ratio; and a significant bronchodilator response

NLD—characterized by TLC within the predicted range and no functional findings indicative of obstruction

All values were expressed as mean ± standard deviation. The groups were compared by means of the Student's t-test and ANOVA (for continuous independent variables), and the chi-square test (for nominal variables). Correlations between Raw × Lv and functional parameters were determined by Spearman's test. The distribution of Raw × Lv was lognormal, and Raw × Lv values were transformed for comparison. ROC curve analysis was used in order to correlate functional parameters and the RV/TLC ratio with specific Raw. Statistical analysis was performed with the IBM SPSS Statistics software package, version 20 (IBM Corp., Armonk, NY, USA). The level of significance was set at $\alpha = 0.05$.

RESULTS

A total of 300 patients were included in the present study. Table 1 shows the general characteristics of the 300 patients included in the study, and Table 2 shows pulmonary function test results expressed as mean ± SD.

Clinical diagnoses were divided into four groups: obstructive diseases, interstitial diseases, obesity, and other diseases (Figure 1).

Of the 300 patients included in the present study, 151 (50.3%) were obese, but only 52 (17.3%) had a final diagnosis of obesity without other conditions. In addition, 172 (57.3%) had TLC below the lower limit of normal (RLD), and 128 (42.7%) had TLC within the predicted range (n = 127) or high TLC (n = 1).

RV and the RV/TLC ratio were above the upper limit of normal in 46 (15.3%) and 126 (42.0%), respectively. High Raw × Lv was observed in 97 patients (32.3%). Raw × Lv (and Gaw/Lv) correlated more strongly with $FEF_{25-75\%}$ ($r_s = 0.55$) than with FEV_1/FVC ($r_s = 0.50$) or percent predicted FEV_1 ($r_s = 0.27$; $p < 0.01$ for all). Raw × Lv also correlated significantly with the RV/TLC ratio ($r_s = 0.46$; $p < 0.001$). ROC curve analysis showed that the area under the ROC curve was higher for $FEF_{25-75\%}$ than for FEV_1/FVC or percent predicted FEV_1 (i.e., 0.75; $p < 0.001$) for differentiating between patients with and without high Raw × Lv. An $FEF_{25-75\%}$ of less than 50% had a sensitivity of 40% and a specificity of 89% for detecting high specific Raw. With regard to lung volume measurements, the RV/TLC ratio had the highest area under the curve for characterizing airflow obstruction (0.75; $p < 0.01$). Given that a high RV/TLC ratio and $FEF_{25-75\%} < 50\%$ can each be found in patients with RLD or NLD, they were combined in order to characterize airflow obstruction. A combination of high RV/TLC and $FEF_{25-75\%} < 50\%$ was found in 46 patients. In 14 of those, the aforementioned parameters constituted the only evidence of obstruction.

Table 1. General characteristics of patients with spirometry results suggestive of restrictive lung disease (n = 300).[a]

General characteristics	Results
Age, years	56.2 ± 14.4
Males/females, n/n	117/183
Nonsmokers/smokers/former smokers, n/n/n	187/34/79
Body mass index, (kg/m^2)	31.0 ± 7.9

[a]Values expressed as mean ± SD, except where otherwise indicated.

Table 2. Functional characteristics of patients with spirometry results suggestive of restrictive lung disease (N = 300).[a]

Functional characteristics	Results
VC%	66.2 ± 11.0
FVC%	65.0 ± 10.6
FEV_1%	64.5 ± 10.8
FEV_1/FVC	0.81 ± 0.06
$FEF_{25-75\%}$	74.6 ± 28.8
RV%	99.5 ± 32.0
RV/TLC	0.44 ± 0.10
TLC%	78.6 ± 14.2
Raw× Lv	8.44 (4.17-9.09)

Raw × Lv: specific airway resistance corrected for lung volume. [a]Values expressed as mean ± SD or median (interquartile range).

A significant bronchodilator response was observed in 23 patients (7.7%). The most common clinical diagnoses in those patients were obstructive diseases (n = 12) and obesity (n = 5). Of the 14 patients diagnosed with congestive heart failure (CHF), only 1 (7.0%) had a significant bronchodilator response.

On the basis of one or more of the aforementioned criteria, 120 patients (40.0%) had airflow obstruction. Of the 120 patients with OLD, 64 (53.3%) had TLC below the lower limit of normal and were therefore considered to have CLD. Of the 128 patients with TLC within the predicted range, 72 (56.2%) had no airflow obstruction and were therefore classified as having NLD.

All patterns of lung disease found in the present study and the respective clinical diagnoses are shown in Figure 2. In the four groups of lung diseases there were patients diagnosed with obesity and patients diagnosed with other diseases. Four of the patients who were diagnosed with asthma had RLD, and 17 of the patients in the CLD group had a diagnosis of OLD, asthma being the most common obstructive disease (n = 10).

Of the 72 patients who were diagnosed with NLD, 24 (33.3%) had a clinical diagnosis of obstructive disease: asthma, in 11; COPD, in 8; bronchiectasis, in 3; and bronchiolitis, in 3. Therefore, of the 300 patients included in the present study, 144 (i.e., the aforementioned 24 plus the 120 who were diagnosed with OLD or CLD, accounting for 48.0% of the sample) had obstructive disease.

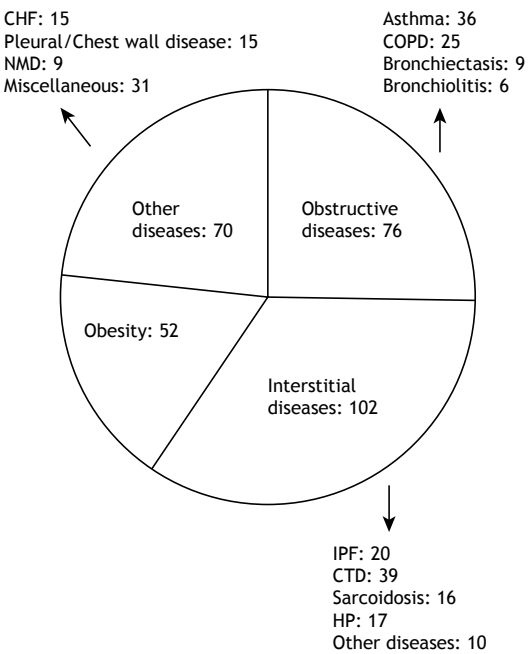

Figure 1. Clinical diagnoses in patients with spirometry results suggestive of restrictive lung disease (N = 300). CHF: congestive heart failure; NMD: neuromuscular disease; IPF: idiopathic pulmonary fibrosis; CTD: connective tissue disease; and HP: hypersensitivity pneumonia.

Several variables, including age, gender, and functional results, were compared among the four major clinical diagnostic groups (Table 3). The patients with a final diagnosis of obesity were younger and had a body mass index above 35 kg/m^2 (79%); therefore, most were referred for preoperative evaluation for bariatric surgery. Expiratory reserve volume (ERV) was lowest in the group of patients who were diagnosed with obesity, who, in comparison with the group of patients diagnosed with interstitial diseases, had higher percent predicted VC, FVC, RV, and TLC; more preserved DLCO; and similar Raw × Lv.

DISCUSSION

The present study confirms the findings of previous studies[2-4] showing that spirometric findings of reduced FVC and FEV_1 associated with a preserved FEV_1/FVC ratio are of limited value in establishing a functional diagnosis. In addition, the study shows that lung volume and Raw measurements provide a more consistent functional characterization.

It is widely recognized that adequate expiratory effort and time are required in order to characterize reduced FVC on spirometry. Incomplete exhalation often results in a restrictive pattern on spirometry. In the present study, all tests were carefully performed and reviewed.

The ATS/ERS task force defined RLD as a reduction in TLC below the 5th percentile of the predicted value and a normal FEV_1/FVC ratio.[1] The reference values for TLC are therefore of great importance. In the present study, we used the values proposed by Crapo et al.[18] Although one study derived reference values for lung volumes in a sample of healthy adults in Brazil,[22] the number of individuals included in that study was small. We cannot exclude the possibility that patients with obstructive diseases that were classified as having RLD or CLD in the present study would have been better classified had there been a more suitable equation for calculating predicted lung volumes. However, cases of asthma with true restriction (reduced TLC) not associated with obesity have been described in the literature, including those with changes in lung function.[23] Such cases are sometimes encountered in clinical practice.

RLD can be due to interstitial diseases, such as pulmonary fibrosis; non-respiratory conditions that secondarily impede lung expansion, such as muscle weakness, pleural diseases, obesity, and kyphoscoliosis; and conditions that directly affect lung function, such as CHF. Several studies have found the prevalence of spirometry-defined RLD to be 7-14%.[24-26] The prevalence of RLD is higher in males, heavy smokers, elderly individuals, individuals with a lower level of education, individuals with diabetes, individuals with CHF, and individuals with a very low or very high body mass index. Heavy smokers commonly have smoking-related interstitial lung disease, which can result in RLD or CLD.[27]

The obesity epidemic does not spare developing countries. For several reasons, obesity introduces confounding factors in the interpretation of lung function. Obesity affects lung volume measurements and spirometric values, particularly by reducing ERV and, consequently, functional residual capacity.[28] In the present study, ERV was significantly lower in the group of patients diagnosed with obesity. Proportional reductions in FVC and FEV_1 resulting in a preserved or slightly increased FEV_1/FVC ratio have been reported in obese individuals. However, although statistically significant, reductions in FVC and FEV_1 are typically small, and FEV_1, FVC, and TLC usually remain within the range of predicted values.[6,28]

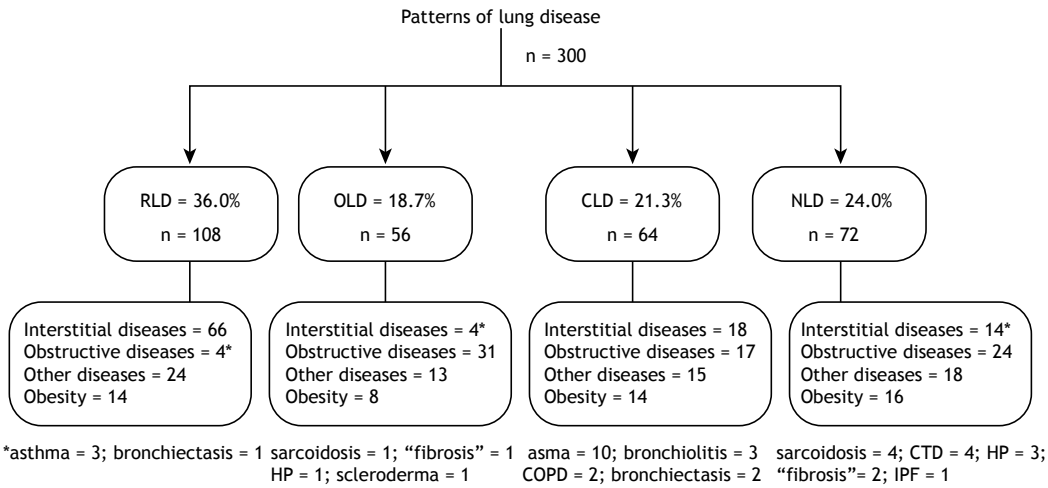

Figure 2. Functional diagnoses (based on lung volume and airway resistance measurements) and corresponding clinical diagnoses in 300 patients with spirometry results suggestive of restrictive lung disease. RLD: restrictive lung disease; OLD: obstructive lung disease; CLD: combined lung disease; NLD: nonspecific lung disease; HP: hypersensitivity pneumonia; CTD: connective tissue disease; and IPF: idiopathic pulmonary fibrosis.

In our study, obese individuals constituted half of the sample. Obesity was found in patients with RLD, OLD, CLD, and NLD. The interaction among obesity, lung function, asthma, and COPD has been the subject of several studies and excellent reviews.[6,29-31] Obesity is associated with an increased risk of asthma.[30,31] In obese individuals, dyspnea can be attributed to obesity itself or asthma, resulting in overdiagnosis and underdiagnosis.[32] Methacholine challenge testing is useful in such cases.[4]

Lung volume measurements can aid in differentiating between RLD and OLD in patients with spirometry results suggestive of restriction.[1] One study showed that the level of agreement between clinical diagnosis and diagnosis based on pulmonary function test results (including lung volume measurements) is low and therefore does not allow differentiation between RLD and OLD.[33]

In the present study, Raw measurements and the combination of $FEF_{25-75\%}$ and a high RV/TLC ratio allowed the diagnosis of airflow obstruction in several cases, the level of agreement between that diagnosis and clinical diagnosis being significant. Given that the total cross-sectional area of the airways decreases dramatically from the periphery to the central regions of the lung, Raw measurements are theoretically less sensitive to peripheral changes. However, measurements of specific Raw can be useful. One study showed that Gaw measurements were more sensitive than spirometry for detecting airflow obstruction in patients with bronchiolitis obliterans syndrome.[10]

In patients with COPD, in whom obstruction is peripheral and mostly mild, the FEV_1/FVC ratio is low and Raw or specific Gaw is within the predicted range; however, in the pulmonary function laboratory setting, the opposite has also been observed. A study conducted nearly 30 years ago showed that a combination of clinical and whole-body plethysmography data detected 18% of airflow obstruction cases.[34] In a classic study of 26 postmortem lungs from sudden death victims, a nearly perfect hyperbolic correlation was found between mean bronchiolar diameter and Raw (r = 0.89), whereas the correlation between mean segmental bronchial diameter and Raw was not significant.[9] In the present study, high Raw × Lv was significantly associated with reduced $FEF_{25-75\%}$ and increased RV/TLC, suggesting a correlation with peripheral airway obstruction. All patients had an FEV_1/FVC ratio within the predicted range.

As occurred with obesity, a final diagnosis of "other diseases" was made in patients with RLD, OLD, CLD, and NLD. Diseases included CHF, pleural disease, chest wall disease (particularly kyphoscoliosis), and neuromuscular disease. Of the 102 patients with interstitial lung disease, only 66 (65.0%) had RLD alone, as confirmed by TLC measurements. The remaining 36 had OLD alone (n = 4), CLD (n = 18), or NLD (n = 14). Combined pulmonary fibrosis and emphysema is a relatively common condition, given that both are smoking-related diseases.[35] In patients with connective tissue disease, bronchiolitis and emphysema associated with interstitial disease and muscle weakness can result in OLD and NLD, respectively.[36,37] In patients with hypersensitivity pneumonia or sarcoidosis, airway involvement is common and can result in OLD.[38,39]

In a study by Hyatt et al.,[4] 68% of the patients with NLD had a final diagnosis of OLD, which is in accordance with the ATS/ERS guidelines stating that proportional reductions in FVC and FEV_1 with TLC within the predicted range are indicative of OLD. However,

Table 3. Anthropometric and functional variables in patients with spirometry results suggestive of restrictive lung disease (N = 300), by clinical diagnosis.[a]

Variable	Clinical diagnosis				p
	Interstitial diseases (n = 102)	Obstructive diseases (n = 76)	Other diseases (n = 70)	Obesity (n = 52)	
Age, years	57.3 ± 12.9*	59.0 ± 15.7*	55.9 ± 14.4	50.2 ± 13.7	0.006
Female gender, %	70*	68*	49	50	0.007
BMI, kg/m^2	28.3 ± 5.9*	29.8 ± 7.0*	28.6 ± 5.8*	41.3 ± 7.1	< 0.001
VC%	64.3 ± 10.9*	69.5 ± 8.6	62.4 ± 12.2*	70.3 ± 10.2	< 0.001
FVC%	63.0 ± 10.8*	67.8 ± 8.0	61.8 ± 11.9*	69.0 ± 9.3	< 0.001
FEV_1/FVC	0.83 ± 0.06	0.77 ± 0.06	0.80 ± 0.05	0.81 ± 0.04	< 0.001
FRC%	70.8 ± 19.1	96.6 ± 23.6*	85.2 ± 18.6	77.0 ± 17.7	< 0.001
ERV%	65.0 ± 36.3*	59.7 ± 41.4*	53.4 ± 27.3*	33.9 ± 18.1	< 0.001
RV%	77.3 ± 23.8*	118.6 ± 29.6	105.7 ± 29.7	99.5 ± 32.0	< 0.001
RV/TLC	0.40 ± 0.09	0.49 ± 0.09*	0.46 ± 0.10*	0.41 ± 0.10	< 0.001
TLC%	70.2 ± 12.9*	88.6 ± 12.8*	77.4 ± 13.0	82.2 ± 10.2	< 0.001
DLCO, %[b]	53.3 ± 16.8*	79.3 ± 19.8	59.1 ± 19.4*	82.9 ± 17.7	< 0.001
Raw × Lv, lognormal	1.42 ± 0.43	2.41 ± 0.43*	2.39 ± 0.70*	1.60 ± 0.37	< 0.001

FRC: functional residual capacity; ERV: expiratory reserve volume; and Raw × Lv: specific airway resistance corrected for lung volume. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]n = 260. *p < 0.05; obesity vs. the remaining groups. Tukey's test.

many of the patients with airway disease had a reduced FEV_1/slow VC ratio. In the present study, such cases were excluded, and, as a result, only one third of all NLD patients were clinically diagnosed with OLD.

Our sample selection strategy limits generalizability of results. Because of the large number of patients routinely treated at the study facilities, selected patients were not consecutive. It is possible that there was discrepancy between functional and clinical diagnoses, given that not all tests for other causes of RLD were performed. However, we believe that the objective of the present study was achieved.

In conclusion, lung volume and Raw measurements are often necessary in order to provide an appropriate characterization of the pattern of lung disease in patients with spirometry results suggestive of restriction. Diseases accompanied by airflow obstruction can result in a restrictive pattern on spirometry.

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Prevalence of latent *Mycobacterium tuberculosis* infection in prisoners

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ABSTRACT

Objective: To determine the prevalence of and the factors associated with latent *Mycobacterium tuberculosis* infection (LTBI) in prisoners in the state of Minas Gerais, Brazil. **Methods:** This was a cross-sectional cohort study conducted in two prisons in Minas Gerais. Tuberculin skin tests were performed in the individuals who agreed to participate in the study. **Results:** A total of 1,120 individuals were selected for inclusion in this study. The prevalence of LTBI was 25.2%. In the multivariate analysis, LTBI was associated with self-reported contact with active tuberculosis patients within prisons (adjusted OR = 1.51; 95% **CI:** 1.05-2.18) and use of inhaled drugs (adjusted OR = 1.48; 95% **CI:** 1.03-2.13). Respiratory symptoms were identified in 131 (11.7%) of the participants. Serological testing for HIV was performed in 940 (83.9%) of the participants, and the result was positive in 5 (0.5%). Two cases of active tuberculosis were identified during the study period. **Conclusions:** Within the prisons under study, the prevalence of LTBI was high. In addition, LTBI was associated with self-reported contact with active tuberculosis patients and with the use of inhaled drugs. Our findings demonstrate that it is necessary to improve the conditions in prisons, as well as to introduce strategies, such as chest X-ray screening, in order to detect tuberculosis cases and, consequently, reduce *M. tuberculosis* infection within the prison system.

Keywords: Prisons; Tuberculin test; Latent tuberculosis, HIV.

INTRODUCTION

It is estimated that there were 9.4 million new cases of tuberculosis worldwide in 2014, 12% of which were HIV-infected, as well as 1.5 million tuberculosis-related deaths.[1] Brazil is one of the 22 countries that collectively account for 80.0% of the global burden of this disease.[1] Minas Gerais ranks fourth among the states with the highest number of tuberculosis cases in Brazil.[2] The main global strategy for tuberculosis control, proposed by the World Health Organization (WHO) and known as Stop TB,[1] highlights, in one of its components, the need to promote tuberculosis control activities targeting prisoners because such individuals are at high risk for latent *Mycobacterium tuberculosis* infection (LTBI) and for developing tuberculosis disease, given that this a serious public health problem in penal institutions.[3-6]

The presence of tuberculosis within the prison system has been described as a threat. Some authors have suggested that it will not be possible to control tuberculosis in the community unless effective measures are taken to combat the disease in prisons. In those places, tuberculosis is not limited to prisoners only, because it also affects the community with which they interact, family members, and prison staff, during and after incarceration.[3,5-9] In Brazil, the incidence of tuberculosis within the prison system is 28-fold higher than that in the general population.[10] The prevalence of LTBI is also high in those places,[5,7-9,11-13] a reality that remains unknown in most Brazilian regions since there are no studies on the subject.

Identifying LTBI in prisoners should be given priority so that preventive measures can be adopted, especially in groups with conditions associated with high risks of developing tuberculosis, such as HIV seropositivity, diabetes mellitus, chronic renal failure, use of immunosuppressants, etc.[6,9,14] The method for identifying suspected tuberculosis cases should be decided upon on a scenario-by-scenario basis, depending on the local context and the existing resources.[3,8,9,15-17] The main screening strategies are based on symptoms, chest X-ray, and the tuberculin skin test (TST).[3,8,9,14,15] Each has advantages and limitations.[3,8,9,15-18] Active surveillance for cases of tuberculosis disease, through screening, should be undertaken not only upon entry into the prison system, but also afterwards. The purpose of this surveillance is to examine individuals who are more likely to develop the disease, such as those with respiratory symptoms (RS), identify active cases, and treat them accordingly. Such measures break the chain of transmission and decrease the incidence of the disease.[6,11,18] The objective of the present study was to estimate the prevalence of and the

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factors associated with LTBI in prisoners in the main prison area in Minas Gerais.

METHODS

Study design

This was a cross-sectional cohort study conducted in the two largest prisons (condemned prisoners) in the main prison area in the state of Minas Gerais, both of which are located in the city of Ribeirão das Neves and house 8.4% of the prisoners in the state. A total of 1,492 inmates were included in the present study (67.0% of the prisoners in the prisons under study and 5.8% of the prisoners in the state)[19-21] between April and June of 2013. The eligibility criteria were as follows: having had no previous treatment for tuberculosis; having no tuberculosis disease at the study outset; and having never undergone a TST.

Administering and reading TSTs

TSTs were performed by the Mantoux method, which consists of administration of 0.1 mL (2 tuberculin units) of *M. tuberculosis* PPD RT23 (State Serum Institute, Copenhagen, Denmark) on the left forearm. TSTs were read 72 h after being administered, by measuring the maximum transverse diameter of induration with a millimeter ruler. An induration of ≥ 10 mm was considered a positive result for HIV-uninfected individuals, whereas an induration of ≥ 5 mm was considered a positive result for HIV-infected individuals.[14] Isoniazid preventive therapy was recommended to all prisoners at risk for developing active tuberculosis.[6] All TSTs were administered and read by trained and certified professionals for research project participation.

Sampling and bacteriological tests

Sputum samples were collected from individuals with a history of cough, regardless of length of history, for smear microscopy and mycobacterial culture. Drug susceptibility testing was performed if culture was positive, in accordance with the WHO laboratory guidelines.

Serological testing for HIV

Serological testing for HIV was offered to all participants, along with pre- and post-test counseling.[22] Positive results by ELIZA were confirmed by the Western Blot method.

Data collection

Educational and awareness-raising activities regarding *M. tuberculosis* infection were developed for the prison health team and the prisoners. The study participants completed a questionnaire addressing sociodemographic characteristics (age, gender, marital status, schooling, occupation before incarceration, and length of incarceration); behavioral characteristics (use of licit and illicit drugs, alcoholism—assessed by the **C**ut down, **A**nnoyed, **G**uilty, and **E**ye-opener screening questionnaire—[23]smoking, and contact with active pulmonary tuberculosis patients inside or outside prison); health history (previous treatment for active tuberculosis, BCG vaccination, HIV infection, presence of diabetes mellitus or other diseases, and use of medications); and symptoms suggestive of pulmonary tuberculosis (cough, cough duration, expectoration, hemoptysis, fever, adynamia, cyanosis, anorexia, weight loss, and night sweats). The health records available in the prisons were consulted to fill in missing information.

Statistical analysis

The selected characteristics were analyzed descriptively by gender, with results being presented as frequencies, and by univariate and multivariate analysis, with results being presented as measures of central tendency and dispersion. The mean differences for continuous variables were compared using the Student's t-test, whereas the proportions of categorical variables were compared using Pearson's chi-square test. The IBM SPSS Statistics software package, version 21.0 (IBM Corporation, Armonk, NY, USA) was used for statistical analysis, and the data were recorded in tables. The magnitude of the association between the selected explanatory variables and the event of interest, that is, LTBI, was estimated by calculating ORs with 95% CIs. Variables with a p value of ≤ 0.25 in univariate analysis, as determined by the Wald test, were manually selected to build a multivariate model via a stepwise regression selection procedure. The significance level required for inclusion in the final model containing the selected variables was set at 0.05 to adjust for potential confounders properly. Only the variables showing a significant independent association with the event of interest remained in the final model. The likelihood ratio test was used to compare the models. The goodness of fit of the final models was assessed using the Hosmer-Lemeshow test.

Ethical guidelines

The study was approved by the Research Ethics Committee of the Federal University of Minas Gerais (Protocol no. 0617.0.203.000-09) on April 5, 2010.

RESULTS

Characteristics of the population

Most of the prisoners agreed to participate in the study (N = 1,431; 96.0%), and, of those, 1,120 (78.0%) were included in the final sample. Of the total number of individuals excluded from the study (n = 311), 68 (21.8%) had undergone a TST previously, whereas 243 (78.1%) had not because of previous tuberculosis (35/243; 14.4%), tuberculosis treatment (4/243; 1.6%), transfer to another prison (13/243; 5.3%), or refusal (191/243; 78.6%; Figure 1).

In the study population (Table 1), the mean age was 29 ± 7 years and most individuals had had less than 7 years of schooling (90.0%). The length of incarceration was longer than 12 months for 57.0%

of the participants (mean, 28 ± 25 months). Contact with active pulmonary tuberculosis patients occurred outside and inside prison, respectively, in 10.0% and 15.0% of the cases. The proportions of smoking and pre-incarceration alcoholism were, respectively, 61.1% and 24.8%. The use of illicit drugs was reported by 75.0% of the inmates. Of those, 97.0% reported using inhaled drugs, 2.0% reported using injected drugs, and 1.0% reported using inhaled and injected drugs.

Diabetes mellitus, chronic renal failure, silicosis, and use of immunosuppressive medications were reported, respectively, by 1.2%, 0.7%, 0.1%, and 2.9% of the prisoners. Serological testing for HIV was performed in 83.9%, and the result was positive in 0.5%.

In the univariate analysis, LTBI was found to be associated with the following variables: gender; marital status; contact with active tuberculosis patients outside prison; pre-incarceration alcoholism; use of inhaled illicit drugs; diabetes mellitus; chronic renal failure; and chronic use of corticosteroids and/or immunosuppressants (Tables 1 and 2).

Prevalence of and factors associated with LTBI

The prevalence of LTBI was 25.2%. In the multivariate analysis, the occurrence of LTBI was associated with self-reported contact with active tuberculosis patients inside prison (adjusted OR = 1.51; 95% CI: 1.05-2.18) and with the use of inhaled drugs (adjusted OR = 1.48; 95% CI: 1.03-2.13; Table 2). The results of the TSTs were negative for all individuals who tested positive for HIV.

Measures of frequency of symptoms suggestive of tuberculosis infection and of tuberculosis disease

At least one of the symptoms suggestive of tuberculosis (cough, weight loss, loss of appetite, and chest pain) were reported by 68.0% of the study population. Cough and weight loss were the most common symptoms, occurring in 27.0% and 25.0% of the participants, respectively. Of those who reported cough, 43.0% had had the symptom for two weeks or more, corresponding to 131 individuals of the total number of prisoners (11.7%). Two cases were diagnosed with tuberculosis, and the strains were susceptible to rifampin, streptomycin, isoniazid, and ethambutol.

DISCUSSION

The magnitude of LTBI within the Brazilian prison system, as previously mentioned, remains mostly unknown. The prevalence observed in this study (25.2%) was higher than those reported for prisons in countries such as the USA (17.0%),[24] Australia

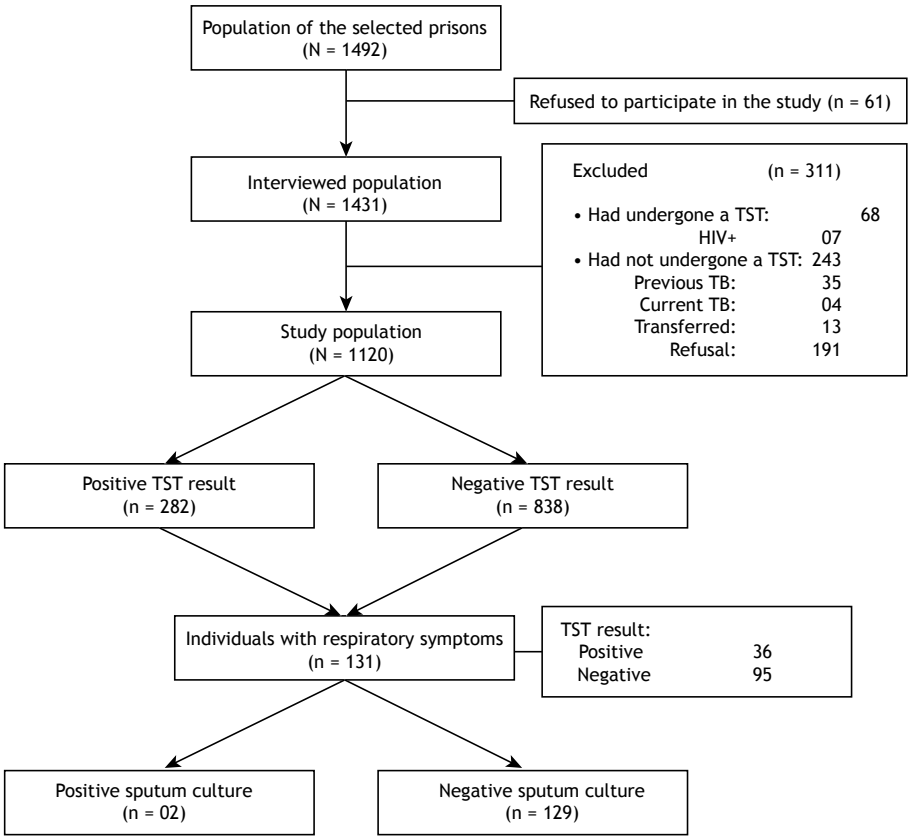

Figure 1. Individuals selected for inclusion in the study, Minas Gerais, 2013 (N = 1,120). TST: tuberculin skin test; and TB: tuberculosis.

Table 1. Descriptive characteristics of the sample of prisoners under study, by gender, Minas Gerais, Brazil, 2013 (N = 1,120).[a]

Characteristics	Gender		p
	Female	Male	
Sociodemographic characteristics			
Age, years			0.165
< 30	53 (53.0)	608 (60.1)	
≥ 30	47 (47.0)	403 (39.9)	
Marital status			< 0.001
Single	80 (80.0)	584 (57.3)	
Married/steady partner	20 (20.0)	436 (40.7)	
Schooling, years			0.089
< 7	85 (85.0)	920 (90.4)	
≥ 7	15 (15.0)	98 (9.6)	
Occupation before incarceration			0.714
Yes	93 (93.0)	938 (92.0)	
No	7 (7.0)	82 (8.0)	
Length of incarceration, months			0.389
≤ 15	55 (55.0)	515 (50.9)	
> 15	45 (45.0)	505 (49.5)	
Behavioral characteristics			
Contact with active TB patients outside prison			< 0.001
Yes	24 (24.0)	83 (8.1)	
No	62 (62.0)	850 (84.1)	
Does not know	14 (14.0)	87 (16.8)	
Contact with active TB patients inside prison			0.054
Yes	7 (7.0)	162 (15.9)	
No	70 (70.0)	754 (73.9)	
Does not know	23 (23.0)	104 (10.2)	
Pre-incarceration alcoholism			0.047
Yes	33 (33.0)	245 (24.0)	
No	67 (67.0)	775 (76.0)	
Smoking			0.203
Yes	67 (67.0)	617 (60.5)	
No	33 (33.0)	403 (39.5)	
Use of inhaled drugs			< 0.001
Yes	49 (49.0)	789 (77.4)	
No	51 (51.0)	231 (22.6)	
Use of injected drugs			0.511
Yes	3 (3.0)	27 (2.6)	
No	97 (97.0)	993 (97.4)	
Health history			
Diabetes mellitus			0.031
Yes	4 (4.4)	9 (1.1)	
No	86 (95.6)	815 (98.9)	
Chronic renal failure			0.027
Yes	3 (3.1)	5 (0.5)	
No	95 (96.9)	1002 (99.5)	
Chronic use of corticosteroids and/or immunosuppressants			0.004
Yes	8 (8.2)	25 (2.4)	
No	89 (91.8)	993 (97.6)	
Presence of at least one symptom suggestive of TB[b]			0.113
Yes	75 (75.0)	686 (67.3)	
No	25 (25.0)	334 (32.7)	
Individuals with respiratory symptoms[c]			0.126
Yes	7 (7.0)	124 (12.2)	
No	93 (93.0)	896 (87.8)	

TB: tuberculosis. [a]Values expressed as n (%). The total number of patients varied according to the amount of ignored information. [b]Cough, expectoration, hemoptysis, dyspnea, chest pain, cyanosis, fever, asthenia, night sweats, recent weight loss, and loss of appetite. [c]Individuals with cough for two weeks or more.

Table 2. Univariate and multivariate analyses of factors associated with latent *Mycobacterium tuberculosis* infection in the study population, Minas Gerais, Brazil, 2014 (N = 1,120).[a]

Factors	LTBI		Univariate analysis		Multivariate analysis*	
	Yes n (%)	No n (%)	p	OR (95% CI)	p	aOR (95% CI)
Sociodemographic factors						
Gender						
Female	26 (26.0)	74 (74.0)	0.843	1	-----	-----
Male	256 (25.1)	764 (77.9)		0.954 (0.597-1.524)		
Age, years						
< 30	166 (25.1)	495 (74.9)	0.934	1	-----	-----
≥ 30	114 (25.3)	336 (74.7)		1.012 (0.768-1.333)		
Marital status						
Married/steady partner	108 (23.7)	348 (76.3)	0.340	1	-----	-----
Single	174 (26.2)	490 (73.8)		1.144 (0.868-1.509)		
Schooling, years						
≥ 7	24 (21.2)	89 (78.8)	0.305	1	-----	-----
< 7	258 (25.7)	747 (74.3)		1.281 (0.798-2.054)		
Occupation before incarceration	260 (25.2)	771 (74.8)	0.917	1	-----	-----
Yes	22 (24.7)	67 (75.3)		0.974 (0.590-1.608)		
No						
Length of incarceration, months	135 (23.7)	435 (76.3)	0.241	1	0.543	1.096 (0.816-1.471)
< 15	147 (26.7)	403 (73.3)		1.175 (0.897-1.540)		
≥ 15						
Behavioral factors						
Contact with active TB patients outside prison	230 (25.2)	682 (74.8)	0.672	1	-----	-----
No	29 (27.1)	78 (72.9)		1.102 (0.702-1.732)		
Yes						
Contact with active TB patients inside prison	193 (23.4)	631 (76.6)	0.030	1	0.026	1.516 (1.052-2.185)
No	53 (31.4)	116 (68.4)		1.494 (1.039-2.147)		
Yes						
Alcoholism						
No	209 (24.8)	633 (75.2)	0.632	1	-----	-----
Yes	73 (26.3)	205 (73.7)		1.079 (0.791-1.470)		
Smoking						
No	98 (22.5)	338 (77.5)	0.097	1	0.207	1.221 (0.896-1.666)
Yes	184 (26.9)	500 (73.1)		1.269 (0.958-1.682)		
Use of inhaled drugs						
No	61 (21.6)	221 (78.4)	0.111	1	0.034	1.483 (1.031-2.133)
Yes	221 (26.4)	617 (73.6)		1.307 (0.940-1.817)		
Use of injected drugs						
No	273 (25.0)	817 (75.0)	0.286	1	0.072	2.249 (0.931-5.430)
Yes	9 (30.0)	21 (70.0)		1.574 (0.684-3.621)		
Health history						
Diabetes mellitus						
No	215 (23.9)	686 (76.1)	0.947	1	-----	-----
Yes	3 (23.1)	10 (76.9)		0.957 (0.261-3.510)		
Chronic renal failure						
No	273 (24.9)	824 (75.1)	0.418	1	-----	-----
Yes	3 (37.5)	5 (62.5)		1.811 (0.430-7.627)		
Use of immunosuppressants						
No	275 (25.4)	807 (74.6)	0.395	1	-----	-----
Yes	6 (18.8)	26 (81.2)		0.677 (0.276-1.663)		
HIV infection						
No	233 (24.9)	702 (75.1)	-----	-----	-----	-----
Yes	0 (0.0)	5 (100.0)				

LTBI: latent *Mycobacterium tuberculosis* infection; TB: tuberculosis; and aOR: adjusted OR. [a]The total number of patients varied according to the amount of ignored information.*Hosmer-Lemeshow test; $\chi^2 = 0.679$; degrees of freedom = 2; p = 0.712.

(14.0%),[25] and Italy (17.9%),[26] and in the state of Mato Grosso do Sul, Brazil (20.8%).[13] However, it was lower than those found in Spain (40.3%)[27] and Switzerland (46.9%),[28] and in other states in Brazil (Bahia, Mato Grosso do Sul, and São Paulo; range, 30.9-61.5%).[29-31] These differences highlight the need for studying LTBI in different regions. There have been no studies showing the prevalence of LTBI in the region where the prisons selected for this study are located. In addition, the results of the present study are important not only to Minas Gerais, but also to other regions in Brazil where the prisons have the same scenarios.

Although there has been considerable debate about the use of the TST in places where there is mass vaccination with BCG, the WHO suggests the use of this test to detect LTBI in places where the TST is recommended, especially in adults. In the present study, 89.2% of the participants had been vaccinated more than 10 years prior (mean, 30 years). Therefore, BCG vaccination probably contributed little to TST positivity, because the response to the TST is nearly nonexistent 8-10 years after vaccination.[32,33] The TST may yield false-negative results in people who live with HIV and are not receiving antiretroviral therapy, because the response to the TST is based on the immune response of the individual[14]; this is probably the reason why LTBI was not identified in the prisoners recently diagnosed with HIV infection.

Several factors create a favorable context for increasing the prevalence of LTBI in prisoners.[11,30,34] As discussed, this population consists predominantly of young adults from socioeconomically disadvantaged communities with high rates of active tuberculosis. Among other elements posing an increased risk for the development of tuberculosis, the rate of alcoholism (24.8%) was lower than that reported in other studies (39.2-44.1%),[30] whereas the rate of smoking (61.1%) was similar.[30] The use of inhaled illicit drugs (97.0%) was higher than that reported by other authors (25.2-45.2%), whereas the use of injected illicit drugs (2.0%) was lower (7.5-13.1%).[27]

The rate of HIV infection found in our study sample (0.5%) was similar to the mean estimated rate for the Brazilian population (0.4%)[35]; however, it was lower than those reported by other authors (1.0-11.3%).[18,27,34,36] Therefore, it is important to detect LTBI in the HIV-infected population so that preventive measures can be taken, because HIV-infected individuals with LTBI have an 8.0-10.0% annual risk of developing active tuberculosis, as do users of inhaled illicit drugs with LTBI. In contrast, HIV-uninfected individuals with LTBI have a 10.0% lifetime risk of developing tuberculosis.[18,37]

In the prisons selected for this study, treatment of LTBI was not given to prisoners with diabetes mellitus, those with chronic renal failure, or those receiving immunosuppressants, although preventive therapy is recommended for these groups, which are considered at high risk for developing tuberculosis.[6,9,14]

Chief among the conditions of the prison environment that can favor the spread of the disease are limited exposure to sunlight,[5,6] overpopulation,[5,6,8,18,30,34] and inadequate ventilation.[5,6,30] To this context, we can add lack of training in tuberculosis control activities among prison health teams[5] and limited access to health care in prisons.[30,34] Therefore, changes should be made to the physical structures of prisons, and training in tuberculosis control should be provided to prison health teams.

In the present study, the likelihood of LTBI was found to be increased when the prisoners reported contact with someone with tuberculosis disease, a finding that has also been observed by several authors.[3,13,34] This might indicate an increased perception by the prisoners of the health risk related to the disease, since there is a high frequency of tuberculosis in the environment.[38] There was an association between the use of inhaled illicit drugs and LTBI, but no association was found between smoking and LTBI; the use of inhaled illicit drugs probably leads to greater tissue damage and greater impairment of mucociliary activity, which is the body's first line of protection when the bacilli enter the airways.[39]

The expected rate of individuals with RS in Brazil is 1.0% in the general population.[6] Among the prisoners included in this study, the proportion of individuals with RS was found to be 11.7-fold higher than that in the general population; however, it was lower than those reported in other studies conducted in Brazil (20.6-36.1%).[13,30,34] This can be explained by the poor infrastructural conditions in the prisons and by the high rates of smoking and use of inhaled illicit drugs.

There is no consensus in the scientific literature regarding an ideal mechanism for identification of people who are more likely to develop tuberculosis among prisoners.[5,6,9,15,16] However, the identification of chronic cough is used as a screening tool.[15,16,36] Some authors have reported that this symptom alone is not relevant to raising a suspicion of tuberculosis in this population.[38] Surveillance for cases of pulmonary tuberculosis should preferably include a combination of symptoms (fever, weight loss, and asthenia, for instance) rather than consider chronic cough alone.[30,40] The symptom approach is the only screening tool available in most resource-poor settings.[5,6,8,15-18] In addition, symptoms suggestive of tuberculosis may be nonspecific where there are high rates of other conditions associated with respiratory diseases, such as smoking.[8]

Minas Gerais, despite having the second largest overall population in the country, has the fourth lowest incidence rate of tuberculosis. It is possible that the prevalence rates of LTBI and active tuberculosis for the prisons selected for this study are not as high as those reported for other prisons because of the local epidemiological context.

One of the limitations of the present study is the fact that data on exposure and outcome were collected at the same time, which makes it difficult to understand

the temporal relationship between them and provides a static view of the dimension of the problem. Another limitation was that the incidence rates of LTBI and tuberculosis disease were not evaluated.

In conclusion, the prevalence of LTBI was high among the prisoners of the two largest prisons in Minas Gerais, as well as being associated with self-reported contact with active tuberculosis patients inside prison and with the use of inhaled drugs. Our findings demonstrate that it is necessary to improve the conditions in prisons, as well as to introduce strategies, such as chest X-ray screening, in order to detect tuberculosis cases and, consequently, reduce *M. tuberculosis* infection within the prison system.

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Staphylococcal superantigen-specific IgE antibodies: degree of sensitization and association with severity of asthma

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ABSTRACT

Objective: To determine the presence of staphylococcal superantigen-specific IgE antibodies and degree of IgE-mediated sensitization, as well as whether or not those are associated with the severity of asthma in adult patients. **Methods:** This was a cross-sectional study involving outpatients with asthma under treatment at a tertiary care university hospital in the city of Rio de Janeiro, Brazil. Consecutive patients were divided into two groups according to the severity of asthma based on the Global Initiative for Asthma criteria: mild asthma (MA), comprising patients with mild intermittent or persistent asthma; and moderate or severe asthma (MSA). We determined the serum levels of staphylococcal toxin-specific IgE antibodies, comparing the results and performing a statistical analysis. **Results:** The study included 142 patients: 72 in the MA group (median age = 46 years; 59 females) and 70 in the MSA group (median age = 56 years; 60 females). In the sample as a whole, 62 patients (43.7%) presented positive results for staphylococcal toxin-specific IgE antibodies: staphylococcal enterotoxin A (SEA), in 29 (20.4%); SEB, in 35 (24.6%); SEC, in 33 (23.2%); and toxic shock syndrome toxin (TSST), in 45 (31.7%). The mean serum levels of IgE antibodies to SEA, SEB, SEC, and TSST were 0.96 U/L, 1.09 U/L, 1.21 U/L, and 1.18 U/L, respectively. There were no statistically significant differences between the two groups in terms of the qualitative or quantitative results. **Conclusions:** Serum IgE antibodies to SEA, SEB, SEC, and TSST were detected in 43.7% of the patients in our sample. However, neither the qualitative nor quantitative results showed a statistically significant association with the clinical severity of asthma.

Keywords: Asthma; Immunoglobulin E; Superantigens; Bacterial toxins; Staphylococcus aureus.

INTRODUCTION

Staphylococcus aureus is a gram-positive bacterium that can colonize the human skin and respiratory tract. Colonization with *S. aureus* might or might not be associated with pathological processes. *S. aureus* produces several toxins related to its colonization ability and pathogenicity. The most important are toxic shock syndrome toxin (TSST), staphylococcal enterotoxin A (SEA), SEB, SEC, SED, SEE, SEG, SEH, and SEI, the activities of which include superantigen activity, pyrogenicity, and potentiation of lethality of other toxins.[1-3]

The superantigen activity of staphylococcal toxins consists of direct stimulation of class II MHC receptors and T cells, independently of antigen presentation by antigen-presenting cells, stimulating the proliferation and activity of CD4 and CD8 T lymphocytes. This mechanism is related to the worsening of allergic diseases by the production of staphylococcal toxin-specific IgE antibodies, as well as by a direct effect on tissue mast cells, leading to their degranulation.[1-3]

In asthma patients, staphylococcal toxins also act as superantigens, stimulating CD4 T lymphocyte proliferation and activity and leading to an increased production of staphylococcal toxin-specific IgE antibodies, causing an allergic-type reaction by biding to mast cells in the respiratory tract. This reaction results in the release of mediators such as histamine, kinins, platelet-activating factor, and arachidonic acid metabolites (prostaglandins and leukotrienes), as well as of chemokines, eliciting immediate and late inflammatory responses (by the recruitment and activation of neutrophils and eosinophils) and culminating in asthma worsening.[1-3]

Staphylococcal superantigens have been shown to play roles in atopic dermatitis, rhinosinusitis, and asthma, being correlated with their severity.[4-8] With regard to asthma, Kowalski et al. found IgE antibodies to SEA, SEC, and TSST in 89.7% of 237 asthma patients (mean levels of 1.096 ± 3.25 kU/L); although there was no significant difference between those with severe asthma and those with non-severe asthma in terms of the prevalence of

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staphylococcal toxin-specific IgE antibodies (81.4% vs. 69.2%), mean levels were higher in the former than in the latter (1.65 ± 3.25 kU/L vs. 0.54 ± 0.72 kU/L).[9] In another study (N = 210), the same authors obtained similar results, the prevalence of staphylococcal toxin-specific IgE antibodies being 76.1% in patients with severe asthma and 71.1% in those with non-severe asthma, mean levels being three times higher in the former than in the latter.[10] Bachert et al. found a significant increase in staphylococcal toxin-specific IgE antibodies in patients with severe asthma when compared with those with mild asthma and controls (N = 70).[11] In a more recent study (N = 387), the same group of authors found a significant increase in staphylococcal toxin-specific IgE antibodies in patients with severe uncontrolled asthma (59.6%) when compared with those with controlled asthma (40.8%) and controls (13.0%). High levels of staphylococcal toxin-specific IgE antibodies have been found to be a risk factor for asthma (OR = 7.6) and severe asthma (OR = 11.09).[12]

In Latin America, there have been no studies correlating staphylococcal superantigens with the severity of asthma. Therefore, we investigated a population of asthma patients treated at a university hospital in the city of Rio de Janeiro, Brazil, and having no risk factors for increased staphylococcal colonization or infection in order to correlate the clinical severity of asthma with the presence of staphylococcal toxin-specific IgE antibodies and degree of IgE-mediated sensitization.

METHODS

Patients

This was a cross-sectional study including adult patients clinically and functionally diagnosed with asthma and receiving outpatient treatment at the Clementino Fraga Filho University Hospital, located in the city of Rio de Janeiro, Brazil. Between 2009 and 2013, consecutive patients were divided into two groups according to the clinical severity of asthma based on the Global Initiative for Asthma criteria[13-15]: the mild asthma (MA) group, comprising patients with mild intermittent or persistent asthma; and the moderate or severe asthma (MSA) group.

According to the Global Initiative for Asthma,[13-15] asthma severity can be evaluated on the basis of the treatment required in order to control the disease. Patients with mild asthma are defined as those requiring only rescue medication, low-dose inhaled corticosteroids/leukotriene receptor antagonists, or a combination of the two. Patients with moderate asthma are defined as those using long-acting β_2 agonists and inhaled corticosteroids at low or moderate doses. Patients with severe asthma are defined as those using long-acting β_2 agonists and inhaled corticosteroids at high doses or other bronchodilators and anti-inflammatory drugs for asthma control.

The criteria for inclusion in the present study were as follows: being an adult patient clinically and functionally diagnosed with asthma,[16,17] regardless of the presence of rhinitis and positive skin test results to aeroallergens.

The exclusion criteria were as follows: presence of COPD, atopic dermatitis, or both; asthma exacerbation in the last four weeks; presence of respiratory infection or use of antimicrobial agents in the last six weeks; use of systemic corticosteroid therapy for seven or more days in the last four weeks; history of immunodeficiency, neoplasia, connective tissue disease, kidney failure, sinonasal polyposis, chronic sinus disease, cystic fibrosis, or bronchiectasis; pregnancy; smoking in the last twelve months; and declining to participate in the study or give written informed consent.

The sample size calculation was based on a study by Kowalski et al.[9] and was performed with a specific statistical calculation program (OpenEpi). For a paired relationship, with a 95% confidence interval and a power of 80%, the required sample size was calculated to be 140 (70 per group).

Procedures

Procedures included the following: clinical history taking; physical examination; routine tests (including blood count, ESR measurement, determination of total IgE levels, parasitological stool examination, chest X-rays, and sinus x-rays); pulmonary function tests (spirometry and PEF measurement); skin prick tests to aeroallergens; and determination of serum levels of IgE antibodies (to SEA, SEB, SEC, and TSST). Spirometry was performed with a spirometer (Jaeger, Würzburg, Germany), in accordance with the American Thoracic Society guidelines[16,17] and the reference values proposed by Knudson et al.[18] A finding of obstruction and positive bronchodilator test results with reversal or significant improvement were consistent with asthma.[16,17] For PEF measurement, a peak flow meter (Mini-Wright AFS; Clement Clarke International, Essex, England) was used, the reference values being those proposed by Nunn and Gregg.[19] Skin tests to aeroallergens were performed with the use of the puncture technique and standard antigens.[20] For determination of serum levels of staphylococcal toxin-specific IgE antibodies, an immunoassay system (ImmunoCAP® 100; Phadia, Uppsala, Sweden) was used.[21] Values above 0.35 U/L were considered positive.[21]

Statistical analysis

In order to compare numerical variables, we used the Student's t-test or the Mann-Whitney test, as appropriate, through the analysis of the Kolmogorov-Smirnov and Shapiro-Wilk coefficients. In order to compare categorical variables, we used the chi-square test or Fisher's exact test, as appropriate. The sample size was calculated in order to provide a power of 80%, and values of $p < 0.05$ were considered statistically significant.

Ethical aspects

The present study was approved by the Research Ethics Committee of the Federal University of Rio de Janeiro Clementino Fraga Filho University Hospital. All participating patients gave written informed consent, and the treatment provided to those who declined to participate in the study was in no way affected by their decision. Data confidentiality and patient anonymity were maintained during data handling and processing.

RESULTS

A total of 142 patients were studied. Of those, 72 (17 with mild intermittent asthma and 55 with mild persistent asthma) were allocated to the MA group and 70 (53 with moderate asthma and 17 with severe asthma) were allocated to the MSA group. The median age was 52.5 years (46 years in the MA group and 56 years in the MSA group), females and White individuals having predominated. In the sample as a whole, the mean body mass index (BMI) was 27.09 kg/m^2, 128 patients had rhinitis, 131 had positive skin test results to aeroallergens, and 99 had a family history of atopy. Only 37 (26.1%) had a history of smoking. Mean percent predicted PEF was 72.59%, mean percent predicted pre-bronchodilator FEV_1 was 71.55%, and mean percent predicted post-bronchodilator FEV_1 was 81.48%. Mean eosinophil count was 4.4%, and mean total IgE levels were 574.92 IU/mL. Table 1 shows the distribution of sociodemographic and clinical variables, and Table 2 shows lung function parameters and laboratory findings in the MA and MSA groups.

Of the sample as a whole, 62 patients (43.7%) tested positive for staphylococcal toxin-specific IgE antibodies: SEA, in 29 (20.4%); SEB, in 35 (24.6%); SEC, in 33 (23.2%); and TSST, in 45 (31.7%). The mean serum levels of IgE antibodies to SEA, SEB, SEC, and TSST were 0.96 U/L, 1.09 U/L, 1.21 U/L, and 1.18 U/L, respectively.

As can be seen in Tables 3 and 4, there were no statistically significant differences between the two groups regarding the frequency of IgE-mediated sensitization and serum levels of staphylococcal toxin-specific IgE antibodies.

DISCUSSION

There were statistically significant differences between the two groups of patients in the present

Table 1. Clinical and sociodemographic characteristics of the study population, by asthma severity.[a]

Characteristic	Group		p*
	Mild asthma (n = 72)	Moderate or severe asthma (n = 70)	
Age, years	45.51 ± 15.36	53.59 ± 12.82	< 0.0001
Weight, kg	66.17 ± 13.20	69.36 ± 15.03	0.2
Height, cm	159.23 ± 8.00	157.1 ± 7.04	0.12
BMI, kg/m^2	26.15 ± 5.26	28.06 ± 5.51	0.03
Gender			
Male	13 (18.1)	10 (14.3)	0.54
Female	59 (81.9)	60 (85.7)	
Race			
White	57 (79.2)	52 (74.3)	0.49
Mulatto or Black	15 (20.8)	18 (25.7)	
Presence of rhinitis	71 (98.6)	57 (81.4)	0.001
Family history of atopy	52 (72.2)	47 (67.1)	0.51
History of smoking	17 (23.6)	20 (28.6)	0.5
Positivity for aeroallergens	69 (95.8)	62 (88.6)	0.1

BMI: body mass index. [a]Values expressed as mean ± SD or n (%). *Student's t-test (for age) and chi-square test or Fisher's test (for the remaining parameters).

Table 2. Lung function parameters and laboratory findings in the study population, by asthma severity.[a]

Characteristic	Group		p*
	Mild asthma (n = 72)	Moderate or severe asthma (n = 70)	
PEF, mL	339.38 ± 96.51	276.64 ± 102.11	< 0.0001
PEF, %	78.52 ± 20.40	66.48 ± 21.65	0.01
Pre-BD FEV_1, %	80.03 ± 16.59	62.83 ± 21.88	< 0.0001
Post-BD FEV_1, %	90.28 ± 16.01	72.43 ± 23.01	< 0.0001
Eosinophils, n	331.96 ± 252.94	283.84 ± 209.93	0.31
Eosinophils, %	4.93 ± 3.63	4.03 ± 3.23	0.08
IgE, IU/mL	578.18 ± 728.68	571.57 ± 772.11	0.41

BD: bronchodilator. [a]Values expressed as mean ± SD. *Student's t-test or Mann-Whitney test.

Table 3. Frequency of IgE-mediated sensitization to staphylococcal toxins in the study population, by asthma severity.

IgE	Result	Group				p*
		Mild asthma (n = 72)		Moderate or severe asthma (n = 70)		
		n	%	n	%	
SEA	positive	12	16.7	17	24.3	0.26
	negative	60	83.3	53	75.7	
SEB	positive	15	20.8	20	28.6	0.28
	negative	57	79.2	50	71.4	
SEC	positive	14	19.4	19	27.1	0.27
	negative	58	80.6	51	72.9	
TSST	positive	21	29.2	24	34.3	0.51
	negative	51	70.8	46	65.7	
Any toxin	positive	31	43.1	31	44.3	0.88
	negative	41	56.9	39	55.7	

SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B; SEC: staphylococcal enterotoxin C; and TSST: toxic shock syndrome toxin. *Chi-square test or Fisher's test.

Table 4. Serum levels of staphylococcal toxin-specific IgE antibodies in the study population, by asthma severity.

IgE	Group						p*
	Mild asthma (n = 72)			Moderate or severe asthma (n = 70)			
	Mean	SD	Median	Mean	SD	Median	
SEA, U/L	1.07	0.72	0.91	0.88	0.66	0.69	0.41
SEB, U/L	1.41	1.66	0.74	0.84	0.44	0.67	0.7
SEC, U/L	1.16	1.14	0.5	1.25	0.98	0.71	0.43
TSST, U/L	1.33	1.04	0.75	1.06	0.74	0.85	0.9

SEA: staphylococcal enterotoxin A; SEB: staphylococcal enterotoxin B; SEC: staphylococcal enterotoxin C; and TSST: toxic shock syndrome toxin. *Chi-square test or Mann-Whitney test.

study regarding their clinical and sociodemographic characteristics (including age, BMI, and prevalence of rhinitis). The fact that the patients in the MSA were significantly older than were those in the MA group might be due to the fact that asthma tends to be more severe in older individuals, especially those in whom the onset of asthma occurred at an older age.[13-15] Our finding of a significantly higher BMI in the MSA group is consistent with the literature, obesity having been reported to be a risk factor for and an aggravator of asthma. Recent studies have established a relationship between obesity-induced changes in the gastrointestinal and respiratory microbiome and the etiopathogenesis of obesity-related asthma.[13-15,22] Our finding of a higher prevalence of rhinitis in the MA group suggests that atopy was more common in the MA group than in the MSA group, supporting the concept that atopic manifestations tend to be less common in asthma patients with disease that is more severe.[13-15]

With regard to the lung function parameters assessed in the present study, absolute and percent predicted PEF were significantly lower in the MSA group, as were percent predicted pre-bronchodilator FEV_1 and percent predicted post-bronchodilator FEV_1. These findings were expected and are consistent with the literature, showing the usual correlation between clinical severity and lung function parameters.[13-15]

Of the 142 patients studied, 62 (43.7%) tested positive for staphylococcal toxin-specific IgE antibodies, IgE antibodies to TSST being the most prevalent. Our findings are different from those of two studies in the literature and similar to those of two other studies. In one study, Kowalski et al. found an 89.7% prevalence of positivity for staphylococcal toxin-specific IgE antibodies in severe and non-severe asthma patients; in another study, they found a 76.1% prevalence in patients with severe refractory asthma and a 71.1% prevalence in patients with non-severe asthma.[9,10] In one study, Bachert et al. found a 38.1% prevalence of positivity for staphylococcal toxin-specific IgE antibodies in patients with asthma (independently of disease severity) and a 62% prevalence in patients with severe asthma; in another study, they found a 59.6% prevalence in patients with severe uncontrolled asthma and a 40.8% prevalence in patients with controlled asthma,[11,12] the latter prevalence being closer to that found in the present study.

Patients with associated chronic infections or other processes facilitating colonization or infection with *S. aureus*, such as sinonasal polyposis, bronchiectasis, chronic bronchitis, and atopic dermatitis, were not included in the present study. By facilitating colonization or infection with *S. aureus*, the aforementioned conditions can lead to increased quantities of staphylococcal toxins in the body, resulting in increased IgE-mediated sensitization and, consequently, a heterogeneous population of asthma patients. The studies conducted by Kowalski et al.[9,10] and Bachert et al.[11,12] had no

such exclusion criteria and included patients with chronic sinus disease and sinonasal polyposis, as was the case with the most recent study by Kowalski et al.,[10] who nevertheless found no statistically significant differences between asthma patients with polyposis and those without regarding their levels of staphylococcal toxin-specific IgE antibodies. This might explain the differences between our results and those obtained by the two aforementioned groups of authors regarding the degree of IgE-mediated sensitization. In addition, specific characteristics of our population might have influenced our results. To date, there have been no studies assessing colonization with *S. aureus* in the Brazilian population; therefore, it is currently impossible to determine whether or not it is lower than that in the European population. The same is true for the presence or absence of *S. aureus* in the respiratory microbiome of healthy individuals and asthma patients. It is also impossible to determine whether the allergic immune response to staphylococcal toxins is lower in asthma patients in Brazil than in those in other countries.

In the present study, there were no statistically significant differences between the two groups regarding the frequency of staphylococcal toxin-specific IgE antibodies. The same was true for the serum levels of IgE antibodies to those toxins. Our results are qualitatively similar to but quantitatively different from those obtained by Kowalski et al.,[9,10] who found significantly higher levels of staphylococcal toxin-specific IgE antibodies in patients with severe asthma. In addition, our results are qualitatively and quantitatively different from those obtained by Bachert et al.[11,12] These differences are also due to the exclusion criteria used in the present study, which were not used in any of the aforementioned studies, and to specific characteristics of our study population, as previously mentioned.

In the present study, IgE antibodies to SEA, SEB, SEC, and TSST were found in 62 (43.7%) of the 142 asthma patients analyzed, and neither the frequency of staphylococcal toxin-specific IgE antibodies nor the serum levels of those antibodies were associated with the clinical severity of asthma. These results are extremely relevant because this is the first study on this topic in Latin America, the results of which differed from those of previous studies conducted in Europe, indicating a "negative" association.

The limitations of the present study lie in the fact that it was a single-center study conducted at a tertiary care university hospital, in a single demographic area of Brazil. Our primary objective was to evaluate the influence of serum levels of staphylococcal toxin-specific IgE antibodies and their association with asthma severity, meaning that this was not a population prevalence study of IgE sensitization in asthma patients and healthy individuals in our region. If that had been the case, a much larger sample size would have been required, and it would have been impossible to obtain that in a single-center study. Therefore, in the present study, each group served as the control group for the other, without the use of a third group, comprising healthy individuals.

Multicenter studies in Brazil and other Latin American countries are needed in order to determine more accurately the role of IgE-mediated sensitization to staphylococcal toxins as an aggravator of asthma, as well as to determine its prevalence in asthma patients and healthy individuals. Such studies should include asthma patients with and without diseases that can lead to increased colonization or infection with *S. aureus*.

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Gel pillow designed specifically for obstructive sleep apnea treatment with continuous positive airway pressure

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ABSTRACT

Objective: To determine whether the use of a gel pillow with side cutouts designed to accommodate a continuous positive airway pressure (CPAP) mask and reduce head temperature improves the efficacy of and adherence to auto-CPAP therapy. **Methods:** Twenty-three consecutive CPAP-naïve patients with obstructive sleep apnea were enrolled in the study. Patients were given an auto-CPAP machine with an appropriate CPAP mask and were instructed to use CPAP for 15 nights. They were instructed to sleep with their own pillow (the control pillow) from nights 1 to 5 and with either a foam pillow or a gel pillow, both of which had side cutouts, for 5 consecutive nights each, in random order. After night 15, auto-CPAP machine data were downloaded and patients rated their satisfaction with each pillow on a visual analog scale. **Results:** Twenty-two patients completed the protocol. The pressures administered, residual apnea-hypopnea index, air leaks, and mean duration of CPAP use did not differ among the periods during which each pillow was used. Patients were significantly more satisfied with the gel pillow than with the control pillow and the foam pillow ($p = 0.022$ and $p = 0.004$, respectively), their level of satisfaction with the gel pillow correlating significantly with excessive daytime sleepiness ($r^2 = 0.19$; $p = 0.0443$). **Conclusions:** Among obstructive sleep apnea patients treated with nasal CPAP, the use of a gel pillow with side cutouts appears to have no impact on treatment effectiveness. Nevertheless, such patients seem to prefer a gel pillow over other types of pillows.

Keywords: Sleep; Continuous positive airway pressure; Sleep apnea, obstructive; Masks.

INTRODUCTION

Sleep represents one third of the human life. In addition to improving cognitive performance, mood state, and quality of life, good sleep quality and enough sleep have a major impact on health conditions and life expectancy.[1] It has been demonstrated that short sleep duration is associated with hypertension, stroke, diabetes, and possibly other diseases.[2] Sleep can be disturbed by environmental conditions, such as noise, temperature, and humidity, and can be influenced by several psychiatric, neurological, or clinical disorders.[3] One of the most common sleep disorders is obstructive sleep apnea (OSA),[4] which is characterized by recurrent episodes of complete or partial obstruction of the upper airway during sleep. OSA can cause excessive daytime sleepiness, as well as predisposing to a variety of diseases and reducing life expectancy.[5,6]

The first-line treatment of OSA is continuous positive airway pressure (CPAP) therapy administered during sleep, usually through a nasal or oronasal mask, in order to maintain the upper airway fully open.[7] The benefits of CPAP therapy include resolution of respiratory disturbances and an increased amount of sleep during CPAP application.[8]

In many OSA patients, adherence to CPAP therapy is less than optimal.[9] Several mechanisms might be responsible for air leak during CPAP therapy. For example, mouth opening can lead to large air leaks, which in turn can cause drying of the oronasal passageway, eye irritation,[10] increased noise, device malfunctioning (particularly if it is an auto-CPAP machine), and, consequently, intolerance to positive airway pressure therapy. Patients commonly complain that the CPAP device is cumbersome and does not allow them to move freely while in bed. In fact, body posture changes can cause mask displacement and excessive air leaks if the mask hits the pillow upon which the head of the patient lies.[11] Therefore, turning in bed can be difficult. In addition, head movements should be avoided, and this can increase the sensation of heat around the head, which can further increase patient discomfort and disrupt sleep. Discomfort during sleep can lead patients to remove the CPAP mask early in the night, and this can lead to the reappearance of respiratory disturbances. The use of comfortable, appropriately shaped pillows during CPAP application could minimize air leaks, improve patient mobility, improve the quality of sleep, and increase CPAP use, thus increasing the benefits of CPAP therapy.

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We hypothesized that the use of a gel pillow that could accommodate the CPAP mask when patients lie on their side and that could reduce head temperature and sweating[12] would improve the efficacy of and adherence to auto-CPAP therapy. Our primary objective was to determine whether the specially designed pillow was associated with fewer air leaks during auto-CPAP application and fewer respiratory disturbances, as automatically detected by the device. A secondary objective was to determine whether gel pillows with side cutouts were preferred by OSA patients receiving auto-CPAP and whether their use was associated with increased short-term adherence to therapy.

METHODS

Individuals 18 years of age or older referred to our sleep laboratory for suspected OSA were screened for inclusion in the present study. Out-of-center nocturnal cardiorespiratory polygraphic recordings were performed. Patients with at least moderate OSA (an apnea-hypopnea index [AHI] ≥ 15 events/h) were advised to initiate treatment with CPAP and were invited to participate in the study. The first 23 patients who agreed to participate were included in the study. One patient did not complete the study. All 23 lived near the sleep laboratory (i.e., less than 30 km from it). Sample size was powered to estimate patient satisfaction, expressed as a change of at least 2 points in the visual analog scale (VAS) score, with a power of 90%, an error probability of 0.05, and an SD of 2 for the change in VAS score. The study protocol was approved by the Research Ethics Committee of the University of Palermo, located in the city of Palermo, Italy. All participating patients gave written informed consent.

After medical history taking, all patients who had psychiatric disorders or diseases that might interfere with the outcomes of CPAP therapy were excluded, as were those who did not give written informed consent. Patients presenting with impairments or comorbidities considered likely to interfere with adherence to instructions, including neuromuscular disease, unstable psychiatric disease, cognitive impairment, myocardial infarction, unstable angina, heart failure, stroke, and lung disease, were excluded.-

Pillows tested

Three pillows were tested in the present study: each patient's own pillow (control pillow); a foam pillow (Technogel Italia S.r.l., Vicenza, Italy); and a gel pillow produced with a combination of memory foam and a stable, plasticizer-free polyurethane gel (Technogel Italia S.r.l.). The last two pillows had a rectangular design and side cutouts designed specifically for CPAP therapy, and both measured 66 cm in length × 40 cm in width × 14 cm in height (Figure 1).

Protocol

In all patients, the body mass index was calculated as weight in kilograms divided by height in meters squared (kg/m^2). The subjective level of sleepiness was assessed with the Epworth Sleepiness Scale.

Nocturnal cardiorespiratory polygraphic recordings were performed with a Somté recorder (Compumedics Inc., Abbotsford, Australia). Nasal airflow was detected through nasal cannulas and a pressure transducer. Respiratory disturbances were manually analyzed. Apneas were identified by cessation of nasal airflow for at least 10 s and were defined as obstructive when thoracoabdominal movements persisted. Hypopneas were identified by a ≥ 30% decrease in the amplitude of the airflow signal for at least 10 s and a > 3% decrease in SaO_2.

Respiratory event index was calculated as the number of apneas and hypopneas per hour of monitoring time. The percentage of sleep time spent at SaO_2 < 90% was automatically calculated by the software accompanying the Somté recorder (Compumedics Inc.). In accordance with the recommendations of the American Academy of Sleep Medicine, moderate OSA was defined as an AHI ≥ 15 events/h, whereas severe OSA was defined as an AHI > 30 events/h, with a predominance of obstructive events.[10]

After adaptation to breathing while receiving CPAP therapy, the patients were instructed on how to run an auto-CPAP machine (S9; ResMed, Abingdon, UK) and were requested to use it at home for 15 nights.

All patients were informed of the diagnosis by the medical staff, who, in addition to describing the disease and the consequences of ineffective treatment,

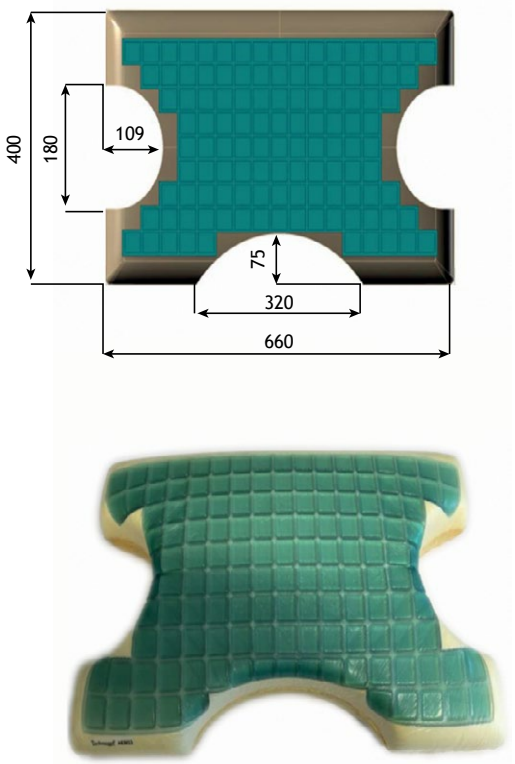

Figure 1. Characteristics of the gel pillow used in the present study. All measurements are expressed in mm.

provided information regarding the follow-up of patients with OSA. Subsequently, the nursing staff assisted patients in identifying the most suitable mask and held a morning session of education and training on CPAP therapy. In addition, patients were given an oximeter for continuous nocturnal SaO_2 recordings and a data logger for monitoring environmental pressure, temperature, and humidity. Patients were instructed to sleep with the control pillow from nights 1 to 5 and with either the foam pillow or the gel pillow from nights 6 to 15. The order of use of the last two pillows was randomized, with patients starting alternatively with the gel pillow or the foam pillow.

Patients returned all instruments after night 15. Auto-CPAP machine data were downloaded in order to assess the pressures administered, residual AHI, air leaks, and adherence to CPAP therapy. The recorded SaO_2 values and environmental conditions were analyzed. Patients were asked to rate their experience with each of the three pillows by marking a point on a VAS consisting of a 10-cm line, the left and right ends of which corresponded to total dissatisfaction and total satisfaction, respectively.

Statistical analysis

Means were compared by the nonparametric Kruskal-Wallis test. Linear regression was used in order to assess the relationships between variables. Data are reported as mean ± SD. Values of $p < 0.05$ were considered significant. Statistical analysis was performed with JMP software, version 8.0 (SAS Institute Inc., Cary, NC, USA).

RESULTS

All patients but one completed the protocol. The characteristics of the 22 patients who completed the protocol are shown in Table 1.

There were no significant differences in environmental temperature, humidity, or barometric pressure among the periods during which each pillow was used. With regard to the outcomes of auto-CPAP therapy, the three periods were similar in terms of residual AHI, delivered pressure, and air leaks (Table 2). However, when the only patient who used an oronasal mask slept with the control pillow, the amount of air leak was highest (21.6 L/min), decreasing significantly with the use of the gel pillow (6.0 l/min) and the foam pillow (7.2 L/min).

Duration of auto-CPAP use did not differ among pillow types (Table 2) and did not correlate with VAS scores. Figure 2 shows the mean VAS scores for each pillow type. The gel pillow was associated with a significantly higher satisfaction level than were the control pillow ($p = 0.022$) and the foam pillow ($p = 0.004$). Although the VAS score for the gel pillow was significantly correlated with excessive daytime sleepiness ($r^2 = 0.19$; $p = 0.0443$), those for the remaining pillow types were not (Figure 3). All participants reported a satisfaction level > 5 with the gel pillow.

DISCUSSION

This is the first study to examine whether a particular pillow shape and material are associated with increased efficacy of and adherence to CPAP therapy. CPAP therapy plays an important role in improving health status, prolonging survival,[13] and improving quality of life in patients with OSA.[14] Recent studies have suggested that it is important to maintain CPAP until the last hours of the night, when rapid eye movement sleep is most abundant, given that rapid eye movement-related respiratory disturbances are dangerous because of their health consequences.[15,16] Therefore, measures aimed at improving treatment adherence and efficacy are required.

We found no differences between the gel pillow and the control pillow regarding treatment effectiveness and short-term adherence to treatment. However, the gel pillow was better accepted by the participating patients.

Although the side cutouts in the pillow were designed to eliminate one of the factors responsible for air leaks, they neither reduced air leaks nor improved respiratory disturbances, as well as having had no impact on patient comfort. However, in most of our patients, there was minimal air leak during the night, as well as a low residual AHI. This was possibly due to careful instructions and adaptation to CPAP before initiation of treatment. In addition, all but one of the participating patients used a nasal CPAP mask, which

Table 1. Characteristics of the study sample and results of nocturnal polygraphic recordings.[a]

Male/female, n/n	Age, years	BMI, kg/m²	AHI, n/h	$TSat_{90}$, %	ESS score
19/3[b]	53.3 ± 7.6	33.7 ± 6.5	47.1 ± 19.4	24.0 ± 23.6	10.3 ± 4.7

BMI: body mass index; AHI: apnea-hypopnea index; $TSat_{90}$: percentage of sleep time spent at $SaO_2 < 90\%$; and ESS: Epworth Sleepiness Scale. [a]Data are presented as mean ± SD, except where otherwise indicated. [b]Data presented as n.

Table 2. Data downloaded from auto-CPAP machines.[a]

Variable	Control pillow	Foam pillow	Gel pillow
Residual AHI, n/h	3.7 ± 2.9	2.7 ± 1.9	2.7 ± 1.8
90th percentile pressure, cmH_2O	12.3 ±2.7	11.8 ± 2.8	12.2 ± 2.7
Air leak, L/m	10.4 ± 6.2	11.1 ± 8.5	10.8 ± 7.0
Mean duration of use, min/day	395 ± 93	373 ± 74	386 ± 80

AHI: apnea-hypopnea index. [a]Data presented as mean ± SD.

allowed them to turn on their sides easily with no increase in air leak.

Patients with OSA syndrome can generally switch between nasal and oronasal masks without changing machine pressure, although there are individual differences that can be clinically significant. Most patients with OSA syndrome prefer a nasal mask as the interface for initiation of CPAP. However, in comparison with CPAP titration with a nasal mask, CPAP titration with an oronasal mask is characterized by increased mask leak, increased residual respiratory disturbance, increased arousal indices, decreased slow wave sleep, and decreased total sleep time on the titration night.[17,18] In the present study, the only patient who used CPAP via an oronasal mask had increased air leak when using the control pillow, air leak being markedly decreased with the use of the foam pillow and the gel pillow. Therefore, it is possible that specially designed pillows are indicated for patients treated with CPAP via an oronasal mask. Studies involving an adequate sample of patients receiving CPAP therapy via an oronasal mask are needed in order to test this hypothesis.

Although the gel pillow was not associated with changes in the efficacy of CPAP therapy in the present study, it improved sleep comfort, particularly in the sleepiest patients. However, the duration of CPAP use tended to be the same irrespective of the type of pillow. Nevertheless, each pillow was tested for only 5 nights. In the long term, improved comfort might result in longer sleep duration and prolonged CPAP use. In fact, many OSA patients have reported that they remove their CPAP mask if they wake up in the middle of the night. A higher degree of comfort during the night might improve sleep continuity and treatment adherence in the long term. It is not clear why the sleepiest patients in the present study were the ones who liked the gel pillow the most. Among OSA patients, the sleepiest are those who have the worst quality of life and who are at the highest risk for accidents and health consequences, such as systemic hypertension.[19] On the basis of our findings, we believe that the sleepiest of OSA patients, in whom OSA is typically most severe, should be advised to

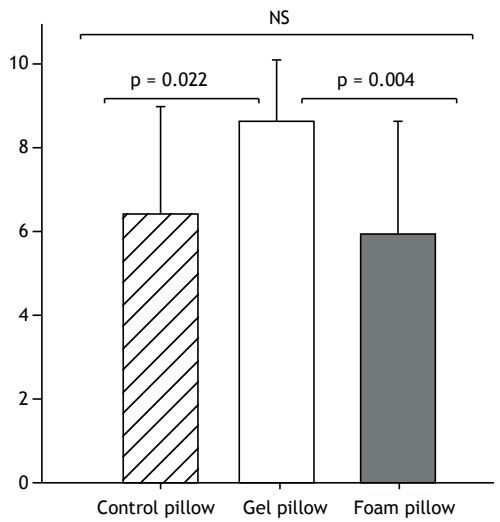

Figure 2. Patient satisfaction with the pillows tested (vertical bars), as expressed by visual analog scale scores. Data are presented as mean ± SD. NS: not significant.

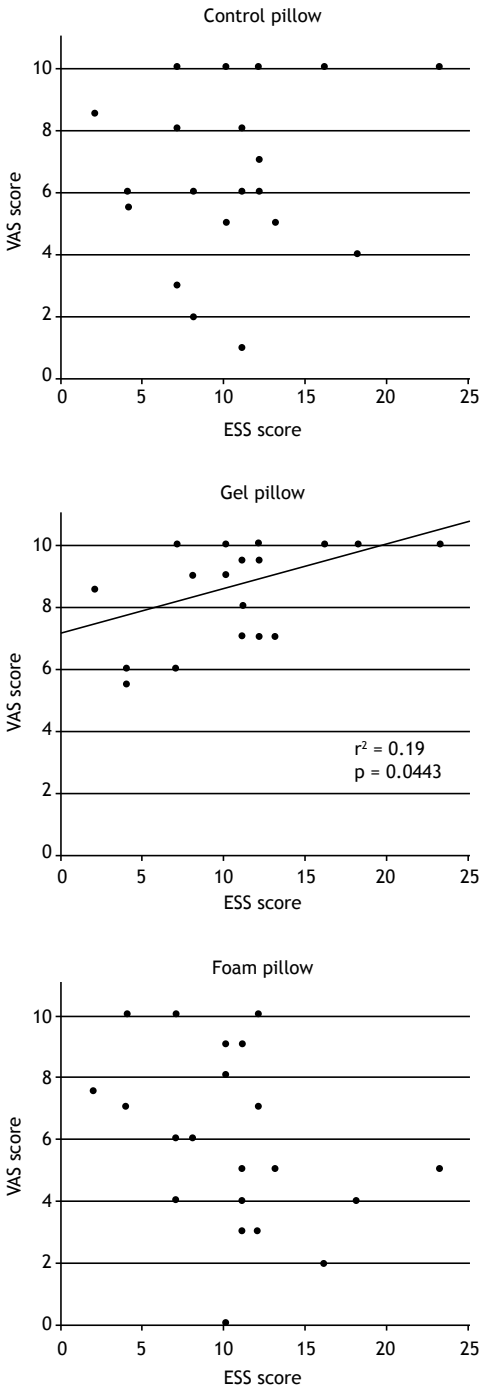

Figure 3. Linear regression between sleepiness, as expressed by Epworth Sleepiness Scale (ESS) scores, and patient satisfaction with the pillows tested, as expressed by visual analog scale (VAS) scores.

use gel pillows when receiving CPAP, in an attempt to improve treatment adherence.

One limitation of the present study is that only one participant used an oronasal mask for CPAP therapy. Although data for that patient suggest that pillows with side cutouts play a beneficial role in CPAP therapy, further studies are warranted. Another limitation is that we relied on data downloaded from auto-CPAP machines in order to evaluate treatment effectiveness. Such data cannot be exactly reproduced by means of direct assessment; however, according to the American Thoracic Society, they might reflect the results of direct assessment, particularly when they indicate very good or very poor treatment outcomes,[20] as was the case in our patients.

In conclusion, among patients treated with CPAP, most of whom used a nasal mask, the use of a gel pillow designed to accommodate the CPAP mask did not improve treatment efficacy. However, the gel pillow was preferred over the other pillows tested, particularly by the sleepiest of our group of OSA patients. Our data do not allow us to draw conclusions in this regard. Further studies are needed in order to test this hypothesis and determine the long-term benefits of different types of pillows.

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ORIGINAL ARTICLE

Effects of indacaterol versus tiotropium on exercise tolerance in patients with moderate COPD: a pilot randomized crossover study

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Study carried out at Pavilhão Pereira Filho, Santa Casa de Porto Alegre, Porto Alegre (RS) Brasil.

Abstract

Objective: To compare a once-daily long-acting β_2 agonist (indacaterol 150 µg) with a once-daily long-acting anticholinergic (tiotropium 5 µg) in terms of their effects on exercise endurance (limit of tolerance, Tlim) in patients with moderate COPD. Secondary endpoints were their effects on lung hyperinflation, exercise-related dyspnea, and daily-life dyspnea. **Methods:** This was a randomized, single-blind, crossover pilot study involving 20 patients (mean age, 60.9 ± 10.0 years; mean FEV_1, 69 ± 7% of predicted). Spirometric parameters, Transition Dyspnea Index scores, Tlim, and exertional dyspnea were compared after three weeks of each treatment (with a one-week washout period between treatments). **Results:** Nineteen patients completed the study (one having been excluded because of COPD exacerbation). Improvement in Tlim from baseline tended to be greater after treatment with tiotropium than after treatment with indacaterol (96 ± 163 s vs. 8 ± 82 s; p = 0.06). Tlim significantly improved from baseline after treatment with tiotropium (having increased from 396 ± 319 s to 493 ± 347 s; p = 0.010) but not after treatment with indacaterol (having increased from 393 ± 246 to 401 ± 254 s; p = 0.678). There were no differences between the two treatments regarding improvements in Borg dyspnea scores and lung hyperinflation at "isotime" and peak exercise. There were also no significant differences between treatments regarding Transition Dyspnea Index scores (1.5 ± 2.1 vs. 0.9 ± 2.3; p = 0.39). **Conclusions:** In patients with moderate COPD, tiotropium tends to improve Tlim in comparison with indacaterol. No significant differences were observed between the two treatments regarding their effects on lung hyperinflation, exercise-related dyspnea, and daily-life dyspnea. Future studies, including a larger number of patients, are required in order to confirm our findings and explore mechanistic explanations.

(ClinicalTrials.gov identifier: NCT01693003 [http://www.clinicaltrials.gov/])

Keywords: Pulmonary disease, chronic obstructive; Exercise; Bronchodilator agents.

INTRODUCTION

Bronchodilators have consistently been shown to result in long-term improvements in clinical outcomes (symptoms, exercise capacity, and airflow limitation) and are currently considered the therapeutic mainstay for patients with COPD.[1] According to current guidelines, all symptomatic patients with COPD should be prescribed a short-acting bronchodilator to be used on an as-needed basis. A long-acting bronchodilator should be added and used regularly if symptoms are inadequately controlled with short-acting bronchodilator therapy or if patients are at an increased risk for poor outcomes, such as frequent exacerbations and disease that is more severe.[1,2]

Until recently, a long-acting anticholinergic (LAMA) was preferred over a long-acting β_2 agonist (LABA) because most of the effects of once-daily LAMAs appeared to be superior to those of twice-daily LABAs.[3-8] The advent of once-daily LABAs (ultra-LABAs) changed that, studies comparing once-daily LAMAs with once-daily LABAs having demonstrated the clinical benefits of the latter.[9,10] However, no studies have compared once-daily LABAs with once-daily LAMAs regarding clinical outcomes during exercise, including exercise tolerance, dyspnea, and dynamic hyperinflation. Therefore, we conducted a pilot study aimed at comparing a once-daily LABA (indacaterol) with a once-daily LAMA (tiotropium) in terms of their effects on exercise tolerance in patients with moderate COPD. Indacaterol and tiotropium were also compared in terms of their effects on lung hyperinflation, exercise-related dyspnea, and daily-life dyspnea.

METHODS

This was a phase IV, randomized, single-blind (i.e., with single-blind masking of outcome assessors), placebo-controlled, two-period, crossover pilot study conducted at a single center specializing in respiratory care (ClinicalTrials.gov identifier: NCT01693003).[11] The study protocol was approved by the local research ethics committee.

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Patients were randomly assigned to receive three weeks of treatment with 150 µg of inhaled indacaterol (Onbrize® Breezhaler®; Novartis, Basel, Switzerland) delivered via a capsule-based dry powder inhaler (DPI), followed by another three weeks of treatment with 5 µg of inhaled tiotropium (Spiriva® Respimat®; Boehringer Ingelheim, Ingelheim, Germany) delivered via a soft mist inhaler (SMI), with a one-week washout period between the two treatment periods; or three weeks of treatment with 5 µg of inhaled tiotropium (Spiriva® Respimat®; Boehringer Ingelheim) delivered via an SMI, followed by another three weeks of treatment with 150 µg of inhaled indacaterol (Onbrize® Breezhaler®; Novartis) delivered via a capsule-based DPI, with a one-week washout period between the two treatment periods (Figure 1). After a screening visit (on day 7), all long-acting bronchodilators were discontinued. Patients were allowed to use short-acting bronchodilators, being instructed to use two puffs every 4 h as rescue medication. They were also allowed to use inhaled corticosteroids, provided that the dose, schedule, and formulation remained unchanged.

At the baseline visit, patients underwent clinical evaluation, pulmonary function testing, and incremental symptom-limited cardiopulmonary exercise testing (CPET). At visits 1 through 4, patients underwent constant-rate CPET to the limit of tolerance (Tlim), at ~80% of the maximum load reached during incremental CPET. Activity-related breathlessness was assessed at baseline with the Baseline Dyspnea Index (BDI), and changes in daily breathlessness were assessed with the Transition Dyspnea Index (TDI),[12] being recorded at the end of each treatment period (Figure 1).

Patients

Patients presenting with stable COPD ($FEV_1/FVC < 0.7$ and 50% < post-bronchodilator FEV_1 < 80% of predicted) and a long smoking history (> 20 pack-years) were enrolled. The exclusion criteria were as follows: cardiovascular or neuromuscular disease potentially affecting exercise tolerance; recent exacerbation (in the last month); long-term oxygen therapy or resting SaO_2 < 90%; and treatment with oral corticosteroids.

Procedures

All spirometric tests were performed with a calibrated pneumotachograph (Vmax29®; SensorMedics, Yorba Linda, CA, USA). Spirometric variables were measured at the baseline visit (before and 20 min after inhalation of 400 µg of albuterol via a metered dose inhaler); at visits 1 and 3 (after a one-week long-acting bronchodilator washout period and before CPET); and at visits 2 and 4 (2 h after administration of the study medications and before CPET). A constant-volume body plethysmograph (Vmax Autobox®; SensorMedics) was used in order to measure RV, functional residual capacity, and TLC. Single-breath DLCO was measured using a Vmax System (SensorMedics). All pulmonary function tests were performed in accordance with international standards.[13-15] The variables obtained were expressed as absolute and percent predicted values.[16-18]

All exercise tests were performed on an electromagnetically braked cycle ergometer (Corival; Lode, Groningen, the Netherlands), with the use of a computer-based breath-by-breath CPET system (Vmax29®; SensorMedics). HR was determined from the R-R interval of a 12-lead electrocardiogram, and SaO_2 was measured by pulse oximetry. All CPET variables were presented as 20-s averages. Participants rated their shortness of breath and leg effort using the 0-10 Borg scale[19] every 2 min. During incremental CPET, the workload was increased every 1 min from a baseline of 2 min of loadless pedaling at a rate of 5-10 W/min to Tlim. Incremental load increases were highest in patients with FEV_1 > 1 L. Constant-rate CPET was performed with loadless pedaling for 2 min at a pedaling frequency of 60 ± 5 rpm, immediately followed by loaded pedaling at ~80% of the maximum workload achieved during incremental CPET. Assuming that resting TLC remains constant during exercise, we considered that changes in inspiratory capacity (IC) reflected changes in end-expiratory lung volume, i.e., end-expiratory lung volume = TLC − IC.[20] IC maneuvers were performed every 2 min. Exercise responses were compared at peak exercise and at "isotime", i.e., the longest exercise duration common to all constant-rate cardiopulmonary exercise tests performed by a given individual.

The BDI and TDI were used in order to measure daily-life dyspnea, and both have three domains: 1) functional impairment, which determines the impact of breathlessness on the ability to carry out activities; 2) magnitude of task, which determines the type of task that causes breathlessness; and 3) magnitude of effort, which establishes the level of effort that results in breathlessness. The BDI domain scores range from 0 (very severe impairment) to 4 (no impairment) and are summed to determine the total score, which can range from 0 to 12. The TDI domain scores range from −3 (major deterioration) to +3 (major improvement). The sum of all domains yields the total score, which can range from −9 to +9.[12] The minimal clinically important difference for the TDI score is 1.[21]

Safety

Safety assessments included adverse events and serious adverse events at the end of each treatment period. HR correction of the QT interval was performed using Bazett's correction.

Statistical analysis

Data are reported as mean ± SD or median (range), except where otherwise indicated. Generalized estimating equations were used in order to test for significant differences between treatments at different visits and time points. Paired t-tests were used in order to compare TDI scores after each treatment and calculate the sample size required to detect a significant difference ($p < 0.05$) between treatments

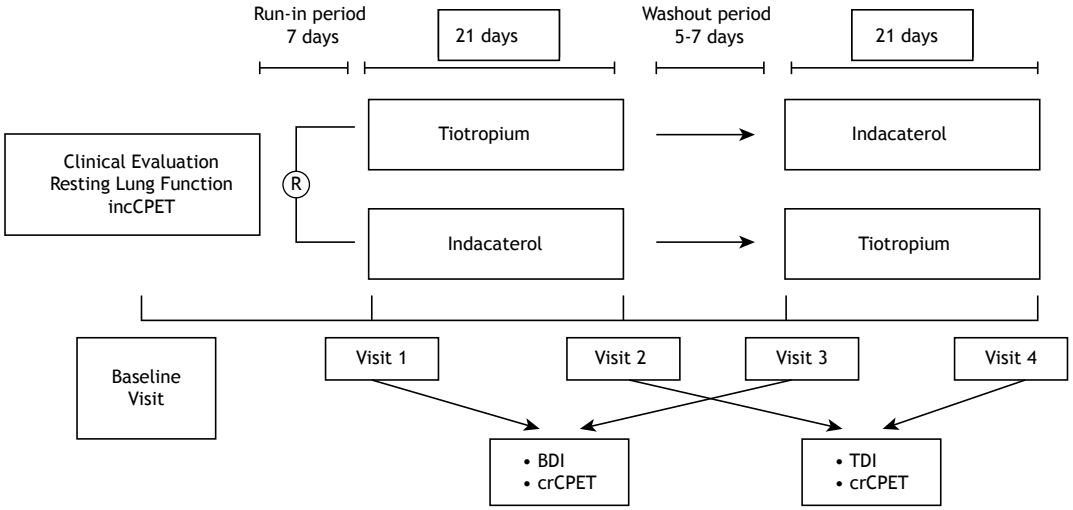

Figure 1. Flowchart of the study design. incCPET: incremental cardiopulmonary exercise testing; crCPET: constant-rate cardiopulmonary exercise testing; BDI: Baseline Dyspnea Index; and TDI: Transition Dyspnea Index.

regarding improvement in exercise tolerance (with a type II error of 20%). The chi-square test was used in order to compare categorical data. Differences were considered significant if $p < 0.05$.

RESULTS

Of the 69 patients who were screened, 20 were randomized. Of those, 19 (95%) completed the study. One patient (in the group of patients assigned to receive indacaterol first) was excluded because of COPD exacerbation (during treatment with indacaterol).

The baseline demographic, anthropometric, and clinical characteristics of the patients studied are described in Table 1. A Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study is shown in Figure 2.

Effects on spirometric variables and daily-life dyspnea

After three weeks of treatment, FEV_1 was significantly improved from baseline in both groups (Table 2). However, in addition to having resulted in greater improvement in FEV_1, indacaterol significantly improved FVC when compared with tiotropium. There were no significant differences between indacaterol and tiotropium regarding TDI scores (1.5 ± 2.1 vs. 0.9 ± 2.3; $p = 0.39$) or the proportion of patients in whom TDI scores were ≥ 1 (58% vs. 37%; $p = 0.19$).

Effects on exercise responses

Improvement in Tlim from baseline (the primary study outcome) tended to be greater after treatment with tiotropium than after treatment with indacaterol (96 ± 163 s vs. 8 ± 82 s; $p = 0.06$; Figure 3). Additionally, Tlim significantly improved from baseline after treatment with tiotropium (having increased from 396 ± 319 s to 493 ± 347 s; $p = 0.010$) but not after treatment with indacaterol (having increased from 393 ± 246 s to 401 ± 254 s; $p = 0.678$). A sample size of 28 was estimated to be required in order to detect a significant difference in exercise tolerance between the two treatments.

There were no differences between the two treatments regarding the magnitude of improvement in Borg dyspnea scores (at isotime and peak exercise) or lung hyperinflation, as estimated from serial measurements of IC (at rest, isotime, and peak exercise). Lung hyperinflation was found to have improved significantly after treatment with bronchodilators (2.00 ± 0.33 L vs. 2.09 ± 0.31 L; $p = 0.03$) at all time points analyzed (i.e., at rest, isotime, and peak exercise). The same was true for exercise-related dyspnea ($p = 0.067$).

Safety

The overall incidence of adverse events was exactly the same in both treatment groups (i.e., 58%), the majority of the events being mild in severity. No serious adverse events (hospitalization or death) were reported during the study period. There was no difference between indacaterol and tiotropium in terms of their effects on the resting corrected QT interval (445 ± 48 ms vs. 439 ± 47 ms; $p > 0.05$), post-bronchodilator values being no different from baseline values (456 ± 34 ms).

DISCUSSION

This was a pilot study designed to collect preliminary data regarding the comparative effects of indacaterol 150 µg (the lowest available dose in most countries) and tiotropium 5 µg on exercise tolerance in patients with moderate COPD. Previous studies[22,23] have demonstrated that indacaterol 300 µg results in significant improvement in exercise tolerance and lung hyperinflation at rest and during exercise when compared with placebo in patients with moderate to severe COPD. Surprisingly, the present study showed that a lower dose of indacaterol (150 µg) in a subset of patients with less severe disease did not increase

exercise tolerance from baseline. In contrast, tiotropium 5 µg significantly improved exercise tolerance from baseline, a finding that is consistent with those of previous studies in which a different drug dose and delivery system were used (i.e., 18 µg of tiotropium delivered via a DPI).[24-27]

In the present study, both drugs resulted in significant improvement in lung hyperinflation and exercise-related dyspnea, as previously described for tiotropium (18 µg delivered via a DPI)[12,19-21] and indacaterol (300 µg),[17,18] with no significant difference between the two treatments. However, it is possible that our small sample size did not allow us to detect individual drug effects on the aforementioned variables or differences between the two treatments.

Table 1. Baseline characteristics of the patients studied (N = 19).[a]

Variable	Result
Demography and anthropometry	
Age, years	60.9 ± 10.0
Male/female, n/n	9/10
BMI, kg/m^2	24.8 ± 3.5
Smoking history, pack-years[b]	45 (6-108)
Pulmonary function	
Pre-BD spirometry	
FEV_1, L	1.86 ± 0.62
FEV_1, % of predicted	67.4 ± 8.6
FVC, L	3.26 ± 0.83
FVC, % of predicted	94.1 ± 10
FEV_1/FVC	57 ± 8
Post-BD spirometry	
FEV_1, L	1.89 ± 0.58
FEV_1, % of predicted	68.7 ± 7.4
FVC, L	3.27 ± 0.8
FVC, % of predicted	94.6 ± 11.2
FEV_1/FVC	58 ± 8
Plethysmography	
IC, L	2.15 ± 0.9
IC, % of predicted	66.3 ± 20.5
TLC, L	5.67 ± 1.4
TLC, % of predicted	109.1 ± 12.7
IC/TLC	0.37 ± 0.1
RV, L	2.36 ± 0.73
RV, % of predicted	122.5 ± 33.6
DLCO, mmol/min/kPa	4.4 ± 1.4
DLCO, % of predicted	67.4 ± 18.3
Symptoms	
mMRC score	2.3 ± 1.1
BDI score	8.4 ± 2.4
Peak incremental CPET	
VO_2, mL/min	1,083 ± 349
VO_2, % of predicted	74.7 ± 16.6
V_E, L	42.4 ± 14.9
V_E/MVV	0.69 ± 0.17
SaO_2, %	96 ± 2
HR, % of predicted	79 ± 12
VO_2/HR, mL/min/bpm	8.45 ± 2.12
Borg scale, dyspnea score[b]	4 (0.5-10)
Borg scale, leg effort score[b]	7 (1-10)

[a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as median (range). BD: bronchodilator; IC: inspiratory capacity; mMRC: modified Medical Research Council; BDI: Baseline Dyspnea Index; CPET: cardiopulmonary exercise testing; VO_2: oxygen uptake; V_E: minute ventilation; and MVV: maximal voluntary ventilation.

Although both treatments improved FEV_1 from baseline, the magnitude of change was greater for indacaterol. Similar findings have previously been described.[10,28] With regard to clinical outcomes, a clinically relevant improvement in total TDI and Saint George's Respiratory Questionnaire scores is more likely to be achieved with indacaterol 150 µg than with tiotropium 18 µg in patients with moderate to severe COPD.[29] However, tiotropium has been reported to afford greater protection against exacerbations.[30] In the present study, indacaterol resulted in greater improvement in FEV_1 than did tiotropium (Table 2). However, it did not result in improved exercise tolerance, probably because constraints on tidal volume expansion as a result of lung hyperinflation constitute the main mechanism related to dyspnea and exercise capacity, independently of the magnitude of airflow obstruction.[20,26,31] Nevertheless, because of its small size, our sample was probably underpowered to detect differences between the two treatments regarding this physiological variable. Therefore, other mechanisms to explain improved exercise tolerance after treatment with tiotropium should be considered and further investigated.[32] For instance, it is impossible to rule out that our small sample size randomly included primarily patients who were more likely to benefit from one specific pharmacological class of bronchodilators. Polymorphisms of β_2-adrenergic receptors can result in differences in pharmacological responses to bronchodilators.[33,34] This underscores the need for further, larger studies. If our findings are confirmed, adequately powered studies will be required in order to investigate physiological and molecular mechanistic aspects.

The present study has methodological limitations that should be noted. First, because this was an exploratory study including only a small number of patients, the results should be interpreted with caution. Our sample was possibly underpowered to detect differences in important outcomes, such as dyspnea and lung hyperinflation, and our main findings should be confirmed in studies including a larger number of patients. Second, because the present study included only patients with moderate COPD, the results should not be generalized to patients with mild or severe COPD. Finally, we used a low dose of indacaterol and a full dose of tiotropium delivered via an SMI. The dose of indacaterol used in the present study (i.e., 150 µg) did not improve exercise tolerance as did the dose used in other studies (i.e., 300 µg).[22,23] In fact, it has been shown that indacaterol is more beneficial to resting pulmonary function at higher doses (> 200 µg) than at lower doses (of 50 µg and 100 µg); however, in comparison with placebo, even lower doses of the

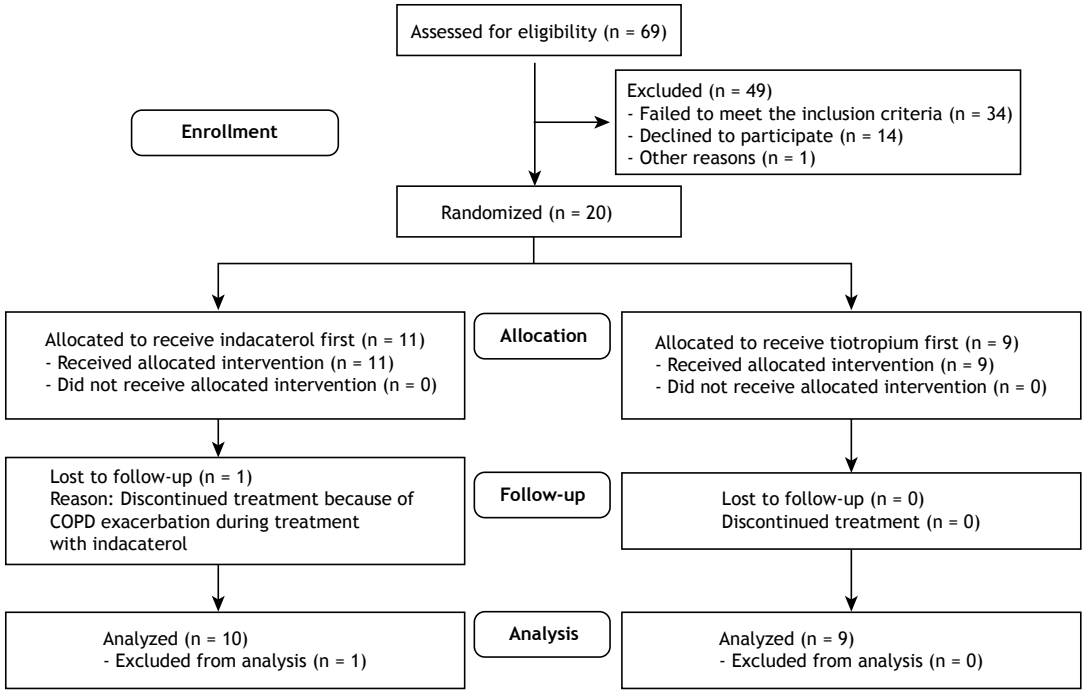

Figure 2. Consolidated Standards of Reporting Trials (CONSORT) flow diagram of the study.

Table 2. Lung function parameters at baseline and after three weeks of treatment with indacaterol or tiotropium.[a]

Variable	Indacaterol		Diff	Tiotropium		Diff
	Baseline	Post-treatment		Baseline	Post-treatment	
FEV_1, L	1.62 ± 0.12	1.82 ± 0.12*	0.20	1.69 ± 0.13	1.79 ± 0.14*	0.10
FEV_1, % of predicted	56 ± 2	63 ± 2*	7†	58 ± 2	61 ± 2	3
FVC, L	2.94 ± 0.2	3.15 ± 0.17*	0.21†	3.06 ± 0.19	3.12 ± 0.2	0.06
FVC, % of predicted	80 ± 2	87 ± 2*	7†	84 ± 2	86 ± 2	2
FEV_1/FVC, %	55.5 ± 2.0	57.6 ± 1.6*	2.1	55.0 ± 1.9	56.9 ± 1.9*	1.9

[a]Data presented as mean ± SE. BD: bronchodilator; and Diff: difference between mean post-treatment values and mean baseline values. *p < 0.05 baseline vs. post-treatment. †p < 0.05 comparison between treatment changes.

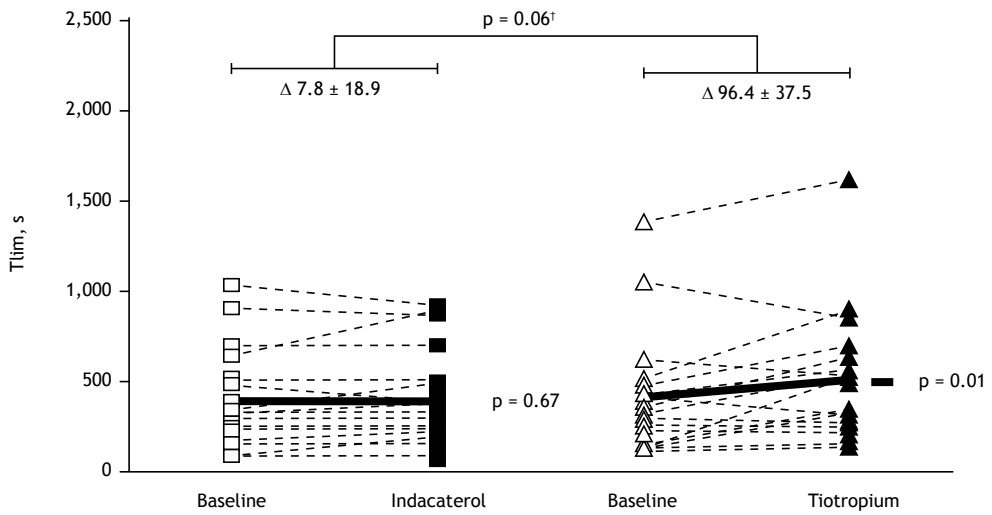

Figure 3. Individual values (dashed lines) and mean values (solid lines) of changes from baseline in the limit of tolerance (Tlim) during constant-rate cardiopulmonary exercise testing after three weeks of treatment with indacaterol (squares) or tiotropium (triangles). *p < 0.05 from baseline. †p = 0.06 for between-treatment difference.

drug result in significant improvement.[28] In contrast, it has been shown that 5 µg of tiotropium delivered via an SMI and 18 µg of the same drug delivered via a DPI are comparable in terms of their effects on lung function[35,36] and clinical outcomes (rescue medication use, death, and exacerbation rate).[30] Given that the doses of indacaterol approved for use in different countries vary from 75 µg to 300 µg and that the only dose of SMI-delivered tiotropium approved for use in COPD patients is 5 µg, we sought to compare doses that are more commonly used in clinical practice.

In conclusion, although treatment with tiotropium at a daily dose of 5 µg resulted in a significant improvement in exercise tolerance in patients with moderate COPD, treatment with indacaterol at a daily dose of 150 µg did not. No significant differences were observed between the two treatments regarding their effects on lung hyperinflation, exercise-related dyspnea, and daily-life dyspnea. Further studies, including a larger number of patients, are required in order to confirm our findings and explore mechanistic explanations.

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Is there a rationale for pulmonary rehabilitation following successful chemotherapy for tuberculosis?

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ABSTRACT

The role of tuberculosis as a public health care priority and the availability of diagnostic tools to evaluate functional status (spirometry, plethysmography, and DLCO determination), arterial blood gases, capacity to perform exercise, lesions (chest X-ray and CT), and quality of life justify the effort to consider what needs to be done when patients have completed their treatment. To our knowledge, no review has ever evaluated this topic in a comprehensive manner. Our objective was to review the available evidence on this topic and draw conclusions regarding the future role of the "post-tuberculosis treatment" phase, which will potentially affect several million cases every year. We carried out a non-systematic literature review based on a PubMed search using specific keywords (various combinations of the terms "tuberculosis", "rehabilitation", "multidrug-resistant tuberculosis", "pulmonary disease", "obstructive lung disease", and "lung volume measurements"). The reference lists of the most important studies were retrieved in order to improve the sensitivity of the search. Manuscripts written in English, Spanish, and Russian were selected. The main areas of interest were tuberculosis sequelae following tuberculosis diagnosis and treatment; "destroyed lung"; functional evaluation of sequelae; pulmonary rehabilitation interventions (physiotherapy, long-term oxygen therapy, and ventilation); and multidrug-resistant tuberculosis.The evidence found suggests that tuberculosis is definitively responsible for functional sequelae, primarily causing an obstructive pattern on spirometry (but also restrictive and mixed patterns), and that there is a rationale for pulmonary rehabilitation. We also provide a list of variables that should be discussed in future studies on pulmonary rehabilitation in patients with post-tuberculosis sequelae.

Keywords: Tuberculosis/complications; Tuberculosis/rehabilitation, Tuberculosis/therapy; Quality of life; Diagnostic imaging; Respiratory function tests.

INTRODUCTION

The World Health Organization (WHO) estimated that 3.3% of the new cases of tuberculosis and 20% of the previously treated cases of the disease are due to multidrug-resistant tuberculosis (MDR) strains of *Mycobacterium tuberculosis* worldwide in 2014. The highest prevalences of MDR tuberculosis (MDR-TB) have been reported in Eastern European and Central Asian countries, although relatively high prevalence rates have been described in Latin America. As of today, the "world record" MDR-TB prevalence has been described in Belarus (34% among new cases and 69% among retreatment cases), where 29% of the cases are reported to be extensively drug-resistant tuberculosis (XDR-TB).[1]

It is unfortunately well known that outcomes of MDR-TB and XDR-TB cases (particularly those with a resistance pattern beyond XDR-TB) are poor, since the treatment success rate is below 20% and the failure and death rates combined are 49%.[2,3]

The WHO has recently published two core documents addressing the critical importance of preventing the emergence of drug resistance, both underlining the relevance of managing MDR-TB adequately.[1,4-8] The WHO action framework "Towards tuberculosis elimination for low-incidence countries" presents eight priority action areas, two of which are focused on, namely, (action #5) optimizing the management of MDR-TB and (action #7) investing in research on new diagnostic tools and drugs.[1,4,6,9]

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However, the scientific and programmatic focus is presently on diagnosis and treatment of the disease, whereas post-cure follow-up is seen as an approach to evaluate the proportion of relapse, particularly in MDR-TB/XDR-TB cases.

The role that tuberculosis plays as a public health care priority, as well as the importance of diagnostic tools being available in order to evaluate the patients thoroughly, by means of their functional status—via spirometry, plethysmography, and determination of DLCO —arterial blood gas analyses, their capacity to perform exercise—via the six-minute walk test (6MWT)—the description of their lesions—via chest X-rays (CXRs) and CT—and their quality of life (QoL)—via the Saint George's Respiratory Questionnaire (SGRQ)—justifies the effort to consider what needs to be done when patients have completed their treatment successfully. This vision has ethical, clinical, organizational, programmatic, and economic implications.

To our knowledge, the follow-up of tuberculosis patients who completed their treatment has never been reviewed in a comprehensive manner in the literature. Therefore, the objective of the present study was to review the available evidence on this topic and to draw some conclusions regarding the future role of the "post-tuberculosis treatment" phase, which will potentially have an impact on several million cases every year around the globe.

METHODS

We carried out a non-systematic review of the literature based on a PubMed search using specific keywords, including various combinations of the terms "tuberculosis", "rehabilitation", "MDR-TB", "pulmonary disease", "obstructive lung disease", and "lung volume measurements". The reference lists of the most important studies were also retrieved in order to improve the sensitivity of the research. Manuscripts written in English, Spanish, and Russian were selected. The main areas of interest that we identified in order to describe the topic were as follows:

1. Tuberculosis sequelae following diagnosis and treatment of tuberculosis
2. Destroyed lung
3. Functional evaluation of sequelae
4. Pulmonary rehabilitation (PR) interventions, such as physiotherapy, long-term oxygen therapy (LTOT), and ventilation
5. MDR-TB

After describing each of these areas of interest, we will provide a summary of the evidence compiled from the literature search (Table 1) and concluding remarks.

TUBERCULOSIS SEQUELAE FOLLOWING DIAGNOSIS AND TREATMENT OF TUBERCULOSIS

Although the potential role of PR has been clearly underlined in a study discussing the role of the new WHO recommendations on shorter treatment regimens,[10] the concept that rehabilitation is a component of tuberculosis treatment is as old as that of sanatoria.[11,12] In 1964, Chapman and Hollander wrote that, based on their experience with 454 patients with active tuberculosis "placed on a program of intensive physical exercise, combined with chemotherapy," "the concept of minimum exercise and prolonged bed rest in the hospital and a prolonged convalescent period after discharge is no longer justified."[12]

In 2006, a group of authors in India[13] prospectively studied the clinical presentation and predictors of outcome in 116 patients with acute exacerbations of COPD who had to be admitted to the ICU and found that 28.4% of those had had pulmonary tuberculosis previously. Among those patients, some required invasive mechanical ventilation and a few died. The authors concluded that "an intriguing relationship" existed among smoking, pulmonary tuberculosis, and COPD "which merits further study."[13]

In 2010, Jordan et al.[14] wrote that "the global prevalence of bronchiectasis, a recognized sequela of tuberculosis, is unknown, but is by no means insignificant. The pathophysiology of chronic airflow obstruction in both of these diseases is poorly understood, but it is associated with an accelerated rate of loss in pulmonary function."

Hassan and Al-Jahdali[15] reported that "in addition to its acute clinical consequences, patients with pulmonary tuberculosis may be left with significant long-term sequelae," "associated with considerable morbidity, mortality, and health expenditure," and commented that both obstructive and restrictive functional abnormalities were present.

Shah and Reed[16] described, among the commonest complications of tuberculosis, "mycetomas developing within residual tuberculosis cavities, impaired pulmonary function, or focal neurologic deficits from tuberculomas," and, therefore, "public health tuberculosis programs and health systems require additional resources to provide comprehensive tuberculosis and post-tuberculosis care."

Bansal and Prasad[17] commented that "COPD, interstitial lung disease, tuberculosis, and lung cancer together are the leading causes of morbidity and mortality," which are "increasing all over the world"; they also stated that "early fatigue and breathlessness" make patients "socially isolated and depressed". Functional disability and repeated hospitalizations reduce the efficiency of the patients at home and at work place, being associated with increased expenditures and utilization of health care systems, which results in a socioeconomic burden. PR, an evidence-based, multidisciplinary, and comprehensive non-pharmacological intervention, has emerged as a recommended standard of health care for patients suffering from respiratory diseases. PR is advised for patients with chronic lung conditions who have dyspnea or other respiratory symptoms, reduced exercise tolerance, restriction in activities, or impaired health status despite optimal pharmacological treatment. Early leaders observed two centuries ago

Table 1. Summary of the major studies that performed functional evaluation of patients with pulmonary tuberculosis.

Authors, year	Study location (name)[a]	Participants[b] (n)	Cases investigated	Investigations performed	Major functional findings	Conclusions
Rhee et al., 2013[21]	South Korea	595	History of PTB	Spirometry; CXR, CT	Mean number of lobes involved: 2.59 ± 0.05. Pleural thickening in 54.1% of the patients. Significant correlation between the number of lobes involved and FVC% ($r = -0.24$; $p < 0.001$) and FEV_1% ($r = -0.21$; $p < 0.001$)	Decreased lung function, exacerbations, and progressive decline of FEV_1 were found in the patients with PTB "destroyed lung"
Lee et al., 2003[22]	South Korea	21 CAO patients vs. 11 COPD patients	History of PTB	Spirometry	Patients with CAO due to PTB vs. COPD patients: FVC (2.14 ± 0.73 L vs. 2.60 ± 0.69 L; p < 0:05); FVC% (57.97% ± 14.8% vs. 70.07% ± 14.8%; p < 0:01); and response rate/% of change in post-bronchodilator FEV_1 significantly lower in CAO patients	Bronchodilator therapy useful to treat CAO
Báez-Saldaña et al., 2013[23]	Mexico	127	Cured PTB	CXR and spirometry	123 patients (96.85%) with CXR abnormalities; degree of CXR abnormalities independently associated with decreases in FVC (0.07 L; 95% CI: −0.01 to −0.04); FEV_1 (0.07 L; 95% CI: −0.10 to −0.05); FVC% (2.48; 95% CI: −3.45 to −1.50); and FEV_1% (2.92; 95% CI: −3.87 to −1.97)	Spirometric results associated with degree of abnormalities on CXR assessed by a simple scoring method
Willcox et al., 1989[25]	USA	71	History of PTB	Spirometry	48 (68%) with evidence of airway obstruction	Treated PTB is a cause of significant CAO.
Hnizdo et al., 2000[26]	South Africa	2,137 (1 TB episode); 366 (2 TB episodes); and 96 (with 3 or + TB episodes)	History of PTB	Spirometry	Chronic airflow impairment (FEV_1 < 80% of predicted) detected in 18.4% (1 TB episode); 27.1% (2 TB episodes); and 35.2% (3 or + TB episodes)	PTB can cause chronic impairment of lung function, which increases incrementally with the number of TB episodes
Amaral et al., 2015[27]	Multinational (BOLD study)[c]	14,050	Self-reported history of TB	Spirometry	Airflow obstruction: aOR = 2.51 (95% CI: 1.83-3.42); and airflow restriction: aOR = 2.13 (95% CI: 1.42-3.19)	History of TB associated with both airflow obstruction and spirometric restriction
Jung et al., 2015[28]	South Korea (KNHANES study 2008-2012)[c]	14,967	History of PTB and no sequelae on CXR	CXR and spirometry	822 (5.5%) with a history of PTB vs. 14,145 (94.5%) without TB history: FVC% (84.9 vs. 92.6), FEV_1% (83.4 vs. 92.4), and FEV_1/FVC% (73.4 vs. 77.9); among the subjects with normal CXR, those with a history of PTB (296; 2.3%) had significantly lower FEV1% and FEV1/FVC than did those without a TB history (90.9 vs. 93.4 and 76.6 vs. 78.4, respectively)	Subjects with prior PTB, inactive lesions on CXR, or no TB sequelae can show impaired pulmonary function and increased respiratory symptoms
Sailaja et al., 2015[29]	India	56	Cured PTB	Spirometry	Obstructive, restrictive, and mixed patterns in 35 (62.50%), 9 (16.07%), and 12 (21.42%), respectively	PTB causes significant impairment of lung function, predominantly airway obstruction due to lung destruction and inflammation.
Pefura-Yone et al., 2014[30]	Cameroon	177	Cured PTB	Spirometry	Distal airflow obstruction ($FEF_{25-75\%}$ < 65%) in 67 (62.9%); and at least one chronic respiratory sign in 110 (62.1%)	$FEF_{25-75\%}$ < 65% is a useful instrument to evaluate post-TB distal airflow obstruction
Ralph et al., 2013[31]	Papua New Guinea	200 PTB patients vs. 40 healthy subjects	Smear-positive TB (direct sputum examination)	Spirometry, 6MWT, and SGRQ	Mean pre-treatment FEV_1% predicted (controls vs. PTB patients) = 92 vs. 63 (p < 0.0001); 6MWD = 497 m vs. 408 m (p < 0.0001); SGRQ = 0 vs. 36.9 (p < 0.0001) In PTB patients, post-treatment FEV_1% = 71 (p < 0.0001 vs. controls); 6MWD = 470 m (p = 0.02); and SGRQ = 4.3 (p < 0.0001).	Early TB detection and treatment are key in minimizing residual impairment.
Vecino et al., 2011[32]	USA	123	Culture-positive PTB	Spirometry	Spirometry at 20 weeks of treatment and at the end of it; mean changes: FVC = −0.02 L (95% CI: −0.09 to 0.06 L); FVC% predicted = −0.02% (95% CI: −2.17% to 2.12%); FEV_1 = 0 L (95% CI: −0.05 to 0.06L); FEV_1% predicted = −0.11% (95% CI: −1.82% to 1.60%)	Pulmonary impairment after PTB treatment was not associated with duration of delay in TB diagnosis and treatment and did not significantly change during follow-up

Table 1. Continued...

Authors, year	Study location (name)[a]	Participants[b] (n)	Cases investigated	Investigations performed	Major functional findings	Conclusions
Lee et al., 2011[33]	South Korea (KNHANES II study 2001)[c]	3,687	Evidence of PTB on CXR	CXR and spirometry	Evidence of PTB on CXR is associated with airflow obstruction (aOR = 2.56; 95% CI: 1.84- 3.56)	PTB is an independent risk factor for obstructive lung disease in never-smokers.
Chung et al., 2011[34]	Taiwan	115	Culture-positive PTB	Spirometry	Nadir of pulmonary function occurs approximately 18 months after treatment completion; risk factors for pulmonary function deterioration were positive sputum smear microscopy, extensive pulmonary involvement, prolonged anti-TB treatment, and reduced improvement on CXR after treatment	In patients with significant respiratory symptoms and multiple risk factors, pulmonary function tests should be used to monitor progression of functional impairment
Lam et al., 2010[35]	China (Guangzhou Biobank Cohort Study)	8,066 (73.6% women and 26.4% men)	Evidence suggestive of inactive PTB on CXR	CXR and spirometry	Prevalence of prior TB was 24.2%; prior PTB is associated with airflow obstruction (aOR = 1.37; 95% CI: 1.13-1.67); smoking did not modify this relationship	Prior TB is an independent risk factor for airflow obstruction, regardless of smoking status
Baig et al., 2010[36]	Pakistan	92	History of PTB with chronic exertional dyspnea with or without cough. Evidence of inactive PTB on CXR	Spirometry	26 (55.3%) with obstructive pattern [severe/stage III in 18 (69.2%); moderate/stage II in 6 (23.0%); mild/stage I in 2 (5.9%)]; 14 (29.7%) with restrictive pattern; and 7 (14.8%) with mixed pattern	COPD can occur as one of the chronic complications of PTB
Pasipanodya et al., 2007[37]	USA	107 PTB patients and 20 LTBI patients	Culture-positive PTB or PTB and extrapulmonary TB patients who had completed at least 20 weeks of therapy	Spirometry	PTB survivors are more likely to have abnormal spirometric results than patients with LTBI (aOR = 5.4; 95% CI: 2.98-9.68)	Pulmonary impairment after TB is associated with disability worldwide and demands more aggressive prevention strategies and post-treatment evaluation
Menezes et al., 2007[38]	Mexico, Venezuela, Brazil, Uruguay, Chile (PLATINO study)[c]	5,571	Self-reported history of PTB	Spirometry	Airflow obstruction in 30.7% of the sample; history of PTB is associated with airflow obstruction (aOR = 2.33; 95% CI: 1.50-3.62)	History of PTB is associated with airway obstruction
de la Mora et al., 2015[39]	Mexico	70	Cured PTB	Spirometry and CAT	Nonreversible chronic obstruction in 24 (34.3%): mean post-bronchodilation FEV_1 = 1.32 ± 0.6 L and 57.30% ± 9.95% of predicted; CAT score = 15.1 ± 10.4	Functional abnormalities are frequent in PTB patients
Di Naso et al., 2011[53]	Brazil	37 (25 DS-PTB vs. 12 MDR-PTB)	Cured PTB	CXR, spirometry, 6MWT, and pressure manometry	Severe combined respiratory disorder was the more prevalent in MDR-PTB patients (9/12); MDR-PTB group (vs. DS-PTB) showed significantly lower values in FVC% of predicted (43.58 ± 16.03 vs. 72.06 ± 14.95); FEV_1% of predicted (33.08 ± 15.64 vs. 66.13 ± 19.87); MIP (49.58 ± 12.55 cmH_2O vs. 68.40 ± 22.78 cmH_2O); MEP (59.08 ± 12.23 cmH_2O vs. 87.20 ± 27.30 cmH_2O); and 6MWD (334.75 ± 104.07 m vs. 484.21 ± 74.01 m)	Patients with MDR-PTB who have undergone multiple treatments have more severe respiratory and functional impairment than do patients who have had just a single treatment
de Vallerière et al., 2004[54]	South Africa	33 MDR-PTB	Cured PTB	CXR and spirometry	Cavitation was present in more than half of the patients. 31 (94%) with abnormal lung function tests: restrictive pattern in 14 (42%); combined pattern in 13 (39%); obstructive pattern in 4 (12%); and normal spirometric results in 2 (6%)	Residual lung damage in MDR-PTB patients who completed treatment is common and extensive

PTB: pulmonary tuberculosis; CXR: chest X-ray; CAO: chronic airway obstruction; TB: tuberculosis; aOR: adjusted odds ratio; 6MWT: six-minute walk test; SGRQ: Saint George's Respiratory Questionnaire; 6MWD: six-minute walk distance; LTBI: latent TB infection; CAT: COPD assessment test; DS-PTB: drug-susceptible PTB; and MDR-PTB: multidrug-resistant PTB. [a]BOLD: Burden of Obstructive lung disease; KNHANES: Korean National Health and Nutrition Examination Surveys; and PLATINO: *Proyecto Latinoamericano de Investigación en Obstrucción Pulmonar*. [b]All studies included both men and women. [c]Population-based studies.

that exercise is an important element in the care of patients with lung and heart diseases, especially in tuberculosis.[17]

In a recent American Thoracic Society (ATS)/European Respiratory Society (ERS) statement, as well as in a guideline from South Africa for the management of COPD, tuberculosis is clearly among the diseases that require the use of PR.[18,19]

"DESTROYED LUNG"

Late diagnosis is often responsible for extensive bilateral lesions, usually due to bronchiectasis, scarring, parenchymal deformation, lung volume loss, and pleural thickening, which might develop to the so-called "destroyed lung" (Figure 1).[20]

Two studies described the effect of "destroyed lung" on the pulmonary function of patients treated for pulmonary tuberculosis, both carried out in South Korea.[21,22] Rhee et al.[21] studied 595 tuberculosis patients from 21 hospitals between 2005 and 2011. The mean extension of the lesions was 2.59 ± 0.05 lobes, and pleural thickening was observed in 54.1% of the patients. Various lung function parameters were reduced (mean values): FVC = 2.06 ± 0.03 L (61.26% ± 0.79% of predicted); FEV_1 = 1.16 ± 0.02 L (49.05% ± 0.84% of predicted); FEV_1/FVC ratio = 58.0% ± 0.70%; bronchodilator response = 5.70% ± 0.34%; and number of exacerbations/year = 0.40 ± 0.04. The number of lobes involved significantly correlated with FVC, FEV_1, and the number of exacerbations/year. The use of long-acting muscarinic antagonists or long-acting β_2 agonists plus inhaled corticosteroids achieved bronchodilator effects. Initial FEV_1% and the number of exacerbations during follow-up were independent factors affecting FEV_1 deterioration in the multivariate analysis.

Lee et al.[22] investigated lung function and post-bronchodilator response in 21 patients with "destroyed lung"-related chronic airflow obstruction against a cohort of COPD patients matched by sex, age, and pulmonary function parameters. The mean FVC values (both in L and in % of predicted) of the patients with "destroyed lung" were significantly lower than those of the COPD patients (2.14 ± 0.73 L vs. 2.60 ± 0.69 L and 57.9% ± 14.8% vs. 70.0 ± 14.8%, respectively). The tuberculosis patients presented with significantly lower FVC and post-bronchodilator FEV_1 than did the COPD patients. In addition, among the tuberculosis patients, those with wheezing symptoms showed significantly lower $FEF_{25-75\%}$ and higher airway resistance than did those without wheezing. Tuberculosis patients with wheezing responded better to the bronchodilator than did those without it. The authors concluded that bronchodilator therapy could be useful in those patients.

In Mexico, 127 cured tuberculosis patients underwent spirometry and CXR; 123 (96.85%) exhibited some degree of radiographic abnormalities.[23] The extent of lung damage was measured by dividing the lung parenchyma into four quadrants and scoring it from 0 to 5; the mean number of radiographic abnormalities was 6.45 ± 4.14. In that sample, 30 patients (24%) showed an obstructive spirometric pattern, and 22 (17%), a restrictive pattern; only 15 (12%) had a positive bronchodilator test, and 21 (17%) had an SpO_2 < 90%. The adjusted multilinear regression model showed that the degree of radiographic abnormalities was independently associated with a decrease in the absolute values of FVC (0.07 L; 95% CI: −0.01 to −0.04) and FEV_1 (0.07 L; 95% CI: −0.10 to −0.05; $p < 0.001$); as well as in their % of predicted values (FVC = 2.48%; 95% CI: −3.45 to −1.50; and FEV_1 = 2.92%; 95% CI: −3.87 to −1.97). That study showed that spirometric values were associated with the degree of radiographic abnormalities assessed by a simple scoring method.

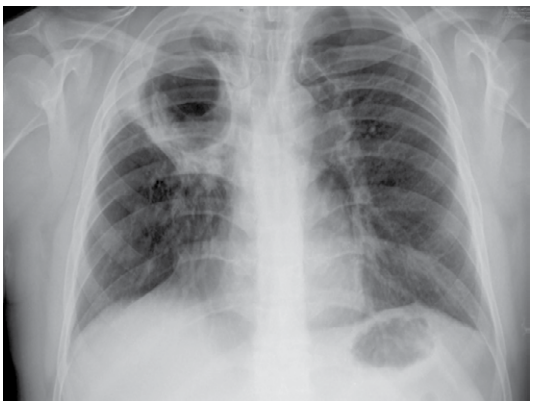


Figure 1. Chest X-ray of a 39-year-old male patient with a history of pan-susceptible tuberculosis treated for six months in 2007. The patient was considered cured. Later in time, he reported a six-month history of cough, mild dyspnea, but no fever. Tuberculosis relapse was ruled out; sputum smear microscopy and culture were negative. The image shows a giant cavity in the right upper lobe and some fibrotic changes.

FUNCTIONAL EVALUATION OF SEQUELAE

Several studies investigated the mechanical lung function in tuberculosis patients (Figure 2).

Already in 1961, Hallet and Martin[24] described the diffuse obstructive pulmonary syndrome (measured via the maximal expiratory flow rate) in 34% of 710 tuberculosis patients admitted to a sanatorium during a one-year period. The factors significantly associated with the incidence of that syndrome were age, severity of tuberculosis, and some comorbidities (bronchial asthma, pulmonary malignancy, frequent and protracted chest colds, and silicosis). The authors concluded that the measurement of maximal expiratory flow rate is a useful tool in determining diffuse obstructive pulmonary disease.

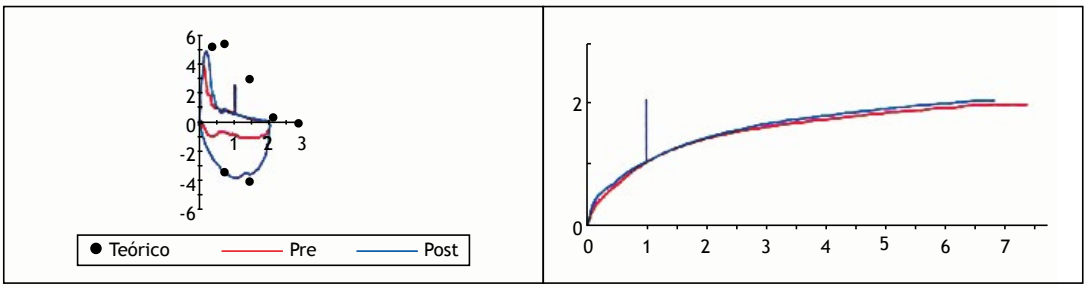
Willcox and Ferguson[25] investigated 71 patients previously treated for tuberculosis up to 16 years prior. Evidence of airway obstruction was found in 48 (68%) of the patients. An inverse relationship between the extent of the disease on the original CXRs and FEV_1

was identified. The authors identified a similar inverse relationship of the amount of sputum produced with FEV_1 and with the extent of the disease on the CXRs. The authors concluded that treated tuberculosis is a cause of COPD.

In a large study in South Africa,[26] a cohort was followed in order to study the chronic effect of initial and recurrent pulmonary tuberculosis: 27,660 black South African gold miners who had reliable pulmonary function test results between January of 1995 and August of 1996 were retrospectively followed for the incidence of tuberculosis to 1970. In that cohort of miners, 2,137; 366; and 96 had had, respectively, one, two, and three or more episodes of tuberculosis. The mean time between the diagnosis of the last episode of tuberculosis and lung function testing was 4.6 years (range: 1-372 months). The loss of lung function was the highest within the first 6 months after tuberculosis being diagnosed and stabilized after 12 months, when the loss was considered chronic. The estimated mean deficits in FEV_1 after one, two, and three or more episodes of tuberculosis were 153 mL, 326 mL, and 410 mL, respectively, whereas the corresponding deficits in FVC were 96 mL, 286 mL, and 345 mL. The loss of lung function was similar in HIV-positive and HIV-negative individuals. The proportion of individuals with chronic airflow impairment (FEV_1 < 80% of predicted) was 18.4%, 27.1%, and 35.2%, respectively, in those with one, two, and three or more episodes of tuberculosis. The authors concluded that tuberculosis can cause chronic impairment of lung function, which increases with the number of episodes of the disease, and that early diagnosis and treatment of tuberculosis coupled with the prevention of HIV, silica dust exposure, silicosis, and poverty are important interventions.

In a recent multicenter, cross-sectional, general population-based study,[27] the association of having a history of tuberculosis with airflow obstruction and spirometric abnormalities was studied in adults. A self-reported history of tuberculosis was associated with airflow obstruction (adjusted OR = 2.51; 95% CI: 1.83-3.42) and spirometric restriction (adjusted OR = 2.13; 95% CI: 1.42-3.19). The authors concluded that a history of tuberculosis was associated with both airflow obstruction and spirometric restriction, and should be considered as an important cause of obstructive disease and impaired lung function, particularly where tuberculosis is common.

In a study performed in South Korea between 2008 and 2012,[28] lung function impairment and persistency of respiratory symptoms were studied in 14,967 adults with and without a history of pulmonary tuberculosis. The adults were also divided into two groups: those showing residual sequelae on CXR and those without showing it in order to determine the risk factors for airflow obstruction. Among the population studied, 822 participants (5.5%) had been treated for pulmonary tuberculosis (mean) 29.0 years prior to study initiation. The individuals with a history of tuberculosis, when compared with those without that, presented with significantly lower FVC% (84.9 vs. 92.6), FEV_1% (83.4 vs. 92.4), and FEV_1/FVC% (73.4 vs. 77.9). Among the 12,885 subjects with no sequelae on CXR, those with a history of pulmonary tuberculosis (n = 296; 2.3%) had significantly lower FEV_1% (90.9 vs. 93.4) and FEV_1/FVC% (76.6 vs. 78.4). Subjects with a history of pulmonary tuberculosis but no sequelae on CXR reported a significantly higher frequency of cough and physical activity limitations due to pulmonary symptoms than those without that history ($p < 0.001$ for both). A history of pulmonary tuberculosis (OR = 2.314), along with older age, male gender, asthma, and smoking were independent risk factors for airflow obstruction. Finally, the study suggested that inactive tuberculosis lesions on CXR (OR = 2.3) were risk factors for airflow obstruction in subjects with a history of pulmonary tuberculosis. The authors concluded that the patients treated for tuberculosis should undergo regular lung function testing and stop smoking in order to prevent chronic airway disease.



	Pre-Bronch			Post-Bronch		
	Real	Teórico	%Teórico	Real	%Teórico	%Cambio
--ESPIROMETRÍA--						
FVC (L)	1.99	2.85	69	2.04	71	+2
FEV1 (L)	1.05	1.63	64	1.06	65	+1
FEV1/FVC (%)	53	71	74	52	73	-1

Figure 2. Spirometry of the same patient shown in Figure 1. FEV_1/FVC ratio was below 70%. FEV_1 was decreased and unresponsive to bronchodilator. FVC was also diminished. Fixed airway obstruction was detected, and mild restriction was considered. The final diagnosis was pulmonary sequelae of tuberculosis. Espirometría: spirometry; teórico: predicted; pre/post bronch: pre-/post-bronchodilator; real: observed; and cambio: change.

In a recent study,[29] 56 treated tuberculosis patients who were considered cured underwent simple spirometry, and pre- and post-bronchodilator FEV_1, FVC, FEV_1/FVC ratio were recorded. Obstructive, restrictive, and mixed patterns were identified in 62.50%, 16.07%, and 21.42% of the patients, respectively.

In a cross-sectional study[30] involving 177 individuals who had been previously treated for tuberculosis in Cameroon between 2012 and 2013, spirometry was performed in order to evaluate the clinical impact of low $FEF_{25-75\%}$. Distal airflow obstruction (DAO) was defined by an $FEF_{25-75\%} < 65\%$ and a FEV_1/FVC ratio ≥ 0.70. At least one chronic respiratory sign was present in 110 (62.1%) of the participants, and DAO was identified in 67 (62.9%). Duration of symptoms prior to the diagnosis of tuberculosis > 3 months (adjusted OR = 2.91) and presence of DAO (OR = 2.22) were independent determinants that were significantly associated with persisting respiratory signs. The authors concluded that $FEF_{25-75\%} < 65\%$ is a useful instrument to evaluate post-tuberculosis DAO.

In Papua New Guinea, presently a hot spot for MDR-TB, a study[31] evaluated morbidity during treatment and residual pulmonary disability in pulmonary tuberculosis cases undergoing spirometry, 6MWT, and evaluation of QoL (SGRQ). The authors evaluated 200 pulmonary tuberculosis patients (at baseline and after 6 months of treatment) and 40 healthy volunteers. The distance walked in the 6MWT (6MWD) was 497 m in controls vs. 408 m in tuberculosis patients at baseline ($p < 0.0001$) and 470 m after 6 months ($p = 0.02$), whereas the SGRQ score was zero in controls vs. 36.9 in tuberculosis patients at baseline ($p < 0.0001$) and 4.3 after 6 months ($p < 0.0001$). The mean predicted FEV_1 was 92% in controls vs. 63% among tuberculosis patients at baseline ($p < 0.0001$) and 71% after 6 months ($p < 0.0001$). After six months of treatment, 27% of the tuberculosis patients still showed at least moderate-to-severe pulmonary function impairment, and 57% had respiratory symptoms, although most of them achieved "successful" treatment outcomes and self-reported good QoL. More advanced disease at baseline (longer illness duration and worse results on CXR at baseline) and HIV-positive status predicted residual disability. The authors concluded that early detection and treatment of tuberculosis are key in minimizing residual impairment.

Post-pulmonary tuberculosis impairment was studied after 20 weeks of tuberculosis treatment and again on, or after, treatment completion.[32] The median duration between the first and the second spirometry was 15 weeks. The mean change in FVC was −0.02 L (95% CI: −0.09 to 0.06 L), and that in FVC% of predicted was −0.02% (95% CI: −2.17% to 2.12%), whereas that in FEV_1 was 0 L (95% CI: −0.05 to 0.06), and that in FEV_1% of predicted was −0.11% (95% CI: −1.82 to 1.60). Pulmonary impairment was not related to the delay in tuberculosis diagnosis or treatment, older age, or smoking habits.

The relationship between previous tuberculosis and the risk of COPD was studied in South Korea in a population-based investigation[33] involving 3,687 individuals performing spirometry and CXR. Among those, 294 subjects had radiological evidence of previous tuberculosis with no evidence of active disease. Radiological evidence of previous tuberculosis was independently associated with airflow obstruction (adjusted OR = 2.56) after adjustments for sex, age, and smoking history. Previous tuberculosis was still a risk factor (adjusted OR = 3.13) with the exclusion of ever-smokers or subjects with advanced radiological lesions. Among the never-smokers, the proportion of subjects with previous tuberculosis on CXR increased as obstructive lung disease became more severe. The authors concluded that previous tuberculosis is an independent risk factor for COPD, even in never-smokers.

The trends toward deterioration of pulmonary function and its risk factors were studied in 115 patients with pulmonary tuberculosis after treatment completion.[34] A model with a locally weighted scatterplot smoothing technique was used in order to evaluate the trends toward changes in pulmonary function. The median interval between the end of antituberculosis treatment and pulmonary function testing was 16 months. The nadir of pulmonary function occurred approximately 18 months after completion of the treatment. The risk factors associated with pulmonary function deterioration included positive sputum smear microscopy, extensive pulmonary involvement prior to antituberculosis treatment, prolonged antituberculosis treatment, and poor radiographic improvement after treatment. The authors concluded that pulmonary function testing should be used as a follow-up tool in order to monitor the progression of functional impairment, especially within the first 18 months after the completion of antituberculosis treatment.

A study in China[35] investigated the relationship between history of tuberculosis, smoking, and airflow obstruction in a population sample of 8,066 participants in the Guangzhou Biobank Cohort Study. The participants underwent spirometry, CXR, and a structured interview on lifestyle and exposures. Prior tuberculosis was defined as the presence of radiological evidence suggestive of inactive tuberculosis. In that sample, 24.2% of the individuals had a history of tuberculosis. After controlling for sex, age, and smoking exposure, prior tuberculosis remained independently associated with an increased risk of airflow obstruction (OR = 1.37; 95% CI: 1.13-1.67). Further adjustments for exposure to passive smoking, biomass fuel, or dust did not alter that association. Smoking did not modify the association between prior tuberculosis and airflow obstruction. The authors concluded that prior tuberculosis is an independent risk factor for airflow obstruction, which might partly explain the high prevalence of COPD in China.

In a study in Pakistan,[36] the prevalence of COPD was studied in 47 patients previously treated for pulmonary

tuberculosis and reporting chronic exertional dyspnea with no other apparent cause. Of the 47 patients, 26 (55.3%) showed an obstructive pattern on spirometry (severe in 18, moderate in 6, and mild in 2), whereas 14 (29.7%) were found to have a restrictive pattern, and 7 (14.8%) revealed a mixed obstructive and restrictive pattern.

In a case-control study,[37] the lung function of 107 prospectively identified patients with pulmonary tuberculosis who had completed at least 20 weeks of therapy was compared with that of 210 patients with latent tuberculosis infection (LTBI). Impairment was present in 59% of the tuberculosis patients and in 20% of the LTBI control subjects. In comparison with the controls, the pulmonary tuberculosis patients showed significantly lower FVC, FEV_1, FEV_1/FVC ratio, and mid-expiratory phase of FEF. A VC < 50% of predicted was found in 10 (9.40%) and 1 (0.53%) of the patients, respectively, in the pulmonary tuberculosis and LTBI groups. In addition, a VC between 20% and 50% of the predicted was found in 42 (39%) and in 36 (17%) of the patients in the same groups, respectively. After adjusting for risk, tuberculosis survivors were 5.4 times more likely to have abnormal lung function test results than were LTBI patients (p > 0.001; 95% CI: 2.98-9.68). Lung damage was more common in cigarette smokers; however, after adjusting for demographic and other risk factors, that difference was not significant. The authors concluded that "microbiological cure is the beginning, not the end of their illness".

In a population-based multicenter study conducted in five Latin American cities including 5,571 subjects, a self-reported history of pulmonary tuberculosis was clearly associated with varying degrees of airflow obstruction, defined by a post-bronchodilator FEV_1/FVC ratio < 0.7. In that study, 30.7% of subjects with a history of tuberculosis presented with airflow obstruction vs. 13.6% of those without that history. The association between a self-reported history of tuberculosis and the presence of airflow obstruction remained unchanged even after adjustments for confounding variables (adjusted OR = 2.33; 95% CI: 1.50-3.62).[38]

In Tijuana, Mexico,[39] 70 cured pulmonary tuberculosis patients were evaluated in order to determine the prevalence and the severity of COPD and its impact on QoL. Among those patients, 24 (34.3%) had nonreversible chronic airway obstruction (mean post-bronchodilation FEV_1 = 1.3 ± 0.6 L). In addition, patients with chronic airway obstruction had a COPD assessment test score of 15.1 ± 10.4—a score ≥ 10 points indicates a significant impact on QoL. The authors concluded that functional abnormalities are frequent in tuberculosis patients, and those with chronic airway obstruction are often symptomatic and experience a significant impact on their QoL.

PR INTERVENTIONS

The mechanisms behind lung damage following tuberculosis and its treatment have been described by Zhuk,[40] who underlined the advantages of PR and who identified that about 50% of those patients undergo PR programs during hospital admissions in Russia.

In an experience in Japan,[41] the effectiveness of PR was evaluated for a mean period of 3.9 weeks in 37 inpatients with pulmonary tuberculosis sequelae. The PR program included relaxation, breathing retraining, exercise training, respiratory muscle training, and educational support. Mean VC improved significantly (n = 37), from 1.48 L to 1.59 L, whereas FEV_1 (n = 37) improved from 0.93 L to 1.02 L, as well as did PaO_2 (n = 35), from 67.1 Torr to 72.4 Torr. The gain in the 6MWD (n = 29) increased from 303 m to 339 m, and MIP (n = 17) increased from 38.5 cmH_2O to 47.5 cmH_2O. There were also improvements in activities of daily living, dyspnea symptoms, and QoL. The effects of PR were independent from previous thoracic surgery for tuberculosis, pattern of ventilatory impairment, findings on CXR, or degree of respiratory insufficiency. The study results suggested that PR is effective in improving pulmonary function, exercise tolerance, symptoms, and QoL in patients with pulmonary tuberculosis sequelae.

A group of authors in Colombia[42,43] investigated the effects of PR on aerobic capacity and health-related QoL in patients with sequelae of pulmonary tuberculosis who participated in an eight-week PR program within a public hospital. The studies included a pre- and post-test design without a control group, and it involved 8 participants intentionally selected from a public program. The program included physical training (upper and lower limb strengthening and aerobic component), education on tuberculosis, and training on activities of daily living. A treadmill-based training protocol for the lower limbs was established, starting with an initial intensity load of 60%, and then increasing up to a load of 85%; the peak oxygen consumption (VO_{2peak}) was set at 90%. The training sessions were carried out three times a week for eight weeks, and each lasted one hour, which included initial examination, warm-up, exercise protocol, and stretching exercises. Outcome measures (VO_{2peak}, 6MWD, and two QoL questionnaires—the Medical Outcomes Study 36-item Short-Form Health Survey [SF-36] and SGRQ)—were performed prior to the first training session and at eight weeks. Comparing baseline and final results, the VO_{2peak} increased by 1.7 mL/kg/min (p = 0.039), and the 6MWD increased by 63.6 m (p = 0.014). The QoL questionnaire scores increased as well: SF-36 physical domain score increased by 6.98 points (p = 0.039), whereas the SGRQ score increased by 13 points (p = 0.039). The authors concluded that the PR program in that sample of patients with pulmonary tuberculosis sequelae resulted in significant improvements in both aerobic capacity and QoL.

A single-blinded randomized controlled study was performed at a clinic in Khayelitsha, Western Cape, South Africa, in order to assess the effects of a six-week home-based PR program in patients receiving treatment for pulmonary tuberculosis.[44] The program included

baseline and post-rehabilitation measurements of lung function (spirometry), of exercise tolerance (6MWT and Borg exercise exertion scale), and of health-related QoL (EuroQoL—EQ-5 D—questionnaire) in 34 patients receiving outpatient treatment for tuberculosis and in 33 controls. When compared with the controls, there were improvements in the lung function (FEV_1 and FVC), exercise tolerance, and QoL in the tuberculosis patients, although statistical significance was not reached at the end of the six-week PR program. The authors concluded that the rationale for using a PR program for patients with pulmonary tuberculosis is valid and that further evidence is needed.

In a prospective nonrandomized open trial over a nine-week period conducted in Japan,[45] the effect of PR on patients with post-tuberculosis lung disorders was compared with that on patients with COPD. The post-tuberculosis group comprised 32 patients (25 of whom had undergone thoracoplasty; mean age = 71 ± 5 years; and mean FEV_1 = 0.84 ± 0.29 L) who were compared with 32 age-matched and FEV_1-matched COPD patients (controls). First, the two groups were compared regarding their exercise tolerance (6MWT). Then, the patients were trained to undergo a nine-week outpatient PR program. Improvements were assessed by using clinical dyspnea ratings, a daily activity score, and the results of the 6MWT. When age and FEV_1 were matched, the 6MWD did not differ between the study and control groups. After the PR program, significant improvements were observed in the two groups regarding the Medical Research Council dyspnea scale, transition dyspnea index, and daily activity scores, as well as in the 6MWD—study group = 42 m ($p < 0.01$) vs. control group = 47 m ($p < 0.01$). The gain in the various parameters was comparable between the groups. The authors concluded that the PR program is as beneficial in post-tuberculosis patients with lung disorders as in COPD patients if the severity of the disability is similar.

LTOT AND VENTILATION

The importance of LTOT was investigated in Japan, together with the relevance of tuberculosis as a disease that demands post-treatment rehabilitation.[46] The importance of ventilation to improve the performance of tuberculosis patients with sequelae was also studied.

In a study involving 7 patients with pulmonary tuberculosis sequelae and severe restrictive ventilatory defect,[47] nasal intermittent positive pressure ventilation (NIPPV) was applied during exercise in order to determine whether arterial blood gas measurements, breathlessness, and exercise endurance could be improved. The authors reported that NIPPV significantly prolonged exercise endurance time and decreased breathlessness in all of the patients, as well as it significantly improved arterial blood gas measurements.

Yang et al.[48] described the positive effects of respiratory support with a poncho (wraparound) ventilator and mouthpiece intermittent positive pressure ventilation on a 44-year-old patient affected by severe restrictive lung disease secondary to right phrenic nerve crush/pneumoperitoneum and left pneumonectomy/decortication for bilateral lower lobe tuberculosis. The patient developed dyspnea, coryza, and somnolence. With the assistance of the two respiratory devices, the patient was able to complete her education, get married, and lead a fulfilling life in the community.

PHYSIOTHERAPY

In 2004, Strelis et al.[49] proposed a vibration massage-based method to prevent early postresection complications after surgical interventions due to tuberculosis. The method included the use of a light vibromassage apparatus that allowed systemic physiotherapy involving electric vibroacupressure of the whole circumference of the chest. In that case-control study,[49] early postresection complications were significantly less frequently observed in the study group than in the control group (60 vs. 50 patients). The procedure reduced the likelihood of development of a number of pleuropulmonary events (atelectasis, nonspecific pneumonia, residual postresection pleural cavity, and bronchial fistulas) and enhanced the functional status of the patients.

MDR-TB

The majority of the studies included in the present review article reported drug-susceptible cases. Only three studies discussed PR interventions in MDR-TB patients.

In a cross-sectional cohort study in Brazil,[50] respiratory function, functional capacity, and QoL were investigated in 18 patients who had been treated for pulmonary MDR-TB for 18 months or more. The subjects underwent the following assessments: forced spirometry, CXR, 6MWT, bioelectrical impedance analysis, MIP, and MEP. They also completed a health-related QoL questionnaire. Spirometric evaluation showed that 78% of the subjects had abnormal ventilatory patterns. All of the subjects presented with significantly decreased MIP and MEP, despite the fact that their nutritional status was within the normal range. In 72% of the subjects, the 6MWD was lower than expected, and residual lesions were present in 100%, whereas 78% reported a worsening in their QoL. The authors concluded that patients achieving MDR-TB cure present impaired respiratory function, as well as mildly reduced functional capacity and QoL, suggesting that a portion of these patients might require PR.

In a cross-sectional study in India,[51] 130 MDR-TB patients who had initiated treatment were evaluated between 2002 and 2006. During the study period, 24 patients died, and 63 (59%) could be traced, of whom 51 were alive. Those patients had completed a mean post-treatment period of 24.0 ± 14.7 months (range, 6-63 months), 40 (78%) had persistent respiratory symptoms, and 50 (98%) had residual sequelae on CXR (40% of which being severe). Abnormal pulmonary

function test results were observed in 45 (96%) of the patients, predominantly with a mixed type ventilatory impairment in 31 (66%), a pure restrictive pattern in 9 (19%), and a pure obstructive pattern in 5 (11%). The authors concluded that functional impairment and radiological lesions are common in patients after completing MDR-TB treatment.

In a case report in Colombia,[52] a patient with MDR-TB underwent a PR program. After the completion of the program, there was improvement in the 6MWD (from 240 m to 350 m), in the Medical Research Council dyspnea scale score (from 4 to 1), and in the Borg scale (from 7 to 0). Furthermore, his upper and lower limb muscle strength increased from 3 to 4. The authors concluded that a period of PR lasting 8-10 weeks was sufficient to improve his functionality.

A cross-sectional study carried out in Brazil[53] compared functional and respiratory changes between patients with a single episode of tuberculosis and MDR-TB patients who had had multiple episodes before receiving an effective treatment. The MDR-TB group showed significantly lower values in FVC (72.06% ± 14.95% vs. 43.58% ± 16.03% of predicted), FEV_1 (66.13% ± 19.87% vs. 33.08% ± 15.64% of predicted), and 6MWD (484.21 m ± 74.01 m vs. 334.75 m ± 104.07 m). The study[53] demonstrated the existence of significant functional limitations in MDR-TB patients who had undergone multiple tuberculosis treatments and strengthened the importance to prevent treatment noncompliance and subsequent rescue regimens.

In a study in Limpopo Province, South Africa,[54] 33 MDR-TB patients performed spirometry: 14 (42%) had a restrictive pattern, 4 (12%) had obstructive disease, and 13 (39%) showed a combined pattern, although no further studies were performed to corroborate the presence of restriction. In the linear regression analysis, FEV_1 and FVC (both in % of predicted) were negatively associated with the time between the first diagnosis of tuberculosis and the completion of treatment (mean time, 51.8 months). The authors concluded that residual lung damage in MDR-TB patients is common and that extensive efforts should be made in order to ensure rapid diagnosis and treatment.[54]

FINAL CONSIDERATIONS

The present review of the evidence available in the literature suggests that tuberculosis is definitively responsible for lung function sequelae, most of which causing an obstructive pattern, although restrictive and mixed patterns are also present.

Unfortunately, few studies are available in the literature investigating the physiopathology of obstruction, the potential need for PR, and the effects of a PR program. The majority of the studies investigated the functional status by spirometry, a few of them by plethysmography, whereas evidence based on determination of DLCO, arterial blood gas analyses, walk tests, and QoL is anecdotal.

The vast majority of the available studies included patients with drug-susceptible tuberculosis. Details on the characteristics of tuberculosis are rarely complete; in particular, information on the microbiological confirmation of the cases (culture or, at least, sputum smear microscopy) is rarely reported, since most studies tend to focus on the physiopathological aspects of the patients studied. We have found no studies in which a diagnosis of tuberculosis was based on rapid molecular assays, such as Xpert™ MTB/RIF as of today.[55]

Very few studies reported MDR-TB cases, and we have found no studies that investigated whether there is any difference in sequelae between drug-susceptible tuberculosis and MDR-TB cases. The latter patients need a much longer period of treatment (18-24 months in comparison with the tentative 6 months for drug-susceptible cases) and have usually completed more than one previous course of treatment with first or second-line antituberculosis drugs. The impact of the shorter tuberculosis regimen (known as the Bangladesh regimen), which has a duration comparable to that for drug-susceptible tuberculosis (9 months), could not be evaluated since it was recommended by WHO only in May of 2016.[10,56,57]

Interestingly, although all studies identified that tuberculosis plays a significant role in deteriorating pulmonary function, the additional role of smoking as a factor that creates additional lung function impairment needs to be studied further.

It is recommended that any future evaluation of tuberculosis and MDR-TB sequelae include complete information on

a) The characteristics of the patients (age, sex, ethnicity, etc.)
b) A complete description of the disease, including history of previous treatments, bacteriological status, pattern of drug resistance, and history of current treatment (drugs and regimen) with an emphasis on adverse events and their management
c) A complete description of the physiopathological status of the patients, including spirometry (and bronchodilator response), assessment of lung volumes (plethysmography or others), determination of DLCO, arterial blood gas analysis, 6MWT, radiological evaluation (ideally including CXR), and QoL evaluated with a general instrument and a specific respiratory instrument (SGRQ or others)
d) Rationale and consistence of the proposed PR plan, with clear pre- and post-test comparisons and evaluation of costs
e) Ideally, further studies should include the number of patients who need PR, since this will help to estimate the need for PR planning

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Prominent bronchial vasculature, hemoptysis, and bilateral ground-glass opacities in a young woman with mitral stenosis

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A 22-year-old woman presented with hemoptysis and dyspnea (respiratory rate, 38 breaths/min; peripheral oxygen saturation, 81%; pro-brain natriuretic peptide, 2,073 ng/L), together with bilateral inspiratory crackles and a diastolic murmur over the apex. Imaging showed multifocal consolidations surrounded by ground-glass opacities, predominantly in the upper lobes (Figure 1), and no pulmonary embolism. Bronchoscopy showed no endobronchial blood but prominent bronchial vasculature on the left (Figure 2). The BAL fluid was consistently bloody (90% hemosiderin-laden alveolar macrophages). Echocardiography detected severe mitral stenosis with classic "hockey stick" morphology of the anterior leaflet and mild aortic regurgitation. The mean pulmonary artery pressure was 48 mmHg, and the pulmonary artery wedge pressure was 32 mmHg. Mitral valve reconstruction was performed.

Mitral stenosis can provoke hemoptysis.[1] Dilatation of the bronchial vasculature may be the first hint of elevated left atrial pressure. The bronchial venous plexus arising from the bronchial arterial circulation is connected to the pulmonary venous circulation. Two thirds of the blood from the venous plexus returns to the pulmonary veins and then to the left atrium.[2,3] An increase in pulmonary venous pressure leads to a reverse flow of blood from the pulmonary veins to the bronchial venous plexus, visible as engorged bronchial vasculature.

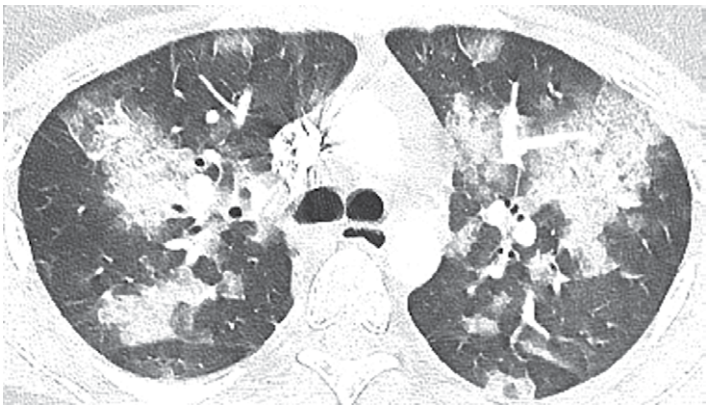

Figure 1. CT of the chest at the level of the main carina showing bilateral consolidations surrounded by ground glass opacities.

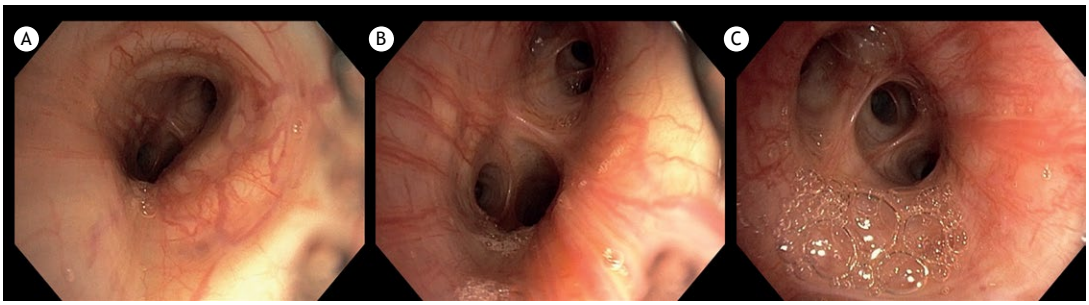

Figure 2. In A, fiberoptic bronchoscopy image of the main left bronchus. In B, a closer view at the left secondary upper lobe carina. In C, a close-up of the left superior bronchus showing the lingula and left upper lobe segments. The bronchial mucosa is edematous and contains a network of prominent blood vessels. The vessels are dilated and engorged to a variable extent. At some sites, the vessels are focally distributed, although most parts of the bronchial mucosa exhibit a dense and partially confluent vascular network.

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CASE REPORT

Applications for a hybrid operating room in thoracic surgery: from multidisciplinary procedures to image-guided video-assisted thoracoscopic surgery

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ABSTRACT

The concept of a hybrid operating room represents the union of a high-complexity surgical apparatus with state-of-the-art radiological tools (ultrasound, CT, fluoroscopy, or magnetic resonance imaging), in order to perform highly effective, minimally invasive procedures. Although the use of a hybrid operating room is well established in specialties such as neurosurgery and cardiovascular surgery, it has rarely been explored in thoracic surgery. Our objective was to discuss the possible applications of this technology in thoracic surgery, through the reporting of three cases.

Keywords: Thoracic surgery, video-assisted; Bronchoscopy; Thoracoscopy; Radiology, interventional.

INTRODUCTION

A hybrid operating room (HOR) can be defined as a high-complexity operating room, fully equipped for minimally invasive procedures, such as video-assisted and robotic-assisted procedures, and provided with imaging tools, such as angiography, ultrasound, CT, or magnetic resonance imaging.[1] Such tools allow intraoperative image acquisition, assisting in planning and performing minimally invasive procedures. In this context, the integration of imaging and surgery is maximized, greatly increasing the accuracy of these procedures.[2] In some specialties, such as neurosurgery,[3] cardiac surgery,[4] and vascular surgery,[5] the importance of an HOR is well established, allowing various compound procedures, such as percutaneous angioplasty plus myocardial revascularization during the same anesthesia. However, the role of an HOR in other specialties has yet to be defined.

Thoracic surgery is a specialty that can also benefit from the use of an HOR. The increasing use of video-assisted thoracoscopic surgery and robotic-assisted surgery associated with the frequent diagnosis of semisolid lesions, which are difficult to palpate/visualize intraoperatively, is a context in which the integration of imaging methods in the operating room can be very useful. Although few series have reported the use of an HOR in such circumstances, their results are promising.

The objective of the present article was to illustrate, through the reporting of three cases, some of the potential applications for an HOR in thoracic surgery: the performance of multiple diagnostic and therapeutic procedures during the same surgical session; and the intraoperative localization of nodules, in addition to determination of resection margins.

CASE REPORTS

Case 1

A 73-year-old male smoker presented with a mass in the right upper lobe associated with mediastinal lymph node enlargement. The radiological profile was consistent with primary lung cancer; however, during endoscopic aspiration biopsy of the subcarinal lymph node, uncertainty arose as to whether it was a granulatomous lesion. Therefore, we opted for performing a transthoracic biopsy, as well as a mediastinal lymph node biopsy by mediastinoscopy, and, depending on the results of the intraoperative analysis, we would also insert an indwelling central catheter for chemotherapy. The patient was brought to the HOR, and the procedures were performed sequentially. Elective endotracheal intubation, large-bore peripheral venous access, and an indwelling urinary catheter were used. The patient was initially placed in the right lateral decubitus position for performance of a percutaneous Tru-Cut needle biopsy, with the gantry (Artis Zeego; Siemens, Munich, Germany) set to tomographic mode, by the interventional radiology team. Pathological examination of the biopsy specimen revealed that it was a carcinoma. The patient was then placed in the supine position to undergo mediastinoscopy with biopsy of the lesion, with the gantry set to fluoroscopic mode. Examination of a frozen section biopsy specimen confirmed an epidermoid carcinoma in the infracarinal lymph node. In view of the diagnosis of (stage N2) lung cancer, we proceeded to

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insert a catheter for chemotherapy, again with the gantry set to fluoroscopic mode.

The use of an HOR in the case described here allowed all procedures to be performed sequentially, in the same setting, without need for transportation. In addition, we would have the flexibility of adding pulmonary lobectomy if the pulmonary mass was positive for cancer and did not extend into the mediastinum. We were planning to use the gantry set to tomographic mode during mediastinoscopy in order to assist the intraoperative localization of the mediastinal lesion. However, the vertical size of the video mediastinoscope system precluded free rotation of the gantry, which is required to generate the tomographic image, and it was possible to use only fluoroscopy, which dispenses with complete rotation of the gantry.

Case 2

A 62-year-old man presented with a history of metastatic rectal cancer to the liver at diagnosis. He was treated with neoadjuvant chemotherapy, followed by resection of a liver lesion and ablation of another liver lesion. Subsequently, the patient received chemotherapy and radiotherapy of the rectal lesion, followed by resection of the rectal tumor. Finally, he received adjuvant chemotherapy totaling 12 cycles, including the initial treatment. Eighteen months after diagnosis, the patient developed two pulmonary lesions in the left upper lobe, suggestive of metastases. Those lesions remained stable for more than 6 months. After that period, they showed slight growth and a new finding was observed in the right lower lobe. The patient was considered for pulmonary metastasectomy; however, he wanted the surgical procedure to be performed minimally invasively. The lesion on the right side was deep; therefore, it would be impossible to resect it by video-assisted thoracoscopic surgery without lobectomy. After a multidisciplinary meeting, we opted for performing right-sided radiofrequency ablation and left-sided video-assisted thoracoscopic surgery. The procedures were performed in an HOR, with the patient undergoing general anesthesia and elective endotracheal intubation. The patient was initially placed in the left lateral decubitus position for right-sided radiofrequency ablation using a radiofrequency ablation probe (ACT2030; Covidien, Boulder, CO, USA), with the gantry (Artis Zeego) set to tomographic mode, and the procedure was uneventful. Subsequently, the patient was placed in the right lateral decubitus position and underwent left-sided video-assisted thoracoscopic surgery. One of the nodules was identified on the lung surface; the other, because of its greater depth, was identified intraoperatively with the gantry set to tomographic mode, a technique that some authors have termed image-guided video-assisted thoracoscopic surgery[6] (iVATS, Figure 1). Pathological examination of the two resected lesions confirmed that they were metastatic adenocarcinomas of colorectal origin, both of which had free margins.

The availability of an HOR not only allowed the scheduling of the minimally invasive procedures in the same surgical session, but also made it possible to locate the slightly deeper lesion easily and safely, through the use of iVATS.

Case 3

A 57-year-old female smoker (with a smoking history of 40 pack-years) presented with a coronary artery CT finding of a 1.7-cm semisolid nodule with a 3-mm solid component. Three months later, the solid component had increased to 5 mm (Figure 2A). The lesion was located close to the transition between the upper segment of the left lower lobe and the basal pyramid. Because the lesion was deep and showed a predominant ground-glass pattern, difficulties were to be expected in locating it intraoperatively, as well as in establishing resection margins. Therefore, we planned to inject the nodule with lipiodol (Guerbet, Villepinte, France), making it radiopaque, which would allow its radiological identification during resection.[7] In the HOR, the patient underwent general anesthesia with elective endotracheal intubation and was placed in the right lateral decubitus position, already in the surgical position. An interventional radiologist identified the nodule using a CT scanner and injected it with 0.3 mL of lipiodol, which resulted in excellent fluoroscopic and tomographic views of the nodule (Figure 2B). Subsequently, a guidewire was inserted to assist in guidance on the lung surface anatomy. A muscle-sparing thoracotomy at the 5th intercostal space was then performed, after which anatomic segmentectomy of the upper segment of the left lower lobe, including part of the basal segments, was performed, as well as hilar lymphadenectomy. The intersegmental resection line was developed through stapling, being guided by the radiopaque image produced by the CT scanner and by fluoroscopy (Figure 2C), in order to achieve adequate margins for the whole nodule (Figures 2D and 2E). Surgery and anesthesia were uneventful, and the patient was extubated at the end of the procedure. Pathological examination revealed a lepidic-predominant adenocarcinoma with a minimum margin of 15 mm.

The use of the HOR imaging tools, as well as lipiodol injection of the nodule, was effective in guiding the localization not only of the nodule, but also of the resection margin. The addition of lipiodol was effective, allowing satisfactory identification of the nodule, and seemed more advantageous than using a guidewire alone, given that the wire often moves during lung mobilization and does not indicate to the surgeon the exact position of the lesion.

DISCUSSION

In this initial experience with an HOR, we obtained satisfactory results; however, it is important to emphasize that there is a learning curve with the use of its tools. First, the use of the imaging equipment

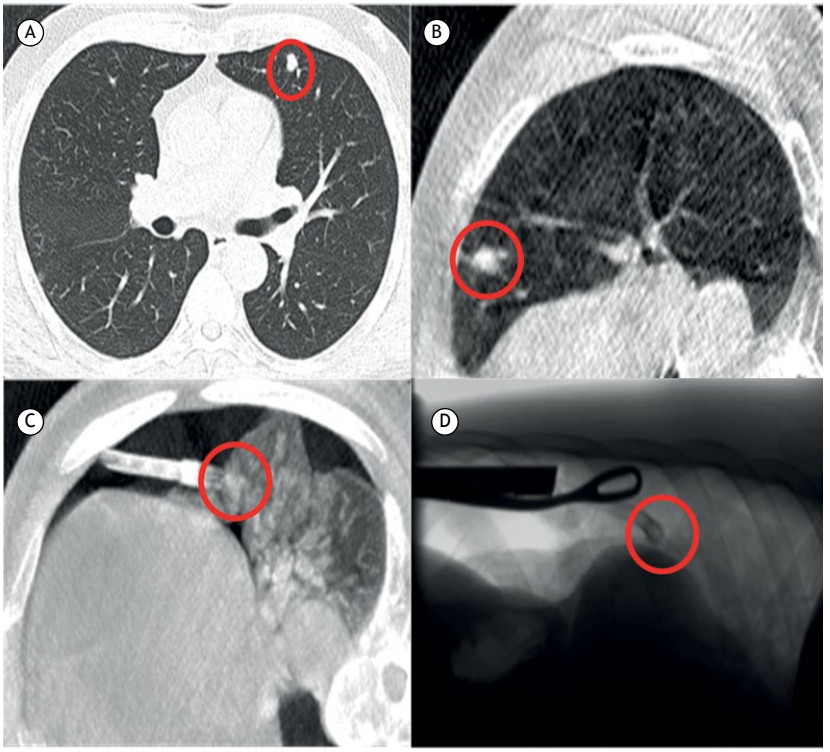

Figure 1. Case 2: In A, a preoperative CT scan. In B, an intraoperative CT scan identifying a nodule in the inflated lung. In C, an intraoperative CT scan identifying a nodule in the collapsed lung. In D, intraoperative fluoroscopy with a probe in place, ensuring the achievement of clear margins for the previously localized nodule.

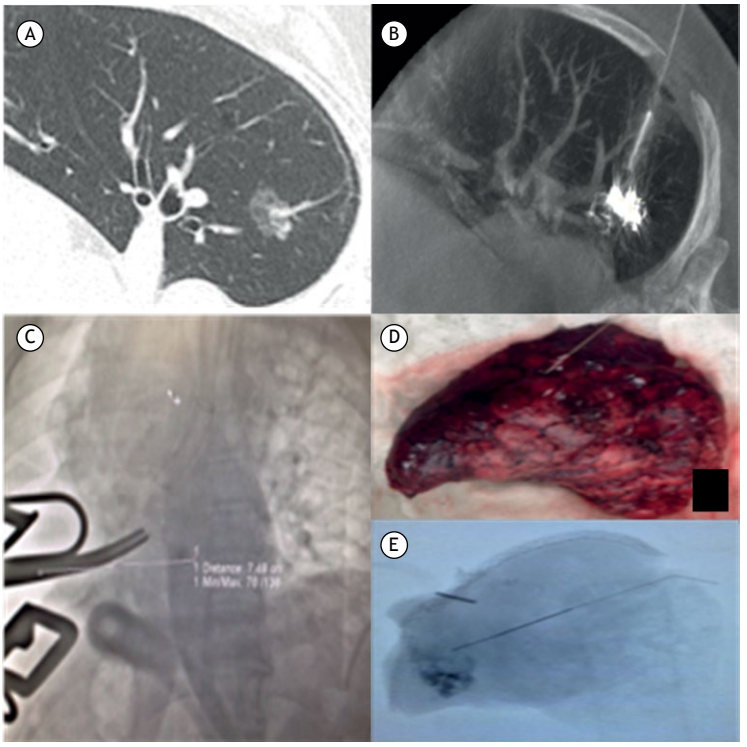

Figure 2. Case 3. In A, a CT scan showing a semisolid nodule. In B, an intraoperative CT scan demonstrating lipiodol uptake in the nodule. In C, intraoperative fluoroscopy with a probe ensuring the achievement of clear margins for the nodule with lipiodol uptake. In D, a photograph of the surgical specimen taken from the upper segment of the lower lobe. In E, an X-ray of the surgical specimen, identifying the nodule with lipiodol uptake and adequate margins.

poses a challenge to radiologists, given that the interface is a little different from that usually found in interventional radiology services. Second, patient positioning, as well as equipment and staff positioning, requires special care,[8] since gantry rotation should be taken into account. The patient's arms should be placed at his/her sides so as not to exceed the width of the operating table; and the use of cushions/pads is essential, because the operating table is flat and very limited in terms of mobilization. Finally, as in any surgical procedure, a larger number of cases would increase the consistency and efficiency of the procedures, consequently reducing room time, which was relatively long in the examples described here.

The decisive factor for the success of the cases reported here was certainly the integration of the surgical, anesthetic, and radiological teams. Only after considerable discussion regarding the needs and contributions of each team were we able to proceed to perform the procedures. The key issue for debate is certainly positioning, because the initially most suitable positioning for surgery was not appropriate for radiology. The first change in positioning was a cause of concern for the anesthetic team, who was vigilant for injuries. Further discussions were held at each adaptation, until positioning was approved by all.

In conclusion, in the three cases reported here, the use of an HOR was feasible and seems to have facilitated performing the procedures. Most importantly, through these cases, we could envision potential applications for an HOR in thoracic surgery, in particular, the possibility of performing diagnostic and therapeutic procedures in the same setting and the possibility of resecting very small lesions accurately. In the current context of increased use of minimally invasive sublobar resections and of an increase in early diagnosis of lung cancer by screening programs, the tools offered by an HOR are promising and merit scientific research.

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An uncommon chest mass: oleothorax

Bruno Hochhegger[1], Gláucia Zanetti[2], Edson Marchiori[2]

TO THE EDITOR:

We would like to report a case of unilateral oleothorax, which generated great diagnostic difficulties and was initially interpreted as a lung mass, probably of neoplastic origin. An 84-year-old woman presented with a dry cough that had persisted for four months. A chest X-ray showed a mass in the right hemithorax (Figure 1A). Due to the suspicion of lung cancer, the patient underwent CT, and the scans demonstrated a posteriorly heterogeneous mass in the upper right hemithorax (Figure 1B). Aspirated viscous pleural fluid proved to be mineral oil. Upon further discussion, the patient vaguely remembered that she had been treated for tuberculosis and had received an oil injection 60 years prior.

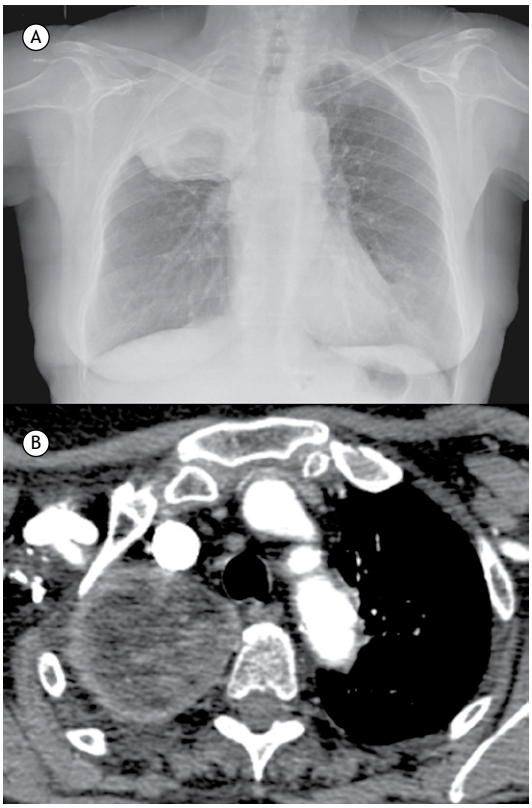

Figure 1. In A, a chest X-ray showing a mass in the apical region of the right hemithorax. In B, an axial CT scan demonstrating that the mass was heterogeneous and well delimited, with no evidence of parietal invasion.

Oleothorax, the intrapleural or extrapleural instillation of mineral or vegetable oil into the pleural space, was widely used from the early decades of the 20th century until the late 1940s. Oleothorax was a form of collapse therapy used to inhibit the multiplication and dissemination of pulmonary *Mycobacterium tuberculosis*. In addition to exerting a mass effect on the adjacent lung, such substances were caustic and produced obliterative pleuritis, which justified their use in tuberculous empyemas. The amount of oil used varied widely (100-2,000 mL). The recommended length of therapy was up to 2 years, with removal of the oil thereafter.[1-5] However, asymptomatic patients were often lost to follow-up, and the oil was never removed, as in our case.

Failure to drain the oil has been associated with diverse complications, some occurring decades later. Long-term complications of oleothorax include bronchopleural fistula, pleurocutaneous fistula, airway obstruction, recurrent tuberculous empyema, chest wall abscess, respiratory distress from an expanding mass, among others. Extrapleural oleothorax produced fewer complications than did the pleural variety. With the advent of effective antituberculosis chemotherapy, the technique was largely abandoned in the 1950s.[2-5] The most characteristic CT finding of oleothorax is an encapsulated pleural collection with three levels: a superior level with air, an intermediary level with lipid content (−30 to −150 Hounsfield units), and an inferior level with positive densities.[3-5]

A diagnosis of oleothorax might be achieved from CT images, and the recognition of this pattern is important because the patient might not know or remember the details of the antecedent intervention performed many years prior. In summary, oleothorax should be included in the differential diagnosis of thoracic masses, particularly in elderly patients.

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A rare case of hemorrhagic pneumonia due to *Cladosporium cladosporioides*

Sérgio Grava[1,2], Francisco Antonio Dias Lopes[3], Rodrigo Silva Cavallazzi[4], Melyssa Fernanda Norman Negri Grassi[5], Terezinha Inez Estivalet Svidzinski[1,2,5]

TO THE EDITOR:

Recently, a letter published in the European Respiratory Review brought attention to a pulmonary infection caused by *Cladosporium cladosporioides* in an immunocompetent woman.[1] Based on this interesting note, we were able to diagnose a case of alveolar hemorrhage by *C. cladosporioides* in a previously healthy patient, reinforcing the importance of this fungus in the respiratory tract. To the best of our knowledge, this is the first case of hemorrhagic pneumonia due to *C. cladosporioides*, and the second report of pulmonary parenchymal infection associated with *C. cladosporioides* affecting a non-immunocompromised patient, whose main symptom was hemoptysis.

We report the case of a 59-year-old male outpatient who presented with hemoptysis for two weeks. He was an active smoker and had a 25-pack-year smoking history. He had been working in a restaurant in Japan for 5 years and reported having ingested a large number of raw peanuts just before the hemoptysis episodes. He came back to Brazil to have this symptom investigated. Clinical examination revealed a good general condition of the patient, no fever or respiratory distress. Auscultation was clear; chest X-ray revealed haziness in the right upper lobe, whereas CT of the chest showed a large opacity with a ground-glass halo in the posterior segment of the upper right lobe (Figures 1A and 1B). Immunological tests were normal, and HIV serology was negative. Fiberoptic bronchoscopy revealed blood in the right upper lobe bronchus; BAL fluid was bloody, and its culture was negative for bacteria and fungi. The patient was started on a seven-day course with amoxicillin/clavulanate; no clinical difference was observed. Two weeks later, the patient presented with dyspnea, wheezing, cough, and increased hemoptysis. Surgical lung biopsy was performed and the histopathological examination of the specimen revealed alveolar hemorrhage. Culture from the biopsy fragments was carried out by using ten tubes containing Sabouraud glucose agar without cycloheximide, which were incubated in the darkness at 25ºC and examined daily. Fourteen days later, there was growth of a pure culture presenting as dark green colonies with black reverse. Microculture assays showed conidiophores branching terminally and laterally, allowing the identification of the fungus as *C. cladosporioides* (Figures 1C and 1D). There was no growth of any type of bacteria in the cultures.

The patient was treated with itraconazole 400 mg daily. Within two months, there was clinical improvement with remission of dyspnea, hemoptysis, and cough, as well as radiological improvement (Figure 1E). At 3 years of follow-up, chest X-rays revealed good resolution (Figure 1F), and there was no clinical relapse at this writing.

Hemoptysis is most commonly caused by bacterial infections or fungi such as *Aspergillus* spp. *Exophiala dermatitidis* is a dematiaceous fungus that was described in a case of hemoptysis.[2] However, *Cladosporium* spp. apparently have yet to be reported as a cause of hemoptysis.

Cladosporium spp. are dematiaceous fungi which are found in a wide variety of habitats; they can be isolated from foods, such as peanuts.[3] They are saprophytic fungi, although they can cause human infections opportunistically; this genus has already been isolated from subcutaneous phaeohyphomycoses,[4] keratomycosis,[5] and from cerebrospinal fluid.[6] Specifically regarding the human respiratory tract, studies have reported *Cladosporium* spp. associated with hypersensitivity pneumonitis,[7] pulmonary fungus ball,[8] obstruction of the left main bronchus by a mucoid lesion,[9] and intrabronchial lesion.[10] Recently, *C. cladosporioides* was isolated from lung parenchyma.[1]

The pathogenic mechanism by which this fungus causes disease is still unclear. Apparently, fungus particles reach the lungs by inhalation, since *C. cladosporioides* is present in the microflora of peanuts.[3] It is possible that melanin, which is present in dematiaceous fungi, allows the maintenance of the fungus in lung tissue. It is a protective advantage against phagocytic cells during their oxidative burst.

Initially, we suspected of bacterial pneumonia, but previous antibiotic treatment had been administered without remission of clinical symptoms. In addition, laboratory investigation of BAL fluid was negative for bacteria. However, Figure 1B shows an opacity with a ground-glass halo in a limited area of the right lung, compatible with pneumonia and alveolar hemorrhage. Therefore, based on clinical and radiological aspects, as well as positive cultures for *C. cladosporioides* from surgical biopsy fragments, we decided to start treatment with itraconazole, the drug of choice for the treatment of infections caused by dematiaceous fungi.[1,10] There was an excellent response to the treatment. Our findings reinforce the concern that Castro et al.[1] reported, which was that pulmonary phaeohyphomycosis is a challenge to the clinician and deserves attention. It seems that infections of the respiratory tract due to

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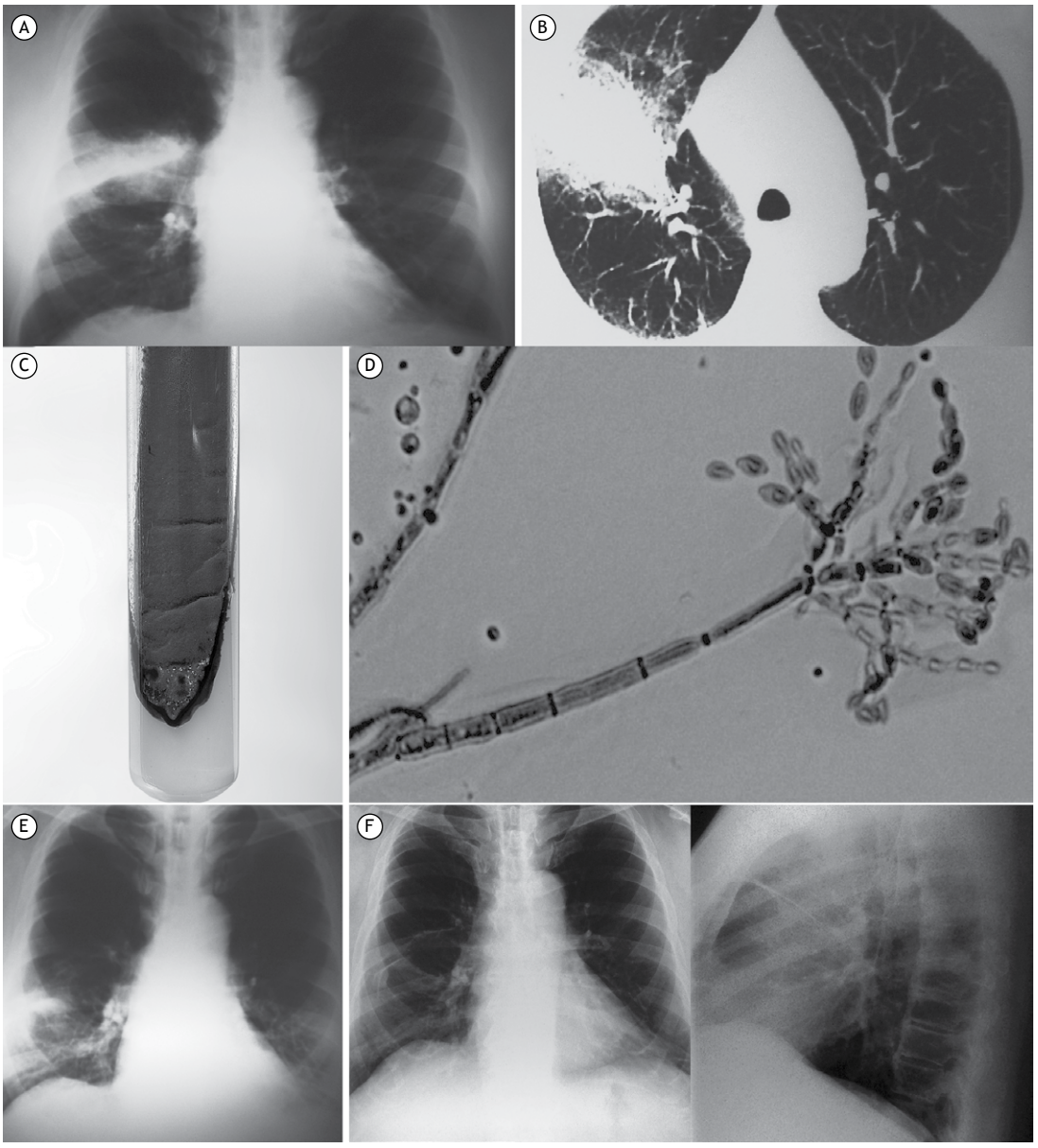

Figure 1. In A, a chest X-ray taken prior to treatment showing opacities in the right upper lobe. In B, a chest CT scan taken prior to treatment with itraconazole showing an opacity with a ground-glass halo in the right upper lobe. In C, a *Cladosporium cladosporioides* colony in a culture from a lung biopsy fragment showing typical microscopic features characterizing *C. cladosporioides*. In D, a photomicrograph of the mycological culture on a glass slide stained with cotton blue (magnification, ×400). In E, chest X-ray showing good resolution after two-month treatment with itraconazole. In F, three-year follow-up chest X-rays showing complete resolution of the infection

Cladosporium spp. have been increasingly identified. The clinical manifestations are very similar to those of bacterial infections, and the clinician needs to differentiate between them, diagnose it, and treat it appropriately.

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Cervical computed tomography in patients with obstructive sleep apnea: influence of head elevation on the assessment of upper airway volume

Shailendra Singh Rana[1], Om Prakash Kharbanda[1]

We read with great interest the article by Souza et al.[1] describing cervical CT in patients with obstructive sleep apnea (OSA) and the influence of head elevation on the assessment of upper airway volume. We congratulate the authors for choosing such a meaningful topic and using cone beam CT. In their study,[1] CT scans were obtained with the head of the patient in two positions (neutral and at a 44° upward inclination). Polysomnography was used for diagnosing the patients with OSA. During polysomnography, the nasal pressure transducer channel or the thermistor channel (oral or nasal) are used for monitoring airflow from the nasal cavity. The nasal cavity is bounded anteriorly by the nostrils and posteriorly by the posterior border of the nasal septum (choanae).[2] The nasal airway comprises nearly two thirds of airway resistance during normal breathing,[3] and engorgement of nasal turbinate blood vessels, septum deviation, polyps, and other mucosal abnormalities due to chronic inflammation worsen nasal obstruction.[4]

The anatomical definition of upper airway includes both the pharynx and the nasal cavity.[5] The combination of nasal obstruction and that of the oropharynx leads to a two-fold increase in the risk of having OSA, when compared with patients with no nasal obstruction.[6] In the study by Souza et al.,[1] airway volume was measured from the hard palate to the base of the epiglottis using CT images, which means that the nasal volume was not considered. These methodological limitations of the study would lead to an erroneous representation in the title of the study and in the discussion of the results. The authors may like to dwell upon this inconsistency for the benefit of readers.

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In our study of cervical CT in patients with OSA, we found that airway volume increased by 7.9 cm^3 (17.5%) when a 44° upward inclination was compared with a neutral head position.[1] Our results are reliable and show statistically significant differences despite the small sample size.[1] The title states that the study involved cervical CT rather than CT with analysis of nasal airway volume. In the studies of imaging evaluation of airway volume cited in the Discussion section of our study, airway volume was measured from the hard palate to the base of the epiglottis for analysis of interventions involving a mandibular advancement splint, maxillomandibular advancement surgery, and continuous positive airway pressure.[1] The clinical foundation for our study was provided by a previous study of OSA patients undergoing polysomnography; in that study, which was cited in our article, the apnea-hypopnea index was measured at baseline (i.e., during standard polysomnography) and after elevation of the head of the bed, the latter having resulted in a reduction in the apnea-hypopnea index.[1] A possible functional explanation is that head elevation contributes to upper airway patency, prevents rostral fluid shift, and averts tongue collapse, reducing upper airway resistance, changing upper airway critical pressure, affecting gravitational effects, and altering neuromuscular activity.[1]

The late Professor Bruno Carlos Palombini (a notable Brazilian pulmonologist) coined the term "*viaerologia*" ("airwayology") to describe an integrated view of airway diseases in the fields of pulmonology, otorhinolaryngology, gastroenterology, and sleep medicine, advocating the importance of the anatomical and functional attributes of the airway.[2] Diseases such as OSA have multiple phenotypic characteristics, which were not fully addressed in our study.

In patients with OSA, the role of the nasal cavity is extremely important not only in clinical analysis but also in the apnea-hypopnea index. Nasal anatomic factors, appropriately pointed out by Rana & Kharbanda, can cause significant resistance and be a contributing factor to OSA. To our knowledge, there have been no studies involving nasal imaging with and without head elevation in patients with OSA. The anatomical region of interest in our study is similar to that used in most studies involving imaging evaluation of upper airway volume and evaluating therapeutic interventions. In addition, we sought to assess upper airway volume in a site in which collapsibility is increased; CT scans of patients sitting upright or lying supine have shown that the highest degree of variation with the postural change was in the oropharynx.[3] Anatomical changes with fixed nasal obstruction, such as septal deviation and polyps, are likely to vary little with postural changes. However, patients with intranasal edema might benefit from head elevation, a hypothesis that should be explored in future studies.

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Pulmonary rehabilitation in severe COPD with hyperinflation: some insights into exercise performance

Luiz Alberto Forgiarini Junior[1], Antonio Matias Esquinas[2]

We would like to highlight the importance of the study entitled "Exercise performance and differences in physiological response to pulmonary rehabilitation in severe chronic obstructive pulmonary disease with hyperinflation",[1] which was recently published in the JBP. The authors evaluated the impact of pulmonary rehabilitation on exercise tolerance in severe COPD with hyperinflation. That study showed improvement in oxygen consumption, reduced carbon dioxide production, and decreased respiratory drive; however, patients with post-exercise hyperinflation did not improve their maximal performance. We congratulate the authors for the important findings, but some key issues need to be taken into account for a proper clinical extrapolation.

First, it is unclear whether those patients with hyperinflation after exercise had peripheral muscle weakness prior to pulmonary rehabilitation or not. Second, we wonder if hyperinflation was significantly higher in those patients when they were compared with those who responded to pulmonary rehabilitation. In this line, a possible evaluation tool would be the handgrip, as demonstrated by Burtin et al.,[2] who evaluated patients with COPD and showed what could be a tool associated with a prognosis of mortality in this population. Third, the authors evaluated the chest wall with the utilization of optoelectronic plethysmography; however, the respiratory muscles were not evaluated directly, which would be an interesting factor given the fact that there are studies demonstrating that the sensation of dyspnea might be associated with respiratory muscle weakness, and the training of these muscles, in association with pulmonary rehabilitation, results in a reduction in dyspnea indices.[3] We consider that other alternatives for COPD patients with chronic hyperinflation after exercise and submitted to a pulmonary rehabilitation program should be evaluated in this population. As an example, Monteiro et al.[4] showed that the use of expiratory positive airway pressure in patients with moderate and severe COPD can reduce dynamic hyperinflation after a submaximal exercise. Similarly, Wibmer et al.[5] evaluated the lung volumes in COPD patients undergoing exercise with the use of positive expiratory pressure via a nasal mask; the authors demonstrated a significant reduction in dynamic hyperinflation during a walking exercise.

We must highlight the importance of the study by Albuquerque et al.,[1] since COPD patients with post-exercise hyperinflation are a reality in outpatient rehabilitation, and adequate knowledge of the response to treatment is of great importance so that new studies with a focus on actions that can alleviate this condition can be carried out. In addition, further prospective clinical trials need to confirm such data.

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We would like to thank Forgiarini Junior and Esquinas for their comments, precisely because they address highly relevant issues related to the chances of success of pulmonary rehabilitation in severe COPD. Our colleagues properly pointed out that patients with more advanced COPD may also have peripheral muscle weakness, which can be a limiting factor for maximal performance gain through cardiopulmonary rehabilitation. In our study, the COPD patients who showed no improvement following rehabilitation had higher leg fatigue values before the intervention than did those who showed improvement. In addition, the degree of fatigue was not reduced after training in the patients who did not respond to rehabilitation. It is in fact to be assumed that peripheral skeletal muscle involvement in those patients is not restricted to the legs but is also present in the arms.[1] For this reason, handgrip evaluation can identify patients with a potentially poorer response in terms of exercise capacity after cardiopulmonary rehabilitation.

In addition to the impact of various organs as limiting factors in COPD, no static or dynamic assessment of the respiratory muscles was performed in our study. Ventilatory weakness can certainly be an additional factor contributing to a greater sensation of dyspnea, as our colleagues pointed out. However, in such patients, one of the major problems is mechanical inefficiency secondary to dynamic air trapping during exercise. Because of this inefficiency, even with strength being generated by the ventilatory muscles, there is no proportional increase in ventilatory flow. As a result, fatigue occurs mainly in the inspiratory muscles in this state of inefficiency and high ventilatory demand.[2] Undoubtedly, complementary therapies that reduce air trapping and result in increased exercise tolerance during rehabilitation, such as the use of noninvasive ventilation, mentioned by our colleagues, should be considered for such groups of patients with severe COPD, especially for those with pulmonary hyperinflation.

We do agree that future studies addressing these multiple limiting factors in COPD are extremely important[3] so that our patients can achieve a decrease in their sensation of dyspnea and an improvement of endurance in their activities of daily living.

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Examples: Journal Articles

1. Neder JA, Nery LE, Castelo A, Andreoni S, Lerario MC, Sachs AC et al. Prediction of metabolic and cardiopulmonary responses to maximum cycle ergometry: a randomized study. Eur Respir J. 1999;14(6):1204-13.

Abstracts

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

Chapter in a Book

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

Official Publications

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

Theses

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

Electronic publications

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

Homepages/URLs

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

Other situations:

In other situations not mentioned in these author instructions, authors should follow the recommendations given by the International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Updated October 2004. Available at http://www.icmje.org/.

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NACIONAIS

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XIV Congresso Brasileiro de Endoscopia Respiratória
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Local: Rio de Janeiro - RJ
Informações: eventos@sbpt.org.br
Fone: 0800 61 6218

XVII Curso Nacional de Atualização em Pneumologia
Data: 20 a 22 de abril de 2017
Local: Othon Palace Copacabana - Rio de Janeiro/RJ
Informações: 0800616218 ou eventos@sbpt.org.br

XX Congresso da Sociedade Brasileira de Cirurgia Torácica
Data: 03 a 06 de maio de 2017
Local: Windsor Barra – Rio de Janeiro/RJ
Organização: Método Eventos
Informações: Beatriz Lemgruber (21) 25485141

INTERNACIONAIS

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Local: Washington, D.C/USA
Informações: www.thoracic.org

SEPAR 2017
Data: 2-5 de junho de 2017
Local: Madrid Marriott Auditorium Hotel & Conference Center, Madrid/Espanha
Informações: www.separ.es

ERS 2017
Data: 09-13 de Setembro de 2017
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1. Lindberg A, Szalai Z, Pullerits T, et al. Fast onset of effect of budesonide/formoterol versus salmeterol/fluticasone and salbutamol in patients with chronic obstructive pulmonary disease and reversible airway obstruction. Respirology 2007 Sep;12(5): 732-9. **2**. Tashkin DP, Rennard SI, Martin P, et al. Efficacy and safety of budesonide+/formoterol in one pressurized metered dose inhaler in patients with moderate to very severe chronic obstructive pulmonary disease. Results of a 6-month randomized clinical trial. Drugs 2008;68(14): 1975-2000. **3**. Rennard SI, Tashkin DP, McElhattan J, et al. Efficacy and tolerability of budesonide/formoterol in one hydrofluoroalkane pressurized metered-dose inhaler in patients with chronic obstructive pulmonary disease: results from a 1-year randomized controlled clinical trial [supplementary material]. Drugs. 2009;69(5):549-65. doi: 10.2165/00003495-200969050-00004. **4**. Hampel F C, Martin P. Mezzanotte W S. Early bronchodilatory effects of budesonide/formoterol pMDI compared with fluticasone/salmeterol DPI and albuterol pMDI: 2 randomized controlled trials in adults with persistent asthma previously treated with inhaled corticosteroids. Journal of Asthma 2008; 45: 265-272. **5**. Bula do produto.

SYMBICORT® SPRAY 6/100 mcg/inalação e **SYMBICORT® SPRAY** 6/200 mcg/inalação (fumarato de formoterol di-hidratado/budesonida) suspensão aerossol. **Indicações: SYMBICORT® SPRAY** está indicado no tratamento da asma nos casos em que o uso de uma associação (corticosteroide inalatório com um beta-2 agonista de ação prolongada) é apropriado e no tratamento regular de pacientes adultos com Doença Pulmonar Obstrutiva Crônica (DPOC) de moderada a grave, com sintomas frequentes e histórico de exacerbações. **Contraindicações: Hipersensibilidade à budesonida, ao formoterol ou a outros componentes da fórmula. Cuidados e Advertências: Advertências:** É recomendado que a dose seja titulada quando o tratamento de longo prazo é descontinuado e este não deve ser interrompido abruptamente. Para minimizar o risco de candidíase orofaríngea, o paciente deve ser instruído a lavar a boca com água após administrar as inalações de **SYMBICORT® SPRAY**. Uma deterioração súbita e progressiva do controle da asma é um risco potencial e o paciente deve procurar suporte médico. Pacientes que necessitaram de terapia corticosteroide de alta dose emergencial ou tratamento prolongado de altas doses recomendadas de corticosteroides inalatórios podem exibir sinais e sintomas de insuficiência adrenal quando expostos a situações de estresse grave. Administração de corticosteroide sistêmico adicional deve ser considerada durante situações de estresse ou cirurgia eletiva. **SYMBICORT® SPRAY** deve ser administrado com cautela em pacientes com graves alterações cardiovasculares (incluindo anomalias do ritmo cardíaco), diabetes mellitus, hipocalemia não tratada ou tireotoxicose. Pacientes com prolongamento do intervalo QTc devem ser cuidadosamente observados (para maiores informações vide bula completa do produto). Estudos clínicos e meta-análises indicaram que o tratamento da DPOC com corticosteroides pode levar a um risco aumentado de pneumonia. No entanto, o risco absoluto para a budesonida é pequeno. Não foi estabelecida uma relação causal com os produtos contendo budesonida. Gravidez: categoria C de risco de gravidez. Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. A administração de **SYMBICORT® SPRAY** em mulheres lactantes deve ser apenas considerada se os benefícios esperados para a mãe superarem qualquer possível risco para a criança (para maiores informações vide bula completa do produto). **Interações medicamentosas:** o metabolismo da budesonida é mediado principalmente pela CYP3A4, uma subfamília do citocromo P450. Portanto, inibidores desta enzima, como o cetoconazol ou suco de grapefruit (pomelo), podem aumentar a exposição sistêmica à budesonida. A cimetidina apresenta um leve efeito inibidor sobre o metabolismo hepático da budesonida. Fármacos como a procainamida, fenotiazina, agentes antihistamínicos (terfenadina), inibidor da monoaminooxidase (MAO) e antidepressivos tricíclicos foram relacionados com um intervalo QTc prolongado e um aumento do risco de arritmia ventricular. Os bloqueadores beta-adrenérgicos (incluindo os colírios oftálmicos) podem atenuar ou inibir o efeito do formoterol (para maiores informações vide bula completa do produto). **Reações adversas**: as reações adversas que foram associadas à budesonida ou ao formoterol são apresentadas a seguir. Comum: palpitações, candidíase na orofaringe, cefaléia, tremor, leve irritação na garganta, tosse, rouquidão. Incomum: taquicardia, náusea, câibras musculares, tontura, agitação, ansiedade, nervosismo e perturbações do sono (para outras reações adversas, vide bula completa do produto). Posologia: a dose de **SYMBICORT® SPRAY** deve ser individualizada conforme a gravidade da doença. Quando for obtido o controle da asma, a dose deve ser titulada para a menor dose que permita manter um controle eficaz dos sintomas. ASMA: **SYMBICORT® SPRAY** 6/100 mcg/inalação: Adultos (a partir de 18 anos de idade): 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. Adolescentes (12-17 anos): 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. Crianças (6-11 anos): 2 inalações duas vezes ao dia. Dose máxima diária: 4 inalações. **SYMBICORT® SPRAY** 6/200 mcg/inalação: Adultos (a partir de 18 anos de idade): 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. Adolescentes (12-17 anos): 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. DPOC: **SYMBICORT® SPRAY** 6/200 mcg/inalação: Adultos (a partir de 18 anos de idade): 2 inalações duas vezes ao dia. Dose máxima diária: 4 inalações. Não foram estabelecidas a segurança e eficácia de **SYMBICORT® SPRAY** 6/100 mcg/inalação para o tratamento de DPOC. Instruções de Uso: vide bula completa do produto. **Superdose**: A superdosagem de formoterol irá provavelmente provocar efeitos típicos dos agonistas beta-2-adrenérgicos: tremor, cefaléia, palpitações e taquicardia. Poderá igualmente ocorrer hipotensão, acidose metabólica, hipocalemia e hiperglicemia. Pode ser indicado um tratamento de suporte e sintomático. A administração de uma dose de 90 mcg durante três horas em pacientes com obstrução brônquica aguda e quando administrada três vezes ao dia como um total de 54 mcg/dia por 3 dias para a estabilidade asmática não suscitou quaisquer problemas de segurança. Não é esperado que uma superdosagem aguda da budesonida, mesmo em doses excessivas, constitua um problema clínico. Quando utilizado cronicamente em doses excessivas, podem ocorrer efeitos glicocorticosteroides sistêmicos (para informações de superdosagem grave vide bula completa do produto). **Apresentações**: **SYMBICORT® SPRAY** 6/100 mcg/inalação: Suspensão aerossol 6/100 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **SYMBICORT® SPRAY** 6/200 mcg/inalação: Suspensão aerossol 6/200 mcg/inalação em embalagem com 1 tubo contendo 120 doses. USO ADULTO E PEDIÁTRICO (vide posologia e bula completa). VIA INALATÓRIA. VENDA SOB PRESCRIÇÃO MÉDICA. SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. Para maiores informações, consulte a bula completa do produto. Tel. SAC: 0800-0145578. www.astrazeneca.com.br **SYMBICORT® SPRAY**. MS – 1.1618.0250 (SYM_SPR002_min).

CONTRAINDICAÇÕES: HIPERSENSIBILIDADE À BUDESONIDA, AO FORMOTEROL OU A OUTROS COMPONENTES DA FÓRMULA. **INTERAÇÕES MEDICAMENTOSAS:** OS BLOQUEADORES BETA-ADRENÉRGICOS (INCLUINDO OS COLÍRIOS OFTÁLMICOS) PODEM ATENUAR OU INIBIR O EFEITO DO FORMOTEROL.

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