ISSN 1806-3713

JBP
Jornal Brasileiro de Pneumologia

PUBLICAÇÃO OFICIAL DA SOCIEDADE BRASILEIRA DE PNEUMOLOGIA E TISIOLOGIA

Volume 45, Number 3
May | June
2019

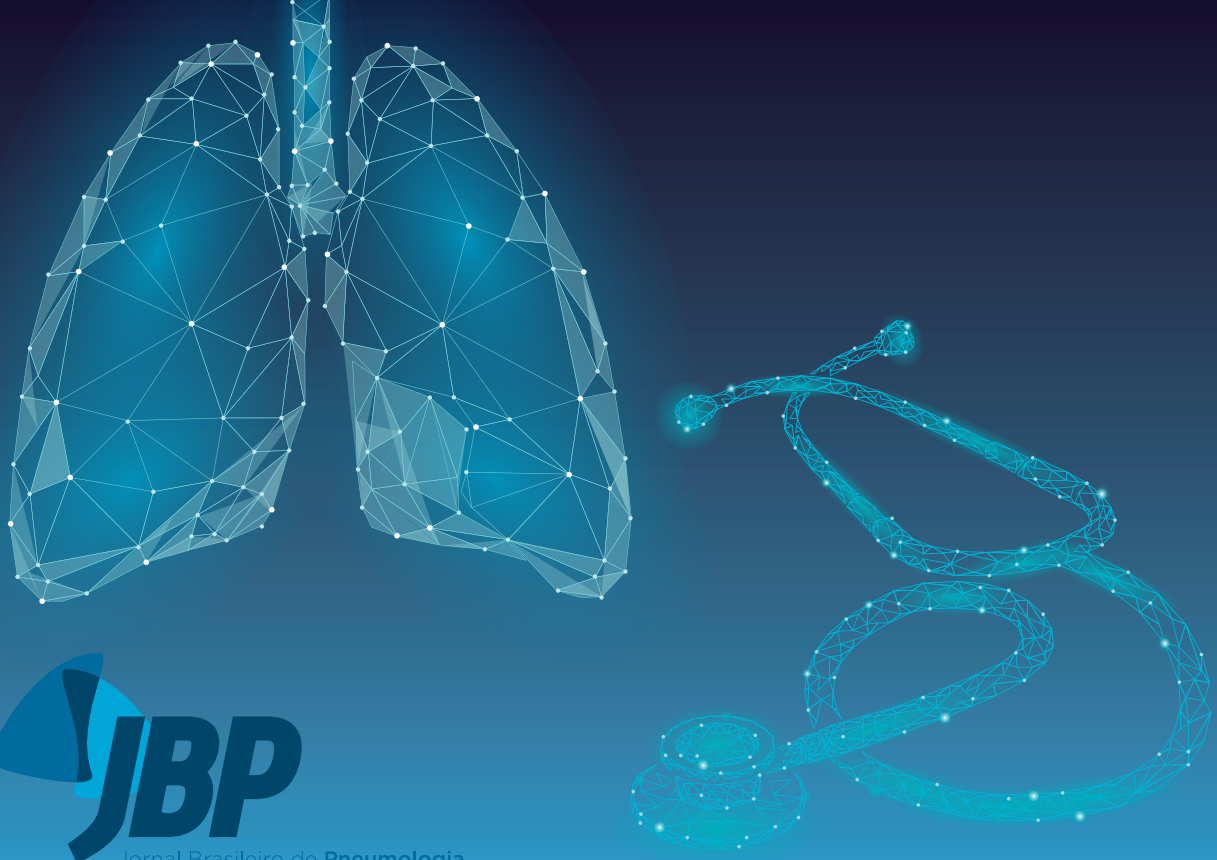
Volume 45, Number 3 May | June 2019

HIGHLIGHT

Update on the approach to smoking

Impact of lipoabdominoplasty on diaphragm and lung function

Molecular profile of non-small cell lung cancer in Brazil



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1937

ISSN 1806-3713

JBP Jornal Brasileiro de Pneumologia

Continuous and Bimonthly Publication, J Bras Pneumol. v. 45, n. 3, May/June 2019

Associação Brasileira de Editores Científicos ABEC

Publicação Indexada em:
Latindex, LILACS, Scielo Brazil, Scopus, Index Copernicus, ISI Web of Knowledge, MEDLINE e PubMed Central (PMC)

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ISSN 1806-3713

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ADMINISTRATIVE SECRETARIAT OF THE BRAZILIAN JOURNAL OF PULMONOLOGY
Address: SCS Quadra 01, Bloco K, Asa Sul, salas 203/204. Edifício Denasa, CEP 70398-900, Brasília, DF, Brazil. Tel. +55 61 3245-1030/+55 0800 616218.
Assistant Managing Editor: Luana Maria Bernardes Campos.
E-mail: jbp@jbp.org.br | jbp@sbpt.org.br
Circulation: 4.000 copies
Distribution: Free to members of the BTS and libraries
Printed on acid-free paper

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ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Continuous and Bimonthly Publication, J Bras Pneumol. v. 45, n. 3, May/June 2019

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ISSN 1806-3713

Jornal Brasileiro de Pneumologia

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J Bras Pneumol. 2019;45(3):e20190130
http://dx.doi.org/10.1590/1806-3713/e20190130

Celebrating World Asthma Day in Brazil: is the glass half full or half empty?

Marcia Margaret Menezes Pizzichini[1,a], Álvaro Augusto Cruz[2,3,b]

Asthma is a chronic respiratory disease that affects 339 million individuals worldwide, of whom approximately 20 million are in Brazil.[1] Since 1998, the Global Initiative for Asthma has globally celebrated "World Asthma Day", which has the objective of raising awareness of the disease and how it affects patients' lives. In Brazil, the Brazilian Thoracic Association has long promoted "National Asthma Day" and "World Asthma Day". This year, "World Asthma Day" will be celebrated on May 7 and will have "STOP for Asthma" as its theme, STOP being an acronym for Symptoms (symptom assessment); Test (asthma control); Observe (and assess); and Proceed (with and adjust treatment). This is a time for reflection, and it seems natural to ask, "What is the asthma scenario like in Brazil today? Is the glass half full or is it half empty?"

For the optimists, among whom we include ourselves, the glass is half full. Not everyone, like us, lived those days when, in order to treat asthma, we only had theophylline, oral corticosteroids, and short-acting $ß_2$ agonists for use orally or by nebulization. Those were the days when emergency rooms and wards were crowded with asthma patients. It is indisputable that there have been great advances in knowledge about the pathophysiology of asthma in recent decades, and that, consequently, there has been a remarkable increase in the number of asthma control medications available. However, the benefits of these advances in knowledge do not extend to all in need.

Paradoxically, asthma control remains suboptimal worldwide.[2,3] In 2011, a survey involving several countries in Latin America interviewed 2,169 adult patients with asthma or parents of children with asthma; only 9% of the Brazilian respondents had controlled asthma.[3] With regard to the past 12 months, 27% of the Brazilian respondents reported having been hospitalized and an additional 47% reported having received emergency room treatment.[4] More recently, study of data from a previous survey[5] of 12,000 Brazilian adults who agreed to complete the study questionnaire showed that 4.1% of the respondents had a previous diagnosis of asthma. Of those, 52.1%, 36.4%, and 12.3% had uncontrolled asthma, partially controlled asthma, and controlled asthma, respectively. Only 32.4% of the respondents reported full adherence to the prescribed treatment regimen. When compared with controls, asthma patients had poorer quality of life and a higher number of hospitalizations in the past 6 months. Work productivity (rates of absenteeism and presenteeism) was lower in asthma patients than in controls.[5]

Do these results indicate that the glass is half empty? Yes, as far as asthma control is concerned. Pulmonologists, clinicians, and pediatricians involved in asthma management in Brazil face several challenges that vary in complexity according to local and patient socioeconomic and cultural conditions, patient beliefs and attitudes, the resources available in health care facilities to investigate and confirm the diagnosis of asthma, the level of patient access to the prescribed medications, physician work overload, etc. In addition, asthma is a complex and heterogeneous disease, for which inhaled drug treatment in itself poses a challenge regarding the correct use of inhalers and treatment adherence, as well as regarding the choice of the most appropriate inhaler and the most appropriate dose for a given patient.[6] That said, we should add that the vast majority of asthma patients are found in primary and pediatric care clinics. For this vast majority of asthma patients, treatment is simple, with rapid resolution of symptoms, since asthma is a concordant disease (i.e., the more severe the symptoms are, the more severe is the airway inflammation), which usually responds well to symptom-guided treatment.[7]

If most asthma patients have treatment-responsive asthma[7] and can easily control it, then why does asthma control remain suboptimal? In addition to several voluntary and involuntary factors involved in treatment adherence,[6] a plausible possibility is poor physician-patient communication.[8,9] As in any chronic disease, the physician-patient relationship cannot be just rhetoric; it should be a reality in which the physician sets aside time to establish trust and bond with the patient. Suboptimal asthma control most likely results from the combination of lack of a tailored approach, both biologically and psychosocially, and lack of detailed knowledge about the behavior of patients, with their expectations and fears, as well as from difficulties in choosing the most appropriate inhaler for each patient.[6,10] Poor asthma control rates are red flags calling attention to the need for interventions aimed at developing the skills not only of physicians but also of the entire health care team for understanding the complexity of asthma. Asthma does not have a single diagnostic biomarker, and its treatment requires the use of inhalers. However, in most cases, the disease can be controlled very easily by using medications dispensed free of charge under the Brazilian Popular Pharmacy program.

There are also concrete data showing that the glass is half full. In Brazil, longitudinal trends in asthma health care use have shown that, between 2008 and 2014,

1. Universidade Federal de Santa Catarina – UFSC – Florianópolis (SC) Brasil.
2. Núcleo de Excelência em Asma/Programa para o Controle da Asma na Bahia – ProAR – Salvador (BA) Brasil.
3. Faculdade de Medicina da Bahia, Universidade Federal da Bahia – UFBA – Salvador (BA) Brasil.
a. http://orcid.org/0000-0001-7409-7536; b. http://orcid.org/0000-0002-7403-3871

there was a mean decrease of 43% in the number of asthma-related hospitalizations among patients aged 1 to 49 years, a finding that is consistent with the provision of medications free of charge via the Brazilian Unified Health Care System as of 2008.[11] These data are encouraging but can be greatly improved. In Finland,[12] a program aimed at increasing asthma diagnosis and control that was implemented 27 years ago resulted in a significant reduction in asthma-related costs despite an increase of almost 300% in the number of asthma diagnoses in the same period. Costs per patient decreased by 72%. In addition, the proportion of patients with severe asthma exacerbation decreased from 20% to 2.5%. The success of the program was attributed to the local emphasis, the engagement of all staff involved in asthma management, and the view that asthma is a public health problem whose solution should involve health care professionals and the public through targeted educational campaigns and zero tolerance to underdiagnosis and undertreatment of asthma.[13] It could be argued that Finland is a small, developed country. Several municipal-level experiences in Brazil have shown that the same can successfully be done in our country when there are specialists willing to work in the Brazilian Unified Health Care System to provide referral centers and train primary health care professionals.[14-17]

In conclusion, it is time we stopped looking at the glass and rolled up our sleeves. Let us fill up the glass together. We want a Brazil where everyone can breathe!

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J Bras Pneumol. 2019;45(3):e20190146
http://dx.doi.org/10.1590/1806-3713/e20190146

Should the diaphragm be evaluated after abdominoplasty?

Antonella LoMauro[1,a]

The engine that moves air in and out of the lungs, sometimes referred to as the respiratory pump (or vital pump), relies on skeletal muscles, specifically the respiratory muscles. When the respiratory muscles contract, they create pressure gradients: negative to inhale and positive to exhale. With the exception of the diaphragm, all of the respiratory muscles have a secondary function as postural and chest wall stabilizers, forming and supporting the walls of the chest and abdomen. Quiet breathing is normally achieved by active inspiration, the diaphragm playing the major role, especially when the individual is in the supine position. The other inspiratory muscles contribute to quiet breathing, particularly when the individual is seated or standing. However, when higher levels of ventilation are required (e.g., during exercise) or when there are explosive events, such as coughing and vomiting, the other inspiratory muscles are strongly recruited, together with the expiratory ones. Expiration normally occurs as a passive return to functional residual capacity, and expiratory muscles do not usually contract in healthy subjects at rest.[1,2] Therefore, the diaphragm is the most important respiratory muscle. Diaphragmatic function can be assessed via invasive or noninvasive methods. The invasive methods require the use of esophageal catheters or ionizing radiation and are therefore not routinely used in clinical practice.[3,4] Ultrasound is a well-tolerated, noninvasive modality that allows quantitative measurements of diaphragm thickness and diaphragmatic excursion. When the diaphragm contracts, its normal movement is caudal, creating a piston-like effect to increase abdominal pressure and reduce pleural pressure. M-mode ultrasound allows the amplitude of the excursion of the hemidiaphragms to be quantified. The amplitude of excursion is defined as the maximal distance (from the end-expiratory baseline to the maximum height during inspiration) on the vertical axis of the M-mode ultrasound tracing of the echogenic line running between the liver (or spleen) and the lung, which corresponds to the diaphragm. The portability of an ultrasound device allows that measurement to be made directly at the bedside of patients, even of those who are critically ill. Reduced mobility of the diaphragm can be an indicator of muscle dysfunction.[5-7] Because of the major role played by the diaphragm, its dysfunction can have an impact on survival and quality of life, often being associated with dyspnea, exercise intolerance, and severe sleep disorders, including excessive daytime sleepiness.

The proper functioning of the diaphragm depends on three factors: innervation (i.e., phrenic nerve integrity); contractile muscle function; and the mechanical coupling of the diaphragm to the chest wall. Surgery can affect one or more of these factors, resulting in diaphragmatic dysfunction. Siafakas et al.[8] listed the following pathophysiological mechanisms that impair the function of the respiratory muscles after surgery: impaired neural control of respiratory muscles (e.g., after phrenicotomy); loss of the integrity of the respiratory muscles caused by the surgical incision; respiratory reflex mechanisms (phrenic nerve inhibition); a change in the length/tension relationship of respiratory muscles because of a change in functional residual capacity; a change in thoracoabdominal mechanics (e.g., due to reduction of the rib cage and/or abdominal compliance); the suppressive effects of pharmacological agents used for anesthesia and postoperative analgesia; specific surgical procedures (e.g., cooling during open heart surgery); and surgical procedures involving organs that affect respiratory muscle function (e.g., parathyroidectomy). The authors emphasized that some types of surgical procedures have a favorable effect on respiratory muscle function, whereas others influence it adversely. Abdominal surgery has a negative impact on respiratory muscles, the diaphragm in particular.[9,10] In fact, a shift to predominantly rib cage breathing after abdominal surgery indicates that the intercostal inspiratory muscles are more active than is the diaphragm in the postoperative period. In addition, MIP, MEP, and transdiaphragmatic pressure all decrease after upper abdominal surgery. Those decreases persist for at least 48 h after surgery and may not return to normal until a week after. In particular, the reported incidence of respiratory muscle dysfunction is very low (2-5%) after lower abdominal surgery, whereas it is considerably higher (20-40%) after upper abdominal surgery, the diaphragm being the muscle that is most affected in the latter.

Surgically induced diaphragmatic/respiratory muscle dysfunction can result in a number of postoperative pulmonary complications, including atelectasis and pneumonia, which can increase morbidity and mortality considerably. The study conducted by Fluhr et al.,[11] published in the current issue of the JBP, shows the negative repercussions that lipoabdominoplasty, a common type of cosmetic surgery, has for the diaphragms (and lungs) of healthy women. They showed that diaphragm mobility, assessed by M-mode ultrasound, was reduced in the first 10 days after surgery, as were lung volumes, and that both were restored to preoperative values after one month. Postoperative pain does not seem to seem to be a major indicator of diaphragmatic function, because it was reported by only 35% of women, in whom the amplitude of diaphragmatic excursion was similar to

1. Dipartimento di Elettronica, Informazione e Bioingegneria, Politecnico di Milano, Milano, Italia.
a. http://orcid.org/0000-0002-1408-6971

that observed in the women who reported no such pain. The authors attributed to the plication of the rectus abdominis next to the xiphoid appendix, and to the consequent increased intra-abdominal pressure, the cause for the reduced motion of the diaphragm. Therefore, surgery per se puts the diaphragm at a mechanical disadvantage, presumably because of a reduction in abdominal compliance and an increase in intra-abdominal pressure, also resulting in a restrictive ventilatory defect in patients undergoing this type of surgery.

The study conducted by Fluhr et al.[11] further confirms the need for physicians who deal with patients undergoing lipoabdominoplasty to be aware of the detrimental effects that the procedure has on the respiratory muscles, the diaphragm in particular. Physicians should be especially aware of the possibility that these complications will occur in healthy subjects or after surgical procedures that are not strictly linked to respiratory problems, as in the Fluhr et al. study,[11] as well as the chance that they will be present at hospital discharge despite appropriate postoperative follow-up. Such awareness should lead physicians to take the appropriate measures to minimize the occurrence of complications related to and reduce the magnitude of surgically induced respiratory muscle dysfunction.

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J Bras Pneumol. 2019;45(3):e20190139
http://dx.doi.org/10.1590/1806-3713/e20190139

The importance of molecular characterization in lung cancer

Gilberto de Castro Junior[1,2,a], Guilherme Harada[2,b], Evandro Sobroza de Mello[3,c]

Lung cancer is the second leading cause of cancer and the most common cause of death from cancer worldwide.[1] In the initial diagnostic workup of lung cancer, it is essential to recognize the fact that it is a highly heterogeneous disease. Precise molecular characterization is key to improving understanding of the tumor pathogenesis, to determining the prognosis, and to defining an individualized treatment plan based on predictive biomarkers. Therefore, since 2010, the Association for Molecular Pathology, the College of American Pathologists, and the International Association for the Study of Lung Cancer have collaborated to review the main evidence in molecular analysis and its implications. The most recent guideline, issued in 2018, includes many relevant recommendations and advocates for testing all patients with nonsquamous non-small cell lung cancer (NSCLC) for *EGFR* activating mutations, *ALK* rearrangements, and *ROS1* rearrangements; if adequate material is available, another group of genes—including *BRAF*, *MET*, *RET*, *ERBB2* (*HER2*), and *KRAS*—should be included in an expanded panel.[2] In addition, samples should be tested for predictive biomarkers of response to immune checkpoint inhibitors targeting the programmed death 1/programmed death-ligand 1 (PD-L1) pathway, such as PD-L1 expression in the tumor and inflammatory cells, and the tumor mutational burden should be also determined.

The importance of molecular characterization of NSCLC has been demonstrated in many trials,[3,4] which has had a major impact on clinical practice. Patients in whom the tumors have targetable oncogenic drivers and who have access to matched therapies have been shown to have better response rates, longer progression-free survival, and better quality of life scores than do unselected patients treated with traditional chemotherapy.[3,4] In fact, the Lung Cancer Mutation Consortium analyzed tumors from 1,007 patients with NSCLC and demonstrated absolute gains in overall survival in the patients in whom an oncogenic driver was identified in biopsy samples and who were treated with matched targeted therapies in comparison with those who did not receive genotype-directed therapies (3.5 years vs. 2.4 years, hazard ratio = 0.69; p = 0.006).[5] Another key advance that must be mentioned is related to the superiority of pembrolizumab, an anti-programmed death 1 antibody, in terms of overall survival rates, in comparison with platinum-based chemotherapy, in NSCLC patients with a tumor proportion score for PD-L1 ≥ 50%.[6] Therefore, the identification of these predictive biomarkers is a critical step in the treatment decision-making process for patients with NSCLC.

In the current issue of the JBP, Oliveira et al.[7] describe the frequency of EGFR mutations, ALK rearrangements, and PD-L1 expression in tumor samples evaluated at a surgical pathology laboratory in northeastern Brazil. Using a sensitive, next-generation sequencing technique, the authors found the frequency of EGFR activating mutations to be 22%. Using immunohistochemistry with the D5F3 clone, they detected ALK expression in 10.4% of the samples, and immunohistochemistry with the SP263 clone revealed a surprisingly low (50.9%) rate of PD-L1 positivity. In comparison with other studies conducted in Brazil, the EGFR mutation rate was similar,[8] whereas the level of ALK expression was higher.[9] Selection bias (due to geographic limitations and small sample sizes) could explain those discrepancies. Larger samples evaluated in multicenter studies would be more informative. Regarding PD-L1 expression, the high proportion of tumors testing negative for PD-L1 (49.5%) is remarkable in comparison with the 30% rate observed in the KEYNOTE-189 trial.[10] In one recent study conducted in Brazil, 61.39% of the 1,018 tumors evaluated (with clone 22C3) tested negative for PD-L1 expression,[11] suggesting that the epidemiology of this biomarker could be different among patients in Brazil.

Brazil faces many challenges in terms of broadening access to molecular pathology diagnosis and health technology in general. In addition to the delayed NSCLC diagnosis, a low proportion of patients have access to the recommended molecular testing.[8] Only half of all patients with advanced NSCLC who are diagnosed with lung adenocarcinoma are tested for EGFR activating mutations, and even fewer are tested in the public health care system. Data regarding ALK rearrangements and PD-L1 expression are scarce. All of that has direct impacts on overall survival rates, which differ between patients treated in the public sector and those treated in the private sector,[12] the median overall survival among patients with stage IV adenocarcinoma being 14.2 months for those who underwent molecular testing compared with 8.5 months for those who did not.

Among the barriers to access to molecular testing and matched targeted therapies in Brazil are the continental dimensions of and widespread internal social inequalities within the country, as well as preanalytical issues, such

1. Serviço de Oncologia Clínica, Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
2. Centro de Oncologia, Hospital Sírio-Libanês, São Paulo (SP) Brasil.
3. Laboratório de Anatomia Patológica, Instituto do Câncer do Estado de São Paulo, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
a. http://orcid.org/0000-0001-8765-3044; b. http://orcid.org/0000-0003-4012-3251; c. http://orcid.org/0000-0002-4383-2910

as the poor quality of formalin at many hospitals and inappropriate handling of small tissue samples in pathology laboratories. Access to the latest health technologies is restricted to a few centers, which compromises prevention, diagnosis, and treatment. There is a considerable delay in the approval of new therapies and in the activation of clinical trials by regulatory agencies, making it even more difficult to broaden access to new technologies. Ways to overcome some of the aforementioned obstacles include international collaboration, the creation of larger databases, and education (of physicians and patients), as well as the fostering of positive dialogues among medical societies, pharmaceutical companies, and advocacy groups.[13]

In daily clinical practice, precision oncology, in which pathological and molecular data, such as those related to prognostic and predictive biomarkers, are incorporated into the decision-making process, can identify the best candidates for some molecular-targeted therapies. Therefore, describing the molecular profile of patients with NSCLC in Brazil is essential to broadening access to therapies that are more safe and effective.

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Pulmonary cysts associated with calcified nodules

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

A 57-year-old woman presented with a two-month history of cough and dry mouth. A CT scan of the chest showed multiple cysts and calcified nodules (Figure 1). The patient presented with two basic CT patterns: multiple pulmonary cysts and calcified pulmonary nodules. The differential diagnosis of diffuse pulmonary cysts is extensive and includes neoplastic, inflammatory, and infectious diseases. Primary causes of diffuse pulmonary cysts include lymphangioleiomyomatosis, Langerhans cell histiocytosis (LCH), Birt-Hogg-Dubé syndrome, *Pneumocystis jirovecii* pneumonia, and lymphocytic interstitial pneumonia (LIP). Multiple pulmonary nodules have numerous etiologies. However, the combined presence of multiple pulmonary nodules and calcifications narrows the diagnostic possibilities, which include calcified metastases, amyloidosis, hyalinizing granulomas, epithelioid hemangioendothelioma, rheumatoid nodules, and multiple chondromas, as well as calcifications resulting from residual granulomatous lesions, particularly tuberculous lesions.[1-3]

By defining morphological features and cyst distribution, CT plays an extremely important role in the differential diagnosis of pulmonary cysts associated with calcified nodules, as do associated findings. In most cases, diagnosis is made by combining CT findings and clinical findings (particularly extrapulmonary manifestations), without the need for lung biopsy. CT findings of nodules and cysts in the same patient are consistent with LCH and LIP associated with amyloidosis. Given that patients with LCH present with nodules that are small and do not calcify, a presumptive diagnosis of LIP associated with amyloidosis was made. The fact that some of the calcifications were within the cystic lesions constituted further evidence of LIP associated with amyloidosis. In addition, our patient presented with xerostomia. Evaluation of clinical and laboratory data led to a diagnosis of Sjögren's syndrome. The final diagnosis was Sjögren's syndrome with LIP associated with amyloidosis.

The association of lymphoproliferative disorders (particularly LIP) with amyloid deposits, cystic lung formation, and Sjögren's syndrome is widely recognized. However, the exact nature of this relationship remains unclear.

A rare lymphoproliferative disorder, LIP is most common in patients with immunodeficiency or autoimmune disease, particularly Sjögren's syndrome. In addition to nodules and cysts, other CT findings include ground-glass opacities, peribronchovascular thickening, and ill-defined centrilobular nodules. Patients can be asymptomatic or present with dyspnea, cough, fatigue, and chest pain. In many cases, cysts are incidental findings on routine CT scans or are findings associated with complications such as spontaneous pneumothorax.

Although histopathological examination is usually required for diagnosis, our patient was definitively diagnosed on the basis of imaging and clinical findings, without the need for lung biopsy.

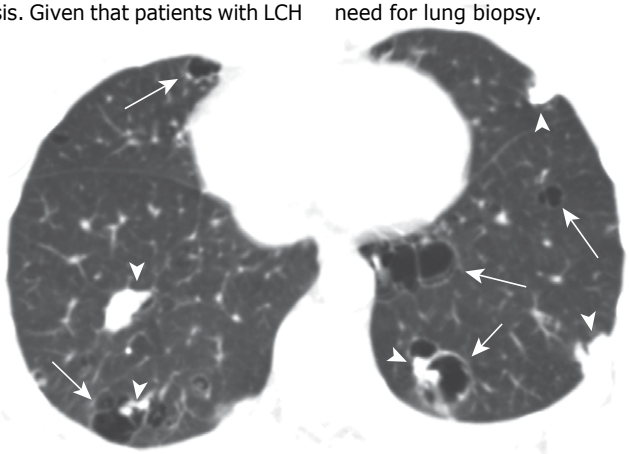

Figure 1. Axial CT scan of the chest with lung window settings at the level of the lung bases, showing multiple cysts (arrows) and calcified nodules (arrowheads). Note that some of the calcifications are within the cystic lesions.

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1. Universidade Federal do Rio de Janeiro, Rio de Janeiro (RJ) Brasil.
2. Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre (RS) Brasil.
a. http://orcid.org/0000-0001-8797-7380; b. http://orcid.org/0000-0003-1984-4636; c. http://orcid.org/0000-0003-0261-1860

How to prepare and present a poster at a conference and communicate your research findings effectively

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

The overall goal of presenting a poster at a conference is to communicate to and receive feedback from your peers who have similar research interests regarding your research findings, as well as to increase your scientific network. To make the most of this opportunity, in an environment where conference attendees are overwhelmed by new information, the presenters of a poster need to communicate their results effectively.

A POSTER IS NOT A MINI MANUSCRIPT

The most common mistake researchers make when designing and presenting a poster is to treat the poster as if it were a mini manuscript. Remember, the goal is to clearly and quickly communicate research content to attendees who are walking around a large area with many posters, with limited time and attention span to read extensive information.

Successfully communicating your study will rely on attracting attendees to your poster and making it easy for them to grasp key messages. Consider the 10-10 rule of thumb for poster viewing: attendees look at posters for 10 seconds and from 10 feet (3 meters) away.[1,2] During those 10 seconds, if they are attracted to the poster, they might want to read more information. Therefore, your key messages must be written using a large enough font size for people to read it from 3 meters away, and the information should be interesting and attractive enough so that attendees will want to come closer, read more, and ask questions.

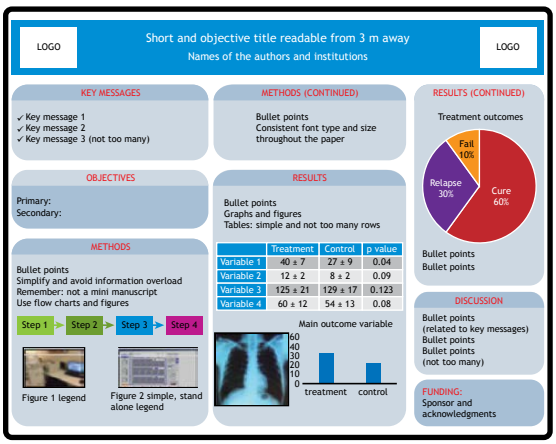

Figure 1. Poster model with examples of headers, figures, graphs, color, and font size.

HOW TO PREPARE YOUR POSTER

The most important advice is to avoid information overload. Remember: it is not a mini manuscript and attendees have limited time. We recommend that you use bullet points, minimize text, and simplify the language, with easy-to-read phrases. Use the active voice and avoid jargon and acronyms whenever possible. The title should be short and informative. The left upper corner is the first area attendees will look after reading the title and scan it during those 10 seconds, so avoid writing an introduction (remember, it is not a manuscript); instead, start with your key messages (Figure 1). Then state your objectives or research question, so that readers will know what your study is about. When describing your methods, avoid unnecessary details, use bullet points, simplify the text, and use flow charts or figures to illustrate the method process. Results are the most important information you will communicate. Use figures and graphs with legible font, clear axis, and, if possible, with the legend embedded in the graph/figure. Finish your poster with a discussion that aligns with your research question.

Check the conference guidelines for poster orientation and size. Use columns and headers to facilitate reading. Resist the temptation to fill all available space, leave some blank space to make the poster more attractive. It is important to use consistent wording, font, font size, and colors.

KEY MESSAGES

- A poster is not a mini manuscript; avoid communicating too much information
- Be mindful of using a small font size that is hard to read from a comfortable distance
- Substitute text for figures and graphs whenever possible
- Practice presenting your poster to your friends and colleagues at least five times
- Be prepared, look and act professionally, and make it worth the effort

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1. Methods in Epidemiologic, Clinical, and Operations Research–MECOR–program, American Thoracic Society/*Asociación Latinoamericana del Tórax*, Montevideo, Uruguay.
2. Divisão de Pneumologia, Instituto do Coração, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
3. Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA.
a. http://orcid.org/0000-0001-6548-1384; b. http://orcid.org/0000-0001-5742-2157

ISSN 1806-3713

Uncovering the beneficial effects of inhaled bronchodilator in COPD: beyond forced spirometry

José Alberto Neder[1,a], Danilo Cortozi Berton[2,b], Denis E O'Donnell[1,c]

BACKGROUND

It is a common clinical observation that many patients with COPD in whom FEV_1 and/or FVC improve less than 200 mL and 12% after the use of inhaled bronchodilator (BD) did report less dyspnea during daily life when exposed to this medication. This state of affairs has shed negative light on the ability of pulmonary function tests to predict a positive clinical response to BDs.

OVERVIEW

A 75-year-old long-term smoker was referred for spirometry with a specific query for diagnosis of COPD—modified Medical Research Council (mMRC) scale score 3. Forced spirometry confirmed moderate-to-severe airflow limitation without a "significant" response to a short-acting BD.[1,2] Despite these negative results, the patient reported marked improvement in daily exertional symptoms (mMRC scale score 1) after 4 weeks of treatment with a combination of a long-acting β_2 agonist (LABA) and a long-acting antimuscarinic agent (LAMA). He was subsequently enrolled in a cross-over, randomized clinical trial contrasting the effects of the same LABA/LAMA combination against placebo. As shown in Figure 1A, the lack of "significant" changes in FEV_1 and/or FVC after the use of the medication coexisted with a marked decrease in gas trapping (lower RV). As lung hyperinflation—⇓ functional residual capacity (FRC)—improved to a greater extent than did thoracic hyperinflation (⇓ TLC), inspiratory capacity (IC) increased significantly. The latter was maintained throughout the exercise test (Figure 1B), being associated with lower dyspnea scores and increased tolerance to physical effort.

Exertional dyspnea arises when the descending motor drive to the inspiratory muscles is increased and the respiratory system fails to meet this increased demand.[3] During exercise, the expiratory time becomes too short to fully exhale what has been inspired. Thus, expiratory flow limitation worsens gas trapping, leading to an upward shift in the operational lung volumes (i.e., those theoretically available for breathing). This, in turn, causes tidal volume to become progressively constrained as it approaches the "ceiling" (TLC).[3] In this context, BDs fundamentally work as pharmacological deflators: there is more room for tidal volume expansion (IC) when the "floor" (end-expiratory lung volume) drops more than the "ceiling" (Figure 1B).

Why is it possible that forced spirometry may fail to show these beneficial effects on lung volumes? FEV_1 is biased to reflect the function of the larger airways, i.e. the "fast component" of expiration, which, by definition, empties first.[4] RV and FRC, in contrast, are strongly influenced by the mechanical properties of the smaller airways which need a longer time to empty (the "slow component").[4] Thus, an improvement in the flow rates of the "slow component" might not be detected by the maximal flow parameters from forced spirometry.[5]

CLINICAL MESSAGE

Adding a slow maneuver to forced spirometry to obtain IC (and, if feasible, measurements of static lung volumes) significantly enhances the clinical usefulness of pulmonary function tests in identifying the COPD patients who are poised to benefit from use of inhaled BDs as pertaining to exertional dyspnea and exercise intolerance.

A	LABA + LAMA		
	Pre	Post (1 hour)	Δ, L (%baseline)
FEV_1, L (%pred)	1.14 (43)	1.06 (40)	-0.08 (-7)
FVC, L (%pred)	3.50 (88)	3.75 (94)	+0.25 (+7)
FEV_1/FVC	0.32	0.28	-0.04
Slow VC	3.75	4.00	**+0.25 (+7)**
TLC, L (%pred)	6.66 (99)	6.27 (93)	**-0.39 (-6)**
FRC, L (%pred)	4.54 (126)	3.83 (106)	**-0.71 (-16)**
RV, L (%pred)	2.91 (118)	2.27 (92)	**-0.64 (-22)**
IC, L (%pred)	2.12 (67)	2.44 (78)	**+0.32 (+15)**

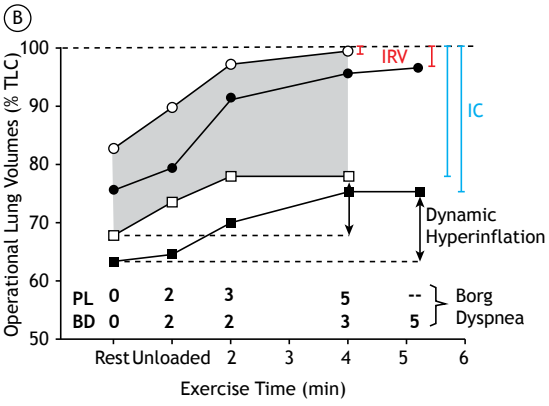

Figure 1. In A, pulmonary function test results (forced and slow expiratory maneuvers and body plethysmography) before and after the administration of a combination of a long-acting β_2-agonist (LABA) and a long-acting antimuscarinic agent (LAMA). In B, results of endurance cardiopulmonary exercise tests performed after the use of placebo (PL; white symbols) and bronchodilators (BD; black symbols) on different days to determine symptom limitation with serial measurements of inspiratory capacity (IC) in order to track end-expiratory (squares) and end-inspiratory (circles) lung volumes. See text for detailed discussion. FRC: functional residual capacity, IC: inspiratory capacity; and IRV: inspiratory reserve volume.

1. Pulmonary Function Laboratory and Respiratory Investigation Unit, Division of Respirology and Sleep Medicine, Kingston Health Science Center & Queen's University, Kingston (ON) Canada
2. Unidade de Fisiologia Pulmonar, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.
a. http://orcid.org/0000-0002-8019-281X; b. http://orcid.org/0000-0002-8393-3126; c. http://orcid.org/0000-0001-7593-2433

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J Bras Pneumol. 2019;45(3):e20180181
http://dx.doi.org/10.1590/1806-3713/e20180181

Molecular profile of non-small cell lung cancer in northeastern Brazil

Ana Claudia da Silva Mendes de Oliveira[1,a], Antonio Vinicios Alves da Silva[2,b], Marclesson Alves[3,c], Eduardo Cronemberger[3,d], Benedito Arruda Carneiro[4,e], Juliana Carneiro Melo[5,f], Francisco Martins Neto[6,g], Fabio Tavora[1,7,h]

1. Programa de Pós-Graduação em Patologia, Universidade Federal do Ceará, Fortaleza (CE) Brasil.
2. Laboratório Argos, Fortaleza (CE) Brasil.
3. Unidade de Oncologia, Pronutrir Oncologia e Nutrição, Fortaleza (CE) Brasil.
4. Lifespan Cancer Institute, Warren Alpert Medical School, Brown University, Providence (RI) USA.
5. Programa de Pós-Graduação em Saúde Coletiva, Universidade de Fortaleza – UNIFOR – Fortaleza (CE) Brasil.
6. Unidade de Cirurgia Torácica, Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza (CE) Brasil.
7. Departamento de Patologia, Hospital de Messejana Dr. Carlos Alberto Studart Gomes, Fortaleza (CE) Brasil.

a. http://orcid.org/0000-0001-6035-8846
b. http://orcid.org/0000-0002-6266-3366
c. http://orcid.org/0000-0002-1662-3398
d. http://orcid.org/0000-0001-8493-7287
e. http://orcid.org/0000-0002-5468-1126
f. http://orcid.org/0000-0003-1734-3352
g. http://orcid.org/0000-0003-2565-365X
h. http://orcid.org/0000-0003-2361-0613

Submitted: 19 June 2018.
Accepted: 7 December 2018.

Study carried out in the Laboratório Argos, Fortaleza (CE) Brasil.

ABSTRACT

Objective: To investigate the histological subtypes and mutational profiles of non-small cell lung cancer in Brazil, looking for correlations among histological subtypes, expression of anaplastic lymphoma kinase (ALK), EGFR mutation status, and programmed death-ligand 1 (PD-L1) expression. **Methods:** We evaluated 173 specimens obtained from patients with lung adenocarcinoma in northeastern Brazil. Expression of PD-L1 and *ALK* was evaluated by immunohistochemistry; *EGFR* mutation status was evaluated by sequencing. We categorized the histological subtypes in accordance with the International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society classification. **Results:** The most common histological subtypes of lung adenocarcinoma were solid predominant (in 46.8%), acinar predominant (in 37.0%), and lepidic predominant (in 9.8%). *ALK* expression was detected in 10.4% of the samples, and 22.0% of the tumors harbored *EGFR* mutations. The most common *EGFR* mutation was an exon 21 L858R point mutation (in 45.5%), followed by an exon 19 deletion (in 36.3%). The tumor proportion score for PD-L1 expression was ≥ 50% in 18.2% of the samples, 1-49% in 32.7%, and 0% in 49.5%. The solid predominant subtype was significantly associated with wild-type *EGFR* status (p = 0.047). Positivity for PD-L1 expression was not found to be significantly associated with *ALK* expression or *EGFR* mutation status. **Conclusions:** Our results suggest that the molecular profile of non-small cell lung cancer in northeastern Brazil differs from those of populations in other regions of the country, with *ALK* positivity being higher than the other biomarkers. Further studies including clinical and genetic information are required to confirm these differences, as well as studies focusing on populations living in different areas of the country.

Keywords: Anaplastic lymphoma kinase; ErbB receptors; B7-H1 antigen; Carcinoma, non-small-cell lung; Brazil.

INTRODUCTION

Approximately 1.8 million new cases of lung cancer are diagnosed annually, accounting for 13% of all cancer cases worldwide. In the United States, there were an estimated 150,000 deaths from lung cancer in 2018.[1] For that same year, data from the Brazilian National Cancer Institute indicate that in Brazil, there were 27,200 deaths from lung cancer, as well as 31,270 new cases of the disease.[2]

Non-small cell lung cancer (NSCLC) accounts for more than 80% of all cases of lung cancer, and this broad category (NSCLC) encompasses a number of subtypes, the most prevalent of which is adenocarcinoma. According to the World Health Organization (WHO) classification, the most common histological patterns of growth in adenocarcinomas are acinar, solid, papillary, micropapillary, and mucinous.[3]

Most lung cancer patients present with metastatic disease and receive chemotherapy, targeted therapies, immunotherapies, or a combination of those modalities. The standard of care for advanced NSCLC was transformed by the identification of oncogenic drivers and the development of tyrosine kinase inhibitors targeting such drivers, including the epidermal growth factor receptor (*EGFR*) and anaplastic lymphoma kinase (*ALK*) oncogenes. The more recent development of immune checkpoint inhibitors, such as anti-programmed death-ligand 1 (anti-PD-L1) and anti-cytotoxic T lymphocyte antigen 4, has also led to major therapeutic advances in this disease as demonstrated by the results of several clinical trials documenting improvements in overall survival.[4,5]

The PD-L1 checkpoint inhibitor (also known as B7 homolog 1) is the major ligand of PD-1, and its expression on the surface of tumors cells upregulates and inhibits the immune response. Some clinical trials in patients with NSCLC have demonstrated a correlation between increased PD-L1 expression on NSCLC cells and enhanced efficacy of single-agent anti-PD-1 or anti-PD-L1 inhibitors,[6,7] as well as of combinations of those with ipilimumab.[8]

Correspondence to:
Fabio Tavora. Avenida Santos Dumont, 5753, apto. 1607, CEP 60175-047, Fortaleza, CE, Brasil.
Tel.: 55 85 3486-3150. E-mail: ftavora@gmail.com
Financial support: None.

The results were independent of molecular alterations in *ALK* or *EGFR*. These advances not only represent major therapeutic breakthroughs but also amplify the importance of identifying the molecular features of tumors in order to guide the therapy and maximize its benefits. In low- and middle-income countries, the enthusiasm for these novel treatments is tempered by the limited access to molecular tools to characterize tumors, as well as by the high costs of targeted therapies and immunotherapies.[9] As a result, a large proportion of patients continue to receive conventional (i.e., non-targeted) chemotherapy, which, in many circumstances, is associated with limited efficacy and significant adverse effects.

Only a few studies have documented the molecular features of NSCLC, including the prevalence of *ALK* rearrangement, *EGFR* mutation status, and PD-L1 expression, in Brazil.[10-12] There is even more limited information regarding patients in underserved regions such as the northeastern region of the country. Here, we report the findings in a sample of 173 specimens of lung adenocarcinoma evaluated at a major referral laboratory of pathology, describing the histological subtypes, *EGFR* status, *ALK* status, and PD-L1 expression.

METHODS

Sample selection

We conducted a retrospective analysis of NSCLC specimens evaluated at a regional referral laboratory for surgical pathology in Fortaleza, Brazil (the Argos Laboratory), between 2015 and 2016. All specimens were fixed in formalin, after which they were stained with hematoxylin and eosin in a routine manner. Cases were reviewed by two independent pathologists with experience in pulmonary pathology and were classified according to the WHO classification system.[3] The morphological patterns, predominant histological subtypes, and available clinical data were recorded. Only non-small cell carcinomas were included in the study, those with sarcomatoid or neuroendocrine differentiation therefore being excluded. The study was approved by the Institutional Review Board of Messejana Heart and Lung Hospital, also located in the city of Fortaleza, and was registered with the National Commission for Ethics in Research (CAAE protocol no. 65315317.0.0000.5039).

EGFR mutation status

For each sample, we selected a representative formalin-fixed, paraffin-embedded block containing at least 10% viable tumor. After proteinase K digestion of the samples, we extracted DNA following standard protocols. Direct DNA sequencing of exons 18 through 21 of the *EGFR* gene was performed as previously described.[13] To detect gene mutations, we employed multiplex polymerase chain reaction in a next-generation sequencing instrument (MiSeq; Illumina, San Diego, CA, USA), as previously described in detail.[13]

ALK expression

For the evaluation of *ALK* expression, all specimens were processed in accordance with the well-established standard operating procedures adopted at the pathology laboratory. In brief, sections were stained in an automated slide staining instrument (Ventana Benchmark GX; Roche Diagnostics, Basel, Switzerland) and incubated with an approved anti-*ALK* rabbit monoclonal primary antibody (clone: D5F3, Cat. #: 790-4796; Roche Diagnostics), after which *ALK* was detected with an amplification kit (OptiView Amplification Kit, Cat. #: 760-099; Roche Diagnostics) and a diaminobenzidine immunohistochemical detection kit (OptiView DAB IHC Detection Kit, Cat. #: 760-700; Roche Diagnostics). Counterstaining was performed with hematoxylin, and negative controls were assessed. Samples were considered positive for *ALK* expression if any cells showed cytoplasmic staining, regardless of the proportion or intensity of staining.

PD-L1 expression

For the evaluation of PD-L1 expression, all specimens were processed in accordance with standard established protocols. Immunohistochemical staining for PD-L1 protein was carried out with the Ventana PD-L1 assay (clone: SP263, Cat. #: 740-4907; Roche Diagnostics) on the Ventana Benchmark GX system, PD-L1 being detected with the kits described for *ALK*. Counterstaining was performed with hematoxylin, and negative controls were assessed. In the interpretation of the results, PD-L1 expression was evaluated on tumor cells. Samples were considered positive for PD-L1 expression on the basis of the proportion of cells showing staining of any intensity, in 10% increments.[14]

STATISTICAL ANALYSIS

Correlations between categorical variables were analyzed with Fisher's exact test (when any cell in a contingency table had an expected count < 5) or Pearson's chi-square test (when none of the cells in a contingency table had an expected count < 5). All reported p-values are two-sided, and tests were conducted at a 0.05 level of significance. Statistical analysis was performed with the Statistical Analysis System, version 9.4 (SAS Institute Inc., Cary, NC, USA).

RESULTS

A total of 173 patients with lung adenocarcinoma were evaluated. The general characteristics of the patients are shown in Table 1. The median age was 67 years (range, 36-93 years), and 103 (59.5%) of the patients were > 70 years of age. Eighty-one (46.8%) of the patients were male.

The most common sampling sites were the lung, pleura, and lymph nodes, which respectively accounted for 125 (72.2%), 23 (13.3%), and 12 (6.9%) of the 173 specimens collected. Most of the specimens were obtained by computed tomography-guided transthoracic biopsy, followed by lobectomy and transbronchial biopsy.

In accordance with the WHO classification of lung tumors,[3] we categorized the invasive adenocarcinoma growth patterns as follows (Table 1): solid predominant, in 81 (46.2%) of the specimens; acinar predominant, in 64 (37.0%); lepidic predominant, in 17 (9.8%); and papillary predominant, in 8 (4.6%).

We analyzed the *EGFR* mutation status in 149 patients. In 116 (77.9%), we detected no *EGFR* mutations (wild-type status). Thirty-three samples (22.1%) were found to harbor mutations in the *EGFR* kinase domain. As shown in Table 2, the main types of *EGFR* mutations were an L858R point mutation in exon 21, seen in 15 (45.5%) of the samples; a deletion in exon 19, seen in 12 (36.3%); and G719X point mutations in exon 18, seen in 3 (9.1%). The histological subtype mostly associated with wild-type EGFR status was the solid predominant subtype ($p = 0.0475$).

Samples were positive for protein expression of *ALK* in 18 (10.4%) of the 173 cases analyzed. Among the *ALK*-positive cases, the histological subtype was acinar predominant in 10 (55.6%), solid in 6 (33.3%), lepidic in 1 (5.6%), and papillary in 1 (5.6%). Cases in which the subtype was mucinous predominant did not display *ALK* rearrangements (Table 3). Figure 1 shows two lung adenocarcinoma samples of the acinar predominant subtype, of which one was negative for *ALK* expression (Figure 1A) and one showed strong, diffuse positivity for *ALK* (Figure 1B).

Table 1. Characteristics of patients with lung adenocarcinoma.

Characteristic	(N = 173)
Age (years), median (range)	67 (36-93)
> 70 years, n (%)	103 (53.5)
≤ 70 years, n (%)	70 (40.4)
Gender, n (%)	
Male	81 (46.8)
Female	92 (53.2)
Histological subtype, n (%)	
Acinar predominant	64 (37.0)
Solid predominant	81 (46.8)
Lepidic predominant	17 (9.8)
Papillary predominant	9 (4.6)
Mucinous predominant	3 (2.0)
Topography, n (%)	
Lung	125 (72.3)
Pleura	23 (13.3)
Lymph node	12 (6.9)
Bone	5 (2.9)
Brain	4 (2.3)
Liver	2 (1.2)
Other	2 (1.2)
Sampling procedure, n (%)	
Biopsy	130 (75.1)
Segmentectomy	21 (12.1)
Lobectomy	18 (10.4)
Other	3 (1.7)

PD-L1 expression was analyzed in 55 of the tumor samples. Of those, 27 (49.1%) were negative for PD-L1 expression and 28 (50.9%) showed some degree of PD-L1 expression. Using the tumor proportion score (TPS) cut-off values employed in clinical trials of atezolizumab,[6] we stratified PD-L1 expression by TPS, which was 0% in 27 (49.1%) of the 55 samples, 1-4% in 2 (3.6%), 5-49% in 16 (29.1%), and ≥ 50% in 10 (18.2%), as shown in Table 4. Figure 2 shows representative images of different extents of (i.e., TPS for) PD-L1 expression in lung adenocarcinoma: 0% (Figure 2A); 10% (Figure 2B); 50% (Figure 2C); and 100% (Figure 2D).

Table 3 shows the associations that gender, age, and histological subtype showed with PD-L1 expression, *ALK* expression, and *EGFR* mutation status. Neither PD-L1 positivity, *EGFR* mutation status, nor *ALK* expression was found to be significantly associated with gender or age. Fisher's exact test showed no significant relationship between positive PD-L1 expression and *EGFR* mutation status ($p = 0.407$) or between positive PD-L1 expression and *ALK* expression ($p = 0.408$).

DISCUSSION

Here, we have attempted to detect associations among PD-L1 expression, *ALK* expression, and *EGFR* mutation status in cases of NSCLC evaluated at a regional referral laboratory for surgical pathology in Brazil. Our findings show that the frequency of PD-L1 expression in patients with nonsquamous NSCLC was 50.9%, higher than the 37.9% reported in another study conducted in Brazil, in which the protocols were similar but different antibodies were used.[15] Previous studies have shown that approximately 30% of patients with advanced NSCLC have a high level of PD-L1 expression (defined as a TPS ≥ 50%).[16,17] In the present study, approximately half of the patients showed some degree of PD-L1 positivity, although only 18.2% had a TPS ≥ 50%. The relatively low proportion of patients with a high level of PD-L1 expression in our study sample could reflect variations in the antibodies, staining platforms, and assay methodologies across studies, as well as a certain degree of arbitrariness in the definition of PD-L1 positivity. There is therefore an urgent need for standardization of PD-L1 testing, which has yet to be addressed. In addition, given that the antibody used in the present study (clone SP263) exhibits staining characteristics for PD-L1 similar to those reported for other anti-PD-L1 antibodies, such as 22C3 and 28-8,[5] we can postulate that our findings are attributable to the unique molecular features of the population studied.

Table 2. Frequency of *EGFR* mutations in primary lung adenocarcinoma.

Mutation	n (%)
Exon 19 deletion	12 (36.3)
Exon 21 L858R point mutation	15 (45.5)
Exon 18 G719X point mutations	3 (9.1)
Exon 20 insertion	2 (6.1)
Exon 18 insertion	1 (3.0)

Knowing the proportional PD-L1 expression in lung tumors in any given population might be important not only for predicting responses to therapy but also for determining the overall prognosis. A recent clinical trial, known as the KEYNOTE-024 trial,[17] showed improved progression-free survival and overall survival in NSCLC patients whose tumors had a PD-L1 TPS ≥ 50%. A recent meta-analysis involving 47 studies and more than 11,000 patients showed a positive correlation between PD-L1 expression and a poor prognosis in lung cancer.[18] It is of note that the association with a poor prognosis was observed only in Asian populations. Given the lack of data for the population of Brazil, the present study might represent a first step toward identifying a specific prevalence.

Populations living in low- and middle-income countries face many challenges in order to gain access to new therapies. Not only are the prices of immune checkpoint inhibitors higher in Brazil but the implementation of biomarker selection also represents a barrier to access to the best immunotherapies.[19] In this context, health care systems are also penalized; in one study, a decision-analytic model showed that the use of PD-L1 expression as a biomarker increases the cost-effectiveness of immunotherapy.[20] Some studies have demonstrated that PD-L1 expression tends to be associated with smoking status, high pathologic grade, positive lymph nodes, and tumor size.[16,21]

The echinoderm microtubule-associated protein-like 4-*ALK* fusion gene was first identified in 2007 by Soda et al.,[22] who estimated its frequency to be 6.7% in patients with NSCLC. Since then, other studies, using immunohistochemistry, have estimated the frequency of *ALK* expression to be 3-7% among such patients.[23,24] The frequency of *ALK* expression in the present study (10.4%) was higher than the 3.2-4.8% previously reported for patients with NSCLC in Brazil.[10,12] To our knowledge, ours is the first study focusing on a population of patients in northeastern Brazil. The few previous studies reporting the prevalence of *ALK* and other biomarkers of NSCLC in Brazil have all focused on the population living in the southeastern region of the country. Socioeconomic disparities between the more developed southeastern region and the northeastern

Table 3. Expression of programmed death-ligand 1, *EGFR* mutation status, and expression of the anaplastic lymphoma kinase oncogene in patients with lung adenocarcinoma, by patient characteristic and histological subtype.

Characteristic	PD-L1 expression		*EGFR* mutation status		*ALK* expression	
	Negative n (%)	Positive n (%)	Wild-type n (%)	Mutated n (%)	Negative n (%)	Positive n (%)
Age						
≤ 70 years	10 (18.2)	8 (14.5)	47 (31.5)	18 (12.1)	65 (37.6)	5 (2.9)
> 70 years	17 (30.9)	20 (36.4)	69 (46.3)	15 (10.1)	90 (52.0)	13 (7.5)
Gender						
Female	17 (30.9)	15 (27.3)	61 (40.9)	20 (13.4)	82 (47.4)	10 (5.9)
Male	10 (18.2)	13 (23.6)	55 (36.9)	13 (8.7)	73 (42.2)	8 (4.6)
Histological subtype						
Acinar predominant	15 (27.3)	8 (14.5)	39 (26.2)	16 (10.7)	54 (31.2)	10 (5.8)
Lepidic predominant	2 (3.6)	1 (1.8)	11 (1.4)	3 (2.0)	16 (9.2)	1 (0.6)
Mucinous predominant	1 (1.8)	1 (1.8)	2 (1.3)	1 (0.7)	3 (1.7)	0 (0.0)
Papillary predominant	1 (1.8)	3 (5.5)	4 (2.7)	3 (2.0)	8 (4.6)	1 (0.6)
Solid predominant	8 (14.5)	15 (27.3)	60 (40.3)	10 (6.7)*	74 (42.8)	6 (3.5)

PD-L1: programmed death-ligand 1; and ALK: anaplastic lymphoma kinase. *p = 0.0475 vs. all other subtypes (chi-square test).

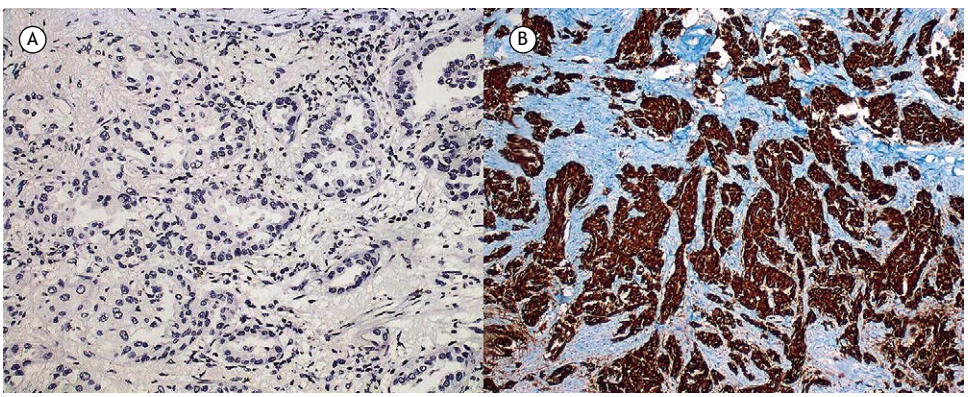

Figure 1. Anaplastic lymphoma kinase (*ALK*) expression in lung adenocarcinomas. In A, invasive lung adenocarcinoma of the acinar predominant subtype, staining negative for *ALK* expression (magnification, ×200). In B, invasive lung adenocarcinoma of the acinar predominant subtype, showing strong, diffuse positive staining for *ALK* (magnification, ×200).

region might have contributed to the higher frequency of *ALK* positivity found in our study. In fact, clinical variables and genetic information should also be considered in order to explain such differences, and further studies certainly will be required.

We found that 22.1% of patients with lung adenocarcinoma harbored *EGFR* mutations, which is similar to the 21.6% reported in another study conducted in Brazil,[12] albeit lower than the 26-33% reported for Latin America at large[25,26] and the 30-50% reported for Asia,[27] whereas it is higher than the 11-17% reported for White patients in the United States and the 8-13% reported for patients in Europe.[28] The most common *EGFR* mutations found in the present study were an exon 19 deletion and an exon 21 L858R point mutation, as has been reported for other populations.[12,21,29]

The associations between PD-L1 expression and *EGFR* mutations vary across studies. Some authors have shown a direct association between high PD-L1 expression and a positive *EGFR* mutation status.[30,31] However, Takada et al.[21] found that PD-L1 expression was significantly associated with a wild-type *EGFR* status. In the present study, we found no association between *EGFR* mutation status and PD-L1 expression. Our results are more akin to those described in a recent meta-analysis conducted by Yang et al.,[32] in which the authors concluded that the relationship between PD-L1 expression and *EGFR* mutation status was variable and not significant.

An association between *ALK* positivity and PD-L1 expression has been demonstrated in some clinical studies,[33,34] although not in others.[35,36] Although we identified a discrete trend toward such an association in the present study, it did not reach statistical significance. The unclear relationship between PD-L1 expression and the activation of oncogenic drivers (*EGFR* and *ALK*) in NSCLC, together with the discrepancies among studies, might be attributable to differences across studies in terms of the baseline clinical characteristics of the patients, heterogeneity among study populations, and the lack of standardization in the definition of PD-L1 positivity.

In attempts to determine whether molecular alterations are able to alter morphology, there have been several studies investigating the associations between *EGFR* mutations and the major histological

Table 4. Expression of programmed death-ligand 1, by tumor proportion score, in cases of primary lung adenocarcinoma (N = 55).

TPS	n (%)
0%	27 (49.5)
1-4%	2 (3.6)
5-49%	16 (29.1)
≥ 50%	10 (18.2)

TPS: tumor proportion score.

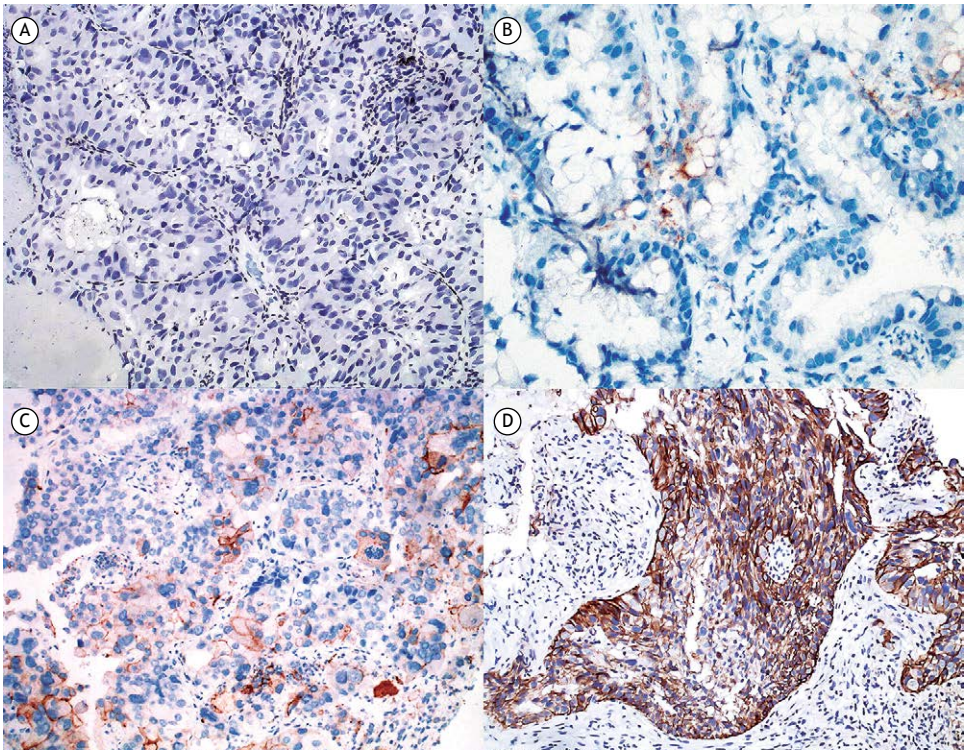

Figure 2. Programmed death-ligand 1 (PD-L1) expression in lung adenocarcinomas. In A, absence of PD-L1 expression in lung adenocarcinoma of the acinar (cribriform) predominant subtype (magnification, ×200). In B, focal positivity for PD-L1 (tumor proportion score [TPS] = 10%) in lung adenocarcinoma of the mucinous predominant subtype (magnification, ×200). In C, moderate positivity for PD-L1 (TPS = 50%) in lung adenocarcinoma of the solid predominant subtype (magnification, ×200). In D, diffuse, intense positivity for PD-L1 (TPS = 100%) in lung adenocarcinoma of the solid predominant subtype (magnification, ×200).

patterns. A study conducted in Japan showed that *EGFR* mutations were significantly associated with the papillary predominant and lepidic predominant subtypes.[37] In a study conducted in China, Song et al.[38] found that the micropapillary predominant and lepidic predominant subtypes were associated with *EGFR* mutations. In a population of patients in the United States, the lepidic predominant subtype was found to be the only histological subtype associated with *EGFR* mutations,[39] whereas the acinar predominant subtype was the only subtype found to be associated with *EGFR* mutations in a population of patients in Brazil.[12] Our finding that the solid predominant subtype was most strongly associated with wild-type *EGFR* status underscores the fact that the relationship between *EGFR* mutation status and the histological subtype of lung adenocarcinoma remains unclear.

Our study has several limitations. First, it was a single-center retrospective study, which makes it impossible to rule out the possibility of bias. Second, because we focused mainly on pathological findings, there is a lack of clinical data, which could have improved the study. Finally, the immunohistochemical analysis of PD-L1 involved the use of only one antibody, which might have been inappropriate if there was heterogeneity in the PD-L1 expression within a tumor sample.

In summary, we have reported the frequency of clinical biomarkers of NSCLC, together with the corresponding pathological findings, in a population of 173 patients in northeastern Brazil. We found no significant associations among those biomarkers. Despite the fact that the frequency of PD-L1 expression and *EGFR* mutation status were consistent with the few data available for Brazil, the frequency of *ALK* expression was higher than that previously reported for populations in Brazil. Further studies are encouraged in order to understand how such biomarkers are distributed throughout this heterogeneous population and, more importantly, how to translate that knowledge into better routine clinical practice.

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J Bras Pneumol. 2019;45(3):e20170395
http://dx.doi.org/10.1590/1806-3713/e20170395

Lipoabdominoplasty: repercussions for diaphragmatic mobility and lung function in healthy women

Sandra Fluhr[1,a], Armèle Dornelas de Andrade[1,b], Emanuel José Baptista Oliveira[2,c], Taciano Rocha[1,d], Ana Irene Carlos Medeiros[1,e], Amanda Couto[1,f], Juliana Netto Maia[1,g], Daniella Cunha Brandão[1,h]

1. Departamento de Fisioterapia, Universidade Federal de Pernambuco, Recife (PE) Brasil.
2. Departamento de Cirurgia Plástica, Hospital Agamenon Magalhães, Recife (PE) Brasil.

a. http://orcid.org/0000-0002-8261-0894
b. http://orcid.org/0000-0001-9430-4395
c. http://orcid.org/0000-0002-9369-3883
d. http://orcid.org/0000-0001-6190-1425
e. http://orcid.org/0000-0003-2958-6079
f. http://orcid.org/0000-0002-7927-9192
g. http://orcid.org/0000-0001-8680-180X
h. http://orcid.org/0000-0001-8805-6815

Submitted: 29 May 2018.
Accepted: 12 August 2018.

Study carried out in the Departamento de Fisioterapia, Universidade Federal de Pernambuco, Recife (PE) Brasil.

ABSTRACT

Objective: To evaluate the impact of lipoabdominoplasty on diaphragmatic mobility (DM) and lung function in healthy women. **Methods:** This was a prospective cohort study using high-resolution ultrasound and forced spirometry to assess DM and lung function, respectively, prior to lipoabdominoplasty, as well as on postoperative day (POD) 10 and POD 30. DM was measured under two conditions: during tidal volume breathing and during a VC maneuver. **Results:** The sample consisted of 20 women, with a mean age of 39.85 ± 7.52 years and a mean body mass index of 26.21 ± 2.0 kg/m^2. Comparing the preoperative and postoperative periods, we found that DM and lung function values were significantly lower after lipoabdominoplasty, the mean DM on POD 10 being 17% and 15% lower during tidal volume breathing and during the VC maneuver, respectively, in comparison with the preoperative mean ($p = 0.009$ and $p < 0.001$, respectively). In addition, FEV_1, FVC, and PEF were significantly lower on POD 10 than in the preoperative period ($p = 0.046$, $p = 0.002$, and $p < 0.001$, respectively), returning to preoperative values by POD 30. **Conclusions:** Lipoabdominoplasty appears to have negative short-term repercussions for DM and lung function in healthy women. However, lung function and DM are both apparently restored to preoperative conditions by POD 30.

(ClinicalTrials.gov identifier: NCT02762526 [http://www.clinicaltrials.gov/])

Keywords: Abdominoplasty; Lipectomy; Ultrasonography; Diaphragm/physiology; Diaphragm/physiopathology; Lung/physiology; Lung/physiopathology; Respiratory mechanics; Spirometry; Respiratory function tests.

INTRODUCTION

Plastic surgery techniques for contouring the abdomen, such as lipoabdominoplasty, are among the most often requested procedures, ranking third among plastic surgery techniques performed worldwide.[1-4] Lipoabdominoplasty, which combines classical abdominoplasty and liposuction, results in a significant reduction in the fat pad and has the added benefits of muscle plication and the removal of skin tissue.[5] It has become common practice among plastic surgeons and has a low incidence of postoperative complications if the clinical condition of the patient is evaluated prior to the procedure.[5,6] However, there have been reports of respiratory comorbidities, including respiratory failure, atelectasis, pneumonia, and bronchospasm, in the postoperative period after lipoabdominoplasty.[7-11] Such complications might be attributable to increased intra-abdominal pressure (IAP), caused by plication of the aponeurosis of the rectus abdominis muscle, which could lead to changes in diaphragmatic mobility (DM),[12] as well as impaired lung function.[10,13] In some of the cases reported, lung function was reduced to half of that observed prior to surgery. Although none of the authors of those reports evaluated DM in the affected patients, other factors have been implicated in a postoperative decrease in lung volumes, such as the administration of anesthetic drugs, visceral manipulation, the incision in the abdominal wall, and patient fear of injury from the surgery.[6,14] Pain exerts an effect on the postoperative evolution of patients submitted to abdominal surgery, with a negative impact on lung function.[15]

The primary objective of this study was to identify respiratory complications in healthy women undergoing lipoabdominoplasty. To that end, we evaluated DM and lung function at several time points.

METHODS

Study design and participants

This was a prospective cohort study conducted in the Physical Therapy Sector of the Laboratory of Physiology

Correspondence to:
Sandra Fluhr. Departamento de Fisioterapia, Universidade Federal de Pernambuco, Avenida Jornalista Anibal Fernandes, s/n, Cidade Universitária, CEP 50670-901, Recife, PE, Brasil.
Tel.: 55 81 3049-3633. E-mail: sandrafluhr@hotmail.com
Financial support: This study received financial support from the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; Grant no 432865/2016-0), *Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco* (FACEPE, Foundation for the Support of Scientific and Technological Development in the State of Pernambuco; APQ 0154-4.08/15), *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Office for the Advancement of Higher Education; Finance Code 001), and *Programa Nacional de Cooperação Acadêmica* (PROCAD, Brazilian Academic Cooperation Program; Grant no 88881068409/2014-01).

ISSN 1806-3713

and Cardiopulmonary Physiotherapy and at the Plastic Surgery Clinic of the *Hospital das Clínicas da Universidade Federal de Pernambuco* (HC-UFPE, Federal University of Pernambuco *Hospital das Clínicas*), as well as at the Plastic Surgery Clinic of the Hospital Agamenon Magalhães, between July of 2015 and March of 2016. The study was approved by the Human Research Ethics Committee of the HC-UFPE Health Sciences Center (CAAE protocol no. 15225913.0.0000.5208) and was registered with ClinicalTrials.gov (identifier: NCT02762526). We employed a non-probability sampling process, in which we screened all individuals who met the eligibility criteria.

Eligibility criteria

We included women between 25 and 55 years of age who underwent lipoabdominoplasty with plication of the rectus abdominis muscle. All had type IV or V abdominal deformity, as described by Bozola.[16] We selected women with no history of respiratory or cardiac comorbidities. We also selected only women with a body mass index ≤ 30 kg/m^2 and a minimum score of 18 on the Mini-Mental State Examination.

We excluded women who were current smokers or had a smoking history greater than 10 pack-years. Women with an FEV_1 < 80% of the predicted value or an FEV_1/FVC ratio < 70% of the predicted value were also excluded.

Surgical procedure

For the surgical procedure, all patients were sedated and received spinal or epidural anesthesia. First, the surgical site was marked. A solution of epinephrine (diluted 1:250,000 in saline) was then infiltrated into the abdominal cavity to initiate liposuction. The aspiration began through the supra-umbilical region, continuing along the sides and through the infra-umbilical region. After the liposuction, the navel was isolated and only the infra-umbilical skin was resected, as in classic abdominoplasty.[5]

Umbilicoplasty was performed by attaching the deep dermis of the navel to the aponeurosis of the rectus abdominis muscle, after which the deep dermis of the neo-umbilicus was attached to the flap with monocryl 3-0 sutures at the intercardinal points. The skin flap was fixed in two planes, with mononylon 3-0 sutures in the subcutaneous tissue and monocryl 5-0 sutures in the dermis, initially with separate single sutures and subsequently with continuous sutures. At the intercardinal and cardinal points of the navel, respectively, we used the modified Allgöwer-Donati suture and single sutures, both with mononylon 4-0 sutures.[17]

Outcome measures

All of the outcomes investigated were measured at three time points: in the preoperative period, on postoperative day (POD) 10, and on POD 30. Initially, medical histories were taken and all of the patients underwent a physical examination. Personal data were collected, and we recorded anthropometric measurements—weight (in kg), height (in m), and body mass index (in kg/m^2)—as well as vital signs—HR, SpO_2, RR, and blood pressure. Patients were asked to remain seated with their arms resting on their legs and to remain quiet during the measurement of vital signs.

DM

We used a high-resolution ultrasound system (SonoAce R3; Samsung Medison, Seoul, South Korea) with a convex 3.5 MHz transducer. The protocol used was that suggested by Testa et al.[12,18] The patients received a verbal command to breathe evenly for the measurement of DM during tidal volume (V_T) breathing and then to perform VC maneuvers (Figure 1), during which each curve relating to the displacement of the dome of the diaphragm (in mm) was measured immediately after acquisition of the images. The maneuvers were repeated in order to obtain five satisfactory images. We considered the average of the three highest values that were within 10% of each other.

Lung function

We used a portable spirometer (Microloop MK8; Micro Medical, Kent, England) in order to evaluate FVC, FEV_1, FEF between 25% and 75% of the FVC ($FEF_{25-75\%}$), PEF, and the FEV_1/FVC ratio. The maneuvers were carried out in accordance with the recommendations of the American Thoracic Society[19] and other guidelines for pulmonary function testing.[20]

Dyspnea

Patients were asked about their perception of dyspnea at rest and during the procedures performed. We applied the modified Borg Scale, in accordance with the American Thoracic Society recommendations.[19]

Pain

We used a visual analog scale[21] consisting of a one-dimensional instrument for graduation of pain intensity level. Pain was assessed at rest during each respiratory evaluation. To avoid measurement bias, all procedures were performed by the same examiner during all phases of the study.

Statistical analysis

The sample size was calculated, on the basis of the results of a pilot study involving 10 patients, with the G*Power3 software package.[22] The calculation was performed by determining the mean difference between the preoperative and POD 10 values (Δ1), as well as between the preoperative and POD 30 values (Δ2) for the most clinically relevant variables: FEV_1, FVC, and DM. For FEV_1, we observed a Δ1 value of 13.4 ± 5 and a Δ2 value of 3.5 ± 3. For FVC, we observed a Δ1 value of 11.8 ± 2.62 and a Δ2 value of 4.7 ± 4.5. For DM, we observed a Δ1 value of 18 ± 7.16 and a Δ2 value of 2.62 ± 3.13. Therefore, we required 5 patients for FEV_1, 7 patients for FVC, and 7 patients for DM, which would give all of those variables a power

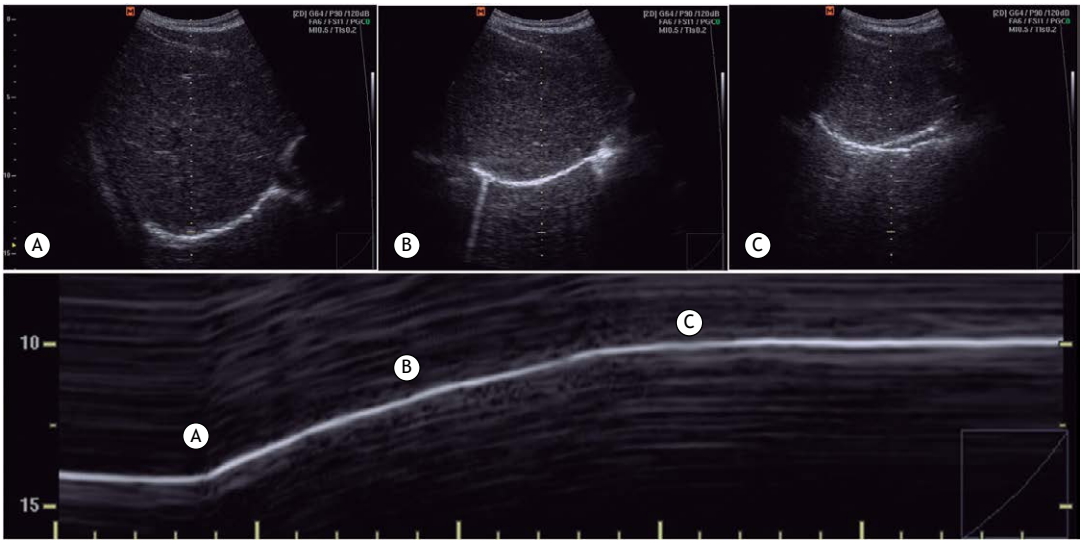

Figure 1. Assessment of diaphragmatic mobility by M-mode ultrasound, with the placement of markers for determining diaphragm displacement during the VC maneuver. A, B, and C indicate the beginning, middle, and end of the inspiratory cycle, respectively.

of 95% and an alpha of 0.05. However, we decided to select 27 patients, considering the potential for losses to follow-up in prospective cohort studies.

The data were analyzed with SigmaPlot software for Windows, version 12.0 (Systat Software, San Jose, CA, USA). To characterize the sample, we calculated descriptive statistics, using means ± standard deviations, means (95% CIs), or medians (interquartile ranges) for quantitative variables.

The Shapiro-Wilk test and Levene's test for homogeneity of variance were applied in order to verify the normality and homogeneity of the data, respectively. For comparisons among time points, we used two-way repeated-measures ANOVA with the Holm-Sidak post hoc test to compare means for quantitative variables with normal, homogeneous distribution. For quantitative variables with a non-normal distribution, we used Friedman's repeated-measures ANOVA on ranks, followed by Tukey's post hoc test for variables that presented a statistical difference. The effect size was determined by calculating the Cohen's d, which involved obtaining the mean difference between the time points and dividing the result by the pooled standard deviation.[23] Pearson's correlation coefficient was used in order to detect associations between DM variables and lung function variables. Statistical analyses were performed with the IBM SPSS Statistics software package, version 20.0 (IBM Corporation, Armonk, NY, USA). Values of $p < 0.05$ were considered statistically significant.

RESULTS

The initial sample comprised 27 women, all of whom underwent lipoabdominoplasty. However, only 20 of those women completed the protocol (Figure 2). Anthropometric variables, clinical characteristics, and vital signs are summarized in Table 1. Two patients were excluded from the analysis of DM, because of technical problems related to the ultrasound examination. On POD 10, DM was relatively low, during V_T breathing and at TLC—9.54 mm (8.42-10.99 mm) and 51.23 mm (41.66-55.89 mm), respectively—those values returning to normal by POD 30—12.37 mm (10.49-14.13 mm) and 63.35 mm (55.19-68.34 mm), respectively—at which point they were comparable to those obtained in the preoperative period—11.56 mm (9.65-13.48 mm) and 60.15 mm (51.95-67.84 mm), respectively. As can be seen in Figure 3, the differences between the preoperative and postoperative values were significant for the images acquired during V_T breathing ($p = 0.009$), as well as for those acquired during the VC maneuver ($p < 0.001$).

Lung function parameters at the three time points evaluated are shown in Table 2. There were significant differences among the time points for FEV_1, FVC, and PEF, all of which were lower on POD 10 than in the preoperative period and were restored to normal values by POD 30. The FEV_1/FVC ratio and $FEF_{25-75\%}$ remained unchanged after lipoabdominoplasty, with no statistical differences between any of the time points. On POD 10, the DM measured during the VC maneuver correlated positively, albeit moderately, with FEV_1 ($r = 0.502$; $p = 0.034$) and PEF ($r = 0.515$; $p = 0.029$), as depicted in Figure 4.

Of the 20 women evaluated, 7 reported pain on POD 10. Three of those women classified their pain as mild and 4 classified it as moderate. One of the women reported moderate pain on POD 30. Two women reported perceived dyspnea on POD 10, the dyspnea being classified as not very intense in one case and very intense in the other. On POD 30, none of the patients reported dyspnea. No differences were observed between the pain/dyspnea reported at rest and that reported during the execution of the maneuvers, in terms of the perception or intensity of

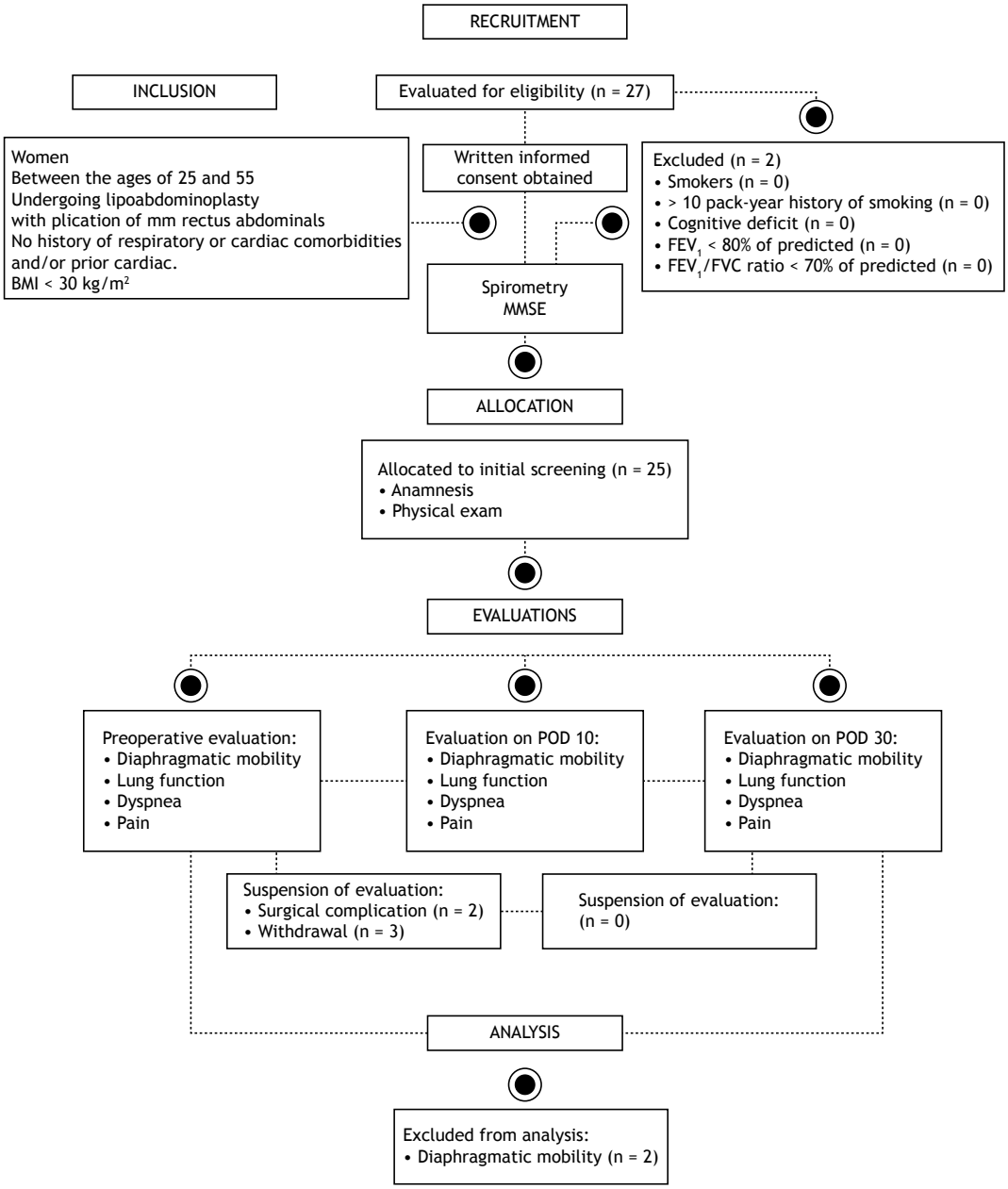

Figure 2. Flowchart of the study design. BMI: body mass index; MMSE: Mini-Mental State Examination; and POD: postoperative day.

pain or in terms of the perception of dyspnea, at any of the time points evaluated.

DISCUSSION

The main findings of our study are that lipoabdominoplasty with plication of the rectus abdominis muscle promoted a reduction in DM and worsening of lung function in healthy women, as evaluated on POD 10. However, both of those parameters showed a tendency to return to preoperative values by POD 30.

In comparison with the preoperative means, the mean DM on POD 10 was 17% lower when measured during V_T breathing and 15% lower when measured during the VC maneuver. By POD 30, the DM had returned to values comparable to those obtained in the preoperative period. However, there is a shortage of studies evaluating DM in this specific population, which precludes any comparisons with our findings.

We hypothesize that there were two main causes of the behavior of DM in our sample. The first potential cause was the influence of plication of the rectus abdominis muscle near the xiphoid process, and the second was the increase in IAP.

Regarding the influence of plication of the rectus abdominis muscle, we suggest that approximation of

the edges of the rectus abdominis muscles can generate a higher tensile strength in the fibers of those muscles, reducing the anteroposterior and transverse diameter of the chest, which will in turn reduce DM, because of the anatomical proximity between the upper insertions of the rectus abdominis muscles and one of the origins of the diaphragm muscle. The rectus abdominis muscle attaches to the fifth, sixth, and seventh costal cartilages and to the xiphoid process, whereas the costal portion of the diaphragm also originates from the last six ribs. In addition, the trunk flexion posture adopted by the patient for just over 10 days after the procedure can increase the magnitude of the changes.[24,25]

The primary action of the transversus abdominis and abdominal oblique muscles is to pull the abdominal wall inward, increasing the IAP. In doing so, they induce cranial displacement of the diaphragm, leading to an increase in pleural pressure and a consequent reduction in lung volume. Although the rectus abdominis muscle must do the same when the ventral abdominal wall has an outward convexity, we would expect that when the convexity is inward, an isolated muscle contraction would pull the wall slightly outward.[26] In an animal study, De Troyer et al.[27] analyzed the selective activation of the rectus abdominis muscle by electrical stimulation, finding that the ribcage and sternum were displaced in caudal and anteroposterior directions, whereas the transverse diameters of the lower ribcage were decreased. Therefore, the plication of the rectus abdominis muscle causes an increase in IAP and pleural pressure, regardless of the shape of the abdominal wall. Despite the limitations of comparison, these findings corroborate the hypothesis put forth in our study: that plication of the aponeurosis generates tension and thus limits thoracic expansion. We hypothesize that the reduction in DM can also be explained by the postoperative increase in IAP, which persisted at least until POD 10, caused by the plication of the rectus abdominis muscle to correct diastasis recti, which prevents the diaphragm from descending.[26] After plication, the abdominal region can properly assist in the lung expansion and IAP values remain within the normal range, which in healthy adults is up to 5 mmHg. Pressures above 15 mmHg can cause further damage to the respiratory system.[28,29] Although our study does not present the IAP values of the women who underwent lipoabdominoplasty, other authors have described the behavior of IAP after similar surgical procedures. Talisman et al.[30] measured IAP oscillations during abdominoplasty in 18 patients and studied the relevance of such oscillations for patient evolution in the immediate postoperative period. Three patients who underwent plication to correct diastasis recti presented an IAP above 24 cmH_2O in the immediate postoperative period and above 20 cmH_2O on POD 1. The authors concluded that such patients are at a higher risk of developing respiratory distress in the immediate postoperative period.

Plication of the rectus abdominis muscle near the xiphoid process and the consequent increase in IAP would place the diaphragm at a mechanical disadvantage, resulting in restrictive lung disease in patients undergoing this type of surgery. In our patient sample, we observed a postoperative decrease in spirometric parameters, the lower values persisting at least until POD 10.

Table 1. Anthropometric variables, clinical characteristics, and vital signs.[a]

Variable	(n = 20)
Age (years)	39.85 ± 7.52
Weight (kg)	67.48 ± 6
Height (m)	1.60 ± 0.07
BMI (kg/m^2)	26.21 ± 2
MMSE score	29.8 ± 0.41
RR (breaths/min)	17 ± 3.42
HR (bpm)	69 ± 9.54
SpO_2 (%)	98.3 ± 1.34

BMI: body mass index; and MMSE: Mini-Mental State Examination. [a]Values expressed as mean ± SD.

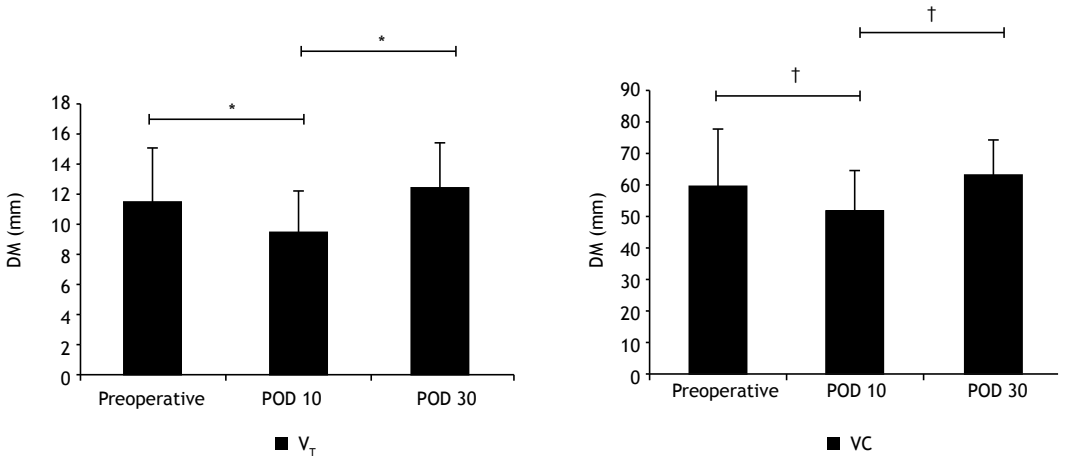

Figure 3. Diaphragmatic mobility (DM), measured during tidal volume breathing (V_T) and during a VC maneuver (VC), at the three time points evaluated. Values of p were calculated with two-way repeated-measures ANOVA followed by the Holm-Sidak post hoc test. POD: postoperative day. *p = 0.009 vs. Preoperative. †p < 0.001 vs. Preoperative.

Table 2. Lung function at the three time points evaluated.[a]

Variable[b]	Preop. (n = 20)	POD 10 (n = 20)		POD 30 (n = 20)			p*
	Value	Value	Cohen's d (Preop. vs. POD 10)	Value	Cohen's d (Preop. vs. POD 30)	Cohen's d (POD 10 vs. POD 30)	
FEV_1/FVC	101.75 (98.25-105.25)	98.65 (94.8-102.5)	0.4	102.07 (94.38-104.77)	0.05	0.51	0.209
FEV_1†	89.5 (84.2-95.0)	83.0 (69.0-91.0)	0.55	87.5 (81.2-94.2)	0.23	0.35	0.046‡
FVC	90.65 (86.49-94.81)	82.25 (76.04 -88.46)	0.74	85.14 (78.6-91.68)	0.50	0.23	0.002‡
$FEF_{25-75\%}$	99.65 (90.08-109.22)	86.05 (73.12-98.98)	0.55	99.21 (90.08-108.35)	0.02	0.61	0.064
PEF	73.60 (67.66-79.54)	57.95 (49.84-66.06)	1.03	71.71 (65.24-78.19)	0.15	1.07	< 0.001‡,§

Preop.: preoperative period; POD: postoperative day; and $FEF_{25-75\%}$: FEF between 25% and 75% of the FVC. [a]Values expressed as mean (95% CI), except where otherwise indicated. [b]All variables shown in percentages of the predicted values. *Two-way repeated-measures ANOVA with the Holm-Sidak post hoc test for variables with a normal, homogeneous distribution; Friedman's repeated-measures ANOVA on ranks with Tukey's post hoc test for variables with a non-normal distribution. †Median (interquartile range). ‡Significant difference between the Preop. and POD 10 values. §Significant difference between the POD 10 and POD 30 values.

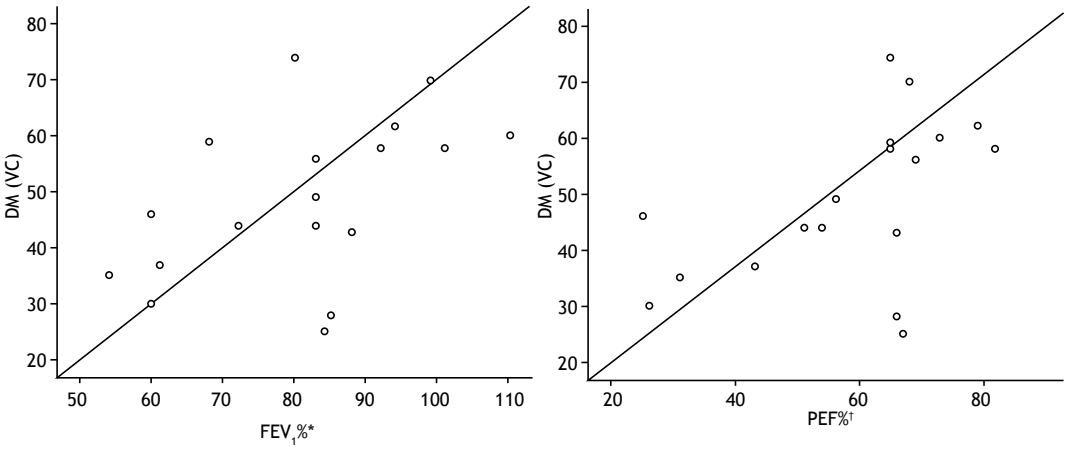

Figure 4. Diaphragmatic mobility (DM) during a VC maneuver on postoperative day 10, in comparison with the FEV_1 and PEF values (both in % of predicted) obtained at the same time point. Values of p were calculated with Pearson's correlation coefficient. *Significant correlation with DM during the VC maneuver (r = 0.502; p = 0.034). †Significant correlation with DM during the VC maneuver (r = 0.515; p = 0.029).

The spirometric data obtained in our study show that there was pronounced worsening of lung function on POD 10, as evidenced by lower FEV_1 and FVC values, and a near-total restoration of normal lung function by POD 30. Similarly, PEF was reduced on POD 10 and returned to preoperative values on POD 30, the difference between POD 10 and POD 30 being significant. During the study period, there were no significant changes in $FEF_{25-75\%}$ or in the FEV_1/FVC ratio. These data suggest that lipoabdominoplasty promotes the development of restrictive lung disease.

To our knowledge, there have been no previous studies evaluating lung function in women undergoing lipoabdominoplasty. However, other authors have studied the behavior of lung function in the postoperative period after abdominoplasty and have observed that lung function worsens in the immediate postoperative period and returns to preoperative levels by POD 30.[10,13,31,32] Our choice to evaluate patients at that time point was based on those studies. Tercan et al.[10] evaluated 14 healthy women who underwent abdominoplasty, observing a significant decline in FVC on POD 10 and subsequent improvement by POD 30, when FVC surpassed the preoperative values, suggesting that correction of diastasis recti abdominis promotes effective containment of the abdominal wall, improving the spirometric parameters over a period > 30 days. Similarly, Helene Junior et al.[13] found that, among patients undergoing abdominoplasty, the values of FEV_1, FVC, $FEF_{25-75\%}$, and PEF were lower on POD 4 than in the preoperative period, the FEV_1/FVC ratio remaining constant, although FEV_1 and FVC were both below normal, suggesting a restrictive pattern. The authors also found that FVC and PEF showed

significant improvement from POD 4 to POD 15, as well as from POD 15 to POD 30, although neither returned to the preoperative value. In one long-term study of 24 patients undergoing total abdominoplasty, Perin et al.[31] evaluated spirometric parameters during the preoperative period and after a mean period of 28 months. The authors found no difference between those two time points in terms of the lung function of the patients. Rodrigues et al.[32] studied the respiratory function of patients who underwent plication of the aponeurosis of the external abdominal oblique muscle and correction of diastasis recti. Those authors observed a ventilatory pattern similar to what occurs in the postoperative period in patients undergoing only correction of diastasis recti, concluding that the use of an L-shaped plication, per se, does not increase IAP; that is, the plication is not responsible for the impairment of lung function after abdominoplasty. The authors attributed the significant postoperative increase in IAP to the use of a compression garment, citing that as the most detrimental factor.

In the present study, we found that, on POD 10, DM during the VC maneuver correlated with lung function. We looked for correlations at that time point because we believe that patients undergoing lipoabdominoplasty show the most limitations in the first 10 days after the procedure. In our patient sample, DM during the VC maneuver showed a moderate positive correlation with PEF and with FEV_1, although DM during V_T breathing did not correlate with any of the spirometric parameters. These data indicate that some of the limitations in lung function in these patients can be explained by the reduction in DM caused by plication, that reduction being more pronounced at maximum effort, given that the sample consisted of healthy women. It is likely that there would be a strong correlation between lung function and DM, including DM during V_T breathing, in a population with pre-existing comorbidities.

In our study, pain, as measured with a visual analog scale, was reported by 35% and 5% of the patients on POD 10 and POD 30, respectively. Although some studies have shown that postoperative pain can be related to reduced lung volumes,[33] we found no correlation between pain and lung function. We also did not consider pain a relevant factor for diaphragmatic dysfunction, because the behavior of spirometric parameters and DM in the patients with pain was similar to that observed in those without. That might be due to the fact that only 7 women reported pain, probably because most of the patients received analgesic medications, as is common practice in the postoperative period. In addition, unlike what occurs during upper abdominal surgery, there is no disruption of muscle fibers during lipoabdominoplasty, which is an important distinction, because muscle injury is the main cause of postoperative pain.[34]

On POD 10, the patients in our sample showed reductions in DM and lung function. However, dyspnea was not an important clinical factor in our study, being reported by only 10% of the patients evaluated.

Our study has some limitations, such as the fact that we did not evaluate respiratory muscle strength or diaphragm thickness. There is therefore a need for further studies involving such analyses, which could better elucidate our findings.

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J Bras Pneumol. 2019;45(3):e20170164
http://dx.doi.org/10.1590/1806-3713/e20170164

Melatonin effects on pulmonary tissue in the experimental model of Hepatopulmonary Syndrome

Adriane Dal Bosco[1,a], Filipe Boeira Schedler[2,b], Josieli Raskopf Colares[2,c], Elisângela Gonçalves Schemitt[2,3,d], Renata Minuzzo Hartmann[2,3,e], Luiz Alberto Forgiarini Junior[4,f], Alexandre Simões Dias[2,3,g], Norma Possa Marroni[2,3,h]

1. Centro Universitário Metodista, Porto Alegre (RS) Brasil.
2. Hospital de Clínicas de Porto Alegre, Porto Alegre (RS) Brasil.
3. Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.
4. Universidade LaSalle, Canoas (RS) Brasil.

a. http://orcid.org/0000-0003-0907-8325
b. http://orcid.org/0000-0002-5518-2601
c. http://orcid.org/0000-0002-0482-204X
d. http://orcid.org/0000-0003-3015-0192
e. http://orcid.org/0000-0001-8667-517X
f. http://orcid.org/0000-0002-6706-2703
g. http://orcid.org/0000-0002-0609-4779
h. http://orcid.org/0000-0001-7856-7953

Submitted: 18 May 2017.
Accepted: 12 August 2018.

Study carried out in the Hospital de Clínicas de Porto Alegre, Porto Alegre (RS) Brasil.

ABSTRACT

Objective: To evaluate the pulmonary alterations of animals with Hepatopulmonary Syndrome (HPS) submitted to Biliary Duct Ligature (BDL), as well as the antioxidant effect of Melatonin (MEL). **Methods:** Sixteen male Wistar rats, divided into four Sham groups: BDL group, Sham + MEL group and BDL + MEL. The pulmonary and hepatic histology, lipoperoxidation and antioxidant activity of lung tissue, alveolar-arterial O2 difference and lung / body weight ratio (%) were evaluated. **Results:** When comparing the groups, could be observed an increase of vasodilation and pulmonary fibrosis in the BDL group and the reduction of this in relation to the BDL + MEL group. It was also observed significant changes in the activity of catalase, ApCO2, ApO_2 in the LBD group when compared to the other groups. **Conclusion:** The use of MEL has been shown to be effective in reducing vasodilation, fibrosis levels and oxidative stress as well as gas exchange in an experimental HPS model.

Keywords: Bile duct; Hepatopulmonary Syndrome; Melatonin; Lung.

INTRODUCTION

The Cirrhosis is presented in the presence of fibrotic nodules in the liver that arise as a result of chronic liver injury.[1] Such alterations can lead to portal hypertension and terminal liver disease, which generate alterations in the vascular system and affecting different organs.[2] In the respiratory system, Hepatopulmonary Syndrome (HPS) and Portopulomonar Hypertension are the two main clinical conditions that affect the lungs.[2] Hepatopulmonary Syndrome is the most common vascular disorder found in patients with cirrhosis, which is responsible for pulmonary vasodilation, hyperdynamic circulation and changes in gas exchange.[2,3]

Abnormalities in gas exchange presented by HPS patients are associated with the presence of arteriovenous shunts, ventilation-perfusion discrepancies and diffusion-perfusion.[2] These changes present in HPS are mostly explained by intra-pulmonary[2] vasodilatation and angiogenesis, and different animal models are used to simulate hepatic cirrhosis, and the Biliary Duct Ligature (BDL) model best simulates the present alterations in the syndrome.[4]

The BDL model is capable of causing gastric changes similar to those found in HPS[2] patients. The angiogenesis process is also present in the BDL model, that is the an alteration present due to the action of the Vascular Endothelial Growth Factor-A (VEGF-A), which is produced by intravascular pulmonary monocytes.[5,6] Pulmonary vasodilation in the HBL experimental model is associated with increased production of Endothelin-1 (ET-1) and Endothelial Nitric Oxide Synthase (eNOS).[6]

Recent studies that investigate the therapeutic potential of Melatonin (MEL) suggest that its antioxidant power can be used in the treatment of HPS, since it has an anti-inflammatory effect[7] and reduces VEGF levels in hepatic carcinoma cells, contributing to the reduction of angiogenesis.[8] Melatonin also has therapeutic effects in animal models of fulminant hepatitis and pulmonary hypertension, reducing oxidative stress and preventing the reduction of the activity of antioxidant enzymes.[9-11] In lung tissue, Melatonin exerts protective effect in animal models of cirrhosis induced by carbon tetrachloride.[12]

Due to the existence of an experimental model that simulates HPS and the potential therapeutic effect of

Correspondence to:
Norma Possa Marroni. Laboratório de Hepatologia Experimental, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Santa Cecília, CEP 90035-903, Porto Alegre, RS, Brasil.
Tel.: 55 51 3359-8760. E-mail: nmarroni@terra.com.br
Financial support: Fundo de Incentivo a Pesquisa do Hospital de Clínicas de Porto Alegre (FIPE/HCPA), Fundação de Amparo a Pesquisado do Rio Grande do Sul (FAPERGS), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

  ISSN 1806-3713

Melatonin in this syndrome, this study aims to evaluate the pulmonary alterations of animals submitted to Biliary Duct Ligature, as well as the antioxidant effect of Melatonin.

METHODS

In this study 16 male Wistar rats were used, weighing 250 g on average. The animals were obtained by the vivarium of University of Brazil Lutheran (ULBRA) and were housed in plastic boxes (47 × 34 × 18 cm) covered with wood shavings, which was stored in a controlled environment with a temperature between 20 and 25 °C. The animals were kept in a light / dark cycle of 12 / 12h, with free availability for water and food. The research project was previously approved by the ULBRA Ethics Committee for Animal Use (ECAU-ULBRA), and all research procedures are in accordance with the rules established by Law Number 11,794 of October 11, 2008 and by the Guideline Brazilian Association of Practice for the Care and Use of Animals for Scientific and Educational Purposes.

The procedure of Common Bile Duct Ligature (CBDL) was used for the development of HPS, according to the one recommended by Kountouras et al.[13] Prior to all surgical procedures, the animals received an anesthetic dose of Xylazine 2% (50mg / kg body weight) and Ketamine (100mg / kg body weight), both injected into the intraperitoneal region. The period for the development of the model was 14 days, followed by another 14 days for the treatment with melatonin. The total period of experiment was 28 days, and at the moment of euthanasia, the animals received a dose three times higher than the one used for the surgical procedure.

Four groups (n = 4) were used in the study: *Sham* group: A simulated CBDL surgical procedure was performed, manipulation of the bile duct with the anesthetized animal and the animals also received intraperitoneal injection of NaCl (0.9%) as on the 15th day after surgery, for 14 days. *Sham+Melatonin* Group (Sham + MEL): The surgical procedure for CBDL was simulated, and administration of Melatonin (20mg / kg) took places by intraperitoneal injection for 14 days, starting on the 15th day after surgery. Biliary Duct Ligature Group (BDL): The animals were submitted to CBDL and NaCl application (0.9%) intraperitoneally for 14 days, starting on the 15th day after surgery. Biliary Duct Ligature Group + Melatonin (BDL + MEL): The animals were submitted to CBDL and received Melatonin (20mg / kg) by intraperitoneal injection for 14 days, starting on the 15th day after surgery.

The laboratory tests were performed at the Laboratory of Clinical Analyzes of the Hospital of Clinics of Porto Alegre (*HCPA - Laboratório de Análises Clínicas do Hospital de Clínicas de Porto Alegre*), and the other analyzes and procedures were performed at the Laboratory of Experimental Hepatology of the Hospital of Clinics of Porto Alegre (*HCPA - Laboratório de Hepatologia Experimental do Hospital de Clínicas de Porto Alegre*).

After 28 days after surgery, the animals were weighed and anesthetized and blood was collected through the Retro-Orbital Bleeding Collection Technique,[14] with the objective of analyzing liver enzyme levels. Subsequently, an anteromedial laparotomy was performed to collect blood from the abdominal aorta for gas analysis, using an ABL 700 Radiometer (*Copenhagen, Denmark*) for the measurement of blood gases. The iontophoresis method was used to measure the Partial Arterial Oxygen Pressure (ApO2), Partial Arterial Carbon Gas Pressure (ApCO2) and Arterial Oxygen Saturation (SaO2). Alveolar Oxygen Pressure (APO2) was calculated by the following formula: APO2 = Inspired fraction of O2 (FiO2) -ApCO2 / 0.8.[15] The Alveolar-Arterial Oxygen Difference (D (A-a) O2) was calculated by the formula: D (A-a) O2 = APO2-ApO2.[15]

After euthanasia, the lung was weighed for later analysis of the Pulmonary Weight / Body Weight relation. A portion of the liver and lower right lung lobe was removed for histological analysis, the remainder of the lung tissue introduced into liquid Nitrogen and stored at -80 °C for further analysis. Pulmonary and hepatic samples collected for histological analysis were inserted in 10% formaldehyde solution for 12 hours, and later inserted in 70% alcohol containers and stained with Hematoxylin and Eosin (HE) staining, the pulmonary samples were also stained of picrosirius. The histological analyzes were performed in a blinded experiment manner by specific pathologists from the *HCPA* Pathology Laboratory.

Frozen lung tissue was homogenized by an Ultra-Turraz homogenizer (*IKA Labortechnik, Staufen, Germany*) in phosphate buffer (140 mM KCl, 20 nM Phosphate, pH 7.4). The lipoperoxidation was measured by the technique of substances that react to Thiobarbituric Acid (TBARS).[16] The activities of the enzymes Catalase (CAT) and Glutathione-S-transferase (GST) were measured through the Spectrophotometer.[17,18]

Averages and Standard Deviations (SD) were calculated. The data were analyzed by Analysis of Variance (ANOVA) followed by Tukey post hoc test. Values were considered significantly different when $p < 0.05$. *Statistical Package for Social Sciences software* (SPSS Inc., Chicago, IL, USA) version 21.0 was used.

RESULTS

The analysis of the liver enzymes activity and liver histology confirmed the diagnosis of Cirrhosis. In Figure 1, it can be seen from the pulmonary histological analysis that the animals of the BDL group presented vessels with increased diameters in relation to the other groups. Since the findings in each group are very similar, only one histological example will be presented for each experimental group.

In Table 1, the results regarding changes in gas exchange through Arterial blood gas test, with values referring to ApO2, ApCO2, SaO2, APO2 and D (A-a)

O2 can be observed, being compared among the four experimental groups. Significant differences were found for ApCO2 and APO2, and the values for the BDL group were significantly increased and decreased, respectively, in relation to the other groups ($p < 0.05$). The Table 2 also shows a significant increase ($p < 0.01$) ratio lung weight / body weight in the BDL group compared to the other experimental groups.

Intrapulmonary vasodilation associated with gastric abnormalities confirms the experimental HPS model. The decrease in intrapulmonary vasodilation, ApCO2 and pulmonary / body weight ratio in the BDL + MEL group, as well as the increase of APO2 in relation to BDL, which suggest a protective effect of MEL in the pulmonary tissue before HPS.

The Table 1 shows results regarding the lipoperoxidation process and activity of the antioxidant enzymes characterized by TBARS, CAT and GST values. The increased values of TBARS in the BDL group compared to the other groups ($p < 0.01$) indicate an increase in lipoperoxidation in this experimental group. The CAT activity was significantly lower in the BDL group ($p < 0.01$), while GST activity in the BDL group was significantly increased in relation to the other groups. The BDL + MEL group presented lower results for the TBARS values in relation to the BDL group, indicating an antioxidant effect of MEL.

In Figure 2, an increase in pulmonary fibrosis, marked by red staining, was found in the BDL group in relation to the other groups. This process was reversed with

Table 1. Blood gases, alveolar-arterial oxygen gradient, and lung / body weight ratio in the four experimental groups.[a]

Variable	Sham	*Sham* + MEL	BDL	BDL + MEL
ApO_2	67±11.3	66.3±12.4	57.2±6.2	71±5.4
$ApCO_2$	45±1.4	48±5.2	56.6±1.5*	48.2±4.5
SaO_2	91±4.2	88.3±6.4	84.8±6.2	92.5±2.6
APO_2	93.4±1.7	89.7±6.6	78.9±1.9*	89.4±5.7
D (A-a)O_2	26.4±13	23.4±5.9	21.7±5.7	18.4±11
Pulmonary weight ratio/ body weight (%)	0.32±0.09	0.34±0,05	0.57±0.05**	0.37±0.02

BDL: Biliary Duct Ligature; *Sham*+MEL: *Sham* and melatonin; BDL+MEL: Biliary Duct Ligature and melatonin. ApO_2: Arterial Partial Oxygen Pressure; $ApCO_2$: arterial partial pressure of carbon dioxide; SaO_2: arterial oxygen saturation; APO_2: alveolar partial pressure of oxygen; and D (A-a)O_2: alveolar-arterial oxygen gradient. [a]Results expressed by average + standard deviation. *p <0.05 vs. group Sham, Sham + MEL, BDL + MEL. **p <0.01 vs. group Sham, Sham + MEL, HBL + MEL.

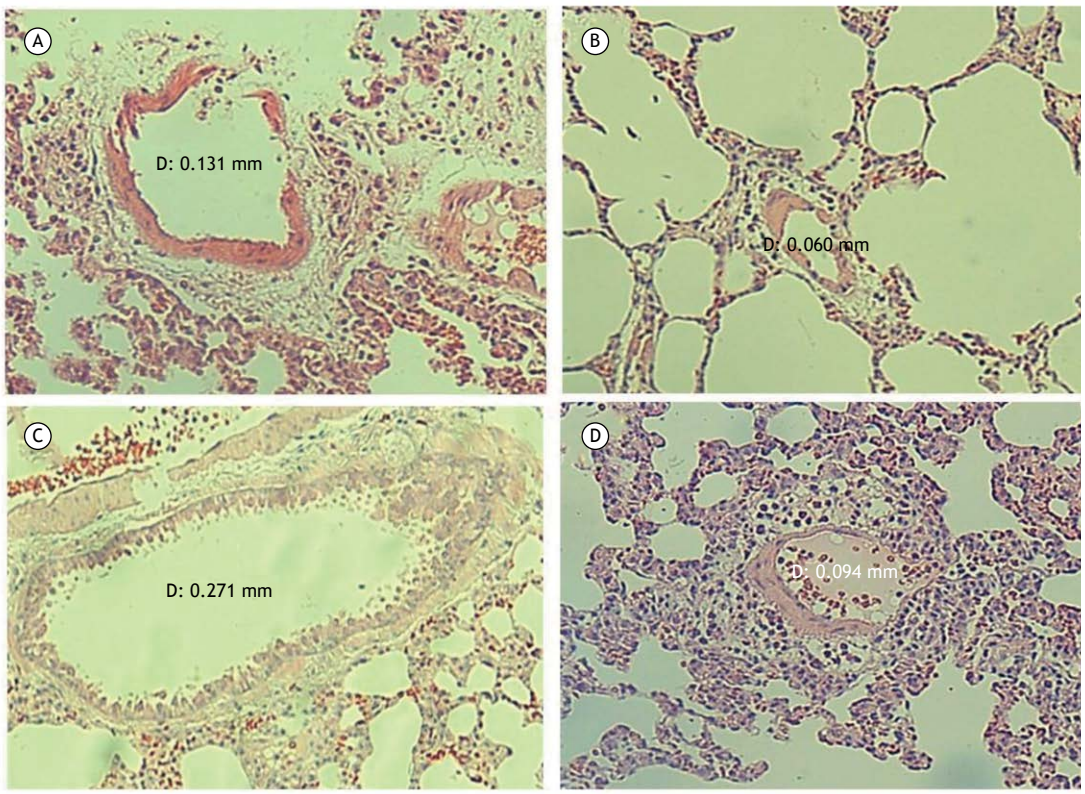

Figure 1. Microscopy images of pulmonary tissue samples stained by hematoxylin and eosin (HE), zoomed 100x. (A) Sham Group; (B) Group Sham and Melatonin; (C) Group Biliary Duct Ligature; (D) Duct Ligature Group Biliary and Melatonin.

the use of Melatonin, with pulmonary fibrosis being shown to be decreased from the BDL + MEL group in relation to the BDL group.

DISCUSSION

The increase in the diameter of the pulmonary vessels, evidenced by the histological analysis by hematoxylin and eosin (HE), in association with the gasometrical alterations, confirms in the present study the induction of HPS through the BDL surgery. The decrease in the diameter of the pulmonary vessels in the BDL + MEL group compared to the group HBL therapeutic effects of Melatonin suggest the vascular adaptation process.

According to our results, the ApCO2 increased in BDL group compared to the other groups, while APO2 decreased in the same group. Vercelino et al.[4] also found an increase in PCO2 in the BDL group when compared to the control group, as well as additional alterations in the BDL group, with changes in ApO2, SaO2 and D (A-a) O2 values. Several studies associate the gasometrical alterations present in HPS with the action of Nitric Oxide (NO) on lung tissue.[19,20] Tieppo et al.[21] found that the antioxidant action of Quercetin is able to reverse the gasometrical alterations in the HPS experimental model, suggesting that the antioxidant is able to regulate the ON levels in the syndrome. Our results demonstrate that Melatonin improves the gas alterations in the BDL model, as well as reverses intrapulmonary vasodilation, suggesting that Melatonin plays a role similar to that of Quercetin in the regulation of ON levels in HPS.

Vercelino et al.[4] also found an increase in lipoperoxidation and antioxidant activity of the SOD

Table 2. Levels of thiobarbituric acid-reactive substances (nmol / mg protein), antioxidant activity of the catalase enzyme (pmol / min / mg prot.) And glutathione-s -transferase (nmol / min / mg prot.).[a]

Variable	Sham	*Sham* + MEL	BDL	BDL + MEL
TBARS (nmol/mg protein)	0.38±0.09	0.37±0.15	0.83±0.13**	0.5±0.08
CAT (pmol/min/mg protein)	8.32±1.27	9.09±1.01	5.17±0.72**	8.52±1.14
GST (nmol/min/mg protein)	2.54±1.05	2.08±1.09	7.78±1.22**	3.95±1.99

TBARS: thiobarbituric acid reactive substances; CAT: catalase; GST (glutation-s-transferase). BDL: Biliary Duct Ligature; *Sham*+MEL: Sham e melatonin; BDL+MEL: Biliary Duct Ligature and melatonin. [a]Results expressed by average + standard deviation. **p <0.01 vs. group Sham, Sham + MEL, BDL + MEL.

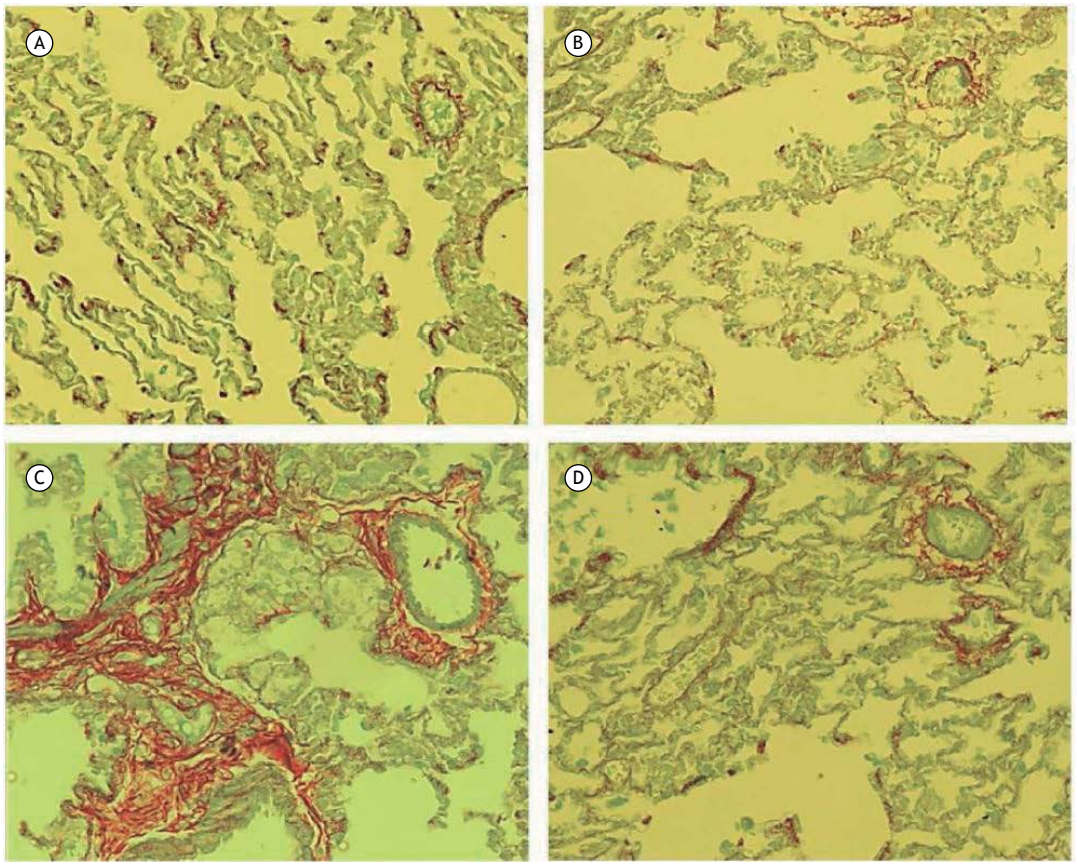

Figure 2. Microscopy images of the pulmonary tissue samples stained by picrosirius, zoomed of 100x. (A) Sham Group; (B) Group Sham and Melatonin; (C) Group Biliary Duct Ligature; (D) Duct Ligature Group Biliary and Melatonin.

enzyme in lung tissue in the BDL group, as well as an increase in lung weight / body weight ratio in animals submitted to BDL surgery, matching with the results in the present study. Maarman et al.[11] found an increase in lung weight and a decrease in body weight in the experimental model of Pulmonary Hypertension, which was reversed by the use of Melatonin. Similarly, as in this study, Melatonin was able to decrease the lung / body weight ratio.

There was an increase in lipoperoxidation in the BDL group and consequent reversal after administration of Melatonin. A decrease in CAT activity and an increase in GST activity were found in the BDL group, which were reverted by the use of Melatonin. The increase of oxidative stress in the body and consequent lipoperoxidation is due to the imbalance between the presence of free radicals and antioxidant agents.[22] Although several results on the activity of CAT and GST antioxidant enzymes are found in the literature, the results of this study demonstrate a decrease in lipoperoxidation and an improvement in the antioxidant system after the use of Melatonin.

Several studies associate the use of Melatonin with the reduction of lipoperoxidation, however the activity of antioxidant enzymes with the use of Melatonin presents diverse results.[11-14] Maarman et al.[11] found a decrease in the activity of SOD and CAT enzymes and plasma lipoperoxidation in animals with Pulmonary Hypertension treated with Melatonin. Taslidere et al.[12] associate the use of Melatonin with decreased lipoperoxidation and increased activity of CAT and Glutathione (GSH) enzymes in rat lung tissue after cirrhosis induced by Carbon Tetrachloride (CCl4). Borges et al.[23] demonstrate that the use of Melatonin decreases muscle lipoperoxidation generated by vigorous exercise, as well as increases SOD activity, but there are no significant changes in CAT and Glutathione Peroxidase (GPx) activity. Similarly, Rosa et al.[24] demonstrated that Melatonin decreases lipoperoxidation and increases SOD activity in the liver of animals submitted to the experimental Sleep Apnea model.

The results of this study, obtained through the histological analysis by picrosirius, suggest that Melatonin was able to reduce the accumulation of pulmonary collagen (Figure 2). Maarman et al.[11] found a decrease in collagen in cardiac tissue after the use of Melatonin in the model of Pulmonary Hypertension, and Rosa et al.[25] found a reduction of hepatic collagen after the use of Melatonin in the experimental model of cirrhosis by CCl4.

The Melatonin's antioxidant activity in liver and lung tissue is associated with the reversal of the main changes in the HPS. This therapeutic effect of melatonin may occur by a direct improvement in antioxidant activity in the lung, or simply by improving liver condition, reversing the systemic changes in the disease. The physical exercise also exerts a decrease in oxidative stress and an increase in antioxidant activity in a systemic way. Since cirrhosis and HPS affect different organs, therapeutic interventions that act on the different body systems are potentially more indicated for the management of the pathology. Therefore, the Melatonin use and the physical exercise practices, associated with traditional and drug interventions well established, may in the future help patients affected by the disease.

This study presents a possible limitation of its experimental design, which impairs the direct clinical transposition of the findings to HPS patients. However, it should be pointed out that the BDL model is the best experimental model for the study of HPS because it promotes true cirrhosis and changes all parameters of arterial gas exchange, increase lipoperoxidation and antioxidant defense.[4]

The MEL antioxidant action in lung tissue has been shown to be effective in reducing vasodilatation, fibrosis, oxidative stress, as well as improving pulmonary weight / body weight ratio, PCO2 and APO2 in the HPS experimental model. These findings suggest an antioxidant effect of MEL on HPS pulmonary damage, being effective in reducing the gasometrical and structural changes caused by the syndrome.

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J Bras Pneumol. 2019;45(3):e20180058
http://dx.doi.org/10.1590/1806-3713/e20180058

Effects of manual chest compression on expiratory flow bias during the positive end-expiratory pressure–zero end-expiratory pressure maneuver in patients on mechanical ventilation

Ana Carolina Otoni Oliveira[1,a], Daiane Menezes Lorena[1,b], Lívia Corrêa Gomes[2,c], Bianca Lorrane Reges Amaral[2,d], Márcia Souza Volpe[3,e]

1. Programa de Residência Integrada Multiprofissional em Saúde do Adulto, Universidade Federal do Triângulo Mineiro – UFTM – Uberaba (MG) Brasil.
2. Departamento de Fisioterapia Aplicada, Universidade Federal do Triângulo Mineiro – UFTM – Uberaba (MG) Brasil.
3. Departamento de Ciências do Movimento Humano, Universidade Federal de São Paulo – Unifesp – Campus Baixada Santista, Santos (SP) Brasil.

a. http://orcid.org/0000-0002-2752-6497
b. http://orcid.org/0000-0002-4193-7946
c. http://orcid.org/0000-0001-7244-2973
d. http://orcid.org/0000-0002-3794-3366
e. http://orcid.org/0000-0003-1179-6254

Submitted: 23 February 2018.
Accepted: 12 August 2018.

Study carried out at the Hospital de Clínicas, Universidade Federal do Triângulo Mineiro – UFTM – Uberaba (MG) Brasil.

ABSTRACT

Objective: To investigate the effects of manual chest compression (MCC) on the expiratory flow bias during the positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) airway clearance maneuver applied in patients on mechanical ventilation. The flow bias, which influences pulmonary secretion removal, is evaluated by the ratio and difference between the peak expiratory flow (PEF) and the peak inspiratory flow (PIF). **Methods:** This was a crossover randomized study involving 10 patients. The PEEP–ZEEP maneuver was applied at four time points, one without MCC and the other three with MCC, which were performed by three different respiratory therapists. Respiratory mechanics data were obtained with a specific monitor. **Results:** The PEEP–ZEEP maneuver without MCC was enough to exceed the threshold that is considered necessary to move secretion toward the glottis (PEF – PIF difference > 33 L/min): a mean PEF – PIF difference of 49.1 ± 9.4 L/min was achieved. The mean PEF/PIF ratio achieved was 3.3 ± 0.7. Using MCC with PEEP–ZEEP increased the mean PEF – PIF difference by 6.7 ± 3.4 L/min. We found a moderate correlation between respiratory therapist hand grip strength and the flow bias generated with MCC. No adverse hemodynamic or respiratory effects were found. **Conclusions:** The PEEP–ZEEP maneuver, without MCC, resulted in an expiratory flow bias superior to that necessary to facilitate pulmonary secretion removal. Combining MCC with the PEEP–ZEEP maneuver increased the expiratory flow bias, which increases the potential of the maneuver to remove secretions.

Keywords: Physical therapy modalities; Critical care; Respiration, artificial; Bodily secretions.

INTRODUCTION

Patients undergoing mechanical ventilation show changes in the airway clearance mechanisms, those changes favoring the retention of pulmonary secretions.[1,2] The accumulation of secretions causes an increase in airway resistance and partial or total airway obstruction, resulting in alveolar hypoventilation, atelectasis, hypoxemia, and increased work of breathing, as well as creating a favorable environment for the proliferation of bacteria and the development of pneumonia.[3,4] All of those changes prolong the time to weaning from mechanical ventilation and worsen patient prognosis.[5] In this context, respiratory therapy, applying manual techniques or using the mechanical ventilator itself, acts with the purpose of facilitating secretion removal and consequently improving the clinical course of critically ill patients.[6,7]

Since the 1980s, there have been studies showing that pulmonary secretion removal depends not only on high expiratory flows but also on the presence of an expiratory flow bias, that is, on PEF being higher than the peak inspiratory flow (PIF) generated in the airways.[8-10] To date, four thresholds have been described for an expiratory flow bias to move secretion toward the glottis: PEF/PIF ratio > 1.1[8,9]; PEF – PIF difference > 17 L/min[11]; PEF/PIF ratio > 4.3[12]; and PEF – PIF difference > 33 L/min.[12] Of those four thresholds, the latter two have a greater potential for reflecting human conditions because they were discovered in an animal study, with the use of secretion from the animals themselves, and because the influence of gravity on secretion movement was taken into account in that study, given that the animals were kept in a semirecumbent position.[12] With regard to the choice between the threshold based on the PEF/PIF ratio and that based on PEF – PIF difference, Volpe et al.[11] showed that PEF – PIF difference has a stronger and more significant correlation with secretion movement than does the PEF/PIF ratio. Therefore, using PEF – PIF difference as the

Correspondence to:
Márcia Souza Volpe. Departamento de Ciências do Movimento Humano, Universidade Federal de São Paulo, Unifesp, Rua Silva Jardim, 136, CEP 11015-020, Santos, SP, Brasil.
Tel.: 55 34 99183-2000. E-mail: marciasvolpe@gmail.com
Financial support: None.

  ISSN 1806-3713

target threshold for an expiratory flow bias to remove secretions seems to be more appropriate.

Among the secretion removal techniques with great potential to generate an expiratory flow bias are the positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) maneuver and manual chest compression (MCC), which can be combined. However, there have been few studies investigating PEEP–ZEEP, and little is known about the ventilation pattern and flow bias generated during it. Herbst-Rodrigues et al.[13] demonstrated that combining PEEP–ZEEP with MCC is a safe technique and that it resulted in increased PEF in myocardial revascularization patients during the immediate postoperative period. Santos et al.[14] compared PEEP–ZEEP vs. MCC, whereas Lobo et al.[15] compared PEEP–ZEEP plus MCC vs. manual hyperinflation, and neither group found PEEP–ZEEP to be superior in terms of improvement in lung compliance[14] or in terms of the quantity of secretions removed.[15] Except for the study by Herbst-Rodrigues et al.,[13] those studies did not investigate the airflows achieved during the maneuver, and none of the three reported the generated blow bias.[13-15] In addition, it is not known whether combining MCC with PEEP–ZEEP makes the maneuver more effective in terms of the flow bias generated.

The objective of the present study was to investigate the effects of MCC on the expiratory flow bias generated by the PEEP–ZEEP maneuver in patients on mechanical ventilation. Because the effects of MCC on PEF can be influenced by operator characteristics, such as hand size and hand grip strength, we compared compressions performed by three different respiratory therapists.

METHODS

This was a quantitative, experimental crossover study involving a convenience sample and conducted at the *Hospital de Clínicas da Universidade Federal do Triângulo Mineiro*, in the city of Uberaba, Brazil. The study was approved by the local research ethics committee (CAAE no. 47299815.8.0000.5154). Written informed consent was obtained from family members.

We included patients who had been on mechanical ventilation for more than 48 h; were between 19 and 68 years of age; were hemodynamically stable—receiving low doses of vasoactive drugs or no vasoactive drugs and having a mean arterial pressure (MAP) > 60 mmHg; had adequate oxygenation ($FiO_2 \leq 0.4$; PEEP ≤ 10 cmH_2O; and $SpO_2 > 90\%$); and showed no signs of respiratory distress. The exclusion criteria were having intracranial hypertension, having pulmonary hypertension, being pregnant, having an undrained pneumothorax, having a rib fracture, and having a chest tube in place.

After being included, the patients were placed in the supine position, underwent the initial evaluation, and were submitted to endotracheal suctioning. The sensor of the CO_2SMO Plus monitor (Dixtal Equipamentos Médicos, São Paulo, Brazil) was then connected, creating a link between the ventilator circuit and the endotracheal tube/tracheostomy tube, in order to obtain respiratory mechanics data.

Thirty minutes after endotracheal suctioning, we collected the baseline data and recorded ventilation for at least one minute, without changing the ventilator settings. The PEEP–ZEEP maneuver was then carried out in four distinct steps, the order in which they occurred being random and computer-generated. Of the 24 randomization possibilities, 12 were maintained so that the four possible steps were balanced in the first and fourth positions. The allocation was concealed until the patient was included in the study. The four steps were as follows: PEEP–ZEEP without MCC; PEEP–ZEEP with MCC applied by respiratory therapist 1; PEEP–ZEEP with MCC applied by respiratory therapist 2; and PEEP–ZEEP with MCC applied by respiratory therapist 3. Between each step, there was a 15-min interval, during which the patient was ventilated at the baseline settings. The three respiratory therapists who applied the maneuvers were part of the study research team and were female.

The PEEP–ZEEP maneuver was carried out during volume assist-control ventilation, with a tidal volume set to achieve a peak inspiratory pressure (PIP) of 30-35 cmH_2O, an inspiratory time of 1.0-1.5 s, a square flow wave, and a PEEP of 15 cmH_2O. At the beginning of the maneuver, PEEP was increased to 15 cmH_2O, and, at the end of the inspiratory phase of the fifth cycle, PEEP was abruptly reduced to zero cmH_2O—a cycle called ZEEP. In each step, the maneuver was carried out twice, totaling ten cycles per step. Figure 1 illustrates the pressure curves and flow curves over time during the PEEP–ZEEP maneuver as applied in the study (two sequential maneuvers).

MCC was applied bilaterally on the lower third of the thorax, in an abrupt/rapid way (compression applied only at the start of expiration), and in synchrony with the reduction of PEEP to zero cmH_2O, which was achieved by observing the curves on the ventilator screen.

The patients were monitored continuously, and the maneuver was interrupted if the patients had an SpO_2 < 90%, a systolic blood pressure < 90 mmHg, an MAP < 60 mmHg, a heart rate < 60 bpm or > 140 bpm, or psychomotor agitation.

The maneuvers were continuously recorded by the device. Hemodynamic data (heart rate, systolic blood pressure, MAP, and diastolic blood pressure) and SpO_2, as well as respiratory mechanics data, were recorded before the first step and 15 min after the end of the fourth step.

The size of the hands of the respiratory therapists who applied MCC was measured by the figure-of-eight method,[16] and the maximum grip strength of the dominant hand was determined with a hydraulic dynamometer (JAMAR; Patterson Medical Co., Danbury, CT, USA). For determination of maximum hand grip strength, the respiratory therapists were placed in a sitting position, with the shoulders adducted, the

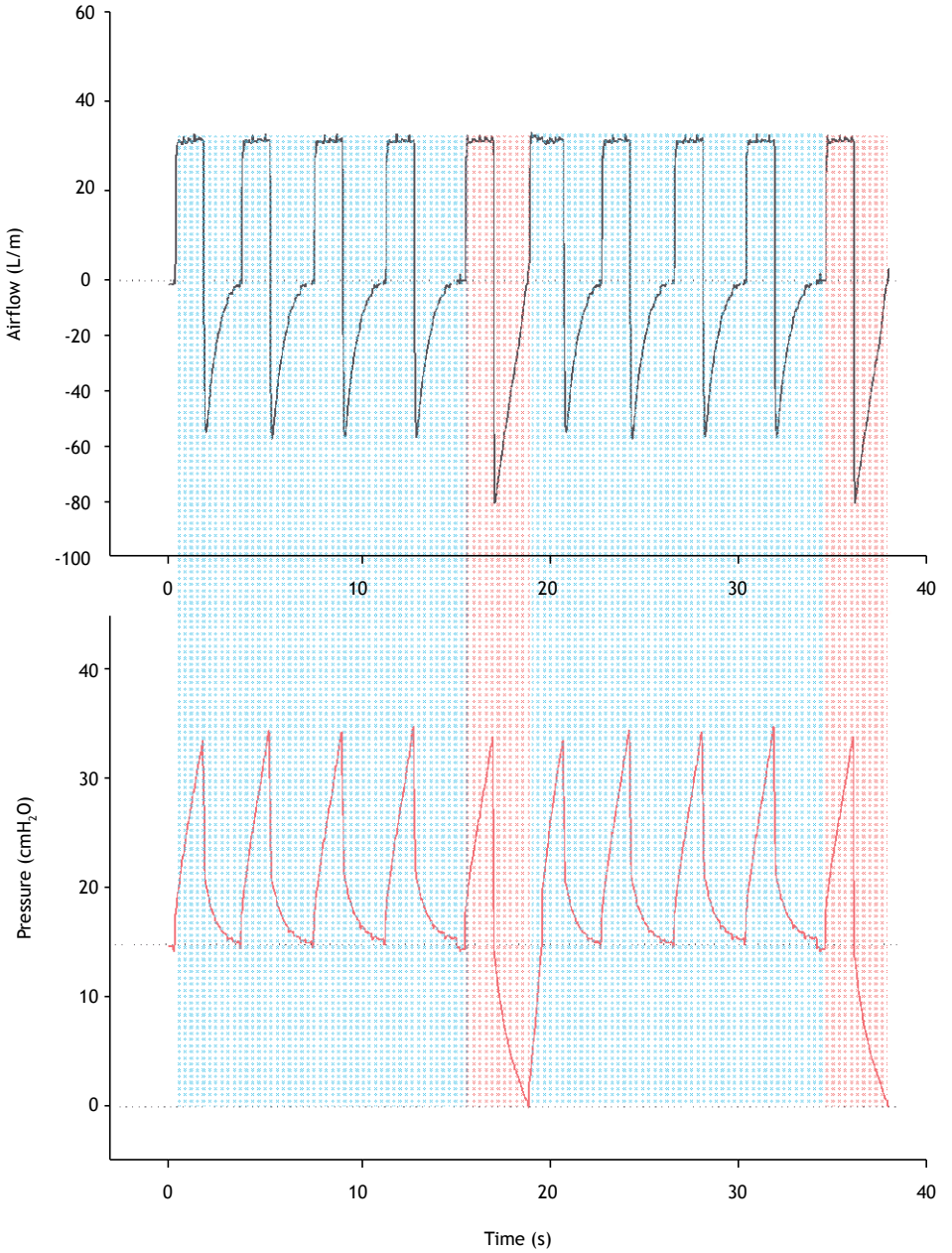

Figure 1. Flow curves (in black) and pressure curves (in red) during two sequential positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) maneuvers during volume-controlled ventilation. After four cycles with PEEP set at 15 cmH_2O (shaded in blue), known as pre-ZEEP, there is a fifth cycle in which PEEP is abruptly reduced to zero cmH_2O (shaded in red), known as ZEEP. Note the increase in peak expiratory flow during the ZEEP cycle as compared with the pre-ZEEP cycles.

elbow flexed at 90°, and the wrist/forearm in a neutral unsupported position, holding the dynamometer.[17] Upon a verbal signal to start, the respiratory therapists gripped the dynamometer. Hand grip strength was measured three times, and the highest value obtained was used for analysis.

Analysis of respiratory mechanics

The system for acquisition of flow, pressure, and volume curves that was used was the CO_2SMO Plus monitor (Dixtal Equipamentos Médicos) connected to a computer with the Analysis Plus software for Windows (Novametrix Medical Systems Inc., Wallingford, CT, USA), which records data at 100 Hz and allows subsequent analysis of the data stored. The analysis of the PEEP–ZEEP maneuver was divided into a pre-ZEEP cycle and a ZEEP cycle. Because the maneuver was applied twice in each step of the study, we selected and analyzed at least six cycles and two cycles, respectively, for the pre-ZEEP cycle and the

ZEEP cycle, in order to obtain the mean values of PIP, inspiratory time, tidal volume, PEEP, PIF, and PEF for the pre-ZEEP cycle and for the ZEEP cycle. The analysis of the pre-ZEEP cycle was performed only during the PEEP–ZEEP maneuver without MCC. For the analysis of respiratory mechanics at baseline and at the end of the fourth step, we randomly selected at least 10 cycles and calculated the mean values of the variables of interest.

The primary outcome was the influence of MCC on the expiratory flow bias (as assessed by PEF – PIF difference) generated by the PEEP–ZEEP maneuver.

Statistical analysis

Data were presented as mean and standard deviation or as absolute and relative values. Comparative analyses of respiratory mechanics variables for the ZEEP cycle vs. the pre-ZEEP cycle (of the PEEP–ZEEP maneuver without MCC), as well as of respiratory mechanics and hemodynamic variables at baseline vs. at the end of the study, were carried out with the paired t-test. We analyzed the influence of MCC with repeated-measures ANOVA, testing four within-factor levels (without MCC, MCC-1, MCC-2, and MCC-3; the last three reflecting the performance of the three different respiratory therapists, respectively). Subsequently, a simple contrast (with Bonferroni correction) was used to determine whether MCC performed by any of the three respiratory therapists was significantly more efficient than was the "without MCC" condition.

Although the study was not designed to explore the influence of respiratory therapist characteristics on the final performance (i.e., accelerate expiratory flow), we conducted an exploratory analysis using within-patient linear regression (having adjusted for PEF without MCC in each patient), in which the following variables were tested as independent variables: maximum hand grip strength; and hand circumference. Values of $p < 0.05$ were considered statistically significant. The statistical analysis was performed with the SPSS Statistics software package, version 20.0 for Windows (IBM Corporation, Armonk, NY, USA).

RESULTS

Twelve patients were included in the study. However, 2 of those 12 were excluded: one for experiencing an increased respiratory rate and respiratory distress during the protocol; and the other because of failures during acquisition of respiratory mechanics data. The characteristics of the 10 patients who participated in the study are described in Table 1.

Figure 2 shows the PEEP–ZEEP maneuver without MCC (in A) and the PEEP–ZEEP maneuver with MCC applied by respiratory therapist 2 (in B) in a patient who is representative of the study sample. The differences between the PEF values generated during the ZEEP cycle illustrate the contribution of the PEEP–ZEEP maneuver and of MCC to the occurrence of an expiratory flow bias.

Table 1. Baseline characteristics of the patients included in the study (N = 10).[a]

Characteristic	Result
Age, years	63 ± 14
Male gender	6 (60.0)
Diagnosis	
Acute respiratory failure	1 (10.0)
Stroke	1 (10.0)
Traumatic brain injury	1 (10.0)
Decreased level of consciousness	2 (20.0)
Cardiopulmonary arrest	5 (50.0)
Level of consciousness or sedation	
Richmond Agitation Sedation Scale[b]	−5 (100.0)
Glasgow Coma Scale[c]	9 ± 3
Duration of ventilation, days	16 ± 7
Ventilator settings	
Ventilation mode	
Pressure-controlled	6 (60.0)
Pressure support	4 (40.0)
Positive end-expiratory pressure, cmH_2O	7.7 ± 0.9
FiO_2	0.34 ± 0.08
Respiratory rate, breaths/min	20 ± 4

[a]Values expressed as n (%) or as mean ± SD. [b]Scale used in 1 patient. [c]Scale used in 9 patients.

Table 2 shows respiratory mechanics variables for the pre-ZEEP and ZEEP cycles of the PEEP–ZEEP maneuver without MCC and for the ZEEP cycle of the PEEP–ZEEP maneuver with MCC applied by each of the three respiratory therapists in the study. A comparison between the pre-ZEEP and ZEEP cycles demonstrates that the abrupt reduction of PEEP to zero cmH_2O increased PEF by 29.4 ± 9.4 L/min, resulting in a significant increase in the PEF/PIF ratio and in PEF – PIF difference (Table 2). With regard to the other variables, only PIP was higher in the ZEEP cycle than in the pre-ZEEP cycle (1.4 ± 1.4 cmH_2O higher), and, as expected, PEEP was lower (and close to zero) in the ZEEP cycle as compared with the pre-ZEEP cycle.

MCC, regardless of the respiratory therapist who performed it, resulted in a significant increase in PEF ($p < 0.001$) and in the expiratory flow bias, as expressed either by the PEF/PIF ratio ($p = 0.004$) or by PEF – PIF difference ($p < 0.001$; Figure 3). The increase caused by MCC was, on average, 7.2 ± 3.3 L/min, 6.7 ± 3.4 L/min, and 0.3 ± 0.2 L/min for PEF, PEF – PIF difference, and the PEF/PIF ratio, respectively. In Table 2, we can see that the increase provided by MCC in the expiratory flow bias occurred because of the increase in PEF generated by the compression, given that no significant difference was found across the PIFs generated during the four steps of the study.

Considering PEF – PIF difference > 33 L/min as the threshold that is most appropriate to represent the target expiratory flow bias during therapy for secretion clearance, we found that, of the 40 maneuvers performed, only 1 (2,5%), which was applied by

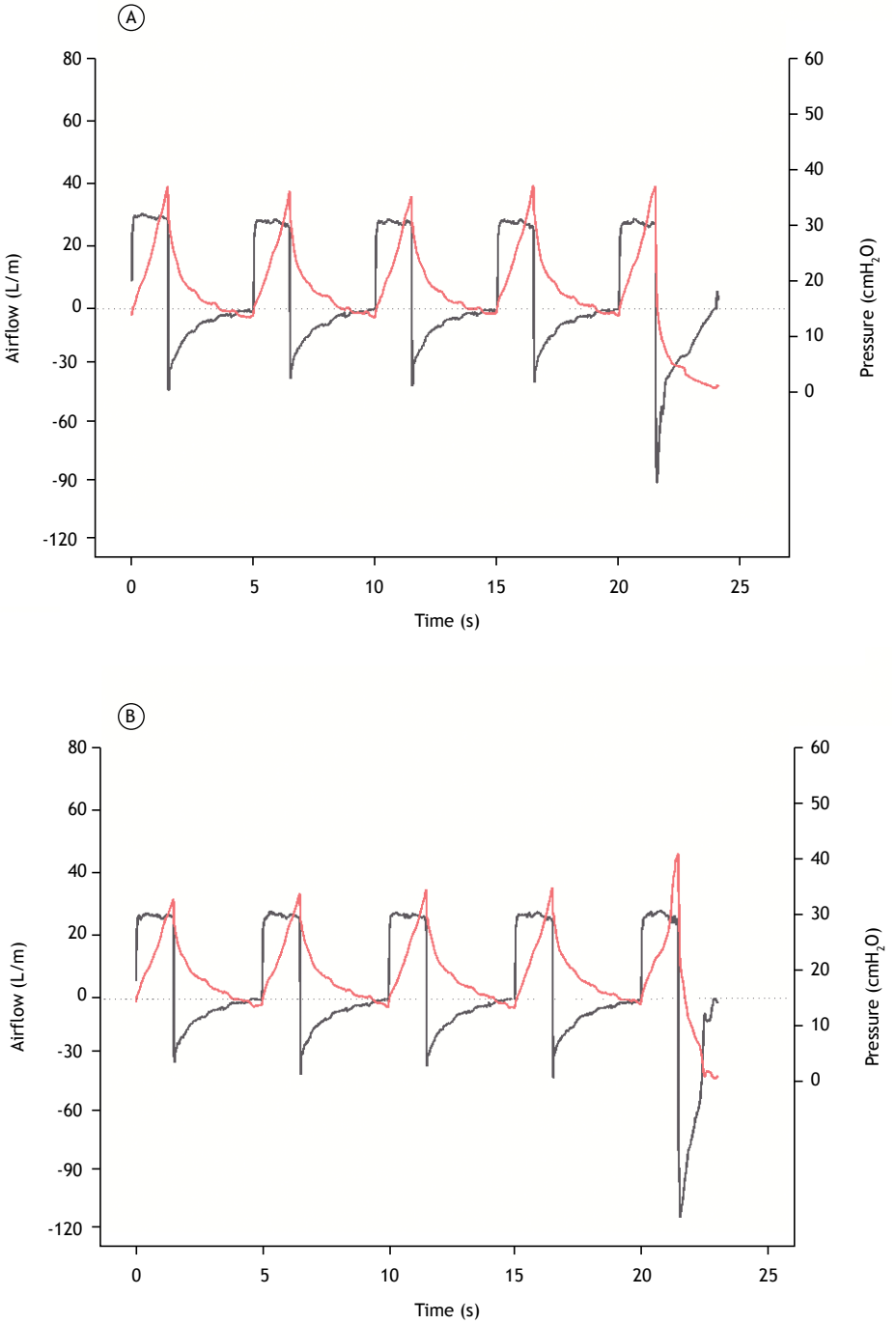

Figure 2. Positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) maneuver without manual chest compression (in A) and PEEP–ZEEP maneuver with manual chest compression applied by respiratory therapist 2 (in B) in a patient who is representative of the study sample. The expiratory flow bias generated during the ZEEP cycle without manual chest compression (in A) was ≈ 60 L/min, whereas, with the addition of manual chest compression (in B), the expiratory flow bias was ≈ 83 L/min.

respiratory therapist 3, did not reach the threshold necessary to move secretion toward the glottis.

Applying MCC in combination with the PEEP–ZEEP maneuver resulted in higher PIP as compared with applying the PEEP–ZEEP maneuver without MCC. Bonferroni analysis revealed that this difference was caused by the MCC applied by respiratory therapist 3 (Table 2, Figure 4). However, the mean PIP during MCC was below 40 cmH_2O, and the PIP was between 40.0 and 45.5 cmH_2O in only 4 of the 30 cycles (Figure 4).

With the exception of dynamic compliance of the respiratory system, which showed an increase when

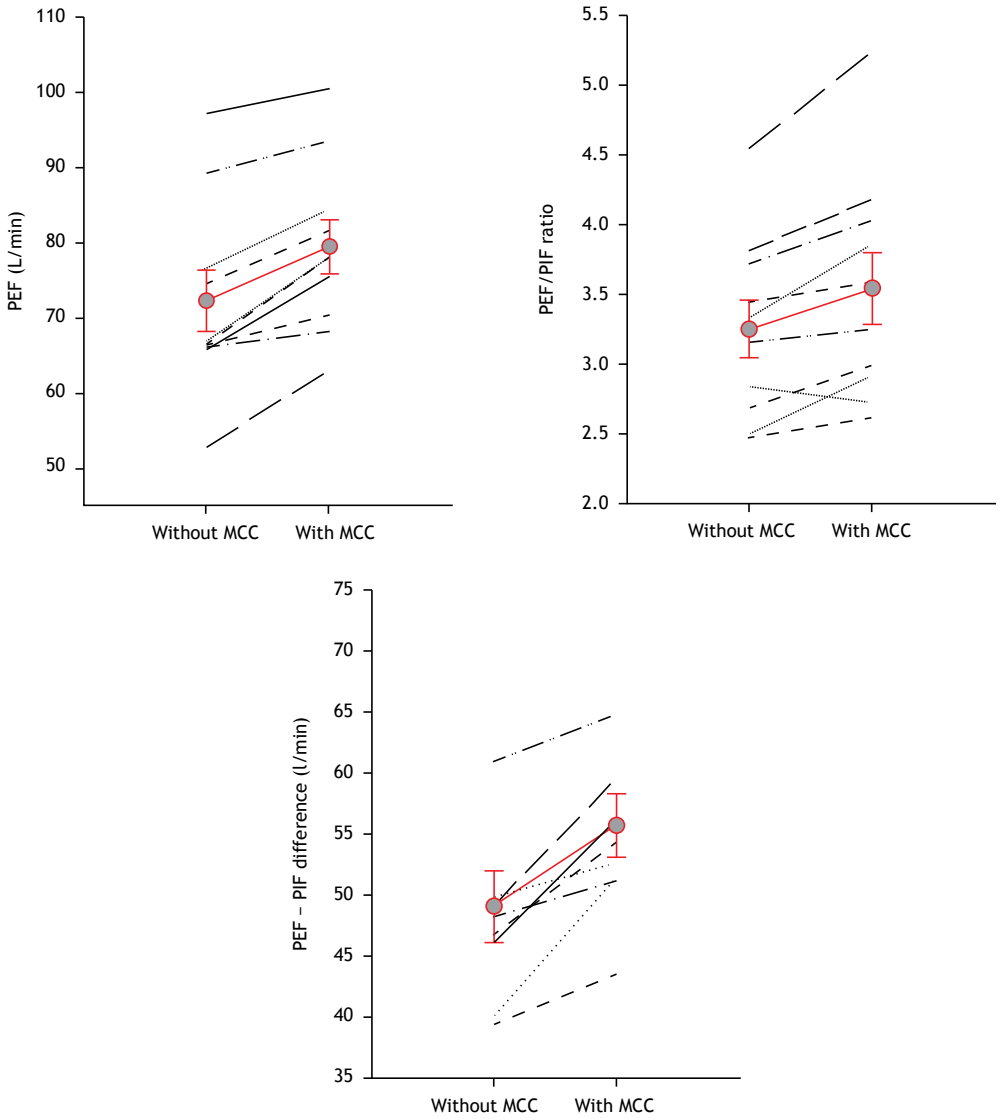

Figure 3. Peak expiratory flow (PEF), PEF/peak inspiratory flow (PIF) ratio, and PEF – PIF difference generated during the positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) maneuver without manual chest compression (MCC) and during the PEEP–ZEEP maneuver with MCC. The values presented for "with MCC" are the means generated by the three respiratory therapists. The black lines illustrate the values reached in each patient in the study, and the red lines represent the means ± standard error.

baseline and end-of-study values were compared, all respiratory mechanics variables and hemodynamic parameters, as well as SpO_2, showed no differences, which demonstrates that the application of the maneuvers was safe in respiratory and hemodynamic terms (Table 1A; JBP online appendix — http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=57).

The values for hand circumference and maximum hand grip strength, respectively, for each respiratory therapist were as follows: 37.5 cm and 25.3 kgf, for respiratory therapist 1; 41.0 cm and 28.6 kgf, for respiratory therapist 2; and 45.0 cm and 17.0 kgf, for respiratory therapist 3. The partial regression coefficients for maximum hand grip strength vs. PEF and PEF – PIF difference were 0.36 and 0.46, respectively; whereas the partial regression coefficients for hand circumference vs. PEF and PEF – PIF difference were −0.21 and −0.33, respectively. These results indicate that hand grip strength is a better candidate (positive correlation) to explain the significant difference in performance among respiratory therapists, whereas hand size has a spurious negative correlation.

DISCUSSION

The major finding of the present study is that MCC potentiated the increase in the expiratory flow bias generated by the PEEP–ZEEP maneuver, making the maneuver potentially more effective in terms of

Table 2. Respiratory mechanics variables for the cycle prior to zero end-expiratory pressure and the zero end-expiratory pressure cycle of the positive end-expiratory pressure–zero end-expiratory pressure maneuver without manual chest compression and for the zero end-expiratory pressure cycle of the positive end-expiratory pressure–zero end-expiratory pressure maneuver with manual chest compression.

Variable	PEEP–ZEEP without MCC		p*	PEEP–ZEEP with MCC			p†
	Pre-ZEEP	ZEEP		ZEEP with MCC			
				RT 1	RT 2	RT 3	
V_T, mL	556 ± 167	519 ± 173	0.145	524 ± 154	532 ± 144	529 ± 153	0.684
PIP, cmH_2O	31.5 ± 3.4	32.9 ± 2.9	0.011	34.7 ± 4.3	36.6 ± 4.3	38.2 ± 3.8§	0.008
PEEP, cmH_2O	13.1 ± 4.7	1.9 ± 0.8	< 0.001	1.7 ± 1.1	1.3 ± 0.9	1.9 ± 1.8	0.552
T_{INSP}, s	1.6 ± 0.1	1.5 ± 0.1	0.065	1.5 ± 0.0	1.5 ± 0.0	1.5 ± 0.1	0.632
PIF, L/min	23.4 ± 6.2	23.1 ± 6.0	0.071	23.0 ± 6.2	23.5 ± 5.7	24.5 ± 7.8	0.331
PEF, L/min	42.8 ± 14.0	72.2 ± 12.9	< 0.001	79.6 ± 12.3‡	82.0 ± 13.1§	76.8 ± 12.8	0.043
PEF/PIF ratio	1.9 ± 0.5	3.3 ± 0.7	< 0.001	3.6 ± 0.7‖	3.6 ± 0.8¶	3.4 ± 1.0	0.019
PEF – PIF difference, L/min	19.4 ± 12.0	49.1 ± 9.4	< 0.001	56.6 ± 7.9**	58.4 ±9.7§	52.3 ± 11.2	0.028

PEEP: positive end-expiratory pressure; ZEEP: zero end-expiratory pressure; MCC: manual chest compression; RT: respiratory therapist; V_T: tidal volume; PIP: peak inspiratory pressure; T_{INSP}: inspiratory time; and PIF: peak inspiratory flow; and PEF: peak expiratory flow. *Comparison between pre-ZEEP and ZEEP (paired t-test). †Comparison among the four ZEEP cycles (repeated-measures ANOVA). ‡p = 0.007; §p = 0.001; ‖p = 0.002; ¶p = 0.011; **p = 0.003. Comparisons between ZEEP without MCC and ZEEP with MCC (Bonferroni correction).

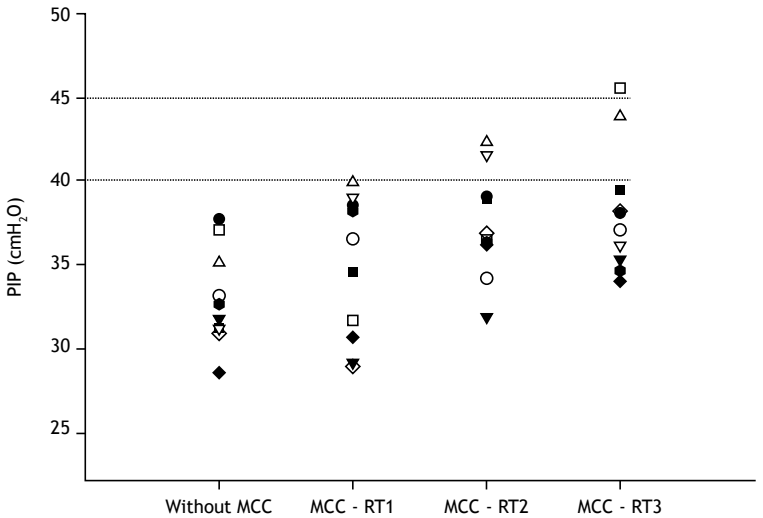

Figure 4. Peak inspiratory pressures (PIPs) generated during the positive end-expiratory pressure–zero end-expiratory pressure (PEEP–ZEEP) maneuver without manual chest compression (MCC) and during the PEEP–ZEEP maneuver with MCC applied by each of the three respiratory therapists (RT1, RT2, and RT3) who participated in the study. The straight lines mark safe threshold PIP values.

pulmonary secretion removal in patients on mechanical ventilation. However, whereas MCC was responsible for increasing the expiratory flow bias by 7 L/min, the contribution of the PEEP–ZEEP maneuver—performed during volume-controlled ventilation—was 49 L/min, which is highly significant and well above the threshold considered necessary for pulmonary secretion clearance (PEF – PIF difference > 33 L/min).

MCC is commonly applied in one of two different ways[18]: slowly and gradually throughout the expiratory phase; or abruptly and rapidly only at the start of expiration. In the present study, we chose the latter because it has been shown to be hemodynamically safe and more effective both in terms of an increase in PEF and in terms of secretion clearance, as compared with the former, in animal studies[18] and in studies of mechanical models.[19]

Comparison across studies in the literature examining the effects of MCC requires caution. In addition to there being two distinct MCC modalities, some studies have been conducted in spontaneously breathing individuals[20,21]; others have compared MCC and vibration (which has an oscillatory component that seems to potentiate the increase in PEF)[20,22]; others have finished MCC with rapid decompression of the chest,[23] which can reduce airway pressure and increase PIF when ventilation modes in which inspiratory flow is free, such as pressure-control ventilation, are used; and others have not described the maneuver in detail,[23] which makes it difficult to interpret their findings. Among the studies investigating the use of abrupt MCC

during mechanical ventilation are studies of mechanical models, animal models, adults, and children; those studies reported that PEF increased significantly by 8.8 L/min,[19] 8.9 L/min,[18] 16.2 to 43.8 L/min,[23-25] and 13.8%,[26] respectively. The increase in PEF caused by MCC in our study was smaller than that found in studies of adults. Such differences are probably explained by the physical characteristics of the MCC operator and mainly by the force applied to the chest and the properties of the respiratory system of the patients studied. In the present study, we found that the grip strength of the dominant hand correlated weakly with the PEF generated during MCC and moderately with the PEF – PIF difference generated during MCC. However, it is noteworthy that the study design, with a small number of respiratory therapists, was not appropriate to establish correlations among the study variables. Wong et al.[27] assessed the effects of vibration (without MCC), percussion, and shaking applied by ten respiratory therapists in an animal model, during mechanical ventilation, and also found no correlation between the size of the hands of the respiratory therapists and the resulting force during the application of these techniques.

The higher PIP generated by respiratory therapist 3 may have been due to poorer synchronization between the application of MCC and the start of expiration. Earlier application of MCC, before the expiratory valve starts to open, translates to a greater increase in PIP. However, the mean PIP generated during the MCC applied by the three respiratory therapists was below 40 cmH_2O, and in only 13% of the cycles did PIP reach values ranging from 40.0 to 45.5 cmH_2O, which is still considered a safe threshold.[28,29] In addition, the increase in PIP occurred because of momentarily decreased rib cage compliance or of increased pleural pressure caused by the compression maneuver and therefore did not increase the transpulmonary pressure.[30] It is very likely that the physiological consequences of the compression maneuver are equivalent to the changes observed in spontaneous cough, or even in trumpet players: despite the large momentary increase in intra-alveolar pressure, there is a concomitant increase in pleural pressures, without there being an increase in the transpulmonary pressure gradient and therefore without risk of damage to the lung parenchyma.[31]Most studies on MCC have not described its effects on the expiratory flow bias.[24,25,32-34] An exception is the study conducted by Gregson et al.,[26] who showed that only when combined with MCC did the manual hyperinflation maneuver result in a PEF/PIF ratio > 1.1. One possible explanation for studies that failed to demonstrate that the use of MCC results in enhanced secretion removal[32,34] is that MCC was used with the specific objective of increasing PEF and there was no awareness of the need to increase the expiratory flow bias. Higher tidal volumes or higher inspiratory pressures are often associated with MCC. These settings can generate high PIFs, which neutralize the PEF increase caused by MCC, making the maneuver only slightly effective or completely ineffective.

The present study has some limitations, such as the small number of patients. However, physiological studies that aim to describe respiratory patterns usually have smaller samples, with 10 to 14 patients. [35,36] A second limitation is that both the PEEP–ZEEP maneuver and MCC can cause peripheral airway closure and expiratory flow limitation, conditions that were not investigated in our study, and therefore prudence is required in applying MCC in patients who are susceptible to these conditions.[23] Finally, the thresholds described for an expiratory flow bias to move secretion toward the glottis have not yet been investigated in humans and should be interpreted with caution. It is of note that the thresholds found in the study by Li Bassi et al.,[12] PEF – PIF difference > 33 L/min and PEF/PIF ratio > 4.3, were obtained in sedated animals on controlled mechanical ventilation, which makes it difficult to use those thresholds in patients on assisted spontaneous ventilation, as is the case of most of the patients in our study. It is also of note that the properties of the secretion of those animals were not analyzed, and secretion viscosity certainly influences the thresholds obtained. In addition, it is not known whether there is a positive linear relationship between the flow bias and secretion removal, that is, whether a greater flow bias (above a certain threshold) translates to greater secretion movement toward the glottis. Therefore, in clinical practice, combining MCC with the PEEP–ZEEP maneuver should not be discouraged solely on the basis of our results.

The results of our study support that the PEEP–ZEEP maneuver, alone or in combination with MCC, is a promising technique for secretion removal; however, the level of evidence remains low. Further studies are needed that use a standardized PEEP–ZEEP maneuver—carried out during volume-controlled ventilation and resulting in a PEF – PIF difference > 33 L/min—and that explore outcomes related to possible adverse effects, such as induction of lung collapse, and outcomes measuring the effectiveness of the maneuver, such as the quantity of secretions removed.

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J Bras Pneumol. 2019;45(3):e20170351
http://dx.doi.org/10.1590/1806-3713/e20170351

Microbiological contamination of nebulizers used by cystic fibrosis patients: an underestimated problem

Barbara Riquena[1,a], Luciana de Freitas Velloso Monte[2,b], Agnaldo José Lopes[3,c], Luiz Vicente Ribeiro Ferreira da Silva-Filho[4,5,d], Neiva Damaceno[6,e], Evanirso da Silva Aquino[7,f], Paulo Jose Cauduro Marostica[8,9,g], José Dirceu Ribeiro[10,h]

1. Novartis Biociências S.A. Brasil, São Paulo (SP), Brasil.
2. Hospital da Criança de Brasília José Alencar, Brasília (DF), Brasil.
3. Departamento de Pneumologia, Hospital Universitário Pedro Ernesto da Universidade do Estado do Rio de Janeiro, (RJ), Brasil.
4. Unidade de Pneumologia, Instituto da Criança, Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP), Brasil.
5. Hospital Israelita Albert Einstein, São Paulo (SP), Brasil.
6. Faculdade de Ciências Médicas, Santa Casa de São Paulo, São Paulo (SP), Brasil
7. Associação Mineira de Assistência a Mucoviscidose – AMAM, Belo Horizonte (MG), Brasil.
8. Hospital de Clínicas, Porto Alegre (RS), Brasil.
9. Universidade Federal do Rio Grande do Sul, Porto Alegre (RS), Brasil.
10. Laboratório de Fisiologia Pulmonar, Centro de Investigação em Pediatria, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas (SP), Brasil

a. http://orcid.org/0000-0003-4145-9945
b. http://orcid.org/0000-0001-5595-351X
c. http://orcid.org/0000-0001-8598-4878
d. http://orcid.org/0000-0001-8431-1997
e. http://orcid.org/0000-0001-5284-3585
f. http://orcid.org/0000-0001-5670-0434
g. http://orcid.org/0000-0003-0252-7570
h. http://orcid.org/0000-0002-3387-5642

Submitted: 27 October 2017
Accepted: 23 September 2018

Study carried out in the Novartis Biociências S.A., São Paulo (SP), Brasil.

ABSTRACT

Objective: Home nebulizers are routinely used in the treatment of patients with cystic fibrosis (CF). This study aims to evaluate the contamination of nebulizers used for CF patients, that are chronically colonized by *Pseudomonas aeruginosa*, and the association of nebulizer contamination with cleaning, decontamination and drying practices. **Methods:** A cross-sectional, observational, multicenter study was conducted in seven CF reference centers in Brazil to obtain data from medical records, structured interviews with patients/caregivers were performed, and nebulizer's parts (interface and cup) were collected for microbiological culture. **Results:** overall, 77 CF patients were included. The frequency of nebulizer contamination was 71.6%. *Candida* spp. (52.9%), *Stenotrophomonas maltophilia* (11.9%), non-mucoid *P. aeruginosa* (4.8%), *Staphylococcus aureus* (4.8%) and *Burkholderia cepacia* complex (2.4%) were the most common isolated pathogens. The frequency of nebulizers' hygiene was 97.4%, and 70.3% of patients reported cleaning, disinfection and drying the nebulizers. The use of tap water in cleaning method and outdoor drying of the parts significantly increased (9.10 times) the chance of nebulizers' contamination. **Conclusion:** Despite the high frequency hygiene of the nebulizers reported, the cleaning and disinfection methods used were often inadequate. A significant proportion of nebulizers was contaminated with potentially pathogenic microorganisms for CF patients. These findings support the need to include patients/caregivers in educational programs and / or new strategies for delivering inhaled antibiotics.

Keywords: Cystic fibrosis; *Pseudomonas aeruginosa*; Nebulizers and vaporizers; Equipment contamination; Decontamination.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease, which predominantly affects Caucasians and is potentially fatal.[1,2] Brazilian incidence estimates vary across the country, from 1/1,587-1/32,258 live births.[3] Chronic respiratory infections are the leading cause of death among these patients and *Pseudomonas aeruginosa* is the pathogen most frequently associated with clinical deterioration.[4]

Home nebulizers are widely used by CF patients as part of their treatment, to deliver mucolytics and antibiotics directly to the lungs.[5,6] Epidemiological studies reported the use of inhaled treatment among 35.8%-82.1% of CF patients, depending on the type of medication.[7] Several studies which assessed contamination of the equipment and frequency of at least one pathogen reported a high rate of nebulizer contamination, around 60%.[8-14] Home nebulizer use was associated with a 28.5-fold greater

Correspondence to:
Barbara Riquena. Novartis Pharma Brasil, Av. Prof. Vicente Rao, 90, Brooklin, CEP 04636-000, São Paulo, SP, Brasil.
Tel.: +55 11 5532-4552. E-mail: publicação.brasil@novartis.com
Financial support: Novartis Biociências SA.

chance of bacterial contamination.[15] Nebulizers might be the primary source of colonization for some patients,[14] since proper cleaning instructions are not adequately followed.[10] Therefore, instead of acting as an auxiliary tool for the treatment of CF, nebulizers can become a harmful device if not properly managed.

International guidelines and, recently, the CF Brazilian guideline point to the importance of proper care with nebulizers.[16,17] Cultural, socioeconomic, and even climatic differences can interfere with the quality of care with nebulizers and consequently their contamination.[12,14] In this way, knowledge of regional particularities is essential, since few studies about the contamination profile of home nebulizers are available in developing countries, mainly in Brazil. This study aims to evaluate the contamination of nebulizers used for CF patients chronically colonized by *P. aeruginosa* and its association with cleaning, decontamination and drying practices.

METHODS

Ethics approval

The study was approved by the independent Ethics Committees of each participating site. Informed consent (assent, for those <18 years old) was signed for each patient before any study procedures.

Study design

Cross-sectional, observational, multicenter study was conducted in seven CF Brazilian reference centers. Data collection was performed from January 2013 to December 2014. Data were obtained from three sources (swab samples from nebulizers, medical records and interviews). Patients were asked to carry the nebulizer to the center during a routine visit. At this point, they were not informed about sample collection to avoid an information bias caused by unusual cleaning of the equipment. Swab samples for culture were collected from interface (mouthpiece/mask) and cup to evaluate nebulizers' contamination. Medical records were revised to collect CF data about diagnosis, Shwachman-Kulczycki score, pulmonary function, age, gender, ethnic groups and patients' body mass index (BMI). During a face-to-face structured interview, patient/caregiver (depending on who was responsible for cleaning the device) answered questions about aspects related to nebulizer hygiene routine, nebulizer use and sociodemographic characteristics. For this study, the nebulizer hygiene process was considered as the following steps: cleaning, disinfection and drying.

Eligibility criteria

Eligible patients were those ≥ 6 years old; diagnosed with CF confirmed by sweat chloride test above 60 mEq/dl or evidence of at least two CF causing mutations and on inhaled antibiotics therapy due to chronic airway colonization by *P. aeruginosa*. Patients should be using nebulizer of the brand PARI® and compressor PRONEB® for at least 3 consecutive months. Patients who did not use the nebulizer for inhaled antibiotic therapy for more than 30 days; who shared the nebulizer with other people; had participated of a similar study in the last 12 months and currently participating in a clinical study were excluded.

Sample collection and laboratory testing

Nebulizer assessment was performed between Day 21 and Day 28 from the OFF period of inhaled antibiotic therapy. Samples were collected via swab using aseptic technique described in laboratory's manual and shipped in Amies culture media, which is a liquid used to maintain the viability of microorganisms during transport.[18] Samples were shipped to a central laboratory and analyzed for the presence of pathogens in CF such as *P. aeruginosa* (mucoid and non-mucoid strains), *B. cepacia* complex, *Stenotrophomonas maltophilia*, *Staphylococcus aureus* (sensitive and resistant to methicillin), *Acinetobacter* sp., *Chromobacter* sp. and fungus. Culture mediums used for bacteria isolation were blood agar[19] and chocolate agar[20] and selective agar to *B. cepacia* complex. Bacterial identification was performed on Vitek 2 or mass spectrometry (Vitek MS), both automated systems.[21-23] Antibiotic susceptibility testing was performed on Vitek 2, using a manual confirmation when applicable in accordance with guidelines from Clinical and Laboratory Standards Institute. The culture mediums used to isolate fungus were sabouraud and mycosel agar. These culture mediums had been previously used in CF.[24,25] Non-fermenting Gram-negative bacilli not identified in the culture mediums were analyzed and identified through molecular biology.[26]

Statistical analysis

Considering the contamination risk of 63%,[12] a sample size of 80 patients would provide a 95% confidence interval (CI) with a margin of error of ± 10.5% to assess the study primary endpoint. However, due to recruitment difficulties, the study was interrupted with 77 patients, which provided a 95% CI with a margin of error of ± 10.7% (still lower than the reference study, which CI was 14.3%, considering the same contamination risk of 63%).

Descriptive analysis was performed through measures of central tendency, measures of dependency and measures of dispersion to quantitative variables, and frequency to qualitative variables. To determine the association among variables were estimated p-value by Pearson's Chi-square test and the odds ratio by binary logistic regression. Data were analyzed using the statistic software Stata MP11 and R Project 2.13.1, using a 95% CI and p-value ≤0.05 as significant.

RESULTS

Patients' demographic and disease characteristics

Demographic and disease characteristics of included patients are shown in Table 1.

Nebulizers' contamination profile

Microbiological contamination profile of home nebulizers was grouped in accordance with nebulizer's part (interface, cup or any part of the device) and pathogen contaminant (bacteria, fungus or any contamination) - Table 2. Assessing any nebulizers' parts there was a prevalence of 71.6% (95%CI = 61.3- 81.9) pathogen contamination in the study. According to nebulizer part, frequency observed was 60.8% (95%CI = 49.7-71.9) in interface and 62.2% (95%CI = 51.2-73.2) in cup.

Bacterial contamination was observed in 56.8% (95%CI = 45.5-68.1) of the cases and fungal contamination in 45.9% (95%CI = 34.5-57.3). Among those with bacterial contamination, Gram-negative bacteria was the most commonly found pathogen (85.7%; 95%CI = 75.1-96.3). The most frequently observed Gram-negative bacterial species were *Pseudomonas* spp. (31.0%; 95%CI = 17.0-45.0) and *Acinetobacter* spp. (21.4%; 95%CI=9.0-33.8). *Staphylococcus* spp. (21.4%; 95%CI = 9.0-33.8) and *Micrococcus* spp. (14.3%; 95%CI = 3.7-24.9) were the most frequent Gram-positive bacterial species. *Candida* spp. was the most frequently observed fungus (52.9%; 95%CI = 36.1-69.7), followed by environmental contaminant fungus (26.5%; 95%CI = 11.7-41.3). Other pathogens of interest and with an important role in clinical practice were also isolated: non-mucoid *P. aeruginosa* (4.8%; 95%CI = 0.0-11.3), *B. cepacia* complex (2.4%; 95%CI = 0.0- 7.0), *S. maltophilia* (11.9%; 95%CI=2.1-21.7) and *S. aureus* (4.8%; 95%CI = 0.0-11.3) - Table 2.

Table 1. Cystic fibrosis patients' profile.

Characteristics	N (%)
Age (years)	15.8 ± 6.5
Gender	
Male	44 (57.9)
Female	32 (42.1)
Ethnic groups	
Caucasian	51 (66.2)
Afro-descendent	11 (14.3)
Mixed (*Pardo*)	13 (17.1)
No information	2 (2.6)
Educational level of the responsible for cleaning the nebulizer	
Never been to school	--
Incomplete elementary school	19 (25.0)
Complete elementary school	6 (7.9)
Incomplete high school	7 (9.2)
Complete high school	25 (32.9)
Incomplete graduation	7 (9.2)
Complete graduation	12 (15.8)
Monthly Family Income (BRL$)	2,972.3 ± 2,975.4
Number of people who cohabit	3.9 ± 1.4
Siblings living in the same residence	0.9 ± 1.0
Rooms in the house	3.7 ± 1.6
Distance between the household and the treatment center (km)	56.3 ± 92.2
BMI (kg/m^2)	18.7 ± 3.65
Height (cm)	155.0 ± 17.2
Weight (kg)	49.7 ± 16.1
Time since CF diagnosis (years)	10.2 ± 5.68
Shwachman-Kulczycki Score	
Excellent	4 (5.2)
Good	10 (13.0)
Medium	11 (14.3)
Moderate	6 (7.8)
Severe	1 (1.3)
Pulmonary function test	
FEV_1 (%)	61.3 ± 22.9
FVC (%)	75.5 ± 24.1
FEV_1/FVC (%)	76.8 ± 19.4

Values are presented as mean ± SD or number (%). BMI: Body mass index; FEV_1: Forced expiratory volume in 1 second; FVC: Forced vital capacity; FEV_1/FVC: ratio of forced expiratory volume in 1 second to forced vital capacity.

Table 2. Contamination profile of home nebulizers of cystic fibrosis patients: type of fungus and bacteria according to place of contamination.

Characteristics	Interface		Cup		Any part of the device	
	N (%)	95%CI	N (%)	95%CI	N (%)	95%CI
Any contamination	45 (60.8)	49.7 - 71.9	46 (62.2)	51.2 - 73.2	53 (71.6)	61.3 - 81.9
Bacterial contamination	35 (47.3)	35.9 - 58.7	37 (50.0)	38.6 - 61.4	42 (56.8)	45.5 - 68.1
Gram-negative bacteria[a]	25 (71.4)	56.4 - 86.4	23 (62.2)	46.6 - 77.8	36 (85.7)	75.1 - 96.3
Pseudomonas spp.[a]	5 (14.3)	2.7 - 25.9	9 (24.3)	10.5 - 38.1	13 (31.0)	17.0 - 45.0
Non-mucoid *Pseudomonas aeruginosa*[a]	--	--	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Mucoid *Pseudomonas aeruginosa*[a]	--	--	--	--	--	--
Other *Pseudomonas*[a]	5 (14.3)	2.7 - 25.9	7 (18.9)	6.3 - 31.5	10 (23.8)	10.9 - 36.7
Acinetobacter spp.[a]	8 (22.9)	9.0 - 36.8	7 (18.9)	6.3 - 31.5	9 (21.4)	9.0 - 33.8
Stenotrophomonas spp.[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Stenotrophomonas maltophilia[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Enterobacter spp.[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	4 (11.9)	2.1 - 21.7
Klebsiella spp.[a]	1 (2.9)	0.0 - 8.5	4 (10.8)	0.8 - 20.8	4 (9.5)	0.6 - 18.4
Sphingobacterium spp.[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	2 (4.8)	0.0 - 11.3
Delftia spp.[a]	1 (2.9)	0.0 - 8.5	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Burkholderia spp.[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 -7.0
Burkholderia cepacia complex[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 -7.0
Other[a]	12 (34.3)	18.6 - 50.0	9 (24.3)	10.5 - 38.1	15 (35.7)	21.2 - 50.2
Chryseobacterium indologenes[a]	5 (14.3)	2.7 - 25.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Sphingomonas paucimobilis[a]	2 (5.7)	0.0 - 13.4	1 (2.7)	0.0 - 7.9	3 (7.1)	0.0 - 14.9
Pantoea agglomerans[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	2 (4.8)	0.0 - 11.3
Aeromonas hydrophila[a]	1 (2.9)	0.0 - 8.5	--	--	1 (2.4)	0.0 - 7.0
Comamonas testosteron[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Moraxella osloensis[a]	1 (2.9)	0.0 - 8.5	--	--	1 (2.4)	0.0 - 7.0
Rhizobium radiobacter[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Serratia marcescens[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Gram-positive bacteria[a]	10 (28.6)	13.6 - 43.6	10 (27.0)	12.7 - 41.3	17 (40.5)	25.7 - 55.3
Staphylococcus spp.[a]	6 (17.1)	4.6 - 29.6	7 (18.9)	6.3 - 31.5	9 (21.4)	9.0 - 33.8
Staphylococcus aureus[a]	2 (5.7)	0.0 - 13.4	2 (5.4)	0.0 - 12.7	2 (4.8)	0.0 - 11.3
Oxacillin-resistant coagulase-negative *Staphylococcus aureus*[a]	1 (2.9)	0.0 - 8.5	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Other *Staphylococcus*[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	6 (14.3)	3.7 - 24.9
Micrococcus spp.[a]	4 (11.4)	0.9 - 21.9	4 (10.8)	0.8 - 20.8	6 (14.3)	3.7 - 24.9
Bacillus spp.[a]	3 (8.6)	0.0 - 17.9	4 (10.8)	0.8 - 20.8	5 (11.9)	2.1 - 21.7
Streptococcus spp.[a]	--	--	1 (2.7)	0.0 - 7.9	1 (2.4)	0.0 - 7.0
Fungal contamination	20 (27.0)	16.9 - 37.1	28 (37.8)	26.8 - 48.8	34 (45.9)	34.5 - 57.3
Candida spp.[b]	11 (55.0)	33.2 - 76.8	14 (50.0)	31.5 - 68.5	18 (52.9)	36.1 - 69.7
Non-*albicans Candida* spp.[b]	9 (45.0)	23.2 - 66.8	14 (50.0)	31.5 - 68.5	16 (47.1)	30.3 - 63.9
Candida albicans[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5
Candida spp.[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5
Environmental contaminant fungus[b]	4 (20.0)	2.5 - 37.5	8 (28.6)	11.9 - 45.3	9 (26.5)	11.7 - 41.3
Other[b]	7 (35.0)	14.1 - 55.9	7 (25.0)	9.0 - 41.0	10 (29.4)	14.1 - 44.7
Cladosporium sp.[b]	3 (15.0)	0.0 - 30.6	3 (10.7)	0.0 - 22.1	4 (11.8)	1.0 - 22.6
Rhodotorula spp.[b]	3 (15.0)	0.0 - 30.6	3 (10.7)	0.0 - 22.1	4 (11.8)	1.0 - 22.6
Aspergillus niger[b]	--	--	1 (3.6)	0.0 - 10.5	1 (2.9)	0.0 - 8.5
Penicillium sp.[b]	1 (5.0)	0.0 - 14.6	--	--	1 (2.9)	0.0 - 8.5

[a]Proportion calculated among number of interfaces (N=35), cups (N=37) and any part of nebulizer (N=42) with bacterial contamination. [b]Proportion calculated among number of interfaces (N=20), cups (N=28) and any part of nebulizer (N=34) with bacterial contamination.

Characteristics of nebulizers´ hygiene

Considering characteristics of nebulizers use, frequency of nebulizer hygiene and method employed, 76 patients answered the interview questions. Patients reported the use of the following medications in nebulizer: dornase alfa (N = 72; 94.7%), tobramycin

inhaler solution (N = 64; 84.2%), hypertonic saline solution (N = 17; 22.4%), colistin (N = 15; 19.7%), bronchodilator (N = 5; 6.6%) and isotonic saline solution (N = 1; 1.3%). All patients reported to use only one drug during each nebulization.

Regarding the nebulizers' parts replacement, most patients had not performed it in the analyzed equipment (N = 48; 63.2%) and the reported reasons were: recommended interval to switch had not been reached (N = 29; 60.4%); lack of knowledge about the necessity (N = 12; 25.0%); forgetfulness (N = 2; 4.2%); and other reasons (N = 7; 14.6%). For those who reported to replace at least one part, cup was replaced in 85.7% (N = 24), hose in 64.3% (N = 18), interface and filter in 60.7% (N = 17) of the cases, other parts in 7.1% (N = 2) and all parts in 21.4% (N = 6). For patients who replaced all parts, half did it after more than six months of use.

Regular nebulizer hygiene was reported by 97.4% of the cases. Among those who reported regular nebulizer hygiene, the cup was the most cited part (N = 74; 100.0%), followed by the interface 79.7% (N = 59), hose 50.0% (N = 37) and filter 12.2% (N = 9). Most patients (71.1%) reported to perform nebulizer hygiene process after each nebulizer's use -Table 3.

Considering each step of nebulizer hygiene, 64 (86.5%) patients performed the cleaning process, 62 (83.8%) patients performed the disinfection process, and 73 (96.0%) patients performed the drying process. Most frequent cleaning process observed was lather and rinse under tap water (N = 49; 76.6%). A wide variety of disinfection methods were reported and the most frequent were immersion in boiling water (24.2%) and immersion in hypochlorite solution (21.0%). The entire process of nebulizer hygiene, using at least one cleaning, disinfection and drying method was reported by most of the study sample (70.3%) -Table 3.

Also, as a secondary objective of the study, the association between educational level and demographic data from patients and/or caregivers and the frequency of nebulizers cleaning was assessed and no significant differences were observed (data not shown).

Relation between nebulizers' cleaning and pathogen contamination

Bivariate analysis of the association between the nebulizers' cleaning and a positive culture for bacteria and/or fungus in the analyzed pieces are described in Table 4. A statistically significant difference in the frequency of contamination was observed for cleaning method (Only tap water = 92.9% of contamination vs. Lather and rinse under tap water = 66.0% of contamination; p = 0.049), performing or not disinfection (Yes = 66.7% of contamination *vs*. No = 100.0% of contamination; p = 0.015) and drying method (With a cloth, paper towels, fan/dryer or compressor/compressed air = 60.5% of contamination *vs*. Only outdoors = 84.4% of contamination; p = 0.028). A multivariate analysis by binary logistic regression for the factors associated to the positivity of culture was performed using the stepwise backward strategy. For this analysis, any contamination in nebulizer and the variables reported in Table 4 were included. The use only of tap water as a cleaning method increased 9-fold chance of contamination (OR = 9.10; 95%CI = 1.01-81.77; p = 0.049) when compared with the use of lather and rinse under tap water. Drying outdoors increased 4.87-fold chance of contamination (OR = 4.87; 95%CI = 1.10-21.61; p = 0.038) when compared with the use of some material, such as a cloth, paper towel, fan/dryer or the compressor/compressed air.

As nebulizer drying process performed outdoor is a recommended practice, the frequency of an inadequate cleaning (none or only tap water) or disinfection (none, sodium hypochlorite or vinegar solution) was assessed. An inadequate cleaning method was observed in 26% of the sample (N = 7), an inadequate disinfection in 40.7% (N = 11), both inadequate cleaning and disinfection methods in 7.4% (N=2) and inadequate cleaning or disinfection methods in 59.2% (N = 16).

DISCUSSION

In this multi-centric Brazilian study, a high prevalence of nebulizer contamination was observed among CF patients chronically colonized by *P. aeruginosa* under inhaled antibiotic on-off therapy. The role of home nebulizers as a source of contamination for patients with CF has been studied since 1987[8-14] but the amount of good and representative data within CF Brazilian patients is scarce. In addition, a high rate of nonconformities was observed in nebulizer use by patients and caregivers. This is an important issue as inadequate cleaning of the nebulizer has been associated with its contamination.[12]

Considering the prevalence of contamination in any part of the device, previous Brazilian studies found estimates from 25.0% to 57.5%, lower than those found in the current study.[10,13] This difference can be possibly attributed to distinct clinical characteristics between populations such as severity of lung function impairment and by patients' behavior. In the present study, adequate care with nebulizers was systematically analyzed and a low rate of appropriate management was observed.

Bacteria were the main pathogenic contaminants identified in the studied devices (56.8% of the patients), mainly the Gram-negative ones. Nevertheless, fungal contamination was also a relevant finding since 40.5% of patients were contaminated by large fungal species variety. Current literature, ranging several countries, has also shown a wide variety of bacterial specimens with heterogeneous results, varying the higher prevalence between Gram-positive and Gram-negative bacteria. *Acinetobacter* spp. and *Pseudomonas* spp. were the most frequently reported Gram-negative bacteria and *Staphylococcus* spp., was the most

Table 3. Hygienization profile of home nebulizers of cystic fibrosis patients.

Characteristics	N (%)
Nebulizer is regularly hygienized	
Yes	74 (97.4)
No	2 (2.6)
Nebulizer parts usually hygienized	
Interface	59 (79.7)
Cup	74 (100.0)
Hose	37 (50.0)
Filter	9 (12.2)
Other	1 (1.4)
Hygienization after each use	
Yes	54 (71.1)
No	20 (26.3)
No information	2 (2.6)
Length of each cleaning/disinfection	
Less than 15 minutes	44 (57.9)
More than 15 minutes	30 (39.5)
No information	2 (2.6)
Cleaning	64 (86.5)
Only tap water	15 (23.4)
Lather and rinse under tap water	49 (76.6)
Disinfection	62 (83.8)
Immersion in boiling water	15 (24.2)
Immersion in sodium hypochlorite solution	13(21.0)
Immersion in boiling water and Immersion in sodium hypochlorite solution	11 (17.7)
Immersion in vinegar solution	5 (8.1)
Immersion in boiling water and Immersion in alcohol	4 (6.5)
Immersion in boiling water and Immersion in alcohol and Immersion in vinegar solution	1 (1.6)
Immersion in boiling water and Immersion in vinegar solution	3 (4.8)
Immersion in alcohol	2 (3.2)
Immersion in sodium hypochlorite solution and Microwave	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in alcohol	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in vinegar solution	2 (3.2)
Immersion in boiling water and Immersion in sodium hypochlorite solution and Immersion in vinegar solution and Immersion in alcohol	1 (1.6)
Immersion in sodium hypochlorite solution and Immersion in alcohol	1 (1.6)
Drying	73 (98.6)
Only outdoors	32 (43.8)
With a cloth	20 (27.4)
With paper towel	19 (26.0)
With a fan/dryer	1 (1.4)
With the compressor/compressed air	1 (1.4)
Hygienization method	
Clean, disinfection and dry	52 (70.3)
Clean and dry	11 (14.9)
Disinfection and dry	10 (13.5)
Only clean	1 (1.4)

frequent Gram-positive bacteria.[8-14] Previous Brazilian studies found *Staphylococcus* spp. as the most frequent pathogen contaminating nebulizers.[10,13] In the present study, the assessment of nebulizer contamination was performed in a significantly larger population, although still pediatric, older than in previous studies. Furthermore, it is well described the relationship of increasing airway colonization by Gram-negative bacteria in CF patients with age increase, associated with a decrease by Gram-positive.[1] This behavior might interfere in the nebulizer contamination and may explain the different results in this field.

Table 4. Association between nebulizers' hygienization and positivity of culture.

Characteristics	Contamination by fungus or bacteria at least in one part of the nebulizer		p-value
	Yes N (%)	No N (%)	
Nebulizer parts usually hygienized[a]			
Interface	40 (70.2)	17 (29.8)	0.380
Cup	51 (71.8)	20 (28.2)	0.378
Hose	24 (66.7)	12 (33.3)	0.262
Filter	5 (55.6)	4 (44.4)	0.221
Other	1 (100.0)	0 (--)	0.536
Length of each hygienization			
Less than 15 minutes	31 (72.1)	12 (27.9)	0.951
More than 15 minutes	20 (71.4)	8 (28.6)	
Hygienization after each use			
Yes	23 (45.1)	28 (54.9)	0.710
No	8 (40.0)	12 (60.0)	
Cleaning			
Yes	44 (72.1)	17 (27.9)	0.839
No	9 (75.0)	3 (25.0)	
Cleaning method			
Only tap water	13 (92.9)	1 (7.1)	0.049
Lather and rinse under tap water	31 (66.0)	16 (34.0)	
Disinfection			
Yes	40 (66.7)	20 (33.3)	0.015
No	13 (100.0)	0 (--)	
Disinfection method			
Immersion in boiling water and/or alcohol and/or microwave only or in association with sodium hypochlorite and/or vinegar solution	30 (69.8)	13 (30.2)	0.595
Immersion in bleach and/or vinegar solution, without other methods	10 (62.5)	6 (37.5)	
Dry			
Yes	50 (71.4)	20 (28.6)	0.277
No	3 (100.0)	0 (--)	
Drying method			
With a cloth, paper towel, fan/dryer or compressor/compressed air	23 (60.5)	15 (39.5)	0.028
Only outdoors	27 (84.4)	5 (15.6)	
Hygienization method			
No hygienization	2 (100.0)	0 (--)	
Clean, disinfection and dry	33 (66.0)	17 (34.0)	
Only clean	1 (100.0)	0 (--)	0.197
Clean and dry	10 (100.0)	0 (--)	
Disinfection and dry	7 (70.0)	3 (30.0)	

[a]Since the answers were not mutually exclusive, each option was analyzed as a dichotomous variable generating different p-values.

Pulmonary infection is the leading cause of death in CF, being most cases associated to *P. aeruginosa* chronic infection. Several different sources can be implicated in airway colonization by *P. aeruginosa*, including nebulizers.[12,14] Despite the high estimates of *Pseudomonas* spp detected in the present study, most cases were related to species other than *P. aeruginosa*. The prevalence of *P. aeruginosa* in the literature ranges from 0%- 38%.[8-14] A low frequency of contamination by this pathogen in our data may probably be associated with the specific profile of our sample. Inclusion criteria definition allowed only patients with chronic colonization by *P. aeruginosa* in regular use of anti-Pseudomonas inhaled antibiotics that could interfere in bacterial growth even in the OFF month of treatment cycle.

Fungal contamination is less explored in available literature and specimens found were not clearly assessed in other studies.[8-14] In our sample, *Candida* spp. was the most common fungus found. Other

studies reported contamination by yeast, specifically by *Candida albicans* (14.0%), which was also observed in our sample (2.9%).[10,12,13] Peckham et al. also conducted a study to analyze specifically the fungal flora of nebulizers of CF adult patients and found a higher frequency of positivity (57.7%) than reported in our study (45.9%).[24]

We found a considerably higher frequency of patients who reported a regular hygiene of the nebulizer compared to other surveys.[5,9,10,13] National and international guidelines emphasizes the necessity to adequate care of nebulizers.[16,17] Cleaning steps must be performed with dish detergent soap and water, disinfection with boiling water, microwave, dishwasher, alcohol or hydrogen peroxide and lately air drying the equipment.[27] A high percentage of patients reported performing all the proposed steps. However, methods not recommended such as cleaning using only tap water, disinfection by sodium hypochlorite or vinegar solution and use of materials for drying were frequently reported. This discrepancy between the high frequency of contamination of nebulizers despite a high self-reported rate of adequate care of the devices points toward the need for better education of patients and caregivers. It is important to emphasize that self-reporting care with nebulizers does not necessarily translate into daily practice. However, in this study a high rate of not recommended nebulizer hygiene actions were observed, further reinforcing the need for improvement in the knowledge of this population. Because this is a multicenter study covering different regions of the country, we consider these data as highly relevant because it characterizes a problem found in all the centers studied and reflects a widespread problem.

A higher frequency of contamination among patients who clean the nebulizer only under tap water, do not disinfect it and dry outdoors was observed. Previous studies found that only the cleaning after each use had significant differences.[8,9] Hohenwarter et al. compared different steam disinfection and drying methods and found recontamination only among those equipment in which an active drying (such as paper or cotton towels) was performed.[6] A multivariate model including these characteristics was built and demonstrated that cleaning under tap water only and drying outdoors were the factors that increase the chance of contamination.

Drying outdoors is a recommended method as category II of evidence level (supported by suggestive clinical and epidemiologic studies). However, in the present study, it was associated with an increase of 4.87-fold chance of contamination. To verify if this association was related to cleaning and disinfection patterns, these frequencies among contaminated nebulizers that were dried outdoors were assessed and most patients reported at least one inadequate method of cleaning or disinfection (59.2%). This study was not designed to test a hypothesis and available recommendations are not based on the highest evidence level, which highlights the need for conducting more studies regarding each particular component of the nebulizer hygiene process. Another Brazilian study assessed the effect of a standardized instruction regarding nebulizers' cleaning and disinfection based on the international recommendations on the frequency of contamination[13,28] and after a single educational intervention, a significant impact was observed, reducing the frequency of contamination by 43%.[13] The proper cleaning of nebulizers can have clinical impact, since lack of cleanliness can reduce nebulizer performance and the equipment can become a potential source of contamination.[29]

There are some limitations in our study. Although this was a multicenter study with CF centers from different regions in Brazil, it was not possible to cover all states of the country. Another limitation refers to the request for patients to bring their nebulizers to assessment by the CF staff. Patients were not aware of the objective of the study before arriving in CF clinic, but we cannot exclude unusual cleaning before visit and information bias due to fear of reporting known misconduct acts to the study team. In addition, no viral agents were tested in this study although the relevance of transmission of this type of pathogen by nebulizer is yet not clear. Finally, data from patients' sputum culture was not assessed. Therefore, the relationship between airway and nebulizer contamination in the present study could not be determined.

In conclusion, high prevalence of contamination in CF nebulizers was observed despite the reports of elevated frequency of nebulizer hygiene. Most patients reported wrong cleaning techniques, emphasizing that CF team should be aware about this problem and intensify educational programs. Airway infection is one of the most important issues in CF management and several strategies should be stimulated to avoid it. The present study highlights that nebulizers are still a potential source of infection for CF patients.

Therefore, better knowledge about this area should be encouraged between patients and caregivers and/or new strategies for inhaled antibiotic delivery, such as dry powder formulations, should be implemented.

AKNOWLEDGEMENTS

This study was funded by Novartis Biociencias S.A., Brazil assistance on study development was provided by ANOVA and manuscript preparation by EUROTRIALS Brazil. The authors thank the staff from the participating clinical centers (Adriana Della-Zuana, Licia Cristine Marinho França, Núbia Cardoso Peixoto, Patricia Yuki Watanabe de Medeiros, Carla Cristina Souza Gomez and Maria Angela Ribeiro) and also from Novartis Biociencias S.A., Brazil (Glaucimar Basaglia, Paula Simões Bruna Abreu Crippa, Nadine Cordeiro Pinho). We are grateful to patients and their relatives for their participation in the study.

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J Bras Pneumol. 2019;45(3):e20180065
http://dx.doi.org/10.1590/1806-3713/e20180065

Reference values for pulmonary volumes by plethysmography in a Brazilian sample of white adults

Thamine Lessa[1,a], Carlos Alberto de Castro Pereira[2,b], Maria Raquel Soares[2,c], Renato Matos[3,d], Virgínia Pacheco Guimarães[4,e], Giancarlo Sanches[5,f], Roberto Helou Rassi[6,g], Israel Maia[7,h]

1. Laboratório de Função Pulmonar, Clínica AMO - Assistência Multidisciplinar em Oncologia, Salvador (BA) Brasil.
2. Laboratório de Função Pulmonar, Centro Diagnóstico Brasil, São Paulo (SP) Brasil.
3. Pneuma - Medicina Respiratória, Criciúma (SC) Brasil.
4. Hospital Madre Teresa, Belo Horizonte (MG) Brasil.
5. Instituto do Pulmão e Alergia, Maringá (PR) Brasil.
6. Pulmonar - Clínica do Aparelho Respiratório, Goiânia (GO) Brasil.
7. Laboratório de Função Pulmonar da Clínica Tórax, Florianópolis (SC) Brasil.

a. http://orcid.org/0000-0001-6301-6315
b. http://orcid.org/0000-0002-0352-9589
c. http://orcid.org/0000-0002-2242-2533
d. http://orcid.org/0000-0002-0429-6828
e. http://orcid.org/0000-0002-9557-4917
f. http://orcid.org/0000-0002-9486-762X
g. http://orcid.org/0000-0001-5153-537X
h. http://orcid.org/0000-0003-3467-5287

Submitted: 13 March 2018.
Accepted: 23 September 2018.

Study carried out in seven centers of Brazil: Clínica AMO, Salvador (BA) Brazil; Centro Diagnóstico Brasil, São Paulo (SP) Brazil; Pneuma-Medicina Respiratória, Criciúma (SC) Brazil; Hospital Madre Teresa, Belo Horizonte (MG) Brazil; Instituto do Pulmão e Alergia, Maringá (PR) Brazil; Pulmonar Clínica do Aparelho Respiratório, Goiânia (GO) Brazil; Clínica do Tórax, Florianópolis (SC) Brazil.

ABSTRACT

Objective: To derive reference values for healthy white Brazilian adults who have never smoked and to compare the obtained values with reference values derived by Crapo and by Neder. **Methods:** Reference equations by quantile regressions were derived in 122 men and 122 women, non-obese, living in seven cities in Brazil. Age ranged from 21 to 92 years in women and from 25 to 88 years in men. Lung function tests were performed using SensorMedics automated body plethysmographies according ATS/ERS recommendations. Lower and upper limits were derived by specific equations for 5 and 95 percentiles. The results were compared to those suggested by Crapo in 1982, and Neder in 1999. **Results:** Median values for total lung capacity (TLC) were influenced only by stature in men, and by stature and age in women. Residual volume was influenced by age and stature in both genders. Weight was directly related to inspiratory capacity and inversely with functional residual capacity and expiratory reserve volume in both genders. A comparison of observed TLC data with values predicted by Neder equations showed significant lower values by the present data. Mean values were similar between data from present study and those derived by Crapo. **Conclusion:** New predicted values for lung volumes were obtained in a sample of white Brazilians. The values differ from those derived by Neder, but are similar to those derived by Crapo.

Keywords: Pulmonary volumes; Pulmonary function tests; Reference values; Pulmonary plethysmography.

INTRODUCTION

The measurements of static pulmonary volumes in practice refer to the measurement of the several lung capacities and volumes. Capacities include: Functional Residual Capacity (FRC) - the volume of air present in the lungs at the end of Tidal Volume (TV) expiration; Total Lung Capacity (TLC) - total volume of air in the lungs at the end of a maximal inspiration; Vital Capacity (VC) - Total expired air volume after maximum inspiration or maximum inspiration after maximum expiration; Inspiratory Capacity (IC) –

volume of air inspired from the end of a normal expiration.[1]

These four capacities can be divided into volumes, with particular interest in the Expiratory Reserve Volume (ERV), the maximum volume of gas that can be exhaled from the FRC, and the residual volume (RV) which is the volume of gas remaining in the lungs after maximal expiration.[1]

The measurement of pulmonary volumes has several clinical applications. The TLC reduction establishes the presence of restriction. Apparent restriction on spirometry (reduction of FVC and FEV_1, with FEV_1 / FVC in the expected range) in many cases it is not confirmed by reduced TLC. This combination is called nonspecific pattern and is commonly observed in obstructive diseases with

Correspondence to:
Carlos Alberto de Castro Pereira. Laboratório de Função Pulmonar, Centro Diagnóstico Brasil, Av. Iraí, 393, Conjunto 34, Moema, CEP 04082-001, São Paulo, SP, Brasil.
Tel.: 55 11 5543-8070. E-mail: pereirac@uol.com.br
Financial support: None.

ISSN 1806-3713

airway closure, obesity and neuromuscular diseases.[2] In patients with reduced TLC, a disproportionately reduced FVC (TLC%-FVC expected> 10%), recently termed "complex restrictive pattern", points to clinical entities that result in impaired pulmonary emptying, such as neuromuscular diseases, obstruction and chest wall disease.[3] Elevated pulmonary volumes, particularly the increase in RV and RV / TLC ratio are common in airflow limitation and may be the only finding abnormal.[4,5] Air trapping is important in evaluating the severity of obstructive diseases and in response to treatment. In many cases, the reduction of RV is observed to a greater degree than the changes observed in spirometry.[6] The FRC reduced at the expense of ERV reduction is a characteristic of obesity.[7] Increase in ERV in obese patients is associated with reduced dyspnea after bariatric surgery.[8] The FRC increases with the degree of airflow obstruction, which results in IC decrease. IC/ TLC ratio <25% predicts a higher mortality in chronic obstructive pulmonary disease (COPD).[9]

The reference values for the static pulmonary volumes show remarkable differences between different authors.[10] These differences may be due to several factors, such as selection of individuals, methodological and technical differences, inclusion of different ethnic groups and types of equation used to calculate the expected values. In sample selection, ideally more than 100 adults of each gender for each ethnic group with a similar frequency distribution in the various age ranges should be included.[10]

Many studies about reference values are old, with small samples included.[11] In Brazil, reference values for pulmonary volumes were derived by Neder et al.[12] in 1999 in 50 men and 50 women of different races and are used in some centers.[12] In Brazil, the Crapo equation is also used as a reference for pulmonary volumes, but its adequacy to our population has never been tested.[2,13]

The aim of the present study was to determine reference values for pulmonary volumes in a white Brazilian multicenter sample and compare the results obtained by the Neder and Crapo equations.

METHODS

The data were obtained in seven Brazilian cities by plethysmographs of the same brand (SensorMedics, Yorba Linda, California) between 2015 and 2017 (Brazil Diagnostic Center, São Paulo; AMO Clinic, Salvador, Madre Teresa Hospital, Belo Horizonte; Matos Clinic, Criciúma; and others). The individuals were selected by verbal invitation, and were more commonly companions or family members of patients or staffs of the study institutions. The volunteers who accepted and agreed to participate in the study initially responded to a questionnaire translated from the American Thoracic Society / Division of Lung Diseases, validated in in Brazil and, when the inclusion criteria were completed, signed a written informed consent form.[14,15]

The project was approved by the Ethics and Research Committee of the Madre Teresa Hospital / Belo Horizonte, Minas Gerais, number 1617108. The project has not been submitted for approval in all centers; however, the complete documentation of all the centers involved was added to the project approved.

The inclusion criteria in the study were:

- Age over 20 years in females and 25 years in males;[16]
- Body Mass Index (BMI) between 18 and 30 kg / m^2;
- Absence of significant respiratory symptoms, current or previous respiratory diseases, cardiac diseases, previous thoracic surgery and relevant occupational exposure;[15]
- Not had smoked for a lifetime. Women, whom cooked in wood stoves, as well as those exposed to cigarette smoke in the bedroom, were excluded;
- Color self-declared white as confirmed by the individual and by observers.

The exams were performed by technicians or medical certificates in pulmonary function by Brazilian Thoracic Society.

The spirometry tests followed the norms suggested by the Brazilian Thoracic Society and the ATS / ERS guidelines.[1,17] The volumes were measured by SensorMedics body plethysmographs with variable pressure, equipped by a pneumotachograph, according to the standards proposed by the American Thoracic Society and European Respiratory Society.[18] The volume signal was calibrated by a 3.0 L syringe connected to the pneumotachograph, according to the manufacturer's recommendations, daily before of the workday start. The mouth pressure transducer and pressures and flows in the plethysmograph were calibrated daily. The functional parameters were expressed as BTPS.[17]

Procedures were conducted in the sitting position while subjects were using a nasal clamp. After detailed instructions regarding the test, the plethysmograph door was closed and the time to temperature equilibrium expected. The patient was then instructed to place the mouthpiece and breathe quietly until a plateau at the endo-expiratory level was reached out. When the breathing was at the Functional Residual Capacity (FRC) level, the shutter was closed and the patient instructed to pant softly, at a frequency between 0.5 and 1.0 Hz. The pressure-flow plots were recorded for airway resistance calculation (not included in the present study). The pressure-volume charts for determination of FRC were obtained by closing shutter at the end of a normal expiration. After opening the obturator, ERV and inspiratory vital capacity were determined. The RV was obtained by subtraction of the FRC and ERV, and the TLC was computed as the sum of the RV and VC. The inspiratory capacity was calculated by TLC-FRC. From these data, the RV / TLC ratio (%) was calculated. The system registers four loops by each maneuver, and at least three maneuvers were performed on all tested, therefore, with 12 loops. When the acceptance criteria were not met, more loops were

performed. At least three maneuvers with ≤5% FRC variability around the average value and vital capacity ≤0.15 L were obtained. The final value noted was the average value. All the curves obtained in the individual tests were sent and reanalyzed by one of the authors (CACP), 46 test cases were excluded (16%) who did not meet the acceptance criteria for inclusion curves. The data from these individuals were not compared to those included.

Initially, the numerical data variables were analyzed descriptively, and expressed by summary measures as median and quartiles (1st quartile, 3rd quartile) and the values of these variables were compared according gender by the non-parametric Mann -Whitney test.

Quantile regressions were used to derive the reference values.[19] The quantile regressions were adjusted (median, 5 and 95 percentile) as dependent variables, each of the functional variables and, as predictors, age, stature and weight as well as quadratic terms for age and stature. Initially, all predictor variables were included in the model. Subsequently, non-significant variables at 5% were excluded one by one in order of significance (retrograde method).

The spirometry values were compared to those expected for the Brazilian population.[20]

The differences between the median values observed and mean predicted values for TLC, VC, RV and RV / TLC by the Neder et al.[12] and Crapo et al.[13] equations were calculated and the significance of the difference calculated by the t test for one sample in both genders. The differences between the TLC values provided by Crapo and Neder and observed in this sample were expressed in graphs and differences calculated by paired t-test.[21]

As recently suggested, to approach the magnitude of a relevant effect in observational studies and by the multiple comparisons, significant p value was considered <0.005, comparing the values found in this study and those calculated by Neder and Crapo.[22]

Statistical analyzes were performed by Stata 12 and SPSS-22 statistical software's.

RESULTS

A total of 244 individuals (122 of each gender) were included in the study. The individuals were tested, in descending order, in São Paulo (62%), Salvador (13%), Criciúma (10%), Belo Horizonte (7%) and other places (8%).

The distribution for anthropometric data is shown in Table 1. Age in males varied from 25-88 years and from 21 to 92 years in females. The median stature in males was 173 cm (156-189 cm) and 160 cm (140-174 cm) in females.

The median values and the dispersion for the main functional data, expressed by quartiles 1 and 3, are shown in Table 2.

The Table 3 presents the final model estimates.

The comparisons between the values observed for the TLC and those predicted by the Crapo and Neder equations are shown in Table 4 and Figure 1. The values predicted by Neder were higher than those found in the present study. For males, the average difference was 0.48 L and for females, 0.24 L (p <0.001 for both).

The average differences between the values predicted by Crapo and those found in the present study for TLC were small and not significant: in males, -0.09 (p=0.12) and for females, -0.02 L (p=0.60).

DISCUSSION

The present study derived by plethysmography new predicted values for pulmonary volumes in adults subjects who never smoked, of white race, in Brazil.

The measurement of TLC is essential for the diagnosis of restrictive lung disease. The VC may be reduced in both, restrictive and obstructive lung disease, in the later due to increased residual volume. The elevated TLC indicates, in general, loss of lung elastic recoil, as occurs in emphysema and in some cases of asthma.[23] Besides that, the measurement of lung volumes is essential for the interpretation of other functional parameters, such as airway resistance, which ranges inversely with the pulmonary volume.

Factors that determine normal lung size include stature, age, gender, body mass, altitude, ethnic group, and physical activity pattern.[24-26] The maximum inspiratory level (TLC) is influenced by the strength developed by the inspiratory muscles, lung elastic recoil, and the elastic properties of the thorax and adjacent structures. Great swimmers, divers and rowers may have increased TLC by increased muscle strength.[27]

The expected values for TLC are considered independent of age because the reduction in pulmonary elastic recoil is compensated by the combined effects of loss of muscle strength and increase in the rib cage stiffness with advancing age.[27] This overlooks the fact that body fat, which on average increases with age, can reduce pulmonary volume.[27] In the present study, only the stature influenced TLC in men; in women, the effect of age was significant, but with opposite effects, as observed by others.[28] Women have a greater tendency to gain weight with age and men tend to lose more lung elastic recoil.[27] In some studies, age influenced TLC in men and women, in others, only in women.[12,13,28,29]

The gas volume at the end of full expiration reflects a balance between the strength of the accessory expiratory muscles, the inherent compressibility of the rib cage, and the closure of the airways.[26] In adults, the RV limiting factor becomes the decreased elastic recoil; this leads to narrowing and eventual closure of the airways. With advancing age, the pulmonary elastic retraction decreases. As a result, both, RV and RV / TLC ratio increase with age. In addition to the loss of elastic recoil, the accumulation of fat and the decrease in the strength of the respiratory muscles lead to the reduction of VC, which is therefore multifactorial.

Table 1. Distribution of the reference population, male and female, by age, stature and body mass index.

Variable	Female n = 122		Male n = 122	
	n	%	n	%
Age (years)				
20-24	4	-	-	-
25-34	30	24.6	27	22.1
35-44	22	18.0	20	16.4
45-54	15	12.3	24	19.7
55-64	24	19.7	19	15.6
65-74	14	11.5	22	18.0
≥75	13	10.6	10	8.2
Stature (cm)				
140-154	29	23.8	-	-
155-164	65	53.3	15	12.3
165-174	28	22.9	61	50.0
175-184	-	-	40	32.8
≥ 185	-	-	6	4.9
BMI (Kg/m^2)				
18-24	60	49.2	49	40.2
25-30	62	50.8	73	59.8

n = number; BMI = body mass index.

Table 2. Main functional data obtained in women and men in a sample of the Brazilian white population.

	Women (n = 122) Md (Q1;Q3)	Man (n = 122) Md (Q1;Q3)	p
FVC (L)	3.30 (2.83 ;3.67)	4.56 (4.00-5.11)	<0.005
FVC% predicted	99.8 ± 12.3	97.3 ± 10.0	
FEV1 (L)	2.65 (2.24; ; 3.03)	3.62 (3.15 ; 406)	<0.005
FEV1% predicted	99.0 ± 10.6	96.0 ± 9.2	<0.005
FEV1/FVC%	80.5 (77.0 ; 85.0)	80.0 (75.0 ; 83.0)	<0.005
FEV1/FVC% predicted	99.4 ± 5.5	98.9 ± 6.2	<0.005
TLC (L)	4.81 (4.46 ; 5.24)	6.71 (6.19 ; 7.14)	<0.005
VC (L)	3.33 (2.85 ; 3.69)	4.65 (4.08 ; 5.22)	<0.005
IC (L)	2.35 (2.07 ; 2.62)	3.27 (2.88 ; 3.56)	<0.005
FRC (L)	2.47 (2.07 ; 2.85)	3.44 (2.95 ; 3.87)	<0.005
ERV (L)	0.87 (0.59 ; 1.08)	1.36 (0.98 ; 1.76)	<0.005
RV (L)	1.59 (1.28 ; 1.89)	1.98 (1.68 ; 2.32)	<0.005
RV/TLC%	33.00 (27.00 ; 39.00)	31.00 (25.75 ; 34.25)	<0.005

FVC = Forced Vital Capacity; FVC% = FVC in % of predicted; FEV1 = Forced Expiratory Volume at the first second; FEV1% = FEV1 in % of predicted; FEV1/FVC% = Forced Expiratory Volume at the first second /Forced Vital Capacity ratio (%); TLC = Total Lung Capacity; VC = Vital Capacity; IC = Inspiratory Capacity; FRC = Functional Residual Capacity; ERV = Expiratory Reserve Volume; RV = Residual Volume; RV/TLC % = Residual Volume / Total Lung Capacity ratio (%).

The fat is an important component of Body Mass Index (BMI), but this index also includes Fat Free Mass (FFM), of which the largest component is muscle. In the present study, IC correlated directly with weight, which can be explained by the correlation of muscle mass with weight, and FRC and ERV correlated negatively with weight, probably due to the effect of deposition of higher central fat, even with the inclusion in the study of individuals with BMI in the normal range. Half of the women and 65% of the men in the present study had BMI values above 25, indicating overweight.

The derived values in the present study were compared to the values suggested by Neder and Crapo.[12,13] Crapo et al.[13] evaluated 122 female and 123 male individuals in Salt Lake City (altitude: 1400m), all Mormons.[13] The selection method was not described. The pulmonary volumes may be higher in inhabitants born at sites with more than 1800 m high, presumably due to increased pulmonary growth due to hypoxemia. Therefore, this factor should not have influenced the values observed in the present study and that of Crapo et al.[13]. Only two male individuals were over 85 years of age. The technique used was the single breath by helium dilution, used to measure of CO diffusion, which may underestimate TLC.[30] The equations were derived by linear regression.

As TLC can range more or less for the respiratory diseases, two-tailed tests (2x SEE) were used by Crapo to establish reference limits, which resulted in wide variation around the predicted value. The TLC limits

Table 3. Estimates of quantile regression models for the 5th, 50th and 95th percentiles of the volume and dispersion variables, according to gender.

Variable	Women	Men
TLC		
P5°	-4.8007 + 0.0559 x stature	-10.7100 + 0.0946 x stature
P50°	-4.3419 + 0.065 x age^2 -0.0007 x age + 0.0483 x stature	-6.1250 + 0.0742 x stature
P95°	-6.435 + 0.0755 x stature	-10.4536 + 0.0097 x age + 0.1020 x stature
VC		
P5°	22.9599 -0.0002 x age^2 -0.2864 x Stature + 0.001 x Estatura^2	-5.2936 -0.0158 x Age + 0.0580 x Stature
P50°	-1.9753 -0.0001 x Age^2 + 0.0353 x Stature	-4.8522 -0.0216 x Age + 0.0615 x Stature
P95°	-4.7539 + 0.0391 x Age -0.0005 x Age^2 + 0.0511 x Stature	-6.5653 -0.0117 x Age + 0.0729 x Stature
IC		
P5°	-3.1738 + 0.0313 x Stature	-3.6500 + 0.0350 x Stature
P50°	-0.8509 -0.0001 x Age^2 + 0.0151 x Stature + 0.0162 x Weight	2.0383 -0.0124 x Age + 0.0239 x Weight
P95°	-41.8650 + 0.0320 x Age -0.0004 x Age2 + 0.5434 x Stature -0.0017 x Stature^2	-3.7778 + 0.0287 x Stature + 0.0361 x Weight
FRC		
P5°	-36.7215 + 0.0567 x Age -0.0004 x Age^2 + 0.4603 x Stature -0.0014 x Stature^2	-3.5400 + 0.0500 x Stature -0.0325 x Weight
P50°	-5.1544 + 0.0115 x Age+ 0.0548 x Stature -0.0264 x Weight	-5.0085 + 0.0617 x Stature -0.0292 x Weight
P95°	-8.2273 + 0.0123 x Age+ 0.0729 x Stature -0.0137 x Weight	-4.2155 + 0.0171 x Age + 0.0444 x Stature
ERV		
P5°	-3.0705 + 0.0214 x Stature	0.9174 -0.0742 x Age + 0.0006 x Age^2 + 0.0001 x Stature^2
P50°	-2.332 + 0.033 x Age -0.0004 x Age^2 + 0.0208 x Stature -0.0123 x Weight	-5.2651 -0.0078 x Age + 0.052 x Stature -0.0257 x Weight
P95°	-2.7024 + 0.0373 x Age -0.0004 x Age^2 + 0.0298 x Stature -0.0212 x Weight	2.2800
RV		
P5°	-56.8440 + 0.0205 x Age+ 0.7111 x Stature -0.0022 x Stature^2	0.9767 + 0.0001 x Age^2
P50°	-3.0184 + 0.0002 x Age^2 + 0.0257 x Stature	0.4146 + 0.0153 x Age + 0.00003 x Stature^2
P95°	-5.8251 + 0.0499 x Age -0.0003 x Age^2 + 0.0448 x Stature -0.0139 x Weight	2.2615 + 0.0002 x Age^2
RV/TLC		
P5°	10.3085 + 0.0041 x Age^2	856.3949 + 0.0027 x Age^2 -9.6007 x Stature + 0.0278 x Stature^2 -0.1902 x Weight
P50°	23.9599 + 0.0033 x Age^2	14.700 + 0.3000 x Age
P95°	27.0370 + 0.3148 x Age	1.021.9540 -0.6122 x Age + 0.0086 x Age^2 -11.1571 x Stature + 0.0318 x Stature^2

TLC = Total Lung Capacity; P5° = 5th percentile; P50° = 50th percentile; P95° = 95th percentile; IC = Inspiratory Capacity; FRC = Functional Residual Capacity; ERV = Expiratory Reserve Volume; RV = Residual Volume; RV/TLC % = Residual Volume / Total Lung Capacity ratio (%).

would be calculated by adding or subtracting 1.08 L from those expected limits for females and 1.61 L for males. However, common pulmonary diseases have a tendency to increase or decrease TLC data, and the calculation of limits by the 5th and 95th percentiles is accepted.[28] By calculating the 5% limits for TLC in males by the Crapo equations (expected-1.30), the lower limit would be 80% of the mean value compared to 84% of the median expected by the current equation. In females, these values would be 82% for the Crapo equation and 86% for the current equation. Therefore, in the present study, the lower limits are closer to the expected value, increasing the sensitivity for the detection of restrictive disorder.

Neder et al.[12] derived reference values in 50 individuals of each gender, aged 20-80 years, randomly selected among employees of a large hospital in São Paulo. The racial profile was variable, with inclusion of 34 non-white individuals. Race has a significant effect on lung volumes.[31] Obese people were not excluded.

Table 4. Difference between values expected by Neder and Crapo and the current values observed for different pulmonary volumes.

Author	Variable	Gender			
		Female		Male	
		Mean difference with current author	p	Mean difference with current author	p
Neder et al.[12]	TLC (L)	0.24	<0.005	0.52	<0.005
	VC (L)	0.38	<0.005	0.46	<0.005
	RV (L)	-0.14	<0.005	0.05	0.22
	RV/TLC (%)	-0.5	0.41	-1.3	0.002
Crapo et al.[13]	TLC (L)	0.02	0.60	-0.09	0.12
	VC (L)	-0.10	0.008	-0.04	0.33
	RV (L)	0.15	<0.005	-0.05	0.18
	RV/TLC (%)	2.3	<0.005	-0.5	0.26

TLC = Total Lung Capacity; VC = Vital Capacity; RV = Residual Volume; RV/TLC % = Residual Volume / Total Lung Capacity ratio (%).

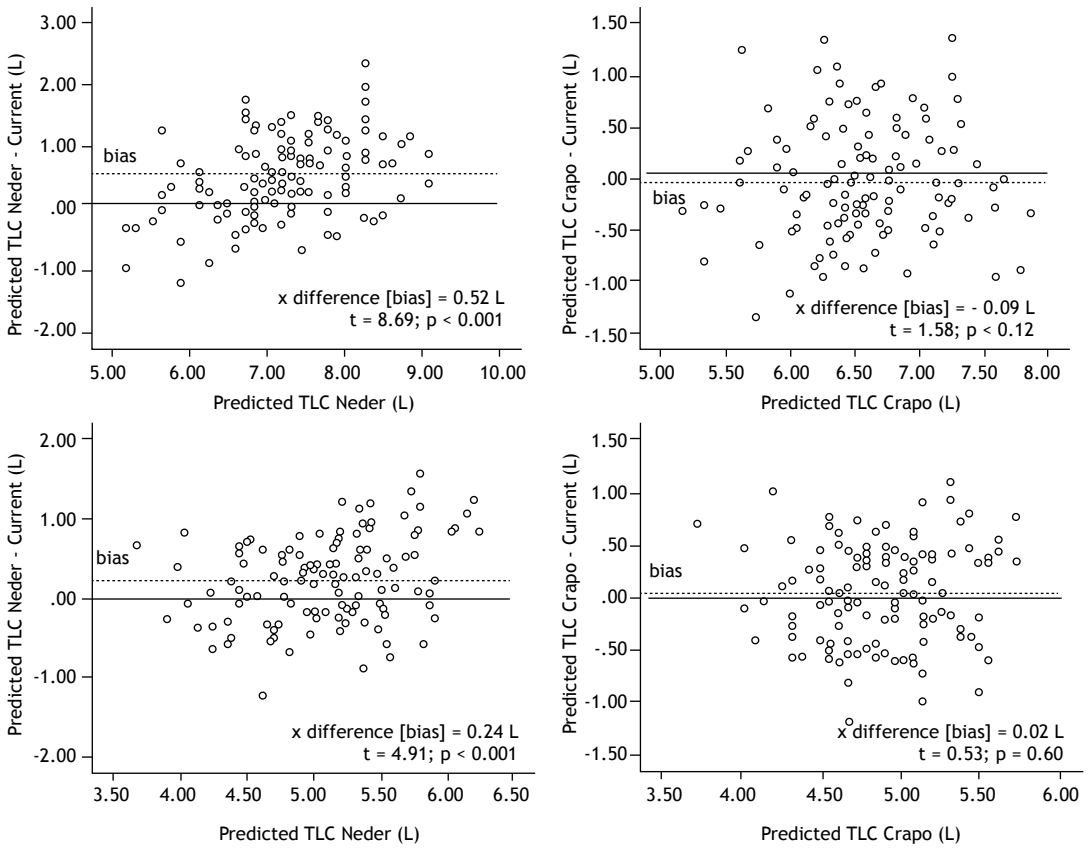

Figure 1. Difference between the values obtained in the present sample and those predicted by Neder (left) and Crapo (right) in males (above) and females (below).

The method used to determine FRC was the N2 washout by multiple breaths. The tests were performed in a Medical Graphics system, which provides greater results compared to other large systems, which may explain the high values found.[32] The physical activity pattern and the body composition were determined and influenced the pulmonary volumes in the univariate analysis, but not in the multivariate analysis. The values were derived by linear equations. As shown in Table 4 and Figure 1, the values of Neder et al.[12] significantly overestimate the values for TLC and VC, when compared to the present study.

Quantile regressions were used in the present study, as in other studies on reference values for pulmonary function.[33] In the classical linear method, by the determination of the least squares line, the dispersion of y values around the regression line (residuals) is considered to have a normal distribution, and the same error occurs for different values of x. However, the covariates may affect the distribution of residuals

in several ways. One advantage of using the quantile regression to estimate the median instead of the usual least squares regression to estimate the average is that the result of quantile regression is more stable in response to *outliers*.[19,34]

The comparison between the current equation and that of Crapo et al.[13] and Neder et al.[12] should take into account that different regression models were used, but the differences cannot be explained by the statistical model. Additional data that should be considered are the type of equipment and the methods used, besides that, the selection criteria and the sample size. A careful revaluation of acceptance criteria of maneuver was applied in the present study by review of all cases.

The present study has limitations. The most obvious is the uncertain extrapolation of data to black race, which is important in Brazil. The participants' level of physical activity was not assessed. In this study, volunteers were invited. For the derivation of reference values for pulmonary function, only non-smokers with no symptoms or cardiorespiratory diseases should be included. For this, a validated respiratory epidemiological questionnaire should be applied. Fulfilled these conditions, the use of volunteers to establish reference values is valid.[10,14] A methodology very similar to the present study was applied in several centers in Canada, in volunteers, to obtain reference values for pulmonary function, including measurement of pulmonary volumes.[35]

In summary, the present study derived new reference values for pulmonary volumes by plethysmography in white individuals in Brazil. The values differ from those expected by Neder and are close to those derived by Crapo.

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J Bras Pneumol. 2019;45(3):e20180085
http://dx.doi.org/10.1590/1806-3713/e20180085

Effects of exercise on sleep symptoms in patients with severe obstructive sleep apnea

Roberto Pacheco da Silva[1,a], Denis Martinez[1,2,3,b], Kelly Silveira da Silva Bueno[1,c], Jhoana Mercedes Uribe-Ramos[2,d]

1. Programa de Pós-Graduação em Cardiologia e Ciências Cardiovasculares, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS) Brasil.
2. Programa de Pós-Graduação em Ciências Médicas, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS) Brasil.
3. Divisão de Cardiologia, Hospital de Clinicas de Porto Alegre, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS) Brasil.

a. http://orcid.org/0000-0002-5580-0465
b. http://orcid.org/0000-0002-3578-0372
c. http://orcid.org/0000-0002-1357-5781
d. http://orcid.org/0000-0003-0907-3831

Submitted: 12 March 2018.
Accepted: 29 October 2018.

Study carried out in the Divisão de Cardiologia, Hospital de Clinicas de Porto Alegre, Universidade Federal do Rio Grande do Sul – UFRGS – Porto Alegre (RS) Brasil.

ABSTRACT

Objective: To investigate the extent to which exercise is associated with symptoms in patients with severe obstructive sleep apnea (OSA). **Methods:** We included subjects with an apnea-hypopnea index (AHI) > 30 events/h who completed validated sleep and exercise questionnaires. We compared symptom frequency/scores between exercisers and nonexercisers, adjusting for the usual confounders. **Results:** The sample included 907 nonexercisers and 488 exercisers (mean age, 49 ± 14 years; mean AHI, 53 ± 20 events/h; 81% men). Nonexercisers and exercisers differed significantly in terms of obesity (72% vs. 54%), the mean proportion of sleep in non-rapid eye movement stage 3 sleep (9 ± 8% vs. 11 ± 6%), and tiredness (78% vs. 68%). Nonexercisers had a higher symptom frequency/scores and poorer sleep quality. Adjustment for exercise weakened the associations between individual symptoms and the AHI, indicating that exercise has a mitigating effect. In binary logistic models, exercise was associated with approximately 30% lower adjusted questionnaire[1] score > 2, tiredness; poor-quality sleep, unrefreshing sleep, and negative mood on awakening. Although the odds of an Epworth Sleepiness Scale score > 10 were lower in exercisers, that association did not withstand adjustment for confounders. **Conclusions:** Exercise is associated with lower frequency/intensity of symptoms in patients with severe OSA. Because up to one third of patients with severe OSA might exercise regularly and therefore be mildly symptomatic, it is important not to rule out a diagnosis of OSA in such patients.

Keywords: Sleep apnea syndromes; Exercise; Sleepiness; Polysomnography.

INTRODUCTION

Excessive daytime sleepiness and daytime fatigue are symptoms of obstructive sleep apnea (OSA).[1] OSA contributes to decreased energy levels and motivation throughout the day.[2] Insufficient motivation is commonly reported as a cause of reduced participation in exercise programs.[3,4] Approximately one quarter of patients with moderate-to-severe OSA are mildly symptomatic.[5] Maintaining a regular exercise program could mitigate the symptoms of OSA.[6,7] One confounder in the OSA-exercise association is exercise intensity.[8] In a previous study conducted by our group, exercise was associated with a 34% lower adjusted risk of severe OSA.[9]

A considerable proportion of individuals with OSA exercise. It has been reported that 34% of patients with moderate-to-severe OSA exercise regularly,[8] as do 21% of those with severe OSA,[9] indicating the relevance of studying the relationship between OSA and exercise.

We tested the hypothesis that regular exercise is associated with sleep quality and OSA symptoms in patients with severe OSA. We examined the database of a university-affiliated sleep laboratory to investigate the relationship between physical activity and sleep symptoms in patients with OSA.

METHODS

This was a retrospective subgroup analysis of a previously published cross-sectional observational study.[9] We examined data related to consecutive patients, ≥ 18 years of age, who were referred to our sleep laboratory between March of 2013 and August of 2015 for the investigation of sleep disorders. At the time of the original study, all of the participants gave written informed consent for the anonymous use of their data.

Questions about sleep quality, together with validated questionnaires on sleep,[10] sleepiness,[11] and psychological symptoms,[12] were employed to assess the variables of interest prior to polysomnography. The quality of sleep was determined with Likert scales, and the level of physical activity was assessed with the International Physical Activity Questionnaire (IPAQ).[13] Participant occupations were also categorized by the level of physical activity required.[14] In addition, the probability of a diagnosis of OSA was assessed with a truncated version of the **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure, **B**ody mass index, **A**ge, **N**eck circumference, and **G**ender (STOP-Bang) questionnaire.[15] To determine the degree of daytime sleepiness, the Epworth Sleepiness Scale (ESS) was

Correspondence to:
Roberto Pacheco da Silva. Divisão de Cardiologia, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, CEP 90035-903, Porto Alegre, RS, Brasil.
Tel.: 55 51 3359-8289. Fax: 55 51 3022-2282. E-mail: roberto.pacheco01@gmail.com
Financial support: None.

[1] A truncated form of the **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure, **B**ody mass index, **A**ge, **N**eck circumference, and **G**ender questionnaire.

ISSN 1806-3713

applied.[15] Incomplete and inconsistent questionnaires were excluded.

Sleep symptoms

Yes/no questions

All patients were interviewed by the researchers. During the interviews, the patients answered four questions—"Do you consider yourself sleepier than other persons?"; "Do you have usually difficulty initiating sleep?"; "Do you have usually difficulty maintaining sleep?"; and "Do you usually wake up feeling unrefreshed?"—in a yes/no format.

Likert scales

Using a 0-10 point Likert scale, the patients responded to the questions "How do you rate your sleep quality?" and "How do you rate your mood?"—a score of 0 indicating the worst (sleep quality or mood) and a score of 10 indicating the best. Sleep quality, as rated by the patients, was dichotomized, a score ≤ 5 being categorized as poor sleep quality and a score > 5 being categorized as satisfactory sleep quality. Scores on another question—"How do you rate your level of tiredness on waking up?"—also ranged from 0 (not tired at all) to 10 (most tired possible).

STOP-Bang questionnaire

The STOP-Bang questionnaire[15] takes into account the following signs and symptoms of OSA: snoring; tiredness; observed apnea; high blood pressure; body mass index > 35 kg/m^2; age > 50 years; neck circumference > 40 cm; and male gender. The questionnaire consists of eight yes/no questions, and a "yes" is equal to a score of 0. The total score therefore ranges from 0 to 8, higher scores indicating a higher probability of a diagnosis of OSA. A score ≥ 3 is associated with high sensitivity for the detection of OSA, and a score of 5-8 is associated with a high probability of moderate-to-severe OSA. Because body mass index, age, and gender were control variables in the multivariate models, we analyzed the STOP portion alone and did not include the Bang score in the comparisons.

ESS

The ESS is a self-report questionnaire, developed in 1991, that assesses the likelihood of the individual falling asleep in eight different situations.[16] The scale has a maximum score of 24 points, and a score > 10 is considered indicative of excessive daytime sleepiness.

Physical activity

Physical activity was assessed with an abridged version of the IPAQ for young and middle-aged adults, which has been translated to Portuguese and validated for use in Brazil.[13] The IPAQ was proposed by an international consensus group with representatives of 25 countries, including Brazil, under the auspices of the World Health Organization.[17] The IPAQ is a self-report questionnaire comprising 8 questions that estimate the time spent weekly in different types of at least moderate physical activity and being physically inactive (sitting). On the basis of the answers on the IPAQ, patients were classified as nonexercisers or exercisers. Individuals reporting at least 10 min of moderate- or high-intensity physical activity per day were classified as exercisers. Exercise was classified, by type, as endurance training, resistance training, or combined training (such as practicing a sport). Exercise intensity was classified as moderate or vigorous. Moderate exercise was defined as that which made the participant breathe somewhat harder than normal. Vigorous exercise was defined as that which made the participant breathe much harder than normal. The frequency of exercise was recorded in days per week, duration of exercise in minutes per day, and time sitting in hours per day. Participants who did not meet the criterion of ≥ 10 min of moderate- or high-intensity physical activity at least once a week were classified as nonexercisers. Incomplete or inconsistent questionnaires were excluded.

Occupational activity

We employed the Physical Demands - Strength Rating from the Dictionary of Occupational Titles[18] to describe the strength requirements of each occupation. The rating is based on worker involvement in activities such as standing, walking, sitting, lifting, carrying, pushing, and pulling. By analogy with the IPAQ, in which only moderate and vigorous levels of exercise are considered in order to classify a person as physically active, participants performing sedentary or light work were classified as inactive, whereas those performing medium, heavy, or very heavy work were classified as active.

Polysomnography

Subjects underwent overnight polysomnography as previously described.[19] In brief, data were recorded from approximately 23:00 to 07:00. Electroencephalogram (electrodes C4-A1, F4-A1, and O2-A1), electrocardiogram (electrode D1 or modified V4), left and right electrooculogram (electrode A1), and submental electromyogram were recorded. Airflow was measured through a nasal cannula connected to a pressure transducer (Ultima PT2 Dual; Braebon Medical Corp., Kanata, Canada). Respiratory effort was assessed by respiratory inductance plethysmography (Ultima Q-RIP; Braebon Medical Corp.), and oxygen saturation by pulse oximetry (XPOD; Nonin Medical, Inc., Plymouth, MN, USA). The recordings were made at room temperatures between 22°C and 26°C. Sleep scoring followed the 2012 American Academy of Sleep Medicine rules.[20]

Apnea was defined as a drop in airflow to ≥ 90% of baseline for ≥ 10 s; hypopnea was defined as a drop in airflow to ≥ 50% of baseline for ≥ 10 s, accompanied by ≥ 3% arterial oxygen desaturation or an arousal. The apnea-hypopnea index (AHI) was

calculated by dividing the total number of apnea and hypopnea events by the total hours of sleep. During the night, snoring was rated, by the technicians, from 0 to 10 on an arbitrary scale. This rating was reconciled with the full-night tracing of snoring by the reviewing physician. Patients diagnosed with respiratory disorders other than OSA were excluded. Polysomnography records including at least 4 h of sleep and indicative of severe OSA, defined as an AHI ≥ 30 events/h, were included. On the night of the study, trained technicians weighed the patients, as well as measuring their blood pressure, neck circumference, and waist circumference. Patients were classified as hypertensive if their blood pressure was ≥ 140/90 mmHg or if they had previously been diagnosed with or treated for hypertension.

Statistical analysis

Data were analyzed using the Predictive Analytics Software package, version 18.0 (SPSS Inc., Chicago, IL, USA). Continuous variables with normal distribution are presented as means and standard deviations. Differences between groups were calculated using Student's t-tests for independent samples. In univariate analyses, binary logistic regression was used in order to determine whether OSA symptoms and scores were significantly associated with the variables of interest—level of physical activity (nonexerciser vs. exerciser) and occupational activity (inactive vs. active)—or with well-known confounders such as male gender, body mass index > 30 kg/m^2, and age > 48 years. In multivariate analyses, the AHI, age, minimum oxygen saturation, age, and sleep quality were analyzed as continuous or dichotomous variables, the latter being dichotomized at the median. As a dichotomous variable, tiredness was based on the participant responses on the STOP portion of the STOP-Bang questionnaire. Due to the numerous comparisons and the large sample size, only results with a < 1% probability of a type I error were considered significant.

RESULTS

From a database of 5,984 sleep studies, we obtained the complete files of 1,395 untreated patients with severe OSA (Figure 1). Table 1 displays the characteristics of the sample according to the exercise status. Exercisers differed from nonexercisers regarding anthropometric features. However, the prevalence of comorbidities was similar between the two groups.

The training characteristics of the exercisers are shown in Table S1 (online supplement on the JBP website). A small proportion of the exercisers (1.6%) reported poorer sleep quality on the nights after a day on which they exercised. The rates of a positive sleep outcome after exercising were similar between men and women, those > 50 years of age, and between patients with a BMI < 30 kg/m^2 and those with a BMI ≥ 30 kg/m^2.

The largest effect size among all polysomnographic and sleep symptom variables, as indicated by Cohen's d, was that of exercise for the AHI (Table 2). Significant differences between exercisers and nonexercisers were also evident for other markers of OSA severity, such as minimum oxygen saturation, time at an oxygen saturation below 90%, and snoring intensity. The duration of non-rapid eye movement stage 3 sleep was longer in the exerciser group, as was that of rapid eye movement sleep. The frequency of symptoms and symptom scores were lower among the exercisers than among the nonexercisers; the STOP score had the largest effect size for symptoms. Fifteen patients had a score of 0 on the STOP portion of the STOP-Bang questionnaire. Between obese and nonobese exercisers, in terms of the frequencies of all symptoms and all symptom scores, the only significant difference found was that the proportion of patients with hypertension was higher among the patients who were obese than among those who were not. Among the nonexercisers, seven symptoms or symptom scores were significantly different between the obese and nonobese patients, the former being consistently more tired and sleepy than were the latter. Hypertension was reported less frequently on the STOP portion of the STOP-Bang questionnaire than was observed in the physical examination/anamnesis (Table 1). That discrepancy was seen more often among exercisers.

The association of exercise with eleven symptoms and symptom scores that were significantly different between exercisers and nonexercisers, as shown in Table 2, was further tested in a binary logistic model. Among those eleven variables, six remained significant, although with small effect sizes (odds ratios) after adjustment for age > 48 years, male gender, obesity, active occupation, and AHI > 43 events/h (Table 3).

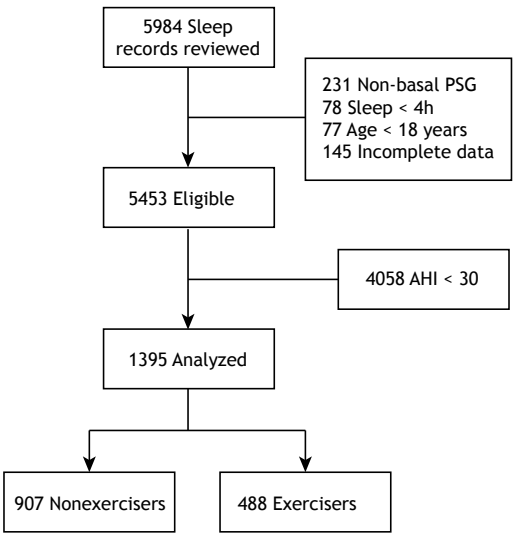

Figure 1. Flowchart of the record selection process. PSG: polysomnography; and AHI: apnea-hypopnea index (events/h).

Table 1. Characteristics of the participants, by exercise status.[a]

Characteristic	Regular exercise		p*
	No (n = 907)	Yes (n = 488)	
Male gender	730 (80.5)	396 (81.1)	0.41
Age (years)	48 ± 14	50 ± 14	0.03
Body mass index (kg/m^2)	34.6 ± 7.4	31.3 ± 5.3	< 0.001
Body mass index > 30 kg/m^2	649 (71.6)	262 (53.7)	< 0.001
Neck circumference (cm)	41.6 ± 4.8	40.5 ± 4.6	< 0.001
Waist circumference (cm)	108 ± 17	104 ± 16	< 0.001
Heart rate (bpm)	85 ± 13	82 ± 13	< 0.001
Systolic blood pressure (mm Hg)	140 ± 19	139 ± 19	0.29
Diastolic blood pressure (mm Hg)	84 ± 13	82 ± 13	0.03
Time sitting per day (hours)	5.9 ± 2.7	5.7 ± 2.4	0.23
Smoking (previous or current)	315 (34.7)	157 (32.2)	0.18
Comorbidities			
Hypertension	538 (59.3)	264 (54.1)	0.06
Diabetes	29 (3.2)	14 (2.9)	0.44
Heart failure	10 (1.1)	8 (1.6)	0.27
Asthma	21 (2.3)	7 (1.4)	0.18
Myocardial infarction	25 (2.8)	15 (3.1)	0.43
Chronic bronchitis	8 (0.9)	4 (0.8)	0.58
Emphysema	5 (0.6)	4 (0.8)	0.39
Renal failure	2 (0.2)	1 (0.2)	0.72
Cancer	7 (0.8)	6 (1.2)	0.28
Arterial disease	15 (1.7)	5 (1.0)	0.24
Stroke	10 (1.1)	7 (1.4)	0.38
Mental disorder	31 (3.4)	14 (2.9)	0.35
Other chronic disease	5 (0.6)	3 (0.6)	0.57

[a]Data are presented as mean ± standard deviation or as n (%). *Nonexercisers vs. exercisers (Student's t-test); significant at p ≤ 0.01.

Figure 2 shows the univariate analyses in which the six sleep quality variables that were significantly different between exercisers and nonexercisers were included as dependent variables in a binary logistic regression to test their association with exercise as the independent variable. Five of those dependent variables remained significantly associated with exercise after adjustment for age > 48 years, male gender, obesity, active occupation, and AHI > 43 events/h, exercisers showing an approximately 30% reduction in the odds of having a sleep symptom or unfavorable symptom score. There was an association between an ESS score > 10 and exercise. However, that association did not withstand adjustment (Figure 2). The ESS score correlated weakly with an AHI > 43 events/h (rho = 0.22; p < 0.001). Although age and AHI were also tested as continuous variables, the versions that were dichotomized at the median were used in the figure for visualization purposes, because the results were similar. Due to its high collinearity with AHI, the minimum oxygen saturation was not included in the models.

To determine whether AHI severity had a dose-response relationship with the frequency of symptoms and symptom scores, we divided the sample into five AHI categories (Table 4). Significantly lower proportions of moderate and vigorous exercisers, for all exercise types, were observed only among the patients with an AHI > 70 events/h. Of the patients in the AHI > 70 events/h category, only 7.8% reported exercising vigorously. In addition, 22.3% of those patients had a normal ESS score. Significant differences among the AHI categories in terms of the frequency of symptoms and symptom scores were seen only for the patients in the AHI 60-70 events/h category, and those differences were small. One single question ("Do you consider yourself sleepier than other persons?") also showed a significant, albeit weak, association with the subcategories of AHI severity (gamma = 0.25; p for trend < 0.001). It is surprising that only 89.4% of the patients in the sample answered "yes" to the snoring question of the STOP portion of the STOP-Bang questionnaire, and that even a few of the patients in the AHI > 70 events/h category answered "no".

DISCUSSION

The present study provides data that underscore the difficulty of detecting OSA on the basis of its symptoms, even in the most severe cases. In addition, we have quantified the differences in symptom frequency and symptom scores between exercisers and nonexercisers.

Table 2. Objective and subjective sleep characteristics, by exercise status.[a]

Characteristic	Nonexercisers (n = 907)	Exercisers (n = 488)	Effect size*	p†
Polysomnography variables (ordered by effect size)				
Apnea-hypopnea index (events/h)	56 ± 21	48 ± 17	0.42	< 0.001
Snoring score (AU)[b]	6.7 ± 1.9	6.1 ± 1.8	0.32	< 0.001
TB90% (min)	50 ± 52	36 ± 43	0.29	< 0.001
N3 sleep (%)	9 ± 8	11 ± 8	0.25	< 0.001
Lowest oxygen saturation (%)	75 ± 10	77 ± 9	0.21	< 0.001
REM sleep (%)	11 ± 6	12 ± 6	0.17	0.006
Total sleep time (min)	396 ± 53	395 ± 53	0.02	0.64
Sleep efficiency (%)	86 ± 10	86 ± 11	0.00	0.57
N1 sleep (%)	6 ± 3	6 ± 3	0.00	0.03
Symptoms and scores				
Total STOP score	3.0 ± 0.9	2.7 ± 1.0	0.32	< 0.001
Total STOP score > 2	664 (73.2)	304 (62.3)	0.28	< 0.001
Snores loudly	814 (89.7)	426 (87.3)	0.13	0.09
Often tired	703 (77.5)	332 (68.0)	0.27	< 0.001
Observed apnea	687 (75.7)	349 (71.5)	0.12	0.07
High blood pressure	475 (52.4)	216 (44.3)	0.18	0.004
Sleep quality score (AU)[b]	5.5 ± 2.4	6.0 ± 2.2	0.26	< 0.001
Unrefreshing sleep	580 (63.9)	263 (53.9)	0.23	< 0.001
Sleepier than other people	515 (56.8)	229 (46.9)	0.22	< 0.001
Difficulty initiating sleep	315 (34.7)	168 (34.4)	0.01	0.97
Difficulty maintaining sleep	384 (42.3)	185 (37.9)	0.10	0.07
Mood score (AU)[b]	5.6 ± 2.6	6.1 ± 2.5	0.20	< 0.001
Low energy on waking score (AU)[c]	2.7 ± 1.7	2.4 ± 1.2	0.20	< 0.001
Total Epworth Sleepiness Scale score[d]	13 ± 6	12 ± 5	0.18	0.002
Epworth Sleepiness Scale score > 10	600 (66.2)	288 (59.0)	0.17	0.005
Stanford Sleepiness Scale score (AU)[c]	2.5 ± 1.3	2.3 ± 1.3	0.15	0.07
Feeling blue score (AU)[e]	0.56 ± 1	0.59 ± 1.1	0.03	0.62
Waking up tired score (AU)[b]	2.5 ± 2.7	2.5 ± 2.7	0.00	0.93

AU: arbitrary units; TB90%: time with oxygen saturation below 90%; N3: non-rapid eye movement stage 3; REM: rapid eye movement; N1: non-rapid eye movement stage 1; and STOP: **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure. [a]Data are presented as mean ± standard deviation or as n (%); [b]Scale of 0-10; [c]Scale of 0-7; [d]Scale of 0-24; [e]Scale of 0-4. *Cohen's d. †Nonexercisers vs. exercisers (Student's t-test); significant at p ≤ 0.01.

Exercise-induced changes in symptoms might reduce the sensitivity of screening instruments, although the magnitude of that effect could not be determined in our study. Our results suggest that exercise reduces sleep symptom frequency and symptom scores by approximately 30% in patients with OSA, an association that withstood adjustment for confounders such as gender, age, and body mass index.

We found no relationship between occupational physical activity level and any of the sleep symptoms studied, indicating that regular exercise is not comparable to hard work in terms of the possible repercussions for OSA symptoms. It is plausible that a higher occupational physical activity level could be responsible for more intense symptoms of fatigue, countering the positive effects of occasional exercise. In our sample, however, that supposition can be dismissed, given that our multivariate analyses confirmed the lack of association between the occupational physical activity level and the symptoms tested.

Even after OSA symptoms are recognized by a patient, years can pass before that patient is diagnosed and treated.[21,22] Patients often underreport their symptoms, and sleep quality is rarely specifically assessed during a clinical consultation. In that context, exercise may contribute to delaying the diagnosis.

Sleepiness is a presumed but nonspecific symptom of OSA. The well-known poor correlation between sleepiness and OSA severity limits the usefulness of the ESS in OSA screening.[23] In our sample of patients with severe OSA, the ESS score correlated only weakly with an AHI > 43 events/h. The question "Do you consider yourself sleepier than other persons?" showed a significant, albeit weak, association with the subcategories of AHI severity. However, a difference in ESS score between those subcategories was observed only for the AHI 60-70 events/h category. Even in the AHI > 70 events/h category, 22.0% of patients still had a normal ESS score.

The diagnosis and treatment of OSA are typically delayed.[21] In exercisers, that delay can be longer

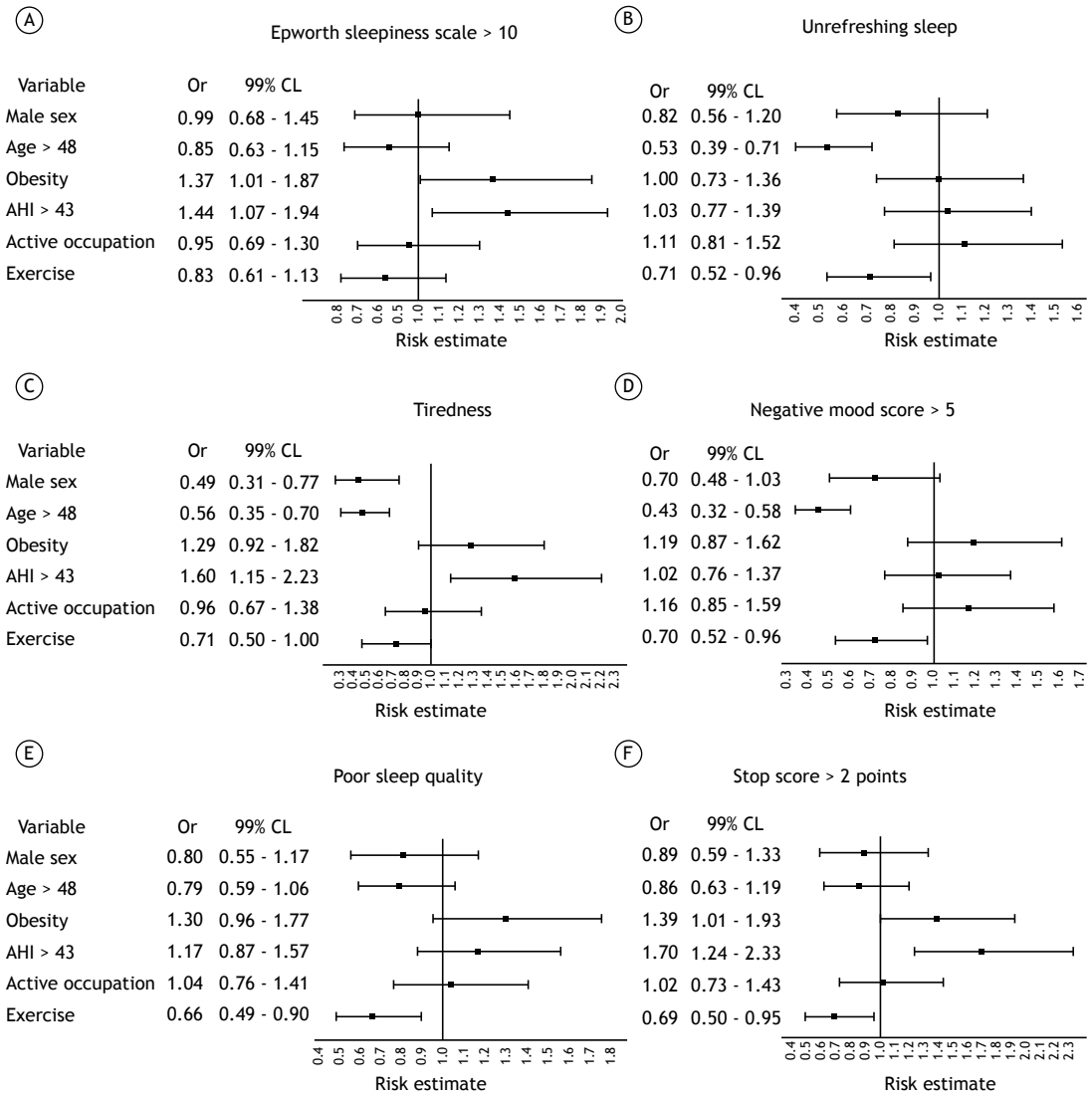

Figure 2. Forest plots of the binary logistic models estimating the odds ratio of six dependent variables. The following symptoms or dichotomized scores are included: a) Epworth Sleepiness Scale score > 10; b) tiredness; c) poor sleep quality (score ≤ 5 on the sleep quality scale); d) unrefreshing sleep; e) negative mood score > 5; and f) **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure (STOP) questionnaire score > 2 points. The variables of interest, regular exercise (nonexercisers vs. exercisers), and occupational activity (inactive vs. active) were adjusted for an apnea-hypopnea index > 43 events/h and for the confounders age > 48 years, male gender, and obesity (body mass index > 30 kg/m^2). Of the symptoms and scores listed above, the only one that did not present a significant association with regular exercise was an Epworth Sleepiness Scale score > 10.

if combined with a reassuring report of intense exercise. This is of particular clinical interest, given that patients with severe OSA could be at a higher risk of cardiovascular events if they engage in vigorous exercise. Of the individuals in the AHI > 70 events/h category, only a small proportion reported exercising vigorously. However, the criterion for vigorous exercise is subjective. Breathing "much harder than normal" might be achieved by such patients at lower levels of effort than by an individual without OSA.

To our knowledge, there have been no previous studies on the symptoms of patients with severe OSA and their commitment to exercise programs. Likewise, a commissioned systematic review[24] concluded that "there is uncertainty about the accuracy or clinical utility of all potential screening tools" in asymptomatic persons. Exercise might be a contributor to this uncertainty. Studies indicating that exercise reduces symptoms such as pain, tiredness, sleepiness, negative mood, and sleep quality[25-28] indirectly corroborate our findings.

Although some sleep hygiene studies have recommended avoiding nighttime exercise, our data suggest otherwise. Only a small proportion of the exercisers reported poorer sleep quality after exercising the night before. That is in agreement with

Table 3. Univariate and multivariate analyses of the association of exercise with symptoms and scores, in order by effect size, as indicated by the adjusted odds ratio.

Binary logistic regression		Odds ratio (99% CI)	
Dependent variable	Symptoms	Model 1 (Univariate)	Model 2 (Adjusted*)
No exercise	Sleep quality score (AU, 0-10)	0.91† (0.86-0.97)	0.93† (0.87-0.99)
	Morning mood score (AU, 0-10)	0.98 (0.90-1.04)	0.97 (0.90-1.04)
	Epworth Sleepiness Scale score (0-24)	1.03† (1.01-1.06)	1.02 (0.99-1.05)
	Low energy on waking score (0-7)	1.16† (1.04-1.30)	1.16† (1.03-1.30)
	Epworth Sleepiness Scale score > 10	1.36† (1.01-1.83)	1.21 (0.89-1.65)
	Score on the STOP questionnaire (0-4)	1.34† (1.15-1.57)	1.21† (1.03-1.42)
	Sleepier than other people	1.48† (1.11-1.99)	1.31 (0.96-1.77)
	High blood pressure	1.39† (1.04-1.85)	1.35 (0.98-1.87)
	Unrefreshing sleep	1.51† (1.12-2.03)	1.44† (1.06-1.96)
	Score on the STOP questionnaire > 2	1.69† (1.24-2.31)	1.45† (1.05-2.01)
	Often tired	1.65† (1.19-2.28)	1.51† (1.08-2.12)

AU: arbitrary units; and STOP: **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure. *Adjusted for age > 48 years, male gender, obesity, active occupation, and apnea-hypopnea index > 43 events/h. †Significant effect.

Table 4. Exercise description and symptom score by strata of severe apnea-hypopnea index.[a,b]

Variable	AHI 30-40 (n = 505)	AHI 40-50 (n = 244)	AHI 50-60 (n = 194)	AHI 60-70 (n = 174)	AHI > 70 (n = 269)	p for trend*
Exercise frequency (days/week)	3.2 ± 1.4†	2.9 ± 1.2†	2.9 ± 1.6†	3.0 ± 1.4†	3.5 ± 1.5†	0.06
Exercise Intensity						
Moderate	146 (34)†	60 (28)†	52 (30)†	38 (23)†	29 (12)‡	< 0.001
Vigorous	79 (22)†	34 (18)†	18 (13)†,‡	10 (7)‡	21 (9)‡	< 0.001
Exercise type						
Endurance	51 (15)†	21 (12)†,‡	10 (8)†,‡	9 (7)†,‡	11 (5)‡	< 0.001
Resistance	62 (18)†	28 (16)†	19 (13)†,‡	17 (12)†,‡	16 (7)‡	< 0.001
Combined	108 (28)†	44 (23)†,‡	40 (24)†,‡	22 (15)‡,§	22 (9)§	< 0.001
Total STOP score	2.7 ± 1.0†	2.9 ± 0.9†	2.8 ± 0.9†	3.2 ± 0.8‡	3.1 ± 0.8‡	< 0.001
Snores loudly	433 (85)†	215 (89)†,‡	172 (89)†,‡	165 (94)‡	255 (95)‡	< 0.001
Often tired	346 (68)†	172 (71)†	142 (74)†,‡	150 (86)§	225 (84)‡,§	< 0.001
Observed apnea	329 (65)†	188 (78)‡	148 (77)‡	146 (83)‡	255 (84)‡	< 0.001
High blood pressure	240 (47)†	125 (51)†	86 (44)†	100 (57)†	140 (52)†	0.09
Sleep quality (AU)[c]	5.9 ± 2.2†	6.0 ± 2.1†	5.8 ± 2.2†	5.5 ± 2.6†,‡	4.9 ± 2.5‡	< 0.001
Unrefreshing sleep	304 (60)†	143 (59)†	114 (59)†	110 (63)†	172 (64)†	0.22
Sleepier than other people	233 (46)†	113 (47)†	104 (54)†,‡	107 (61)‡,§	187 (70)§	< 0.001
Negative mood on waking score (AU)[c]	5.9 ± 2.5†,‡	5.9 ± 2.4†,‡	6 ± 2.5‡	5.5 ± 2.6†,‡	5.2 ± 2.7†	0.001
Low energy on waking score (AU)[d]	2.6 ± 1.4†	2.5 ± 1.3†	2.5 ± 1.2†	2.7 ± 1.3†	2.8 ± 1.3†	0.26
Epworth Sleepiness Scale score	11.4 ± 5.1†	12.1 ± 5.3†,‡	11.5 ± 5.1†	13.2 ± 5.3‡	15.0 ± 5.4§	< 0.001
Epworth Sleepiness Scale score > 10	269 (58)†	154 (63)†	108 (58)†	121 (69)†,‡	209 (78)‡	< 0.001

AHI: apnea-hypopnea index (events/h); STOP: **S**noring, **T**iredness, **O**bserved apnea, high blood **P**ressure; and AU: arbitrary units. [a]Data are presented as mean ± standard deviation or as n (%);[b]Due to rounding and incomplete questionnaires, percentages do not always add to 100%; [c]Scale of 0-10; [d]Scale of 0-7. *ANOVA or chi-square test; significant at p ≤ 0.01; †,‡,§Different symbols represent significantly different subsets of Bonferroni post-hoc test at 0.01 alpha level.

meta-analysis evidence about the effect of nighttime exercise on sleep.[29] Considering meta-analysis data from interventional studies,[29] it is plausible that exercise improves sleep quality in general and is associated with lower OSA severity even in a stratum restricted to severe OSA. Our results are not sufficiently robust to be conclusive, and additional studies are needed in order to confirm our findings.

In the present study, there were significant differences between exercisers and nonexercisers in terms of non-rapid eye movement stage 3 and rapid eye movement sleep, although neither of those variables were included in the multivariate analyses. Including those variables in the models did not change the symptom-exercise associations.

Our study has some limitations. Due to the cross-sectional design of the study, we cannot infer any causal aspects of the OSA-exercise relationship. The external validity of the results is limited due to the exclusive sampling of patients with severe OSA from a population undergoing polysomnography at a sleep laboratory. Although that limits the

generalizability of the data to the overall population of patients with OSA, we decided to study only severe cases in order to obtain a group in which symptoms were more intense than in mild-to-moderate OSA. Although mild OSA is common,[30,31] affecting up to one third of the general population,[32] patients have fewer symptoms or are asymptomatic in the incipient stages of the disease.[33] One additional reason for including only severe OSA cases in the present study is that the cardiovascular consequences of OSA,[34] which could impair exercise capacity, are more often seen in cases of moderate-to-severe OSA.[3] The sample being derived from patients that were referred to a sleep laboratory in general represents a selection bias, also reducing the external validity of the results. Another limitation is that physical activity and sleep symptoms were assessed by means of questionnaires, given that such instruments introduce a measurement bias. In general, studies assessing the measurement properties of physical activity questionnaires indicate that their quality is insufficient to allow a reliable estimate of exercise level when compared with accelerometers.[35,36] Despite these restrictions, the IPAQ has an acceptable level of reliability. It is convenient as well as being widely used in clinical scenarios and in cross-sectional studies in which the bias introduced will be the same in every group. However, the IPAQ may not be suitable for interventional studies due to low reproducibility.[37] In sleep medicine, the STOP-Bang questionnaire is a common tool in OSA screening, despite its low-to-moderate accuracy. We analyzed the STOP portion alone and did not include the Bang score in the comparisons, because body mass index, age, and gender were control variables in the multivariate models.

The strength of the present study is that the data evaluated were derived from overnight in-laboratory polysomnography tests. The statistical power of our results (> 99%) and the choice of a 1% critical limit for the probability of a type I error support the internal validity of the study. The use of effect size to indicate the clinical relevance of a finding adds to the suitability of the statistical analyses.

This report indicates that patients with severe OSA often exercise and that regular exercise is associated with less frequent and less intense symptoms, independently of confounders. Compared with nonexercisers, exercisers appear to be less likely to complain of poor sleep quality and might therefore be more often dismissed by clinicians as not meeting the criteria for a diagnosis of OSA. Because up to one third of patients with severe OSA might engage in exercise on a regular basis, it is important to consider exercisers as potential cases of OSA even if they are only mildly symptomatic.

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J Bras Pneumol. 2019;45(3):e20180169
http://dx.doi.org/10.1590/1806-3713/e20180169

Pediatric Asthma Control and Communication Instrument: translation into Portuguese and cross-cultural adaptation for use in Brazil

Thayla Amorim Santino[1,a], João Carlos Alchieri[2,b], Raquel Emanuele de França Mendes[1,c], Ada Cristina Jácome[1,d], Tácito Zaildo de Morais Santos[1,e], Linda Kahn-D'Angelo[3,f], Cecilia M. Patino[4,g], Karla Morganna Pereira Pinto de Mendonça[1,h]

1. Programa de Pós-Graduação em Fisioterapia, Universidade Federal do Rio Grande do Norte – UFRN – Natal (RN) Brasil.
2. Programa de Pós-Graduação em Ciências da Saúde, Universidade Federal do Rio Grande do Norte – UFRN – Natal (RN) Brasil.
3. Zuckerberg College of Health Sciences, University of Massachussets Lowell, Lowell (MA) USA.
4. Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles (CA) USA.

a. http://orcid.org/0000-0002-5514-762X
b. http://orcid.org/0000-0002-4150-8519
c. http://orcid.org/0000-0003-2226-5096
d. http://orcid.org/0000-0002-5498-3108
e. http://orcid.org/0000-0002-9495-7078
f. http://orcid.org/0000-0003-2050-5893
g. http://orcid.org/0000-0001-5742-2157
h. http://orcid.org/0000-0001-5734-3707

Submitted: 30 May 2018.
Accepted: 10 January 2019

Study carried out in the Universidade Federal do Rio Grande do Norte – UFRN – Natal (RN) Brazil.

ABSTRACT

Objective: To translate the *Pediatric Asthma Control and Communication Instrument* (PACCI) to Portuguese and adapt it for use in Brazil, ensuring the cultural validity of the content and semantic equivalence of the target version. **Methods:** The Brazilian Portuguese-language version of the PACCI was developed according to the most commonly used methodology, which included the following steps: translation; synthesis of the translation; review by the author of the original questionnaire; back-translation; synthesis of the back-translation; review by a native external researcher who is a native speaker of English; approval of the author of the original questionnaire; review by a specialist in Portuguese; review by a multidisciplinary committee of experts to determine the agreement of the items, considering the clarity of each and its appropriateness in the cultural context; cognitive debriefing; and development of the final version. The cognitive debriefing involved 31 parents/legal guardians of children 1-21 years of age with a clinical diagnosis of asthma, as defined by the Global Initiative for Asthma, with the objective of determining the comprehensibility and clarity of the items for the target population. **Results:** The multidisciplinary committee of experts indicated that the items on the questionnaire were clear and comprehensible, with kappa values above 0.61, indicating substantial agreement. In the cognitive debriefing, the parents/legal guardians presented no difficulties in understanding any of the items (agreement > 0.90); therefore, no further changes were needed.**Conclusions:** The translation and cross-cultural adaptation of the PACCI for use in Brazil were successful.

Keywords: Surveys and questionnaires; Translating; Asthma; Child; Adolescent.

INTRODUCTION

Asthma is a chronic lung disease with a global prevalence ranging from 1 to 18% of the population in different countries.[1] In Brazil, asthma is a serious public health problem affecting mainly children and adolescents.[2] The prevalence of asthma in the pediatric population in Brazil is above 20%.[2]

Asthma control is related to the occurrence of disease manifestations.[1,3] The Global Initiative for Asthma (GINA) recommends that the clinical control evaluation be performed with the use of questionnaires.[1] However, these should always provide a valid, reliable, accurate, and equivalent result interpretation.[4]

Some of the questionnaires available to evaluate the clinical control of asthma in children have already been validated for use in the Brazilian population, such as the Childhood Asthma Control Test,[5] the Asthma Control Test,[6] the Asthma Control Questionnaire,[7] and the Control of Allergic Rhinitis and Asthma Test for Children.[8] However, none of these questionnaires were developed to evaluate children under 4 years of age. In addition, these questionnaires[5-8] were developed for use with restricted age groups. For this reason, it may be difficult to use these instruments as evaluation tools in studies involving participants from different age groups.

With that in mind, Okelo et al.[9] developed the Pediatric Asthma Control and Communication Instrument (PACCI). The PACCI is a questionnaire developed for the multidimensional evaluation of the clinical control of asthma in children and adolescents between 1 and 21 years of age. It contains 12 questions, of which one is open-ended, aimed to improve communication between

Correspondence to:
Karla Morganna Pereira Pinto de Mendonça. Departamento de Fisioterapia, Universidade Federal do Rio Grande do Norte, Avenida Senador Salgado Filho, 3000, Caixa Postal 1524, Campus Universitário, Lagoa Nova, CEP 59072-970, Natal, RN, Brazil.
Tel.: 55 84 3342-2022. E-mail: karlamorganna@hotmail.com
Financial support: Thayla Amorim Santino received a master's scholarship from the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES) – Financial Code 001.

parents/guardians and health care professionals. The main purpose of the PACCI is to collect parents/guardians' self-report via a straightforward instrument that is easy to use and understand, even for individuals with a low level of education.[9]

The PACCI is subdivided into five domains: direction (1 item), bother (1 item), risk (3 items), adherence (1 item), and control (5 items).[9] The control domain can be scored in three ways: a sum of the items (from 0 to 19 points); problem index, in which each item is a dichotomous variable (from 0 to 5 points); and categories (controlled or uncontrolled), which is based on the color of the box checked on the right side of the instrument. The other domains receive categorical scores.

The objective of the present study was to translate the PACCI to Portuguese and make a cross-cultural adaptation for use in Brazil, aiming to fill in the gaps currently found in the toolbox of instruments available for the evaluation of the clinical control of pediatric asthma.

METHODS

The present study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (Process No. 1,808,185). All participants gave written informed consent and written informed assent. This is an exploratory methodological study based on recommendations proposed by several international studies.[10-13]

The process was composed of the following steps: 1) authorization from the author of the original questionnaire; 2) forward translation: the questionnaire was translated into Portuguese by two translators (1 and 2) working independently—both were native speakers of Portuguese and fluent in English, whereas one was a specialist in the field of knowledge addressed by the instrument and the other was not; 3) synthesis of the translations: consensus between translators 1 and 2 and the review committee (researchers conducting the study) on the translated versions with the aim of devising a single version; 4) report sent to the author of the original instrument for consideration and approval of steps 2 and 3; 5) back-translation: a translation of the synthesis of the first two translations into English by another two translators (3 and 4) who were native speakers of English and fluent in Portuguese and were blind to the original questionnaire; 6) synthesis of the back-translations: consensus between translators 3 and 4 and the review committee on the back-translations with the aim of devising a single version. In this step, an external researcher who was a native English speaker was asked to compare the final version of the back-translation into English with the original version and evaluate semantic equivalence; 7) report sent to the author of the original instrument for consideration and approval of steps 5 and 6; 8) review by a specialist in Portuguese who ensured an appropriate use of the standard language; 9) review by a multidisciplinary committee of experts to determine the agreement of the items, considering the clarity of each and its appropriateness in the cultural context; 10) cognitive debriefing: once the items were approved by the experts, a comprehension test of a pre-final version of the questionnaire was administered to the target population; and 11) report sent to the author of the original instrument for consideration and approval of the final version in Brazilian Portuguese. A diagram of the translation and cross-cultural adaptation process described above can be seen in Figure 1.

The semantic equivalence between the original and the translated version, as well as the appropriateness in the new cultural context, was achieved in the stages described previously. To ensure content validity, a multidisciplinary committee of experts was convened. This committee was composed of pediatricians and pulmonologists, physical therapists, researchers with experience in translation and cross-cultural adaptation of questionnaires, as well as lay individuals representing the target population (parents/guardians of children and adolescents with asthma), adding up to a total of nine members. To evaluate agreement, the Delphi method was used[14] on SurveyMonkey® virtual platform. The selected experts evaluated each item and response options of the Portuguese version of the PACCI. The degree of agreement was calculated with the kappa test, considering the agreement values proposed by Landis and Koch.[15]

The cognitive debriefing was performed with a sample composed of 31 parents/guardians of children and adolescents between 1 and 21 years of age who had received a clinical diagnosis of asthma according to the GINA.[1] After clarification about the purpose and procedures of the study, the questionnaire was applied in the format of an interview. The interviewer read the questions, without any further explanation, repeating them when necessary; then the participants were asked about how clear and comprehensible each question was. If an item was unclear, the participant was encouraged to suggest possible changes to make it more comprehensible. If an item had a level of understanding below 90%,[16] the previous steps of review by the multidisciplinary committee of experts and cognitive debriefing would be repeated for that item in order to guarantee a greater level of clarity.

The data were analyzed with IBM SPSS Statistics statistical package, version 22.0 (IBM Corporation, Armonk, NY, USA). The data analysis consisted of a descriptive analysis, with measures of absolute and relative frequency, central tendency, and dispersion. The kappa test was used to analyze the degree of agreement among the experts.

RESULTS

During the steps of translation and cross-cultural adaptation previously described, a few adjustments were deemed necessary to ensure clear understanding and adequate cross-cultural adaptation of the items

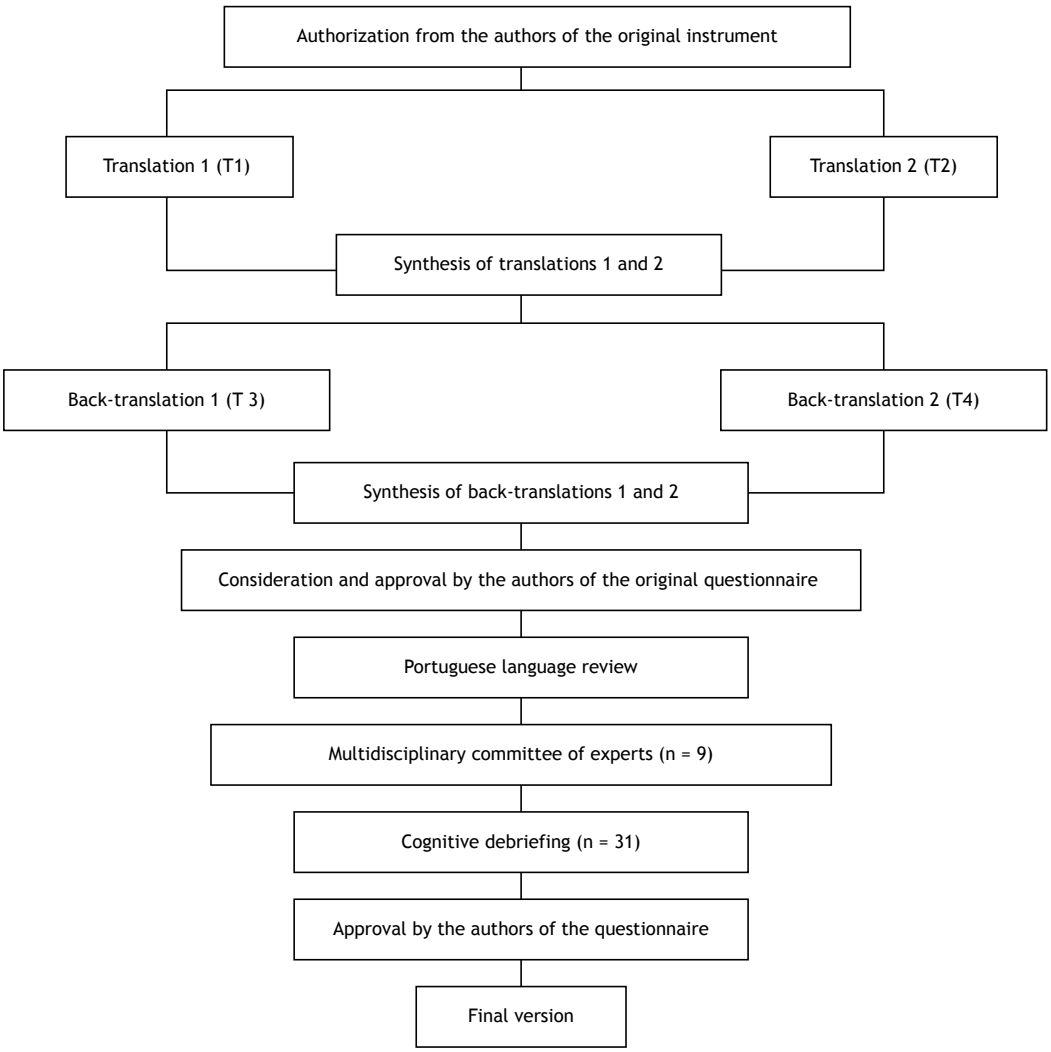

Figure 1. Steps of the translation and cross-cultural adaptation process of the Pediatric Asthma Control and Communication Instrument to Brazilian Portuguese.

and response options of the questionnaire. A report on the possible adjustments was submitted to the author of the original version after each step of the process. After approval from the author, the necessary changes were made. Through this process, it was possible to guarantee that the intention and meaning of the original items were fully preserved in the Portuguese version of the instrument.

Of the nine members of the multidisciplinary committee of experts, three were parents/guardians of children or adolescents with asthma, two were pediatricians, two were physical therapists specializing in respiratory physical therapy, one was a pulmonologist, and one was a researcher with methodological experience in translation and cross-cultural adaptation. As for the educational background of the health care professionals, three had a master's degree and another three had a Ph.D. They had between 7 and 31 years of professional experience. The parents/guardians who participated in the committee had different levels of educational attainment (high school, higher education, and postgraduate education).

Several sections of the original questionnaire use the term "your child"; however, instead of a literal translation, we chose to use the term "*seu(sua) filho(a)*" ["your son/daughter"]. This adjustment was deemed appropriate because the instrument was also intended for parents/guardians of adolescents.

Items 1 to 5 of the original instrument begin with the following sentence: "Since your child's last visit to this doctor's office, has your child:"; however, this has been adjusted to: "*Desde a última consulta médica de seu(sua) filho(a), ele(a)*:" ["Since your son/daughter's last medical visit, he/she"], which made it simpler and more understandable in the new cultural context. In addition, the term "this doctor's office" could be inappropriate, depending on the place where the questionnaire was being applied. The term "hospitalized", used in item 4, was replaced by "*internado*" ["under inpatient

care"], which is more widely used in Brazil and more appropriate in this context. In item 5, the question "Used prednisone (Orapred, steroid pill, steroid liquid or steroid syrup) for asthma?" was changed to "*Usou prednisona/prednisolona (comprimidos de corticoide ou cortisona, corticoide líquido, corticoide xarope ou injeção de corticoide) para sua asma?*" ["Used prednisone/prednisolone (corticosteroid or cortisone tablets, liquid corticosteroid, corticosteroid syrup, or corticosteroid injection) for his/her asthma?"]. Like the Portuguese version of the instrument for adults, the Asthma Control and Communication Instrument (ACCI),[17] the following explanation was added to item 5: "*Essa pergunta não se refere a sua bombinha de uso diário*" ["This question does not refer to your daily use inhaler"]. The review committee proposed this adaptation be made with the purpose of making it clear that the item was not referring to the inhaled corticosteroids of daily use, but to the use of oral and injected corticosteroids used in periods of symptom exacerbation. In item 9 the expression "asthma attack" was replaced with "*crises de asma*" ["asthma crises"], since the original expression is not widely used by the Brazilian population.

Items 6, 7, 8, and 9 of the original version provide examples to help or guide participants in completing the questionnaire. For a better adjustment to the context of the Brazilian population, the final version in Portuguese rephrases the examples in different ways: 1) item 6 refers to the daily medications most frequently used for asthma control in North America. However, some of these medications are not widely known or are unavailable for use in Brazil and were, therefore, replaced with more common ones.The following medications were mentioned originally "Advair, Asmanex, Budesonide, Dulera, Flovent, QVAR, Pulmicort, Singulair and Symbicort", while the translation mentions "*Seretide spray e Diskus (Fluticasona e Salmeterol), Oximax (Mometasona), Budesonida, Formoterol, Fluticaps (Fluticasona), Clenil spray e A (beclometasona), Montelukast e Symbicort/Alenia (Formoterol e Budesonida)*" ["Seretide spray and Diskus (Fluticasone and Salmeterol), Oximax (Mometasone), Budesonide, Formoterol, Fluticaps (Fluticasone), Clenil spray and A (beclomethasone), Montelukast and Symbicort/Alenia (Formoterol and Budesonide)]; 2) item 7 contains examples of asthma symptoms that were mostly translated literally, except for the expressions "shortness of breath", "sputum (spit, mucous, phlegm when coughing)", and "wheezy or whistling sound in the chest". After consideration by the review committee, these symptoms were translated as "*falta de ar*" ["shortness of air"], "*escarro (expectoração, muco, catarro ao tossir)*" ["sputum (expectoration, mucus, catarrh when coughing)"], and "*chiado ou sibilo (assobio) no peito*" ["chest wheezing or hissing (whistling)"], respectively. 3) The original version of item 8 gives examples of the following forms or medicines for symptom relief: "Albuterol/Proventil/Proair/Ventolin/Xopenex via Inhaler/Spray/Pump or Machine/Nebulizer". In an attempt to simplify and mention medications or forms of administration that were more usual for the Brazilian population, the example in Portuguese says: "*Salbutamol/Aerolin/Berotec/Bombinha/Inalador/Nebulização*" ["Salbutamol/Aerolin/Berotec/Pump/Inhaler/Nebulizer"]. This format was also chosen because it bears similarity to the corresponding item of the Portuguese version of the questionnaire for adults, the ACCI.[18]

Adjustments were made to two response options only, in items 2 and 10. Originally, item 2 presented the following response options: "Not bothered/Somewhat bothered/Very bothered". These were changed to "*Nem um pouco incomodado/Um pouco incomodado/Muito incomodado*" ["Not bothered at all/A little bothered/Very bothered]". The response options of item 10 were "Not at all/Slightly/Moderately/Very much/Completely". These were changed to "*Nem um pouco/Levemente/Moderadamente/Muito/Completamente*" ["Not at all/Lightly/Moderately/Very/Completely"].

The analysis of agreement among the members of the multidisciplinary committee of experts showed that all items and response options of the questionnaire had kappa values above 0.61, which indicates a high level of agreement. Therefore, there was no need for a second round of discussions.

During the cognitive debriefing of the pre-final version of the questionnaire, 31 parents/guardians were interviewed, of which 26 (83.9%) were female. Their age ranged from 26 to 60 years. Most of the participants of the cognitive debriefing had children aged 2 to 15 years, most of whom were 5-11 years old (54.8%), eutrophic (71.4%), with moderate asthma (56.7%), which was partially controlled (72.4%). The sample also included representatives with different levels of educational attainment, ranging from incomplete primary education to complete higher education, as well as representatives of various economic strata, according to the Brazilian Economic Classification Criteria[18] of the Brazilian Association of Businesses and Research.

The evaluation of clarity and comprehension of the questionnaire was conducted by the parents/guardians who gave all items scores above 90% for both criteria. No comprehension difficulties were registered in any of the items and no suggestions were made, indicating no change was necessary.

DISCUSSION

The present study shows that the Brazilian Portuguese version of the PACCI and its cross-cultural adaptation were considered appropriate. All the instructions, response options, and items were considered clear and comprehensible by the target population.

Like other instruments that focus on quality of life, such as the Asthma Quality of Life Questionnaire[19] and the Pediatric Asthma Quality of Life Questionnaire,[20] the PACCI was developed in parallel with the adult version of the questionnaire, in this case the ACCI,[21] which evaluates clinical control of asthma in adults.

The ACCI has already been translated and adapted for use in Brazil.[17] Therefore, many relevant aspects reported during the cross-cultural adaptation of the ACCI[17] that could also apply to the PACCI were taken into consideration, since both have similar structures and items.

In order to achieve an appropriate cross-cultural adaptation and to guarantee the construct was properly evaluated by the new population, the cognitive debriefing of the Brazilian Portuguese version of the PACCI was carried out with the participation of individuals from different educational and socioeconomic backgrounds, ensuring a better representation of the target population. There are currently four instruments available in Portuguese to evaluate the clinical control of pediatric asthma.[5,7,8,22] However, this was the only study that included representatives of the different social strata and with different levels of education in an attempt to reflect the great diversity of the Brazilian population. The authors of the Brazilian version of the ACCI,[17] which was originally developed to evaluate the clinical control of asthma in adults, also state, in an article about the process of translation of the instrument, they have included participants with different levels of education. However, they do not mention whether or not the Brazilian version of the ACCI took into consideration the socioeconomic diversity of the population for which the instrument was translated into and cross-culturally adapted.[17]

The PACCI differs from other instruments that focus specifically on the pediatric population and that are currently available in several respects.[5,7,8,22] First, the PACCI is the only questionnaire developed specifically for the evaluation of the clinical control of asthma in children and adolescents across a wide age range, unlike the Childhood Asthma Control Test, for example, which applies to children between 4 and 11 years of age only.[5] Second, the possibilities of score of the PACCI are differentiated. The three different ways to score the questionnaire and the possibility to use the GINA clinical control classification enable it to be adapted to different situations.[1] Also, this instrument can facilitate the communication between physicians and patients because it contains an open-ended question that encourages parents/guardians to express their concerns about their child's asthma.[9]

Another characteristic that distinguishes the PACCI from the other questionnaires currently available to the Brazilian population is that it is based on parents/guardians reports.[9] The use of self-administered instruments in the pediatric population may result in answers of disputed validity and reliability due to the limitations of children's understanding. On the other hand, the self-report of adolescents provides more accurate information than that of parents/guardians. However, one should also consider the emotional and social aspects inherent to each stage of an adolescent's development. In this context, parent reports may be useful, especially in regards to symptoms and the impact of the disease.[23]

The GINA recommends that the assessment of asthma control be composed of two domains: control of manifestations and future risk.[1,24] The other questionnaires currently available focus on symptom control only,[24] whereas the PACCI contains questions related to future risk factors, which makes it more comprehensive than others in relation to these recommendations. The present study is also relevant since the process of translation and cross-cultural adaptation of instruments is the first step to make an instrument available for use in a population with another cultural and linguistic background.[11,13,25] If an instrument is subject to incorrect or incomplete cross-cultural adaptation, misinterpretation of the results may occur either individually, within one country, or when making country comparisons.[26]

The methodology proposed in the present study has been widely used and can be considered a significant factor that leads to a more robust study. The profile of the translators included in the process and the inclusion of representatives of the target population in the multidisciplinary committee of experts should be considered in order to ensure a good semantic, conceptual and contextual equivalence between the versions.[27-30] However, previous studies about the translation and cross-cultural adaptation of specific questionnaires for the evaluation of the clinical control of pediatric asthma do not state whether or not these aspects were considered relevant.[5-7]

Such as previous studies of translation and cross-cultural adaptation of the questionnaires that evaluate the clinical control of asthma for the Brazilian population,[5,8] the cognitive debriefing of the Brazilian version of the PACCI was not multicentric. Therefore, we would recommend that further studies be conducted in the future with the aim of comparing the rates of understanding and clarity of the instrument in other Brazilian regions, especially because Brazil has a large territory with distinct regional characteristics. Nevertheless, during the process of cross-cultural adaptation of the instrument, the use of regional terms was avoided in order to ensure the validity of the content of this version of the PACCI throughout the entire country. Another possible limitation of the present study was the absence of parents/guardians of children and adolescents of all ages within the proposed range (1-21 years of age). However, we believe this aspect did not impact the results of our study, since the respondents' understanding of the Brazilian version of the PACCI would certainly not be influenced by the age of their children.

In conclusion, we can state that the Brazilian Portuguese version of the PACCI was successfully adapted for use in Brazil (annex available on the JBP website: http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=66). In addition, it is semantically equivalent to the original instrument and appropriate in the Brazilian context. The adapted version is also easy to understand and apply, regardless of the level of education and socioeconomic condition of the respondent.

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J Bras Pneumol. 2019;45(3):e20180138
http://dx.doi.org/10.1590/1806-3713/e20180138

Temporal evolution of and factors associated with asthma and wheezing in schoolchildren in Brazil

Gabriela Ávila Marques[1,2,a], Andrea Wendt[2,b], Fernando César Wehrmeister[2,c]

1. Universidade Católica de Pelotas, Pelotas (RS) Brasil.
2. Programa de Pós-Graduação em Epidemiologia, Universidade Federal de Pelotas, Pelotas (RS) Brasil.

a. http://orcid.org/0000-0003-3935-5207
b. http://orcid.org/0000-0002-4640-2254
c. http://orcid.org/0000-0001-7137-1747

Submitted: 28 May 2018.
Accepted: 23 September 2018.

Study carried out in the Programa de Pós-Graduação em Epidemiologia, Universidade Federal de Pelotas, Pelotas (RS), Brazil.

ABSTRACT

Objective: To estimate the evolution of the prevalence of asthma and wheezing among schoolchildren in Brazil from 2012 to 2015, as well as to identify factors associated with both conditions. **Methods:** This was a cross-sectional study using data from the Brazilian National School-Based Adolescent Health Survey for 2012 and 2015. To characterize the evolution of the prevalence of asthma and wheezing, we used linear regression with weighted-least-squares estimation and presented the annual percent change (APC). **Results:** During the study period, there was a reduction in the prevalence of wheezing, from 23.2% in 2012 to 22.4% in 2015 (APC, −0.27). The prevalence of asthma increased from 12.4% in 2012 to 16.0% in 2015 (APC, 1.20). The increase in the prevalence of asthma was greatest in the southern region of the country (APC, 2.17). Having any history of smoking and having consumed alcohol in the last 30 days were factors that influenced the prevalence of wheezing and the prevalence of a self-reported diagnosis of asthma during the two years evaluated. **Conclusions:** There has been an increase in the prevalence of asthma in recent years in Brazil. Our data underscore the importance of improving health strategies and policies aimed at the control of asthma.

Keywords: Asthma/epidemiology; Respiratory sounds; Students.

INTRODUCTION

Asthma is considered a chronic noncommunicable disease (CNCD) whose symptoms appear early in life in approximately half of the cases.[1] Its most common symptom is wheezing, which is caused by bronchospasms and airway obstruction.[2] Although episodes of cough, chest tightness, and wheezing, among others, are important for characterizing the condition, there are no rules as to whether or not these symptoms must be present for it to be considered asthma, which represents a diagnostic challenge.[3] The International Study of Asthma and Allergies in Childhood (ISAAC) reports a mean prevalence of asthma symptoms of 14.1% in adolescents aged 13 to 14 years worldwide, whereas in Latin American countries the rates are above 20%.[4]

In 1988, the prevalence of asthma in Brazil was already among the highest in the world,[5,6] and, apparently, these estimates continue to rise. The prevalence of asthma in children and adolescents evaluated between 1998 and 2008 increased from 8.6% (1998) to 9.1% (2008) for those living in urban areas, and from 4.9% to 5.9% for those living in rural areas.[7] Therefore, it is important to identify the associated factors to gain a better understanding of the disease. These factors are related to environmental, social, and demographic conditions, as well as to lifestyle.[8]

Asthma can also have a negative impact on quality of life, because it results in direct and indirect costs to the population in the form of medical appointments, use of medication, hospitalizations, loss of productivity, work/school absenteeism, and early mortality.[9-13] In Brazil, there was a 36% decrease in the number of hospitalizations between 2008 and 2013; the northern, northeastern, and southeastern regions are the ones with the highest hospitalization rates in the country. In that same period, the cost of hospitalizations reached nearly USD 170 million.[14] A systematic review found that the cost of hospitalizations and medications for the treatment of asthma is of USD 733 per person per year.[15]

Considering this scenario, monitoring asthma and its risk factors is key for the development of enhanced health strategies and policies, especially because the condition can be treated at the primary level of health care.[16] Therefore, the objective of the present study was to estimate the prevalence of asthma and wheezing in schoolchildren in the years of 2012 and 2015, and to verify the factors associated with them.

METHODS

This was a cross-sectional study using data from the 2012 and 2015 editions of the *Pesquisa Nacional de Saúde do Escolar* (PeNSE, Brazilian National School-Based Adolescent Health Survey). The PeNSE is a school-based survey conducted by the Brazilian Ministry of Health in partnership with the Brazilian Institute of Geography and Statistics, whose purpose is to collect information on adolescent health. The survey covers various health-related aspects (socioeconomic condition, family context, eating

Correspondence to:
Gabriela Ávila Marques. Centro de Pesquisas Epidemiológicas, Universidade Federal de Pelotas, Rua Marechal Deodoro, 1160, 3º piso, Caixa Postal 464, Centro, CEP 96020-220, Pelotas, RS, Brazil.
Tel./Fax: 55 53 3284-1300. E-mail: gabriamarques@gmail.com
Financial support: None.

habits, physical activity level etc.). To measure such aspects, we used straightforward self-administered questionnaires that adolescents can easily understand.

The fist edition of the PeNSE happened in 2009 (with schoolchildren from Brazilian state capitals only), followed by the second and third editions in 2012 and 2015, respectively. We chose to use data from these last two surveys because the sample was expanded to other municipalities in addition to the state capitals.[17,18] The 2012 and 2015 editions of the PeNSE surveyed middle school students from public and private institutions located in urban and rural areas throughout Brazil. The PeNSE target population was formed by Year 9 students (former 8th grade) because adolescents of this age group already have reasonable autonomy to complete the self-administered questionnaire. The student sample was composed of children attending schools in the 26 state capitals, the Federal District, and other selected municipalities.[17,18]

Our study used the occurrence of asthma in life and wheezing in the previous 12 months as outcomes, measured through the following questions: a) "Have you ever had asthma in your life?" (yes/no); and b) "Have you had any wheezing (squeaking) in your chest in the last 12 months?" (yes/no). The independent variables were as follows: gender (female/male); age (≤ 13 years, 14 years, or ≥ 15 years); skin color (white, black, brown, yellow, or indigenous); maternal educational attainment (no schooling, complete primary education, incomplete primary education, complete secondary education, or higher education or above). The following questions were used to measure adolescent and parental smoking, respectively: "Have you ever smoked cigarettes, even if only one or two drags?" (yes/no), and "Does any of your parents or guardians smoke?" (none of them, my father or male guardian only, my mother or female guardian only, both my parents or guardians, or I do not know). Alcohol consumption in the previous 30 days was measured by the question: "In the last 30 days, on how many days did you drink at least one glass or dose of alcohol?". For the present study, we considered the consumption of at least one dose of alcohol in the previous 30 days (yes/no). We also calculated an economic index using a principal component analysis based on the ownership of the following goods/services: landline, cell phone, computer, Internet, car, motorcycle, and number of bathrooms with a shower in the household. We also took into consideration whether or not participants had household help. Then, the index was divided into quintiles, the first quintile corresponded to the poorest economic index.

As for the statistical analysis, first, we described the sample of the two editions of the survey, and then we presented the prevalence of asthma and wheezing, as well as their respective 95% CIs. We also presented gross and outcome-adjusted analyses for asthma and wheezing in the two years evaluated, using the Poisson regression and the respective 95% CIs to estimate prevalence ratios (PR). For the outcome-adjusted analyses, the associations between independent variables and outcomes were adjusted for each variable.

To evaluate the evolution of asthma and wheezing in 2012 and 2015, we used a linear regression with weighted-least-squares estimation, and then we presented the annual percent change (APC) for each outcome in each Brazilian region and countrywide. All analyses were conducted with Stata statistical package, version 12.1 (StataCorp LP, College Station, TX, USA), and command *svy*, which considers the study design.

The PeNSE was approved by the Research Ethics Board of the Brazilian Ministry of Health under report number 192/2012 (CONEP/MS Registry number 16805 of March 27, 2012). Student participation was voluntary; students were informed they were free not to participate, or not to complete parts of or the entire questionnaire, should they wish to do so. All student and school details were collected and kept confidential.

RESULTS

The student samples in the 2012 and 2015 editions of the survey were composed of 109,104 and 102,072 individuals, respectively. The 2012 and 2015 sample characteristics are shown in Tables 1 and 2. In both editions, most of the students were female (52.2% in 2012 and 51.3% in 2015), were 14 years of age (45.6% and 51.0% in 2012 and 2015, respectively), and had brown skin color (42.2% in 2012 and 43.1% in 2015). Also in both editions, approximately 20% of the participants reported having some history of smoking and 25% reported having consumed alcohol in the previous 30 days.

Figure 1 shows wheezing prevalence rates in 2012 and 2015. In Brazil, wheezing decreased from 23.2% to 22.4% in 2015 (APC: −0.27). Considering the evolution of wheezing in each Brazilian region, we see that only the northern and center-western regions presented significant reductions, with an APC of −0.47 and −0.43, respectively.

Figure 2 shows the evolution of asthma (reported in life) in both editions (2012 and 2015). In Brazil as a whole, there was an increase from 12.4% to 16.0% in the prevalence of asthma (APC: 1.2). The center-western region was the only one that did not present a significant change in the prevalence of asthma. All other regions showed significant increases, the greatest one in the southern region (APC: 2.17).

Table 3 shows the prevalence ratios of factors associated with wheezing in 2012 and in 2015. After adjustments, in 2012, the following factors showed a positive association with a higher prevalence of wheezing: being a girl (PR = 1.16, 95% CI: 1.12-1.21), having a higher economic index (PR = 1.20; 95% CI: 1.11-1.29), having any history of smoking (PR = 1.39, 95% CI: 1.33-1.45), and having consumed alcohol in the previous 30 days (PR = 1.28, 95% CI: 1.33-1.45). Age was also associated, as older adolescents presented a lower prevalence of wheezing. In 2015, the factors associated with a higher prevalence of wheezing were

Table 1. Description of the 2012 and 2015 samples of the Brazilian National School-Based Adolescent Health Survey.

Variables	2012 n (%)	2015 n (%)
Gender		
Male	52,015 (47.8)	49,290 (48.7)
Female	57,089 (52.2)	52,782 (51.3)
Age, years		
≤ 13	22,443 (22.9)	17,260 (18.3)
14	50,900 (45.6)	51,611 (51.0)
≥ 15	35,761 (31.6)	33,201 (30.7)
Skin color		
White	37,674 (36.8)	33,775 (36.2)
Black	14,513 (13.4)	12,849 (13.4)
Brown	48,237 (42.2)	46,935 (43.1)
Yellow/Indigenous	8,611 (7.6)	8,405 (7.4)
Maternal educational attainment		
No schooling	7,371 (10.1)	5,531 (7.4)
Complete primary education	25,951 (32.2)	18,217 (26.5)
Incomplete primary education	156,975 (18.1)	12,299 (17.1)
Complete secondary education	28,244 (28.8)	23,359 (30.9)
Higher education	13,036 (10.9)	17,232 (18.1)
Economic index		
1st quintile	21,725 (21.4)	22,634 (20.7)
2nd quintile	23,821 (22.2)	19,320 (18.1)
3rd quintile	22,738 (21.2)	22,383 (22.6)
4th quintile	32,056 (29.7)	29,022 (32.4)
5th quintile	8,249 (5.6)	8,239 (6.3)
Any history of smoking		
No	86,113 (80.4)	83,158 (81.6)
Yes	22,784 (19.6)	18,723 (18.4)
Alcohol previous 30 days		
No	80,905 (73.9)	79,364 (76.2)
Yes	27,763 (26.1)	22,597 (23.8)
Parents who smoke		
None	76,809 (70.2)	75,098 (73.2)
One parent only	24,138 (24.2)	20,562 (22.3)
Both	5,022 (5.6)	3,800 (4.5)
TOTAL	109,704 (100)	102,072 (100)

similar to those of 2012; however, having one parent/guardian who smokes (PR = 1.15, 95% CI: 1.09-1.21), or both (PR = 1.24, 95% CI: 1.13-1.37) were also associated this time.

Table 4 describes the prevalence of factors associated with asthma in 2012 and in 2015. After adjustments, in 2012, the following factors were associated with asthma: having yellow or indigenous skin color (PR = 1.16; 95% CI: 1.05-1.28), having a higher maternal educational attainment (PR = 1.25; 95% CI: 1.11-1.40), having a higher economic index (PR = 1.27; 95% CI: 1.14-1.42), having any history of smoking (PR = 1.38; 95% CI: 1.30-1.47), and having consumed alcohol in the previous 30 days (PR = 1.23, 95% CI: 1.16-1.30). In 2015, the factors associated remained the same, except for skin color, which was not associated with the outcome this time, and having parents/guardians who smoke, which was (PR = 1.16, 95% CI: 1.00-1.35).

DISCUSSION

The results of the present study indicate a decrease in the prevalence of wheezing from 23.2% in 2012 to 22.4% in 2015. The data related to asthma, in turn, indicate an increase in the prevalence of this CNCD from 2012 to 2015 (APC: 1.2). The main factors associated with wheezing in 2012 were gender, age, and economic level. Having smoked at least once in life and having consumed alcohol in the previous 30 days also influenced the prevalence of wheezing and the self-reported diagnosis of asthma in both years.

One of the possible explanations for the increase in the prevalence of asthma and a concomitant decrease in wheezing is the different reporting periods for one or another. Also, wheezing is a symptom that is used as a proxy for the disease in epidemiological studies, especially in the period of life between childhood and adolescence, and, unlike asthma, it does not require

Table 2. Distribution of the prevalence of wheezing and asthma in the Brazilian National School-Based Adolescent Health Survey, 2012 and 2015.

Variables	2012		2015	
	Wheezing % (95% CI)	Asthma % (95% CI)	Wheezing % (95% CI)	Asthma % (95% CI)
Gender				
Male	21.4 (20,8-22,0)	12.8 (12.4-13.3)	19.2 (18.5-19.8)	15.6 (15.0-16.1)
Female	24.9 (24.3-25.4)	12.1 (11.7-12.5)	25.4 (24.7-26.1)	16.5 (15.9-17.0)
Age, years				
≤ 13	23.6 (22.7-24.5)	11.7 (11.1-12.4)	24.0 (22.7-25.3)	16.7 (15.6-17.8)
14	23.4 (22.8-24.0)	12.3 (11.9-12.7)	22.5 (21.8-23.1)	15.7 (15.1-16.2)
≥ 15	22.6 (21.9-23.3)	13.1 (12.6-13.7)	21.2 (20.4-22.0)	16.2 (15.5-16.9)
Skin color				
White	23.3 (22.7-24.0)	12.6 (12.1-13.2)	22.3 (21.5-23.1)	16.1 (15.4-16.8)
Black	22.7 (21.6-23.8)	12.0 (11.1-12.8)	20.5 (19.2-21.7)	16.0 (14.9-17.1)
Brown	23.1 (22.5-23.7)	12.0 (11.6-12.5)	22.7 (22.0-23.4)	15.7 (15.1-16.3)
Yellow/Indigenous	24.2 (22.7-25.6)	14.2 (13.1-15.4)	24.2 (22.5-25.9)	17.8 (16.3-19.3)
Maternal educational attainment				
No schooling	22.7 (21.3-24.1)	12.6 (11.5-13.6)	21.3 (19.5-23.0)	14.6 (13.0-16.1)
Complete primary education	23.1 (22.3-23.9)	11.8 (11.2-12.3)	23.1 (22.0-24.2)	15.7 (14.8-16.7)
Incomplete primary education	23.8 (22.8-24.9)	12.3 (11.6-13.1)	23.7 (22.3-25.1)	16.4 (15.2-17.6)
Complete secondary education	23.9 (23.0-24.7)	13.5 (12.9-14.1)	24.3 (23.2-25.3)	17.3 (16.4-18.1)
Higher education	26.3 (25.0-27.7)	16.6 (15.6-17.8)	23.2 (22.0-24.4)	19.1 (18.0-20.3)
Economic index				
1st quintile	20.9 (20.1-21.7)	11.1 (10.5-11.7)	19.7 (18.8-20.6)	13.6 (12.9-14.4)
2nd quintile	22.6 (21.8-23.5)	12.3 (11.7-12.9)	22.2 (21.1-23.2)	16.3 (15.4-17.2)
3rd quintile	23.2 (22.3-24.1)	11.8 (11.1-12.4)	22.6 (21.6-23.6)	16.2 (15.3-17.0)
4th quintile	25.0 (24.2-25.8)	13.1 (12.5-13.7)	24.0 (23.1-25.0)	16.4 (15.7-17.2)
5th quintile	25.6 (24.0-27.2)	17.1 (15.7-18.4)	22.7 (20.9-24.4)	20.3 (18.5-22.0)
Any history of smoking				
No	21.1 (20.7-21.6)	11.4 (11.1-11.7)	20.6 (20.1-21.1)	15.0 (14.5-15.4)
Yes	31.7 (30.7-32.7)	16.7 (15.9-17.4)	30.3 (29.0-31.6)	20.8 (19.8-21.9)
Alcohol previous 30 days				
No	20.9 (20.5-21.4)	11.4 (11.0-11.7)	20.4 (19.8-20.9)	14.9 (14.5-15.4)
Yes	29.7 (28.9-30.5)	15.4 (14.8-16.1)	28.8 (27.8-29.9)	19.6 (18.7-20.5)
Parents who smoke				
None	22.5 (22.0-22.9)	12.2 (11.8-12.5)	21.1 (20.5-21.6)	15.5 (15.0-16.0)
One parent only	24.8 (23.9-25.7)	12.6 (12.0-13.2)	25.2 (24.1-26.3)	17.0 (16.1-17.9)
Both	24.8 (23.0-26.7)	13.8 (12.4-15.2)	28.3 (25.7-31.0)	14.5 (16.2-20.8)
TOTAL	23.2 (22.8-23.6)	12.4 (12.1-12.7)	22.4 (21.9-22.8)	16.0 (15.6-16.4)

a physician to be diagnosed. The increase we found in the prevalence of asthma can be related to an improved access to health care, especially at the primary level, observed in Brazil.[19-21] On the other hand, the decrease in wheezing in 2015 can be related to a better control of the disease, resulting from the asthma medication provided for free via the Popular Pharmacy Program the previous year.[22]

There is evidence of an increasing prevalence of asthma from 1960 onwards, but its causes have yet to be fully understood.[23] A study published in 2009 using ISAAC data showed a high prevalence of asthma in Latin America and stated that it was associated with social and biological mechanisms related to economically unfavorable conditions and urbanization, as well as to exposure to allergens, irritants, and pollutants.[20] In the present study, we found a positive association between a higher economic level and a higher prevalence of wheezing and asthma, which can be explained by a more favorable family financial condition that facilitates access to health care and diagnosis. In addition, a higher maternal educational attainment can mean more access to information about the disease and, as a consequence, a higher quality of reporting by this group of participants.[24] A lower maternal educational attainment is associated with lower incomes and limited access to health care, which leads to lack of medication and undertreatment.[10,25,26]

Having yellow or indigenous skin color has also shown to be a factor associated with asthma in the 2012 edition of the survey. As a consequence of discrimination, ethnic minorities have poorer access to

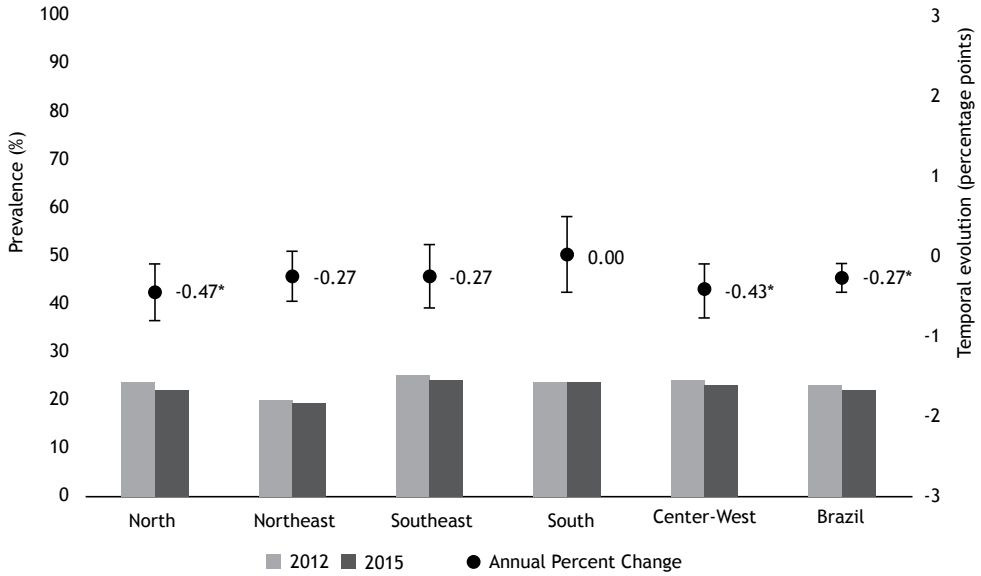

Figure 1. Prevalence of wheezing in 2012 and 2015 per Brazilian region.

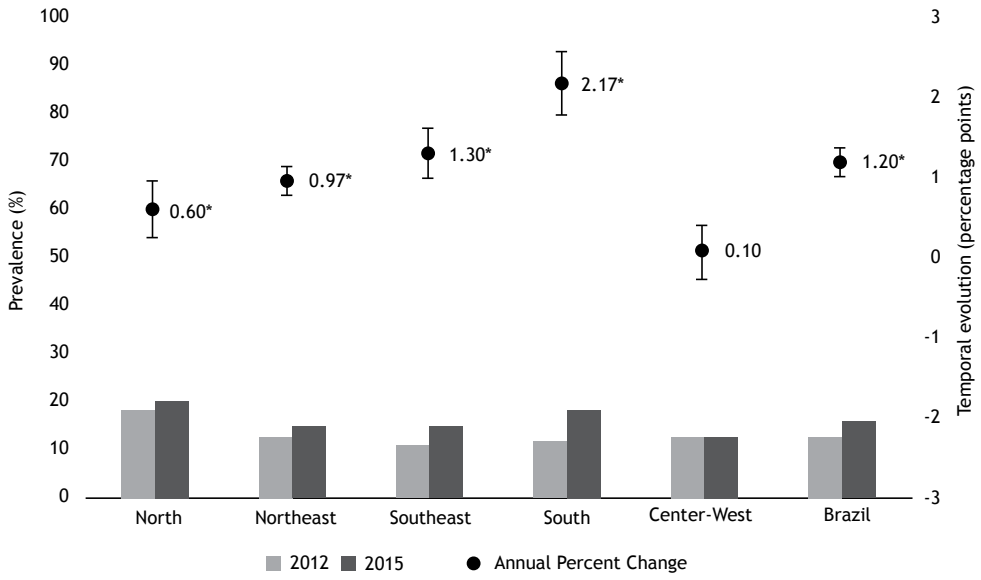

Figure 2. Prevalence of asthma in 2012 and 2015 per Brazilian region.

goods, opportunities, and health care. Therefore, people who are in a situation of socioeconomic marginalization, living in terrible housing conditions in the outskirts of Brazilian cities, are more susceptible to diseases in general, including asthma.[27,28]

As for the biological mechanisms, wheezing was more prevalent in female and younger participants. A group of authors[7] who studied children between zero and 9 years of age found a higher rate of asthma diagnosis in boys, possibly due to physiological disadvantages presented by boys in this age group, such as lower respiratory rates and a higher concentration of IgE (allergen-specific antibodies).[7,29] One of the possible explanations for this is that the risk of boys developing wheezing and asthma reduces in late childhood and, therefore, in adolescence, the disease becomes more prevalent in girls.[30]

Exposure to allergens, irritants, and pollutants might be associated with an increased prevalence of asthma.[2] In the present study, having smoked and having consumed alcohol were also associated with the prevalence of asthma in 2012 and 2015, as well as having one or both parents/guardians who smoked. A study with Hispanic children living in the southwest of the USA found a positive association between exposure to cigarette smoke in places with limited air circulation and asthma.[31] According to the Brazilian guidelines for asthma management,[32] smoking hinders asthma control, whereas quitting smoking improves the quality of life of individuals and decreases the asthma costs

Table 3. Gross and adjusted prevalence ratios of factors associated with wheezing. Brazilian National School-Based Adolescent Health Survey, 2012 and 2015.

Variables	2012				2015			
	Gross	p	Adjusted	p	Gross	p	Adjusted	p
Gender		< 0.001		< 0.001		< 0.001		< 0.001
Male	-		-		-		-	
Female	1.16 (1.12-1.20)		1.16 (1.12-1.21)		1.32 (1.27-1.38)		1.31 (1.25-1.37)	
Age, years		0.130		0.001		0.001		< 0.001
≤ 13	-		-		-		-	
14	0.99 (0.95-1.04)		0.97 (0.93-1.02)		0.94 (0.88-0.99)		0.92 (0.86-0.98)	
≥ 15	0.96 (0.91-1.01)		0.92 (0.87-0.96)		0.88 (0.83-0.94)		0.85 (0.79-0.91)	
Skin color		0.410		0.371		0.003		0.021
White	-		-		-		-	
Black	0.97 (0.92-0.13)		1.00 (0.94-1.06)		0.92 (0.85-0.98)		0.95 (0.89-1.03)	
Brown	0.99 (0.95-1.03)		1.01 (0.97-1.06)		1.01 (0.97-1.07)		1.04 (0.99-1.09)	
Yellow/Indigenous	1.04 (0.97-1.11)		1.06 (1.00-1.14)		1.08 (1.00-1.17)		1.08 (1.00-1.17)	
Maternal educational attainment		< 0.001		0.107		0.077		0.532
No schooling	-		-		-		-	
Complete primary education	1.02 (0.95-1.09)		0.99 (0.92-1.06)		1.08 (0.98-1.19)		1.02 (0.93-1.13)	
Incomplete primary education	1.05 (0.97-1.13)		1.01 (0.94-1.10)		1.11 (1.01-1.23)		1.04 (0.94-1.15)	
Complete secondary education	1.05 (0.98-1.13)		1.00 (0.93-1.08)		1.14 (1.04-1.25)		1.06 (0.96-1.17)	
Higher education	1.16 (1.07-1.26)		1.08 (0.99-1.18)		1.09 (0.99-1.20)		1.01 (0.91-1.13)	
Economic index		< 0.001		< 0.001		< 0.001		< 0.001
1st quintile	-		-		-		-	
2nd quintile	1.08 (1.03-1.14)		1.07 (1.01-1.13)		1.13 (1.06-1.20)		1.12 (1.05-1.19)	
3rd quintile	1.11 (1.05-1.17)		1.10 (1.04-1.16)		1.15 (1.08-1.22)		1.15 (1.08-1.22)	
4th quintile	1.20 (1.14-1.26)		1.18 (1.12-1.24)		1.22 (1.15-1.30)		1.22 (1.15-1.30)	
5th quintile	1.23 (1.14-1.32)		1.20 (1.11-1.29)		1.15 (1.05-1.26)		1.17 (1.06-1.29)	
Any history of smoking		< 0.001		< 0.001		< 0.001		< 0.001
No	-		-		-		-	
Yes	1.50 (1.44-1.56)		1.39 (1.33-1.45)		1.47 (1.40-1.54)		1.35 (1.28-1.42)	
Alcohol previous 30 days		< 0.001		< 0.001		< 0.001		< 0.001
No	-		-		-		-	
Yes	1.42 (1.37-1.47)		1.28 (1.33-1.45)		1.42 (1.35-1.48)		1.25 (1.19-1.32)	
Parents who smoke		< 0.001		0.05		< 0.001		< 0.001
None	-		-		-		-	
One parent only	1.10 (1.06-1.15)		1.06 (1.01-1.10)		1.20 (1.14-1.26)		1.15 (1.09-1.21)	
Both	1.11 (1.02-1.19)		1.04 (0.96-1.13)		1.34 (1.22-1.48)		1.24 (1.13-1.37)	

to the health care system. Not only has tobacco a negative impact on the management of asthma, exacerbating its symptoms, but cocaine, heroin, and alcohol do as well.

Table 4. Gross and adjusted prevalence ratios of factors associated with asthma. Brazilian National School-Based Adolescent Health Survey, 2012 and 2015.

Variables	2012				2015			
	Gross	p	Adjusted	p	Gross	p	Adjusted	p
Gender		0.012		0.273		0.032		0.004
Male	-		-		-		-	
Female	0.94 (0.90-0.99)		0.97 (90.92-1.02)		1.56 (1.00-1.11)		1.09 (1.03-1.15)	
Age, years		0.003		0.174		0.219		0.203
≤ 13	-		-		-		-	
14	1.05 (0.98-1.12)		1.01 (0.94-1.08)		0.94 (0.87-1.01)		0.94 (0.86-1.02)	
≥ 15	1.12 (1.05-1.20)		1.06 (0.99-1.15)		0.97 (0.90-1.05)		0.97 (0.89-1.07)	
Skin color		0.002		0.024		0.069		0.141
White	-		-		-		-	
Black	0.95 (0.88-1.03)		1.01 (0.93-1.10)		0.99 (0.92-1.08)		1.03 (0.94-1.14)	
Brown	0.95 (0.90-1.01)		1.02 (0.96-1.08)		0.98 (0.92-1.04)		1.02 (0.96-1.09)	
Yellow/Indigenous	1.12 (1.03-1.23)		1.16 (1.05-1.28)		1.11 (1.01-1.22)		1.14 (1.02-1.27)	
Maternal educational attainment		< 0.001		< 0.001		< 0.001		0.002
No schooling	-		-		-		-	
Complete primary education	0.94 (0.85-1.03)		0.92 (0.84-1.02)		1.08 (0.96-1.22)		1.02 (0.90-1.16)	
Incomplete primary education	0.98 (0.88-1.09)		0.97 (0.87-1.08)		1.13 (0.99-1.28)		1.06 (0.93-1.21)	
Complete secondary education	1.07 (0.98-1.18)		1.05 (0.94-1.16)		1.18 (1.05-1.33)		1.11 (0.98-1.26)	
Higher education	1.32 (1.19-1.47)		1.25 (1.11-1.40)		1.31 (1.17-1.48)		1.23 (1.07-1.40)	
Economic index		< 0.001		< 0.001		< 0.001		< 0.001
1st quintile	-		-		-		-	
2nd quintile	1.11 (1.03-1.19)		1.08 (1.00-1.17)		1.19 (1.10-1.29)		1.22 (1.11-1.34)	
3rd quintile	1.06 (0.98-1.15)		1.03 (0.95-1.12)		1.19 (1.10-1.28)		1.20 (1.10-1.31)	
4th quintile	1.18 (1.10-1.26)		1.09 (1.01-1.19)		1.20 (1.12-1.29)		1.16 (1.06-1.27)	
5th quintile	1.54 (1.40-1.69)		1.27 (1.14-1.42)		1.49 (1.34-1.64)		1.36 (1.20-1.53)	
Any history of smoking		< 0.001		< 0.001		< 0.001		< 0.001
No	-		-		-		-	
Yes	1.46 (1.38-1.54)		1.38 (1.30-1.47)		1.39 (1.31-1.48)		1.25 (1.16-1.35)	
Alcohol previous 30 days		< 0.001		< 0.001		< 0.001		< 0.001
No	-		-		-		-	
Yes	1.36 (1.29-1.43)		1.23 (1.16-1.30)		1.31 (1.24-1.39)		1.17 (1.09-1.25)	
Parents who smoke		0.045		0.360		0.001		0.042
None	-		-		-		-	
One parent only	1.03 (0.98-1.10)		0.99 (0,93-1.06)		1.10 (1.03-1.17)		1.07 (0.99-1.15)	
Both	1.14 (1.02-1.26)		1.08 (0,96-1.22)		1.19 (1.05-1.35)		1.16 (1.00-1.35)	

Our data show that there was a reduction in the prevalence of wheezing in Brazil, especially in the northern region (APC: −0.27); however, the prevalence of asthma increased in the same region despite the fact

that there was greater coverage of public health care assistance programs and of the Family Health Strategy initiative in 2015 than in 2012.[21] Another study found a higher prevalence of a medical diagnosis of asthma in the north of Brazil (13.5%; 95% CI: 12.7-14.2) than in the other regions.[24] The prevalence of wheezing also decreased in the center-western region, whereas the prevalence of asthma did not present a statistically significant difference. Other authors[21] reported an increase in the prevalence rate from 21.6% in 1998 to 30.1% in 2013 (8.5 percentage points) based on data from visits that took place via private health care plans in that region. This could be understood as a higher quality of treatment provided by the private health care network, though there was also an increase in the participation of the Unified Health System (Brazilian public health care system).

In addition to the northern region, the northeastern, southeastern, and southern regions also presented increases in the prevalence of asthma in life. According to the American Thoracic Society, climate and temperature can be considered irritants that trigger asthma.[33] For asthma patients, places with a wide temperature variation or high levels of pollution can be harmful.[34,35] A study[36] concluded that climate changes can alter local climate patterns—such as maximum and minimum temperatures, and precipitation from rain and storms—and have negative impacts on allergic diseases, which could explain the increased prevalence of asthma in climatically distinct regions. In addition, according to a study with data from throughout Brazil,[37] lower temperatures, such as those found in the south of the country, tend to keep people indoors for longer periods of time, with reduced air circulation, which leads to an increase in allergic processes and respiratory infections. A positive association was also found between the prevalence of active asthma and water deprivation in the northeastern region.[38]

Controlling risk factors—such as smoking, alcohol consumption, and exposure to allergens—is considered important for a better management and treatment of asthma.[39] Therefore, despite the increase in the prevalence of asthma, the decrease in the prevalence of its main symptom suggests a reasonably positive management by the health care system.

We should point out that our study has a few limitations. One of them is the different reporting periods for asthma (in life) and wheezing (in the previous 12 months). Another one is related to the fact that the PeNSE is a school-based survey conducted throughout the country, focusing on several aspects of adolescent health. As such, the establishment of a medical diagnosis or the inclusion of further questions to better define asthma for the entire study population might have been logistically infeasible and certain pieces of information might not have been collected. In addition, the fact that the questionnaire is self-administered may lead to errors since participants might misunderstand some of the questions or make mistakes when choosing the answers. These limitations can prevent this study from being compared with others that focus on respiratory diseases, such as the ISAAC, which describes not only the prevalence but also the severity of asthma, rhinitis, and eczema, and contains more information on risk factors for these diseases.[4,5] However, among the strengths of the our study are the representative sample of schoolchildren from throughout the country and the continuity in asthma monitoring, which can contribute to improving disease control strategies. Despite their limitations, self-reported questions have been used in population studies as a good proxy for the monitoring of wheezing and, consequently, asthma.

Our results suggest a reduction in the prevalence of wheezing but an increase in the prevalence of asthma in the years evaluated. Social and biological mechanisms, such as an economically unfavorable condition, limited access to health care (and consequently poorer diagnoses), lack of medication and treatment, as well as urbanization, climate, and temperature, can lead to a higher prevalence of the disease. A higher prevalence of asthma, in turn, has negative effects on quality of life and increases health costs with medical appointments, use of medication, frequency of hospitalization, loss of productivity, work/school absenteeism, in addition to early mortality. Therefore, a better understanding of the factors associated with the increased prevalence of asthma is essential for the development of health strategies and policies.

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J Bras Pneumol. 2019;45(3):e20170358
http://dx.doi.org/10.1590/1806-3713/e20170358

Translation of the quality-of-life measure for adults with primary ciliary dyskinesia and its application in patients in Brazil

Ana Paula Lima de Queiroz[1,a], Rodrigo Abensur Athanazio[2,b], Mary Anne Kowal Olm[3,c], Bruna Rubbo[4,5,d], Yuri Reis Casal[1,e], Jane Lucas[4,5,f], Laura Behan[4,5,6,g]

1. Escola Bahiana de Medicina e Saúde Pública, Salvador (BA) Brasil.
2. Divisão de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
3. Departamento de Patologia, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
4. Primary Ciliary Dyskinesia Centre, NIHR Biomedical Research Centre, University of Southampton, Southampton, United Kingdom.
5. University Hospital Southampton NHS Foundation Trust, Southampton, United Kingdom.
6. School of Applied Psychology, University College Cork, Cork, Ireland.

a. http://orcid.org/0000-0002-4660-0090
b. http://orcid.org/0000-0002-9399-5275
c. http://orcid.org/0000-0001-9489-4655
d. http://orcid.org/0000-0002-1629-8601
e. http://orcid.org/0000-0002-0488-5307
f. http://orcid.org/0000-0001-8701-9975
g. http://orcid.org/0000-0002-0748-6141

Submitted: 15 October 2017.
Accepted: 2 May 2018.

Study carried out in the Divisão de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brazil.

ABSTRACT

Primary ciliary dyskinesia (PCD) is a genetic disorder that is typically inherited in an autosomal recessive manner. It is clinically characterized by recurrent respiratory infections. However, its repercussions for patient quality of life should not be overlooked. Studies have shown that PCD has a significant impact on the lives of patients, although there are as yet no PCD-specific markers of quality of life. To address that problem, researchers in the United Kingdom developed a quality-of-life questionnaire for patients with PCD. The present communication focuses on the process of translating that questionnaire into Brazilian Portuguese, through a partnership between researchers in Brazil and those in the United Kingdom, as well as its subsequent application in patients in Brazil.

Keywords: Quality of life; Kartagener syndrome; Surveys and questionnaires.

Primary ciliary dyskinesia (PCD) is a genetic disorder that is typically inherited in an autosomal recessive manner. It is characterized by recurrent respiratory infections caused by impaired mucociliary clearance, symptoms often appearing soon after birth.[1] Patients presenting with the triad of dextrocardia, bronchiectasis, and chronic rhinosinusitis are said to have Kartagener's syndrome. Up to 50% of patients with PCD have situs inversus due to abnormal motility of the nodal cilia, which are responsible for the left-right body orientation during embryonic development.[2]

The clinical spectrum of PCD is broad. However, when there are no laterality defects, the level of clinical suspicion of the disorder remains low. The clinical presentation includes neonatal respiratory distress syndrome, evolving to rhinosinusitis, chronic otitis media, recurrent pneumonia, and bronchiectasis. In this context, it is expected that PCD will have a significant impact on the quality of life of patients and their families.[3]

Studies on the psychological and cognitive aspects of PCD have contributed to a better understanding of the psychosocial needs of the affected patients. A pioneering study in this field[4] showed, through the use of questionnaires, that children with PCD had significantly higher scores for internalizing problems, as well as scoring higher for somatic complaints, symptoms of anxiety, and symptoms of depression, when compared with a control group. Parental distress and maternal stress were also significantly more common among parents of patients with PCD.[4]

The authors of a study[5] in which questionnaires were applied to 78 patients diagnosed with PCD (mean age at diagnosis, 9.4 years) concluded that patients with a higher "treatment burden" had a poorer quality of life.

Correspondence to:
Rodrigo Abensur Athanazio. Divisão de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, Avenida Dr. Enéas de Carvalho Aguiar, 44, CEP 05403-900, São Paulo, SP, Brasil.
Tel.: 55 11 2661-5695. E-mail: rathanazio@yahoo.com.br
Financial support: None.

ISSN 1806-3713

Over time, those patients lost interest in treating the disease, showing lower levels of treatment adherence. Therefore, early diagnosis and better treatment strategies—requiring greater physician knowledge about the condition and measures to encourage adherence to the treatments proposed—are paramount.[5]

A recent systematic review[6] on the psychosocial impact of PCD identified 14 studies conducted, variously, in the United States and in several European countries. That review showed that, over time, quality of life decreases in patients with PCD over the years, the disorder having significant effects on the physical aspects of quality of life (limitations in activities of daily living), as well as on its emotional aspects (frustration, anxiety and stress), and social aspects (stigmatization).[6]

The lack of PCD-specific quality of life markers led to the development of a quality-of-life measure for patients with PCD (the QOL-PCD), which was first devised for use in adult patients and later adapted for use in pediatric patients.[7,8] The questionnaire was developed through individual and group interviews with specialists, adult patients with PCD, and parents of pediatric patients with PCD. The questionnaire was later refined following cognitive interviews. The instrument contains 37, 43, 41, and 48 items in its child, adolescent, caregiver, and adult versions, respectively. The English-language version of the QOL-PCD for adults has recently been validated, representing an important step for its use in research and clinical practice.[9]

The objective of this brief communication was to present the process of translating the QOL-PCD from English into Portuguese, conducted through a partnership between Brazilian and British researchers from the referral center for PCD at the University of Southampton, in the United Kingdom. The process of translating and applying the questionnaire is described here. Our aim was to make an instrument in Portuguese available for the psychosocial evaluation of patients with PCD in Brazil and, as a consequence, enable more integrated care to be provided to those living with the disease, as well as to provide an important research tool for this population.

The QOL-PCD was initially translated from English into Portuguese by two researchers, working separately. Both were fluent in English and were native speakers of Brazilian Portuguese. Subsequently, there was a discussion among those two researchers and one of the creators of the original questionnaire. In that discussion, they compared the meaning of the Portuguese translations with the original meaning in English in order to reach an agreement and produce a consensus version. Another researcher, also fluent in English and a native speaker of Brazilian Portuguese, with no access to the original questionnaire, then translated the Portuguese-language version back into English (i.e., performed a back translation). There was then another discussion, among the two researchers who did the translation, the researcher who did the back translation, and the developer of the original questionnaire in English, in order to make the translation as faithful as possible to the original version. In this process, cultural differences between Brazil and the United Kingdom were taken into account, and, in order to adapt the Brazilian version to the social and cultural reality of the country, a few changes were made. Thus,

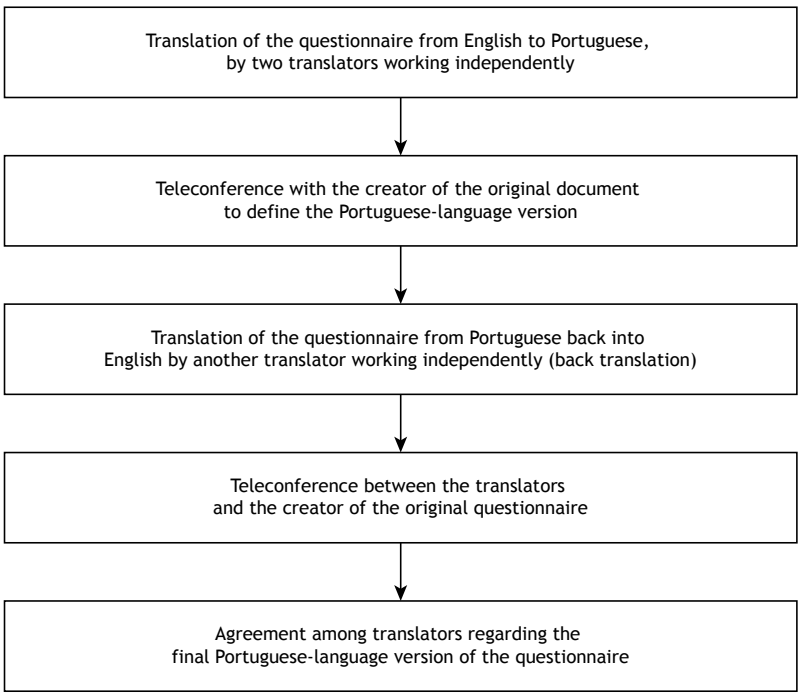

Figure 1. Algorithm for the process of translating the questionnaire from English into Portuguese.

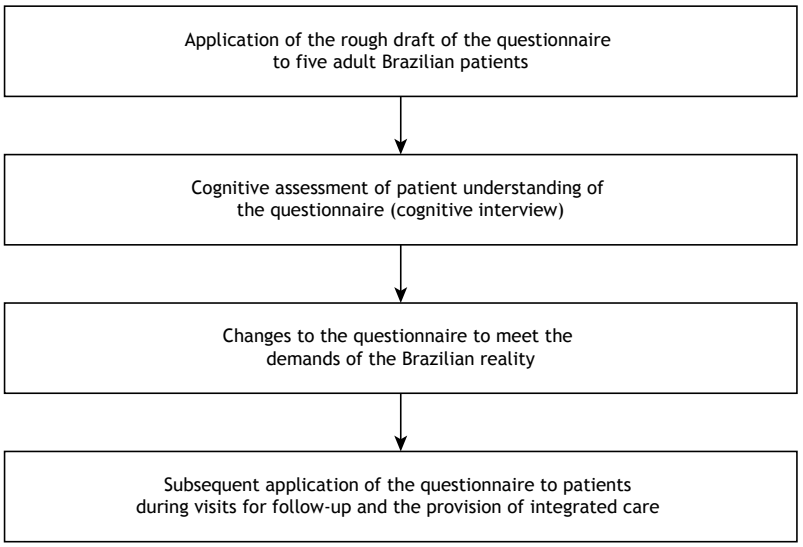

Figure 2. Algorithm for the application of the rough draft of the Portuguese-language version of the quality-of-life questionnaire for patients with primary ciliary dyskinesia, the cognitive interview, and the creation of the final version of the questionnaire.

we created four questionnaires in Portuguese, one for each target population: children, adolescents, adults, and caregivers (Figure 1).

The QOL-PCD for adults includes questions related to three domains of patient quality of life: everyday life; school, work, or activities of daily living; and symptoms. The questions address how patients feel in their everyday life, how they are affected by the disease and the treatment, and whether or not they experience impairment in performing their activities of daily living. The possible responses vary depending on to what the given block of questions refers, such as the frequency or intensity of a certain situation. Greater frequency and more pronounced repercussions for the life of a patient translate to poorer quality of life. The questionnaire can be accessed in full in the online supplement (Appendix 1) on the JBP website (http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=59).

These are examples of the questions in Portuguese:

- *"Na última semana, com que frequência você se sentiu cansado/preocupado/cheio de energia/exausto/triste?"*

- *"Atualmente, quanto tempo por dia você passa fazendo o seu tratamento? Seu tratamento tornou suas atividades diárias mais difíceis de serem realizadas?"*

- *"Pensando no seu estado de saúde na última semana, indique o quão verdadeiro é cada uma das frases para você: 'Eu me sinto confortável ao tossir na frente de outras pessoas'; 'Eu me sinto preocupado por estar em contato com pessoas doentes'; 'Sinto-me sozinho'"*

The questionnaire was applied to adult patients with PCD seen at the Bronchiectasis Outpatient Clinic of the University of São Paulo. All participating patients gave written informed consent. After completing the questionnaire, in compliance with the protocol for validation of the questionnaire outside the United Kingdom, a group of five patients answered the questions of the so-called "cognitive interview". In the individual interview format, the session aimed to evaluate how Brazilian patients process the questions and the response options (i.e., the clarity and objectivity of the questions). Thus, it was possible to evaluate patient understanding when reading the questions and whether or not the meaning was consistent with the intent of the researchers. On the basis of that evaluation, changes were made to the instructions given in the headers, as well as to the questions and response options (Figure 2).

These are examples of the questions asked in the cognitive interview conducted after patients completed the prototype questionnaire in Portuguese:

- *How clear are the response options? Is there a better way to formulate the question?*

- *Are there any questions that do not apply to you or apply to events that have not happened to you? If so, which ones are they?*

- *Are there any questions that sound confusing or are difficult to answer? If so, which ones are they?*

- *Have we forgotten anything important?*

With the results of the cognitive interview in hand, we were able to take into consideration the point of view of the patients with PCD regarding the QOL-PCD. Another discussion among the researchers involved led to new adaptations in the questionnaire to meet the Brazilian demand in the best possible way.

Researchers and clinicians who wish to use the questionnaires for research or clinical purposes should contact the copyright holders (Jane Lucas, Margaret

Leigh, Alexandra Quittner, or Sharon Dell; e-mail to jlucas1@soton.ac.uk) for an agreement between the parties. Once permission is granted, the use of the questionnaires is free of cost.

In conclusion, the translation of the QOL-PCD into Portuguese and its final revisions followed a systematic and interactive approach in collaboration with the researchers who developed the original instrument in English. We believe that this communication is essential to encourage a more widespread use of the questionnaire and its translation into other languages. Here, we present the final version of the QOL-PCD in Portuguese for adults (Appendix 1, http://jornaldepneumologia.com.br/detalhe_anexo.asp?id=59). The use of this questionnaire is important for the appropriate clinical follow-up of patients with PCD. It also serves as an efficacy outcome measure in studies of therapeutic interventions and the natural history of the disease.

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Update on the approach to smoking in patients with respiratory diseases

Maria Penha Uchoa Sales[1,a], Alberto José de Araújo[2,b], José Miguel Chatkin[3,c], Irma de Godoy[4,d], Luiz Fernando Ferreira Pereira[5,e], Maria Vera Cruz de Oliveira Castellano[6,f], Suzana Erico Tanni[4,g], Adriana Ávila de Almeida[7,h], Gustavo Chatkin[3,i], Luiz Carlos Côrrea da Silva[8,j], Cristina Maria Cantarino Gonçalves[9,k], Clóvis Botelho[12,13,l], Ubiratan Paula Santos[14,m], Carlos Alberto de Assis Viegas[15,n], Maristela Rodrigues Sestelo[16,o], Ricardo Henrique Sampaio Meireles[10,11,p], Paulo César Rodrigues Pinto Correa[17,q], Maria Eunice Moraes de Oliveira[18,r], Jonatas Reichert[19,s], Mariana Silva Lima[6,t], Celso Antonio Rodrigues da Silva[20,u]

1. Hospital de Messejana Dr. Carlos Alberto Studart, Fortaleza (CE) Brasil.
2. Núcleo de Estudos e Tratamento do Tabagismo, Instituto de Doenças do Tórax, Universidade Federal do Rio de Janeiro – UFRJ – Rio de Janeiro (RJ) Brasil.
3. Escola de Medicina. Pontifícia Universidade Católica do Rio Grande do Sul – PUCRS – Porto Alegre (RS) Brasil.
4. Disciplina de Pneumologia, Faculdade de Medicina de Botucatu, Universidade Estadual Paulista – UNESP – Botucatu (SP) Brasil.
5. Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte (MG) Brasil.
6. Hospital do Servidor Público Estadual de São Paulo, São Paulo (SP) Brasil.
7. Instituto Nacional de Pesquisas Espaciais, São José dos Campos (SP) Brasil.
8. Pavilhão Pereira Filho da Santa Casa, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.
9. Instituto Nacional de Câncer José Alencar Gomes da Silva, Rio de Janeiro (RJ) Brasil.
10. Faculdade de Medicina, Universidade Estácio de Sá, Rio de Janeiro, (RJ), Brasil.
11. Instituto Estadual de Doenças do Tórax Ary Parreiras - IETAP - Secretaria Estadual de Saúde do Rio de Janeiro, Niterói (RJ) Brasil.
12. Faculdade de Medicina, Universidade Federal de Mato Grosso, Cuiabá (MT) Brasil.
13. Centro Universitário de Várzea Grande – UNIVAG – Várzea Grande (MT) Brasil.
14. Divisão de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
15. Faculdade de Medicina, Universidade de Brasília, Brasília (DF) Brasil.
16. Escola Bahiana de Medicina e Saúde Pública, Salvador (BA) Brasil.
17. Escola de Medicina, Universidade Federal de Ouro Preto, Ouro Preto (MG) Brasil.
18. Hospital Conceição, Porto Alegre (RS) Brasil.
19. Secretaria de Saúde do Paraná – SESA-PR – Curitiba (PR) Brasil.
20. Secretaria de Saúde do Distrito Federal – SESA-PR – Brasília (DF) Brasil.

Submitted: 1 October 2018.
Accepted: 29 October 2018.

Study carried out in the Hospital de Messejana Dr. Carlos Alberto Studart, Fortaleza (CE) Brasil.

ABSTRACT

Smoking is the leading cause of respiratory disease (RD). The harmful effects of smoking on the respiratory system begin in utero and influence immune responses throughout childhood and adult life. In comparison with "healthy" smokers, smokers with RD have peculiarities that can impede smoking cessation, such as a higher level of nicotine dependence; nicotine withdrawal; higher levels of exhaled carbon monoxide; low motivation and low self-efficacy; greater concern about weight gain; and a high prevalence of anxiety and depression. In addition, they require more intensive, prolonged treatment. It is always necessary to educate such individuals about the fact that quitting smoking is the only measure that will reduce the progression of RD and improve their quality of life, regardless of the duration and severity of the disease. Physicians should always offer smoking cessation treatment. Outpatient or inpatient smoking cessation treatment should be multidisciplinary, based on behavioral interventions and pharmacotherapy. It will thus be more effective and cost-effective, doubling the chances of success.

Keywords: Respiratory tract diseases/therapy; Respiratory tract diseases/drug therapy; Tobacco use disorder/epidemiology; Smoking cessation; Counseling; Lung neoplasms.

INTRODUCTION

Smoking is the leading cause of preventable death worldwide, annually accounting for 7 million deaths, 890,000 of which are associated with passive smoking.[1] In Brazil, 156,000 people die each year from smoking-related diseases.[2] Worldwide, there are approximately 1.1 billion smokers, most of whom live in low- and middle-income countries, where the morbidity and mortality burden of smoking is higher.[1]

The prevalence of smoking in Brazil has dropped significantly, as evidenced in the 2006-2017 historical series of the Brazilian "Telephone-based System for the Surveillance of Risk and Protective Factors for Chronic Noncommunicable Diseases Survey", which showed that the prevalence fell from 19.5% to 10.1%, translating to a 48.2% reduction.[3] The survey showed that, although smokers accounted for only 10.1% of general population in 2017 (13.2% of the male population and 7.5% of the female population), there were still 18.2 million smokers ≥ 18 years of age in that year.[3,4]

The Framework Convention on Tobacco Control[5] lists offering smoking cessation treatment as one of the six most cost-effective policies in the MPOWER package, the acronym MPOWER standing for **M**onitoring tobacco use and prevention policies; **P**rotecting people from tobacco smoke; **O**ffering help to quit tobacco use; **W**arning about the dangers of tobacco; **E**nforcing bans on tobacco advertising, promotion and

Correspondence to:
Maria da Penha Uchoa Sales. Rua Gothardo Moraes, 155/1002A, CEP 60177-340, Fortaleza, CE, Brasil.
Tel.: 55 85 99909-8728. E-mail: puchoasales152@gmail.com
Financial support: None.
a. http://orcid.org/0000-0002-4226-6216; b. http://orcid.org/0000-0001-7159-1395; c. http://orcid.org/0000-0002-4343-025X;
d. http://orcid.org/0000-0002-6588-5626; e. http://orcid.org/0000-0002-1377-2072; f. http://orcid.org/0000-0002-1982-4590;
g. http://orcid.org/0000-0002-2587-2759; h. http://orcid.org/0000-0003-3226-8231; i. http://orcid.org/0000-0001-6928-5647;
j. http://orcid.org/0000-0003-2964-1453; k. http://orcid.org/0000-0002-1299-0596; l. http://orcid.org/0000-0002-2646-2639;
m. http://orcid.org/ 0000-0003-4919-884X; n. http://orcid.org/0000-0002-0801-6299; o. http://orcid.org/0000-0003-0961-4474;
p. http://orcid.org/0000-0002-9916-2987; q. http://orcid.org/0000-0001-7108-0640; r. http://orcid.org/0000-0003-4012-8220;
s. http://orcid.org/0000-0003-4461-7582; t. http://orcid.org/0000-0002-5438-3428; u. http://orcid.org/0000-0001-8842-5393

  ISSN 1806-3713

sponsorship; and **R**aising taxes on tobacco.[6] Despite the success of anti-smoking policies in Brazil, including the fact that smoking cessation treatment is offered via the Brazilian Unified Health Care System,[7,8] there are subgroups of smokers with relevant comorbidities who have greater difficulty in quitting smoking, such as patients with COPD, which requires additional efforts to offer smoking cessation treatment and devise strategies for smoking cessation programs.[9]

Smokers with chronic noncommunicable diseases related to smoking need to be treated with maximum efficacy because, if they continue to smoke, the progression of those diseases will have enormous consequences for their lives, including early disability and premature death.[10,11]

The approach to smoking cessation should be directed toward effecting behavioral changes, creating motivation, and instilling the desire to avoid the triggers of cravings, supported by effective pharmacotherapy, as recommended in the main guidelines.[12-14] Physicians should be prepared to provide smoking cessation treatment, patients with chronic lung diseases requiring special attention.

This article had a number of objectives. In addition to reviewing and evaluating the main evidence regarding the health effects of smoking on respiratory diseases, we aimed to alert physicians (pulmonologists in particular) to the fact that smoking cessation should be prioritized in the treatment of lung diseases.[8,15,16]

METHODOLOGY

The chosen search strategy was the method known as integrative review, the purpose of which is to perform keyword searches, as well as to gather articles, systematic reviews, and technical reports, in order to summarize the results and evidence regarding a specific theme.[17-19]

For the present review, the following research questions were formulated:

- What is the evidence regarding smoking cessation counseling in patients with respiratory diseases?
- What is the evidence regarding smoking cessation pharmacotherapy in patients with respiratory diseases?

From the results of online searches of the Brazilian Virtual Health Library, SciELO, and MEDLINE/PubMed databases, we selected 176 articles. We used the following search terms (keywords): smoking; nicotine dependence; effects on respiratory health; pharmacotherapy on cessation; cessation counseling; chronic obstructive pulmonary disease; asthma; tuberculosis; and pneumoconiosis.

CLINICAL EVALUATION OF SMOKERS

Smokers with respiratory diseases have a greater need and urgency to quit smoking; therefore, physicians need to take a proactive role in encouraging such patients to give up the habit and offering smoking cessation treatment. This approach should be taken in conjunction with the treatment of the underlying disease. Therefore, physicians need to be trained not only in the management of the smoking cessation pharmacotherapy but also in cognitive-behavioral therapy techniques.[13,15]

From the first visit, the physician should inform the patient of the fact that quitting smoking is the only measure that will slow the decline in lung function, improve the response to treatment, and reduce the frequency of exacerbations. The approach should be multidisciplinary and should be applied at all levels of health care, for outpatients and inpatients.[20,21]

Smokers with respiratory diseases have peculiarities that can hinder smoking cessation, such as a higher level of nicotine dependence and withdrawal symptoms that are more severe; low motivation; low self-efficacy; excessive concern about weight gain; and a high prevalence of psychiatric disorders. In addition, they require treatment that is more intensive and prolonged.[21,22]

Identifying the predictive factors and knowing the techniques for achieving smoking cessation are fundamental to the approach to smokers. The level of motivation is predictive of the frequency of attempts to quit smoking, and the level of nicotine dependence is predictive of the outcomes of those attempts. The concomitant use of alcohol or drugs can make smoking cessation more difficult.[23,24]

Before starting treatment, smokers should undergo a full clinical evaluation, as detailed in Chart 1.[10,13] The objectives of the anamnesis include analyzing the smoking habits of patients, as well as their level of motivation, dependence, and self-efficacy, together with their experience in previous attempts at smoking cessation, their history of smoking-related diseases, contraindications for specific medications, their beliefs, and their preferences.[10,25] The physician should perform a complete physical examination and order ancillary tests, depending on the local demand and availability.[23] Spirometry and imaging findings can be useful in motivating such patients to quit smoking.[24,26,27]

Knowing the level of nicotine dependence is important to guide the treatment (Chart 2). The most widely used instrument is the Fagerström Test for Nicotine Dependence.[28] In 2012, it was renamed the Fagerström Test for Cigarette Dependence.[29] The instrument most widely used in assessing the level of motivation for quitting smoking is the transtheoretical model of change developed by Prochaska & DiClemente,[30] which is useful for developing a smoking cessation plan (Chart 3).

CURRENT EVIDENCE REGARDING THE EFFECTIVENESS OF COUNSELING AND PHARMACOTHERAPY

Cognitive-behavioral counseling

After identifying the level of nicotine dependence for each patient, the main subsequent step is a brief

Chart 1. Smoking anamnesis and initial clinical examination: what to value?

Smoking history	Information to be collected and tests to be performed
Age at smoking onset and smoking intensity	Age at onset of regular smoking; number of cigarettes/day; frequency of use; and total smoking history, in pack-years
Forms of nicotine use	Conventional cigarettes; hand-rolled cigarettes; clove cigarettes; chewing tobacco; snuff, hookah; e-cigarettes; heat-not-burn tobacco products; cigars; and pipes
Attempts, treatments, abstinence and outcomes	Number of attempts and previous treatments, with or without success; withdrawal symptoms; and relapse and probable causes
Associated factors: smoking triggers	Behavioral (coffee, alcoholic drink, after meals, driving, etc.); emotional (stress, argument, anxiety, depression, etc.); and environmental (living with smokers at home, at work, or during leisure)
Passive smoking	Passive smoking: secondary and tertiary
Level of dependence	Fagerström Test for Nicotine Dependence
Level of motivation	Motivational stage: Prochaska & DiClementi model; and self-efficacy scale: readiness, importance, and trust
Evaluation of anxiety and depression	Hospital Anxiety and Depression Scale; Beck Anxiety Inventory; and Beck Depression Inventory
Clinical comorbidities that alter the course or management of treatment	Oral lesions; peptic ulcer; diabetes; hypertension; heart diseases; lung diseases; nephropathy; liver disease; cancer; history of convulsion; epilepsy; stroke; allergies (cutaneous, respiratory, and drug-related); and skin disorders
Psychiatric comorbidities that alter the course or management of treatment	Anxiety; depression; bipolar disorder; panic disorder; schizophrenia; attention-deficit/hyperactivity disorder; anorexia nervosa; bulimia; and impulse control disorders (food, shopping, pathological gambling)
Consumption of alcohol or other psychoactive substances	CAGE questionnaire; AUDIT; pattern of alcohol use; recent alcohol withdrawal; and pattern of use of marijuana, crack cocaine, and other drugs
Physical activity and body weight	Regular physical activity; sedentary lifestyle; and body mass index
Medications that can affect treatment	Antidepressants; MAO inhibitors; carbamazepine; phenytoin; barbiturates; antipsychotics; cimetidine; pseudoephedrine; oral hypoglycemic agents; insulin; systemic corticosteroids; and theophylline
Individuals/situations that call for caution in the use of drugs	Adolescents; pregnant or breastfeeding women; the elderly; recent cases of acute myocardial infarction or stroke; severe arrhythmia; use of psychotropic drugs; and chronic renal failure (hemodialysis)
Family history	History of smoking in family members; cohabitation with smokers
Physical examination	Complete physical examination, identifying previous or current symptomatology, limited to the therapeutic protocol to be proposed
Ancillary tests	Basic routine: chest X-ray; spirometry; electrocardiogram; complete blood count; and serum/urine biochemistry; and useful tests in the evaluation and follow-up: exhaled carbon monoxide and cotinine level (in serum, saliva, or urine)

Based on data from Reichert et al.[12] and Fiore et al.[13] CAGE: Cut down, Annoyed, Guilty, and Eye-opener; AUDIT: Alcohol Use Disorders Identification Test; and MAO: monoamine oxidase.

counseling session for smoking cessation and the scheduling of follow-up visits with longer sessions.[31]

It is known that self-efficacy and self-esteem affect the capacity of an individual to quit smoking; therefore, constructive advice should be directed at addressing issues related to the level of motivation to quit smoking and to remain abstinent.[31] Whenever possible, the physician should individualize smoking cessation treatment, discussing the relationship that the individual has with the smoking habit and the reasons why the individual continues to smoke, as well as the therapeutic options available in terms of psychological and pharmacological support.[10,13]

In the initial counseling sessions, the physician should be empathetic and should be aware of smoking cessation strategies, in order to help the smoker through this early stage. The negative effects of smoking and the benefits of smoking cessation should be addressed.[31] In the treatment protocol, it is essential to establish a date of cessation; to identify situations in which the risk of relapse is high and teach the skills required in order to cope with them; to explain the withdrawal symptoms; and to follow the patient closely through physician consultations, telephone calls, e-mails, WhatsApp messages, and text messages.[23,30,31] The physician should educate the patient regarding the potential complications of treatment and benefits of quitting smoking. In addition, minimizing fatalistic beliefs and addressing depression improve self-efficacy, self-esteem, and the feeling of having control over the treatment.[31]

In patients with asthma or COPD, it is possible to increase the level of motivation by contextualizing the results of spirometry.[24,26,27] In patients with lung

Chart 2. Evaluation of the level dependence: Fagerström Test for Nicotine Dependence.

1. How soon after you wake up do you smoke your first cigarette?
(3) ≤ 5 min (2) 6-30 min (1) 31-60 min (0) > 60 min
2. Do you find it difficult to refrain from smoking in places where it is forbidden?
(1) Yes (0) No
3. Which cigarette would you hate most to give up?
(1) The first one in the morning (0) Any other
4. How many cigarettes do you smoke per day?
(0) ≤ 10 (1) 11-20 (2) 21-30 (3) ≥ 31
5. Do you smoke more frequently during the first hours after waking than during the rest of the day?
(1) Yes (0) No
6. Do you smoke when you are so ill that you are in bed most of the day?
(1) Yes (0) No
Total score: 0-2 = very low; 3-4 = low; 5 = average; 6-7 = high; and 8-10 = very high.

Adapted from Heatherton et al.[28]

Chart 3. Stages of behavioral change.

Stage	Description of the stage and motivational counseling strategies
Precontemplation	There is no intention to stop in the foreseeable future (next 6 months); nor is there even the realization that the smoking behavior is undesirable. Counseling strategy: patients should be educated about the risks of smoking.
Contemplation	Although there is awareness that smoking is a problem, there is ambivalence about the perspective of changing; the patient plans to quit within the next 6 months. Counseling strategy: patients are receptive to information about how to change their behavior.
Preparation (determination)	There is a readiness to stop smoking, often within the next month, and the patient is determined to do so. Counseling strategy: patients should actively plan a cessation date as a strategy to change the behavior.
Action	Smoking cessation: the patient takes the action that leads to the desired change in behavior. Counseling strategy: patients should change their behavior and quit smoking.
Maintenance (prevention of relapse)	The patient finalizes the change process or relapses. Counseling strategy: patients should learn strategies to resist triggers and prevent relapse.

Adapted from Prochaska & DiClemente.[30]

cancer, an empathic, positive, direct approach is fundamental: "One of the best things you can do to combat lung cancer is to quit smoking. I can help you." In smokers with tuberculosis, application of the directly observed treatment, short-course (DOTS) strategy in combination with smoking cessation treatment has been shown to improve quality of life. For those patients, it is recommended that a brief intervention be provided at the time of diagnosis and that monthly behavioral support be maintained throughout the duration of the tuberculosis treatment.[32]

PHARMACOLOGICAL APPROACH

In patients with COPD, pharmacological intervention combined with intensive behavioral counseling has been shown to be effective, one meta-analysis demonstrating a high quality of evidence in pooled results, with a relative risk (RR) of 2.53 (95% CI: 1.83-3.50).[33] Comparing placebo with the use of monotherapy, that same meta-analysis showed that the chance of smoking cessation doubled when bupropion was used (RR = 2.03; 95% CI: 1.26-3.28), more than doubled when nicotine replacement therapy (NRT) was used (RR = 2.60; 95% CI: 1.29-5.24), and was three times higher when varenicline was used (RR = 3.34; 95% CI: 1.88-5.92).[33]

Nortriptyline, a second-line medication, shows no superiority over placebo in terms of achieving smoking cessation in patients with COPD.[34] Among smokers with asthma, the rate of cessation is low, although there have been few studies of the issue. Possible reasons for that include a lack of motivation to quit smoking, as well as other factors, such as depression, low socioeconomic status, and a low level of education.[35] A randomized clinical trial (RCT) involving patients with asthma showed no difference between the use of varenicline and that of placebo in terms of the smoking cessation rate (RR = 1.25; 95% CI: 0.38-4.14).[36]

With the use of NRT, bupropion, or varenicline, as with any other medication, adverse events may occur. Therefore, all patients using medications for smoking cessation should be monitored. Charts 4 and 5 present the first-line medications for smoking cessation and their main characteristics.

Extended and combined treatment

There are still few data in the literature to support the long-term use of varenicline. An open observational

Chart 4. Mechanism of action, absorption, metabolism, presentation, and recommended dosage of first-line smoking cessation drugs.

Medication	Characteristic	
Nicotine replacement therapy	Mechanism of action	Acts on the nicotinic receptors in the nucleus accumbens, in the ventral tegmental area of the central nervous system
	Absorption	Slow: transdermal patch (75% of the dose is absorbed over 24 h), peak plasma concentration in 40 min, serum levels stable after 8-10 h Rapid: gum and lozenges (50% of the dose is absorbed), peak plasma concentration in 20 min
	Metabolism	Nicotine is metabolized into cotinine in the liver; minimal renal elimination in an unaltered form. Only 5% binds to plasma proteins.
	Formulations	Patch[a]: 21, 14, and 7 mg, boxes of 7
		Gum[a]: 2 and 4 mg, boxes of 30
		Lozenges[a]: 2 and 4 mg, blister packs of 4 or boxes of 36
	Standard dosing schedule	Patch: For moderate-to-high dependence (15-20 cigarettes/day): 21 mg/day for 4 weeks, followed by 14 mg/day for 4 weeks and 7 mg/day for 2-4 weeks Gum and lozenges: 2 mg every 1-2 h for 4 weeks, followed by 2 mg every 2-4 h for 4 weeks and 2 mg every 4-8 h for 2-4 weeks. Maximum: 20 per day. Dose can be increased to 4 mg in the first 4 weeks in subjects with high-to-very high dependence
Bupropion	Mechanism of action	Inhibition of reuptake of dopamine, norepinephrine, and serotonin
	Absorption	Rapid by the digestive system, reaching peak plasma concentration in 3 h, remaining high in patients with renal failure
	Metabolism	Half-life of 21 h; metabolized in the liver, mainly by isoenzyme CYP2B6, which can be affected by several drugs; slow release by the kidneys (87%); many drug interactions (see Table 5)
	Formulations	Bupropion hydrochloride tablets, 150 mg; boxes of 30 or 60
	Standard dosing schedule	1 tablet (150 mg) in the morning, after breakfast, for the first 3 days, followed by 1 tablet (150 mg) in the morning and in the afternoon for 12 weeks
Varenicline	Mechanism of action	Partial agonist of α4β2 nicotinic receptors (competes with nicotine for the receptors and releases dopamine) and dopamine reuptake inhibitor
	Absorption	Almost total absorption after oral administration and with high systemic availability; peak at 3 h and steady state at 4 days
	Metabolism	Minimal; renal elimination (92%), excreted in an unaltered form
	Formulations	Varenicline tartrate tablets, 0.5 mg and 1 mg, boxes containing 11 0.5-mg tablets + 154 1-mg tablets
	Standard dosing schedule	1st week: 1 tablet (0.5 mg) per day for 3 days, followed by 1 tablet (0.5 mg) twice daily for 4 days 2nd to 12th week: 1 tablet (1 mg) twice daily

Based on Reichert et al.[12]; Fiore et al.[13]; Jiménez-Ruiz et al.[22]; van Eerd et al.[33]; Cahill et al.[36]; the European Network for Smoking and Tobacco Prevention[37]; Brazilian National Ministry of Health[38]; and the (U.S.) National Comprehensive Cancer Network.[39] [a]Formulations available in Brazil.

study involving patients with severe to very severe COPD who used varenicline for 24 weeks showed that the smoking abstinence rate, as assessed by an intention-to-treat analysis, was 17.7%.[40] However, when studies involving smokers in general were evaluated, pooled data from four RCTs, involving a collective total of 2,170 individuals, indicated that long-term treatment with varenicline was effective (RR = 3.64; 95% CI: 2.1-1-4.72).[32]

To our knowledge, there have been no RCTs examining the use of reduced doses of varenicline in patients with respiratory diseases. There have been only four RCTs, involving a collective total of 1,266 subjects, showing that varenicline is effective even at doses lower than those recommended for the general smoking population (RR = 2.08; 95% CI: 1.56-2.78).[33] A retrospective study of more than 14,000 patients diagnosed with COPD, with or without cardiovascular or psychiatric comorbidities, showed that, in comparison with the use of NRT, the use of bupropion or varenicline did not increase the risk of cardiovascular or neuropsychiatric events within the first six months of treatment.[41]

The combination of two forms of NRT has the same effectiveness as does the use of varenicline (OR = 1.06; 95% CI: 0.75-1.48).[42] A meta-analysis of the pooled data from two RCTs, involving a total of 787 individuals, showed that the combination of varenicline and NRT (the nicotine patch) was more effective than

Chart 5. Mode of use, precautions, adverse effects, contraindications, efficacy, and combinations of first-line smoking cessation drugs.

Medication	Characteristic	Points to consider
Nicotine replacement therapy	Prescription	Standard prescription
	Mode of use	Initiation: recommended to start on the scheduled cessation date; can be used as pre-cessation therapy on a case-by-case basis Patch: apply to the trunk every morning, in hairless areas that are not exposed to the sun; leave in place for 24 h; no need to remove for bathing; rotate sites Gum: chew until tasting the flavor or feeling a tingling sensation; then leave between the gingiva and the cheek; chew again for 20-30 min Lozenge: slowly move it around in the mouth, without sucking, chewing or swallowing; dissolves in the oral cavity after 20-30 min
	Precautions	Avoid citrus drinks and food for 15-30 min after using nicotine gum or lozenges.
	Adverse effects	Patch: erythema and infiltration of the dermis at the site of application, sialorrhea, nausea, vomiting, diarrhea, and insomnia Gum: gingival lesions, sialorrhea, unpleasant taste, dental softening, nausea, vomiting, dyspepsia, hiccups, and pain in the TMJ Lozenges: hiccups, sialorrhea, unpleasant taste, and dyspepsia
	Contraindications	In the presence of cerebrovascular disease or cardiovascular disease that is severe or acute (< 15 days), discuss with the specialist and assess the risk-benefit ratio. Avoid the use of nicotine gum or lozenges in patients with active peptic ulcer or TMJ pain. In patients who are pregnant or breastfeeding, weigh the risk-benefit ratio of a dose lower than that of smoking and opt for gum or lozenges over the patch.
	Efficacy	Relative risk of success = 2.60 (95% CI: 1.29-5.24)
	Combinations	Patch used in combination with nicotine lozenges or gum Addition of bupropion or varenicline in cases that are more difficult
Bupropion	Prescription	Restricted prescription, in duplicate
	Mode of use	Start 7 days before the scheduled cessation date. Take the second tablet in the afternoon, at least 8 h after the first.
	Precautions	If possible, avoid taking the 2nd dose after 5:00 p.m., to reduce the risk of insomnia. Patients with gastritis should use an antacid and take bupropion with food. Patients with uncontrolled hypertension should not take bupropion.
	Adverse effects	Reduced reflexes (poor performance on tasks that require motor skills), dry mouth, insomnia, dizziness, headache, agitation, anxiety, tremors
	Contraindications and drug interactions	Numerous drug interactions Relative: harmful use of alcohol; and the use of carbamazepine, barbiturates, phenytoin, antipsychotics, antidepressants, cimetidine, theophylline, systemic corticosteroids, oral hypoglycemic agents, and insulin Absolute: epilepsy and seizures (including febrile seizures), recent alcohol withdrawal, cerebrovascular disease, bulimia, anorexia nervosa, panic attacks, use of MAO inhibitors in the last 14 days, < 16 years of age, pregnancy, and breastfeeding
	Efficacy	Relative risk of success = 2.03 (95% CI: 1.26-3.28)
	Combinations	Addition of nicotine replacement therapy or varenicline in cases that are more difficult
Varenicline	Prescription	Standard prescription
	Mode of use	Start 7 days before the scheduled cessation date 0.5-mg tablets only for the 1st week, then 1-mg tablets twice daily for 11 weeks
	Precautions	The bioavailability is not affected by eating or by the schedule of administration; it can be taken after meals.
	Adverse effects	Most common: nausea (in 33%, rarely requiring discontinuation); and vivid dreaming Some patients report dizziness, dry mouth, drowsiness, and flatulence. It can be associated with depressive mood, suicidal ideation, and lack of control of psychiatric disorders, although such symptoms can also occur due to withdrawal syndrome.
	Contraindications and drug interactions	Few drug interactions Use with caution in smokers with severe psychiatric disorders (psychotic outbreak, suicidal ideation/attempt, etc.): discuss with the psychiatrist.
	Efficacy	Relative risk of success = 3.35 (95% CI: 1.89-5.92)
	Combinations	Addition of nicotine replacement therapy or bupropion in cases that are more difficult

Based on Reichert et al.[12]; Fiore et al.[13]; Jiménez-Ruiz et al.[22]; van Eerd et al.[33]; Cahill et al.[36]; European Network for Smoking and Tobacco Prevention[37]; Brazilian National Ministry of Health[38]; and the (U.S.) National Comprehensive Cancer Network.[39] TMJ: temporomandibular joint; and MAO: monoamine oxidase.

was the use of varenicline alone (OR = 1.62; 95% CI: 1.18-2.23).[43]

SMOKING AND RESPIRATORY DISEASES

Asthma and smoking

Asthma is a heterogeneous disease, with a variety of phenotypes, that results from complex interactions between environmental and genetic factors. Prenatal and postnatal exposure to environmental tobacco smoke (ETS) is associated with an increased risk of developing asthma-like symptoms in childhood.[44,45]

In a systematic review with meta-analysis,[44] prenatal and postnatal exposure to ETS were found to be associated with a 30-70% increase in the risk of incident wheezing among children ≤ 2 years of age. In that study, postnatal maternal smoking was shown to have a greater effect on the development of wheezing among such children (OR = 1.70; 95% CI: 1.24-2.35), with a 21-85% increase in the risk of incident asthma, whereas prenatal maternal smoking was shown to have a greater effect on the development of asthma (OR = 1.85; 95% CI: 1.35-2.53).

In another systematic review with meta-analysis,[45] prenatal maternal smoking was found to be associated with an increased risk of wheezing in children < 6 years of age (OR = 1.36; 95% CI: 1.19-1.55), as well as with an increased risk of wheezing or asthma in children ≥ 6 years of age (OR = 1.22; 95% CI: 1.03-1.44). One study showed that postnatal exposure to ETS was associated with wheezing in children < 6 years of age (OR = 1.21; 95% CI: 1.13-1.31 and OR = 1.30; 95% CI: 1.13-1.51 for maternal and paternal smoking, respectively), although it was often impossible to separate the role of postnatal exposure from that of prenatal exposure.[46]

In some patient samples, it has been shown that the likelihood of incident asthma increases after smoking cessation.[47-49] In one such sample, continued smoking during follow-up was also found to increase the risk of incident asthma significantly.[47]

To our knowledge, there have as yet been no studies evaluating asthma mortality attributable to smoking or the fraction of asthma attributable to active and passive smoking in Brazil. In 2013 in Brazil, there were more than 120,000 hospitalizations for asthma and 2,047 asthma-related deaths (5 deaths/day).[50] In individuals with asthma, smoking is associated with an accelerated decline in lung function,[51] as well as with a poor response to inhaled and systemic corticosteroids.[52-55] In addition, smokers with asthma have a lower chance of achieving control of the disease, as well as a higher frequency of asthma exacerbations and hospitalizations due to such exacerbations.[56-59]

An association between marijuana smoking and the worsening of asthma symptoms has been recognized since the 1970s.[60-62] Smoking marijuana also exacerbates bronchial asthma and provokes symptoms consistent with asthma.[60-64] Passive exposure to marijuana smoke (inhalation of toxic substances) worsens the symptoms of asthma.[65] Therefore, individuals with asthma or bronchial hyperresponsiveness should avoid active and passive smoking of tobacco or marijuana.

Smoking cessation reduces asthma symptoms and allows better control of the disease.[66,67] There is some evidence to suggest that smokers with asthma are less likely to quit smoking than are those without asthma.[68,69]

In every patient with asthma or bronchial hyperresponsiveness, the physician should inquire about the smoking status. In the counseling sessions, physicians should emphasize the following:

- Individuals with asthma or bronchial hyperresponsiveness should avoid active and passive smoking of tobacco and marijuana.
- Nonsmokers should be advised not to start smoking.
- Tobacco smokers and marijuana smokers should be informed of the difficulties and risks of continuing to smoke those products and should receive support for smoking cessation.

The main benefits of smoking cessation in individuals with asthma include improving asthma control, reducing exacerbations, slowing the functional decline, and improving the therapeutic response.

COPD and smoking

COPD is a multisystem inflammatory disease that results from the interaction between genetic and environmental factors. Although cigarette smoke continues to be the main cause of the disease, there are regions in which the inhalation of smoke from biomass burning, occupational exposure, and air pollution also play relevant roles.[70-74]

The smoking history, which is related to the development, progression, and severity of COPD, is typically expressed in pack-years. However, the duration of smoking in years, in addition to being easier to evaluate, might correlate better with the risk of the development and progression of the disease.[75]

The prevalence of COPD varies depending on the risk factors, functional criteria, and analytical criteria.[76,77] The estimated prevalence of COPD worldwide and in Brazil is 12% and 15.8%, respectively.[70,78] Because age and smoking have a cumulative effect, it is estimated that 50% of smokers will develop the disease during their lifetime.[79,80]

COPD is now the third leading cause of death worldwide. A study conducted in the capital cities of Brazil found that 65% of the deaths attributable to smoking are in individuals with COPD, ischemic heart disease, lung cancer, or cerebrovascular disease.[81] In another study, comparing mortality rates in Brazil between 1990 and 2015,[82] there was a 31% reduction in the rate of mortality from COPD (from 64.5 to 44.5/100,000 inhabitants), compared with a reduction of only 2.1% in the rate of mortality from lung cancer. In that same period, there was a 36.1%

reduction in the number of years of life lost due to death or disability.

Patients with COPD tend to conceal the fact that they still smoke from their physicians, even when their cotinine or exhaled carbon monoxide level belies their self-reported smoking status.[83] Smokers with COPD have a greater smoking intensity and a higher level of nicotine dependence, requiring more guidance on the risks of and need to quit smoking, than do smokers without COPD.[20,84]

The level of motivation to quit smoking differs little between patients with and without COPD. However, self-efficacy is lower in smokers with COPD, partly because of the high prevalence of anxiety and depression among such individuals.[20,70,84] In a sample of smokers with COPD in Denmark, the factors related to the lower chance of smoking cessation were being < 65 years of age; having mild airflow obstruction; being classified as being in Global Initiative for Chronic Obstructive Lung Disease risk group A; scoring low on the Medical Research Council scale; and having a low socioeconomic status.[85] In that sample, depression was not found to worsen the smoking cessation rate, as was shown in another study.[85]

Smoking cessation is the only intervention that alters the natural history of COPD.[20,70,71] Smokers with COPD are more likely to be questioned about their smoking, receiving more guidance and treatment, than are those without COPD. However, when they are not encouraged to quit smoking or referred to smoking cessation programs, they maintain their smoking habits almost unchanged for several years.[87] Chart 6 summarizes the treatment recommendations and benefits of smoking cessation in patients with COPD.

Lung cancer and smoking

Cigarette smoke contains more than 7,000 compounds.[93] According to the International Agency for Research on Cancer, more than 60 of those compounds have been shown to be carcinogenic in laboratory animals, and there is sufficient evidence that 12 of those are carcinogenic to humans.[94,95] There is a strong correlation between lifetime smoking and genetic changes (DNA methylation and microRNA changes) leading to inactivation of tumor suppressor mechanisms.[71,96-98] Active and passive smoking are responsible for more than 90% of lung cancer cases, with a direct correlation between pack-years of smoking and an increased risk of cancer.[71,97]

Studies conducted in Brazil have shown that the incidence and mortality rates of smoking-related cancer, particularly in the lungs, oral cavity, and larynx, are high in the country.[99,100] A study evaluating the proportion of cancer cases attributed to modifiable risk factors in Brazil estimated that, by 2020, the proportion of cases of lung cancer attributable to smoking will be 83.28% in men and 64.80% in women.[100,101]

In a systematic review of studies involving patients with early-stage lung cancer who continued to smoke,[102] continued smoking among those with non-small cell lung cancer was found to increase the risk of relapse (RR = 1.86; 95% CI: 1.01-3.41) and all-cause mortality (RR = 2.94; 95% CI: 1.15-7.54). In patients with limited small cell lung cancer who continued to smoke, there were also increases in the risk of relapse (RR = 1.26; 95% CI: 1.06-1.50), a second primary tumor (RR = 4.31; 95% CI: 1.09-16.98), and all-cause mortality (RR = 1.86; 95% CI: 1.33-2.59). Among the patients with non-small cell lung cancer ≥ 65 years of age, the authors found that the survival rate was 33% for those who continued to smoke and 70% for those who had quit smoking. The 5-year survival rate in the small cell tumor group was 29% among those who continued to smoke and 63% among those who had quit.

Smokers with cancer live with the pressure to quit smoking exerted by their physician and their family, as well as internal pressure to do so, blaming themselves

Chart 6. Recommendations for the approach to smoking cessation and benefits of cessation in patients with COPD.

Description	Recommendations
Consultation	All patients with COPD should be asked if they smoke and, if so, should be encouraged to stop smoking and referred to a smoking cessation program.
Start of treatment	Smoking cessation treatment alters the natural course of COPD. Begin smoking cessation treatment as part of COPD treatment.
Treatment strategy	Combining behavioral counseling with first-line drugs (nicotine replacement therapy, bupropion, and varenicline) is the most effective approach. Patients who have more difficulty in quitting smoking can benefit from the use of more than one drug or high-dose nicotine patches.
Benefits of smoking cessation	In patients with COPD, the benefits vary depending on age, severity, and comorbidities. Main benefits: Slows the progressive decline in FEV_1 Reduces exacerbation and hospitalization rates Minimizes respiratory symptoms and improves quality of life Reduces the limitations in activities of daily living Improves the ability to perform activities of daily living Improves the control of comorbidities Improves the response to bronchodilators and inhaled corticosteroids

Based on Jiménez-Ruiz et al.[20]; Jiménez-Ruiz et al.[22] van Eerd et al.[33]; the Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease[70]; the U.S. Department of Health and Human Services[71]; Bai et al.[88]; Tonnesen et al.[89]; Godtfredsen et al.[90]; Anthonisen et al.[91]; and Anthonisen et al.[92]

for the development of the disease, although most such smokers want to quit. The threats of physical pain, psychological suffering, and death, as well as future challenges, hamper the decision-making process of some patients and motivate others to quit smoking. The distorted thought of it being too late to quit smoking also hampers that process.[103]

A patient can oscillate between moments of high and low self-efficacy to quit smoking. That requires the physician to be sensitivity to the difference between those two states and to promote an individualized approach focusing on the benefits of smoking cessation, which will promote better treatment outcomes.[103-105] Approximately 50% of all smokers diagnosed with cancer continue to smoke.[106] Smokers with lung cancer are more motivated to quit smoking than are those in the general population, although they can require care that is more intensive and comprehensive.[103]

Smoking is an independent prognostic factor for lung cancer, the only one that is under the direct control of the patient. Although smoking abstinence rates are high after diagnosis, relapse rates are also high. Treatment and prevention of relapse are imperative since the first visit.[103] Chart 7 summarizes the key recommendations in the approach to smoking cessation treatment in patients with lung cancer, as well as emphasizing the benefits of cessation during cancer treatment.

Tuberculosis and smoking

In 2016, there were 10.4 million new cases of tuberculosis and 1.7 million tuberculosis-related deaths, more than 95% of which occurred in low- and middle-income countries.[110] Brazil is among the 22 countries that account for 80% of all cases of tuberculosis worldwide. According to the Brazilian National Ministry of Health, there were 4,374 tuberculosis-related deaths in Brazil in 2014.[111] Tuberculosis and smoking are both more common in low- and middle-income countries. According to the World Health Organization, more than 20% of all new cases of tuberculosis can be attributed to smoking.[112]

Smoking was identified as a risk factor for tuberculosis a century ago.[113] In the last decade, several studies have demonstrated that, even after adjustment for other risk factors, there is a significant association between exposure to tobacco smoke and tuberculosis.[114] There is evidence that active and passive smoking are associated with active tuberculosis, as well as with the treatment response, relapse, and tuberculosis-related mortality.[71,112,114-116] These effects appear to be independent of other risk factors for tuberculosis, such as alcoholism and socioeconomic conditions.[71,112,114]

Tobacco smoke impairs the pulmonary defense mechanisms by effecting structural changes, cellular changes, and an altered immune response. Smoking

Chart 7. Recommendations for the approach to smoking cessation and benefits of cessation in patients with lung cancer.

Description	Recommendation
Consultation	All patients should be asked if they smoke.
Chart	Insert a category for smoking status in the patient chart, as one more vital sign.[a]
Start of treatment	Initiating treatment in the preoperative period increases cessation rates. Smoking cessation treatment should be integrated into the cancer treatment strategy.
Treatment strategy	Counseling alone is indicated only when pharmacotherapy is contraindicated or refused by the patient. Pharmacotherapy is usually similar to that used in the general population. First-line pharmacotherapy, in combination with counseling, is cost-effective and should be offered to all patients who smoke, including those in follow-up treatment. Patients undergoing chemotherapy or radiotherapy should receive counseling and pharmacotherapy.
Nicotine replacement therapy	Pre-cessation nicotine replacement therapy and counseling provide the best results. Consider using combined therapy, extended therapy, and higher doses of nicotine replacement therapy. Patch: its use is inadvisable in patients with graft-versus-host disease. Gum/lozenges: can accentuate mucositis after chemotherapy.
Bupropion	Its use is indicated in patients with depressive symptoms. It can inactivate tamoxifen and is contraindicated in patients with metastases in the central nervous system.
Varenicline	It can exacerbate the nausea caused by chemotherapy.
Benefits of smoking cessation	Greater chances of survival; a lower risk of relapse; a lower incidence of a second smoking-related primary tumor Better treatment efficacy and response; improved quality of life and pain control; and higher self-esteem Lower risk of surgical complications (pulmonary embolism, suture dehiscence, and infections), as well as of complications of radiotherapy and chemotherapy Lower risk of developing or worsening of other smoking-related diseases

Based on Álvares et al.[98]; Cinciripini et al.[105]; Gritz et al. 2014[106]; the American Society of Clinical Oncology[107]; Shields et al.[108]; and Koshiaris et al.[109] [a]Increases the rate at which physicians and other health professionals intervene for smoking cessation.

disrupts the integrity of the airway epithelium, alters mucociliary clearance, and reduces the phagocytic capacity of alveolar macrophages, which increases the likelihood that *Mycobacterium tuberculosis* will reach the alveoli, where tuberculosis infection begins.[71,117-121] Some studies suggest that increased susceptibility to pulmonary tuberculosis is due to reductions in circulating immunoglobulin levels and in the CD4/CD8 ratio, both of which are caused by exposure to tobacco smoke.[71,122-126]

Passive smoking increases both the risk of tuberculosis infection and the occurrence of active tuberculosis, especially in children. That risk is increased up to nine-fold in individuals under 15 years of age, even those who have had no contact with pulmonary tuberculosis in the home. There is a strong dose-response relationship between the risk of tuberculosis and the volume of tobacco smoke (number of cigarettes) to which children are exposed per day.[127,128] Nonsmokers exposed to tobacco smoke in closed spaces are also at an increased risk of developing pulmonary tuberculosis.[129] Among individuals with tuberculosis, those who smoke are at an increased risk of the most severe clinical manifestations, mortality, a delay in achieving sputum negativity, treatment failure, tuberculosis relapse, resistance to antituberculosis drugs, cavitary lesions, greater sputum positivity, and sequelae that are more extensive.[71,114,115,124,130-133]

Among individuals with active tuberculosis who have no previous history of tuberculosis, the risk of death from tuberculosis is nine times higher in smokers than in those who have never smoked.[134]

Smoking control is an important strategy to reduce the number of individuals infected with *M. tuberculosis* and that of those who will develop the disease. Therefore, it is essential to advise smokers with tuberculosis to quit smoking and to support them in that endeavor. When smokers with tuberculosis quit smoking, they reduce their risk of death from tuberculosis by approximately 65% in comparison with those who continue to smoke.[134] The World Health Organization recommends that tuberculosis control programs work in an integrated way with anti-smoking programs, so that smoking cessation treatment is offered to every patient with tuberculosis.[112,114,115]

Environmental respiratory diseases and smoking

Smoking, environmental air pollution, and indoor air pollution are risk factors for mortality, respectively accounting for 7.13, 4.3, and 2.6 million deaths/year, ranking second, sixth, and eighth, worldwide.[135] In Brazil, environmental air pollution ranks eleventh among mortality risk factors.

Diseases related to exposure to particulate matter, gases, and carcinogens occur due to oxidative stress, pulmonary inflammation, systemic inflammation, and DNA damage. The main diseases related to exposure to environmental pollutants are ischemic cardiovascular diseases, neoplasms, COPD, and respiratory infections.[137]

According to the World Health Organization estimates for 2016, environmental air pollution and indoor air pollution in Brazil were respectively responsible for 51,800 and 14,100 deaths, with standardized mortality rates of 24/100,000 population and 7/100,000 population.[138] One group of authors, estimating the level of inhaled particulate matter at 12 mg/cigarette and the exposure to air pollution at 13-30 μm^3 of air/day, concluded that those exposures increase the risk for lung cancer, cardiovascular disease, and lung disease in general.[139]

In one study, lung function values were found to be lower in women (smokers and nonsmokers) who lived in environments with high levels of pollution than in those who lived in environments with lower levels of pollution.[140] The authors also found that exposure of smokers to ETS increases their risk of lung cancer, showing an additive effect.

No differences have been identified among the various profiles of smokers in terms of the risk of diseases related to air pollution. Although the isolated impact of smoking cessation in individuals exposed to pollution is not yet known, the ban on smoking indoors has had a significant impact on reducing morbidity and mortality. A meta-analysis of 44 studies showed significant reductions in the rates of hospitalization and death from respiratory diseases.[141] This suggests that people living in the same city, without changes in pollution levels, could benefit from reduced ETS exposure. The same holds true for the impact of smoking cessation on the reduction in the incidence of respiratory diseases observed in cohort studies.[142]

Occupational respiratory diseases and smoking

The combination of exposure to tobacco smoke and occupational exposure to pollutants is associated with deleterious and sometimes synergistic effects, potentiating injuries to the airways and the pulmonary interstitium. Inhibition of mucociliary clearance and other changes in the airways result in increased retention of inhaled particles, as well as facilitating damage from the inhalation of gases, mists, or chemical vapors. The pro-inflammatory nature and DNA toxicity of both exposures can increase the risk of becoming ill.[97,143,144]

Occupational exposure to pollutants is a major risk factor for morbidity and mortality worldwide, the various occupational risks collectively estimated to be responsible for 1.53 million deaths/year and 75.93 million years of life lost to death or disability.[145] According to data on the global burden of diseases in 2016, exposure to ETS in the workplace alone was associated with 433,200 deaths, having the greatest impact on mortality from ischemic cardiovascular diseases (252,000 deaths), followed by mortality from COPD (52,000 deaths) and mortality from lung cancer (44,400 deaths).[146]

The prevalence of smoking among workers varies depending on the type of company and its activity, being lower among workers in the health and education sectors, whereas it is higher among those in the industrial, janitorial, mining, and construction sectors.[147] In the latter groups of workers, the prevalence is higher than in the general population, in several countries: 53.2% vs. 40.2% in China[148]; 66.3% vs. 43.0% in Turkey[149]; and 49.8% vs. 15.1% in Brazil.[150] Those high rates reveal the impact of smoking among the less educated and those with lower incomes, who make up the workforce that engages in insalubrious activities or is exposed to greater occupational risks.

Smoking cessation reduces the risk of morbidity and mortality, an earlier age at cessation translating to a greater benefit.[151] It is recommended that the occupational health departments of companies develop policies to encourage smokers and their families to quit smoking and to support them in that endeavor, from the hire date onward. Providing treatment to smokers should be incorporated into the routine at primary health care clinics and in companies.[152]

Conducting research with brief questionnaires to monitor the health/illness relationship not only helps identify the need for interventions in the workplace but also can create important motivational moments, thus increasing the chance of success in smoking cessation. Chief among the various motivating factors for quitting smoking presented by groups of workers who are more exposed and more socially vulnerable are the presence of respiratory symptoms and established respiratory disease.[149]

Interstitial respiratory diseases and smoking

In recent years, concern about the harmful effects of smoking has begun to focus on the development of interstitial lung diseases (ILDs). Among such diseases, three are considered to be etiologically related to smoking[153]: ILD accompanied by respiratory bronchiolitis; desquamative interstitial pneumonia; and pulmonary Langerhans cell histiocytosis.

Certain interstitial diseases are more likely to develop in smokers, such as idiopathic pulmonary fibrosis and rheumatoid arthritis-related ILD.[153] Some individuals also develop a combination of pulmonary fibrosis and emphysema. That combination is considered a distinct phenotype of idiopathic pulmonary fibrosis.[154]

It is important to identify the role of smoking in ILD, because understanding the pathogenic pathways could allow the development of new medications. From a clinical point of view, the recognition of a smoking-related phenotype would facilitate early diagnosis and treatment.[155]

One group of authors analyzed four prospective cohort studies, involving a collective total of 11,691 participants, and assessed mortality in subjects with interstitial lung abnormalities.[156] The abnormalities were associated with greater smoking intensity in two of those studies and with higher mortality rates in all four.

Although smoking-related ILDs are less well recognized, there is a well-defined causal correlation, based on epidemiological data, between smoking and the development of an ILD. Smoking cessation is the primary therapy for the control of such ILDs, and the approach requires perfect integration of clinical, functional, radiological, and histopathological data.

Infectious respiratory diseases and smoking

Deposition of the toxic constituents of tobacco smoke in the airways affects the pulmonary defense mechanisms in multiple ways[157]: by impairing mucociliary transport; by increasing bacterial adherence to the respiratory epithelium; and by increasing alveolar and epithelial vascular permeability. In addition, continued exposure to tobacco smoke is associated with significant changes in the nasopharyngeal microflora, which favors colonization by opportunistic pathogens.[158]

One experimental study in mice demonstrated that chronic exposure to ETS increased levels of inflammatory cytokines and TNF-α in the lungs, as well as impairing adaptive immunity, after chronic infection or intranasal immunization with the recombinant P6 protein of *Haemophilus influenzae*.[159] The authors concluded that there is unequivocal evidence that exposure to ETS has long-term effects that are detrimental to the lung microenvironment (promoting inflammation), as well as impairing immunity to infection and the response to vaccination.

In one systematic review,[160] the risk of contracting bacterial pneumonia was shown to be higher in smokers than in former smokers (hazard ratio = 1.37; 95% CI: 1.06-1.78) and nonsmokers (hazard ratio = 1.73; 95% CI: 1.44-2.06). Pneumonia and influenza increase the risk of morbidity and mortality. When the individual is a smoker, the social, medical, and pension costs are further increased. A 2017 study showed that pneumonia is the third leading smoking-related illness in Brazil.[2] Chart 8 summarizes the scientific evidence regarding the exposure and risks of smokers.

Smoking among inpatients with respiratory diseases

The reported prevalence of smoking among hospitalized patients in Brazil ranges from 15% to 22%.[167,168] In one study,[169] the authors found a 25% prevalence of smoking among inpatients at a smoke-free hospital, and 55% of the patients who were smokers experienced withdrawal symptoms during their hospital stay. In a systematic review,[170] the prevalence of smoking among inpatients was found to range from 15% to 27%.

Hospitalization creates a window of opportunity to initiate anti-smoking measures with a high chance of success, especially if there is follow-up after hospital discharge.[12,169] Addressing the issue of smoking among inpatients who are smokers should be part of

Chart 8. Recommendations for the approach to smoking cessation, benefits of cessation, and risks in patients with infectious respiratory diseases.

Description	Recommendation
Consultation	Patients should be encouraged to stop smoking and should be referred to a smoking cessation program.
Risks of active smoking	High risk of varicella-zoster virus pneumonitis Two times higher risk of influenza, with worse clinical evolution Four times higher risk of pneumonia in patients with COPD Higher risk of pneumonia in HIV-infected patients Two times higher risk of community-acquired pneumonia Smoking is an aggravating factor for other respiratory infections
Risks of passive smoking	Exposure to environmental tobacco smoke is associated with *Mycobacterium tuberculosis* infection and active pulmonary tuberculosis
Treatment strategy	Combine behavioral counseling with first-line medications
Benefits of smoking cessation	Reduces the risk of respiratory infections in active and passive smokers, especially children

Based on Feldman,[161] Murin et al.,[162] Lipsky et al.,[163] Wewers et al.,[164] Correa et al.,[165] and Bates et al.[166]

the hospital routine. Although such patients are often highly motivated and amenable, only a minority receive smoking cessation treatment and most of those relapse after hospital discharge.[12,169]

The recommended course of action in the approach to inpatients who are smokers is to offer counseling during hospitalization and follow-up for at least four weeks after discharge, either in person or by telephone. In a meta-analysis of 50 studies,[171] it was concluded that intensive approaches with follow-up after discharge were more effective, because relapse typically occurs during the first month after discharge. Identifying the level of craving and other factors that indicate a greater chance of failure, such as dependence on alcohol or other drugs, allows the treatment of inpatients at higher risk of relapse to be individualized.

In a study evaluating the efficacy of a smoking cessation program for patients hospitalized for respiratory disease or heart disease, 31% of the patients with respiratory diseases were reported to be abstinent at six months after discharge.[172] Patients receiving individual counseling and medication for smoking cessation during hospitalization and after discharge showed greater adherence to the treatment, even after hospital discharge.[173]

Hospital admission should be transformed into an opportunity for smoking cessation. Smokers hospitalized for respiratory diseases should be advised of the benefits of quitting smoking, as well as being assessed in terms of their level of motivation and level of nicotine dependence in order to receive specific treatment, which is similar to that recommended for patients with other conditions. Smoking cessation programs involving teams trained in dealing with smokers have good cost-effectiveness ratios.[174] Follow-up for at least six months after discharge improves outcomes and increases the chance of successful abstinence.[170,175,176] Intensive intervention combined with pharmacological treatment for smoking cessation in patients with respiratory disease, started during hospitalization, is effective, with a high level of evidence.[22]

FINAL CONSIDERATIONS

All patients with respiratory diseases should be asked if they smoke. If so, they should be encouraged to quit smoking and referred for smoking cessation treatment, regardless of their age and disease stage. Smoking cessation treatment, based on cognitive-behavioral therapy and pharmacotherapy, is the first measure to be taken in the treatment of lung diseases and has major benefits: fewer exacerbations and hospitalizations; a reduction in respiratory symptoms; an improvement in quality of life; fewer limitations in activities of daily living; better control of comorbidities; improved response to bronchodilators and inhaled corticosteroids; greater chances of survival; a lower risk of relapse; a lower incidence of a second smoking-related primary tumor; better pain control; improved self-esteem; and a lower risk of complications from surgery, radiotherapy, and chemotherapy.

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Endobronchial ultrasound in esophageal cancer - when upper gastrointestinal endoscopy is not enough

Lília Maia Santos[1,2,a], Márcia Jacomelli[1,b], Paulo Rogério Scordamaglio[1,c], Paulo Francisco Guerreiro Cardoso[3,d], Viviane Rossi Figueiredo[1,e]

TO THE EDITOR:

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) and endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) are minimally invasive, safe, and accurate techniques for sampling hilar and mediastinal lymph nodes and masses.[1] They are considered to be complementary techniques, and the combination of the two can reach virtually all mediastinal nodal stations, being highly accurate for lung and esophageal cancer staging.[1,2]

Esophageal cancer is the sixth leading cause of cancer-related death worldwide, and upper gastrointestinal endoscopy (UGIE) is the gold standard for its diagnosis.[3] Although EUS-FNA is primarily used for locoregional staging of esophageal cancer, being the gold standard method for this purpose, it can be used for diagnosing esophageal cancer in cases in which there is a high suspicion of esophageal malignancy and UGIE does not provide a definitive diagnosis.[2-4] Because EUS-FNA and UGIE are both performed through the esophagus, they can be technically challenging in the presence of a malignant stricture that obliterates the esophageal lumen. Given the proximity between the esophagus and the tracheobronchial tree, EBUS-TBNA can be an alternative diagnostic technique in such cases. Here, we report a case in which EBUS-TBNA was used for diagnosing esophageal carcinoma.

A 60-year-old woman presented with a one month-history of odynophagia, progressive dysphagia, and weight loss (of 10 kg). A CT scan of the neck and chest showed nonspecific thickening and mild heterogeneous enhancement at the pharyngoesophageal junction (Figure 1A). UGIE showed a vegetative lesion that partially obstructed the esophageal lumen and prevented the progression of the endoscope. A biopsy of the lesion showed low-grade squamous epithelial dysplasia. Flexible bronchoscopy showed no endobronchial lesions or signs of extrinsic compression. A positron emission tomography scan revealed circumferential thickening of the cervical esophagus leading to narrowing of the esophageal lumen (standardized uptake value, 9.6), as well as nonspecific subcentimetric mediastinal lymph nodes (subcarinal lymph node standardized uptake value, 2.2), together with no signs of invasion into the adjacent structures (Figure 1B).

Because of the proximity of the lesion to the upper esophageal sphincter and because esophageal stenosis prevented the passage of the endoscope for a second biopsy, an EBUS-TBNA was performed; EBUS showed a heterogeneous lesion with well-defined margins and no vessels at the subglottic level (Figure 1C), and 22-gauge TBNA findings were consistent with squamous cell carcinoma of the esophagus.

The clinical hallmarks of esophageal cancer are progressive dysphagia and weight loss.[3,4] However, patients with esophageal cancer often present with few or no symptoms; dysphagia occurs only when two thirds of the esophageal lumen are involved, which is why diagnosis is often delayed and prognosis is poor.[3-5]

Esophageal cancer can present as a flat, subtle area or as a lumen-obscuring mass.[3] UGIE allows detailed tumor analysis and tissue sampling.[3,4] At least seven biopsies should be performed in the suspected area for adequate sampling, brushings alone being inadequate.[3,4] When UGIE fails to provide a definitive diagnosis, EUS-FNA should be considered.[4] However, EUS-FNA probes are typically large and can therefore be a problem in the presence of a malignant stricture.[4] In addition, although local extension to lymph nodes and esophageal wall invasion are best investigated by EUS-FNA,[2,4] narrowing of the esophageal lumen can prevent the progression of the endoscope in up to 30% of cases,[5] constituting an obstacle to diagnosis and staging by preventing the collection of representative biopsy material through the esophageal lumen. In such cases, EBUS-TBNA can be performed either through the esophagus (because EBUS-TBNA probes are smaller in diameter) or through the tracheobronchial tree, constituting an alternative approach.[2,5]

EBUS-TBNA is indicated for the diagnosis of mediastinal lesions that are adjacent to or in direct contact with the tracheobronchial tree, allowing visualization of mediastinal structures such as lymph nodes, vessels, and the esophagus itself.[1] In addition to mediastinal staging of lung cancer, EBUS-TBNA has been described for esophageal cancer staging and as a diagnostic method for centrally located lung cancer and benign/malignant mediastinal and hilar lymphadenopathy.[1,2]

1. Serviço de Endoscopia Respiratória, Disciplina de Pneumologia, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
2. Serviço de Pneumologia, Hospitais da Universidade de Coimbra, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal.
3. Departamento de Cardiopneumologia, Disciplina de Cirurgia Torácica, Instituto do Coração – InCor – Hospital das Clínicas, Faculdade de Medicina, Universidade de São Paulo, São Paulo (SP) Brasil.
a. http://orcid.org/0000-0003-3215-4701; b. http://orcid.org/0000-0001-8657-458X; c. http://orcid.org/0000-0001-8971-5333;
d. http://orcid.org/0000-0003-0749-4165; e. http://orcid.org/0000-0002-9458-8153

Garrido et al. described the use of EBUS alone (i.e., without biopsy) for assessing tracheobronchial invasion in patients with malignant esophageal stricture.[5] EBUS has been shown to be highly specific and sensitive for detecting tracheobronchial invasion, because it allows high-resolution evaluation of the outermost layer of the tracheobronchial tree.[4] The use of EBUS-TBNA in combination with EUS-FNA has been shown to improve the staging of esophageal cancer by sampling of lymph nodes that are poorly assessed or cannot be assessed by EUS-FNA, including peritumoral lymph nodes.[1,2] EBUS-TBNA contributes to esophageal cancer staging by allowing evaluation of right paratracheal lymph nodes—which are often difficult to visualize with EUS-FNA because of air interposition from the trachea—and hilar lymph nodes that cannot be reached by EUS-FNA.[1,2] When the peritumoral lymph nodes are sampled by EUS-FNA, there is a risk of specimen contamination secondary to piercing the primary tumor; this can be avoided with EBUS-TBNA performed through the tracheobronchial tree.[2]

To our knowledge, there have been no data on the usefulness of EBUS-TBNA for the diagnosis of esophageal cancer by means of sampling performed through the tracheobronchial tree. Our study adds to the literature by reporting on a patient in whom malignant esophageal stricture prevented the collection of representative biopsy material through the esophageal lumen, a definitive diagnosis of primary esophageal cancer being made by EBUS-TBNA performed through the tracheobronchial tree. Given the proximity between the tracheobronchial tree and the esophagus, collaboration between the gastrointestinal endoscopist and the bronchoscopist is intuitive and desired in order to improve diagnostic accuracy.

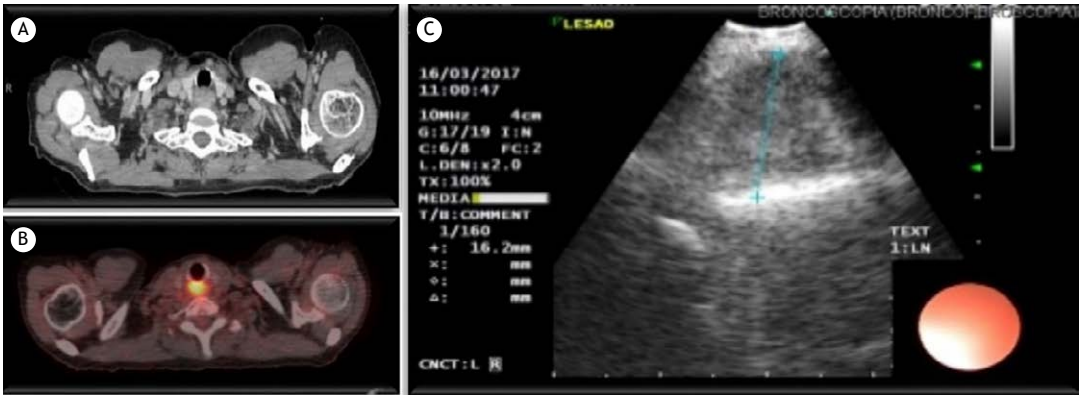

Figure 1. In A, CT scan of the neck and chest showing nonspecific thickening and mild heterogeneous enhancement at the pharyngoesophageal junction. In B, positron emission tomography scan showing circumferential thickening of the cervical esophagus leading to narrowing of the esophageal lumen (standardized uptake value, 9.6). Note the absence of signs of invasion into the adjacent structures. In C, endobronchial ultrasound showing a heterogeneous lesion with well-defined margins and no vessels at the subglottic level, where endobronchial ultrasound-guided transtracheal needle aspiration was performed.

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Lung cancer and parenchymal lung disease in a patient with neurofibromatosis type 1

Alessandro Severo Alves de Melo[1,a], Sérgio Ferreira Alves Jr[2,b], Paulo de Moraes Antunes[1,c], Gláucia Zanetti[2,d], Edson Marchiori[2,e]

TO THE EDITOR:

A 63-year-old nonsmoking female patient with neurofibromatosis type 1 (NF1) was referred to our hospital with a 2-month history of cough and a chest X-ray finding of an opacity in the right lung (Figure 1A). Physical examination revealed multiple cutaneous neurofibromas and *café-au-lait* spots primarily on the trunk. A CT scan of the chest showed a spiculated nodule in the right upper lobe (Figure 1B), as well as mediastinal and right hilar lymph node enlargement, together with cystic and emphysematous changes in the lung parenchyma and skin nodules (neurofibromas) on the chest wall (Figures 1B, 1C, and 1D). Hypodense nodular lesions (metastases) were observed in the liver. Alpha-1 antitrypsin levels were normal. A transbronchial lung biopsy was performed, with histopathological findings of adenocarcinoma. A diagnosis of pulmonary adenocarcinoma with neurofibromatosis-associated diffuse lung disease was established, and the patient underwent chemotherapy. Her condition worsened, and she died 5 months later.

The most common pulmonary manifestations of NF1 are diffuse interstitial lung disease and emphysematous, cystic, and bullous changes in the lung, with a prevalence rate of 10-20%. In addition, NF1 can increase lung sensitivity to cigarette smoke, leading to early emphysema-like changes. Therefore, in addition to being a risk factor for lung cancer, smoking is a potential risk factor for interstitial lung disease in NF1 patients.[1,2] Although NF1 is the most common inherited syndrome predisposing to neoplasia, particularly neural crest-derived tumors, it is not commonly reported in association with lung cancer.[3]

Two major hypotheses have been proposed to explain the association between NF1 and lung cancer.[1] One hypothesis is related to the development of tumors from previous scar tissue or bullae secondary to interstitial fibrosis.[1] The other hypothesis is related to chromosome 17p deletions, the prevalence of which is increased in certain NF1 patients.[1,4] It should be noted that the p53 tumor suppressor gene is located on the short arm of chromosome 17.[1,4] Inactivation of p53 has been implicated in the development of small cell lung cancer in patients with NF1, and p53 mutations have been found in approximately 50% of patients with non-small cell lung cancer.[1,4] This increased risk of lung cancer in never smokers with NF1, as was the case with our

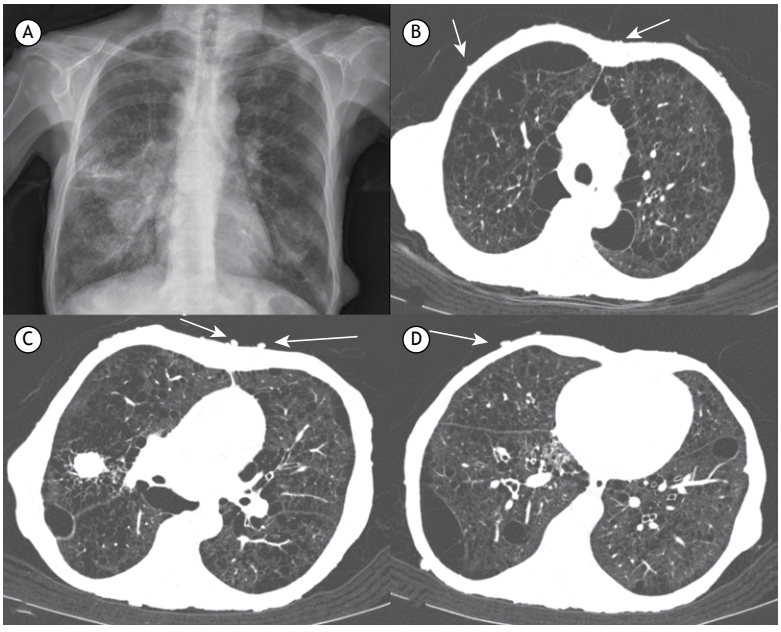

Figure 1. In A, chest X-ray showing an ill-defined opacity in the right lung and mediastinal enlargement, suggestive of lymph node enlargement. Note soft-tissue nodules on the chest wall and shoulders. In B, C, and D, axial CT images of the chest (lung window settings) showing cystic and emphysematous changes predominantly in the upper lobes, as well as a solid nodule with spiculated margins in the right upper lobe. Note nodules (neurofibromas) on the chest wall (arrows).

1. Universidade Federal Fluminense, Rio de Janeiro (RJ) Brasil.
2. Universidade Federal do Rio de Janeiro, Rio de Janeiro (RJ) Brasil.
a. http://orcid.org/0000-0002-9536-6608; b. http://orcid.org/0000-0001-6249-450X; c. http://orcid.org/0000-0001-5070-7053;
d. http://orcid.org/0000-0003-0261-1860; e. http://orcid.org/0000-0001-8797-7380

patient, can be further compounded by tobacco use; studies have demonstrated a higher frequency of p53 mutations in smokers than in never smokers.[1] Because our patient was a never smoker, her case highlights the role of NF1 as a risk factor for the development of lung cancer and the need for assessing pulmonary involvement in patients with NF1, especially those with known interstitial lung disease, by means of low-dose chest CT performed at long intervals for early detection of lung cancer.

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J Bras Pneumol. 2019;45(3):e20180062
http://dx.doi.org/10.1590/1806-3713/e20180062

False-negative newborn screening result for immunoreactive trypsinogen: a major problem in children with chronic lung disease

Magali Santos Lumertz[1,a], Thaiane Rispoli[2,b], Katiana Murieli da Rosa[3,c], Leonardo Araújo Pinto[3,4,d]

TO THE EDITOR:

For patients with cystic fibrosis (CF), specialized multidisciplinary care is important, as is early diagnosis to prevent or delay CF-related complications. Therefore, the CF newborn screening test for immunoreactive trypsinogen (IRT) is essential.[1] Because many patients are asymptomatic when the test is performed, prophylactic and therapeutic interventions can be implemented in a timely manner, and this increases their efficacy.[2,3] However, false-positive and false-negative results can occur, the latter being less common than the former. Both are undesirable for screening and ideally should not occur. Factors associated with false-positive results are more commonly reported in the literature than are those associated with false-negative results; in addition, an increased number of follow-up studies have examined the potential psychosocial impact of false-positive results on children and their families, showing no persistent psychosocial harm despite immediate distress following notification of the positive result.[4] False-negative results can occur in newborns with meconium ileus (MI), which is strongly associated with CF, as well as in those in whom a high cut-off point is used and in those in whom there is a delay between the date of birth and the date of dried blood spot (DBS) sample collection; in addition, false-negative results can be attributed to laboratory errors, including inefficient elution of DBS samples on filter paper.[5,6]

At our multidisciplinary tertiary care center for CF we identified four patients whose IRT test results were interpreted as false negative because they were below the cut-off point used in the laboratory in which the tests were performed. The characteristics of the patients are summarized in Table 1. All four were male and White. Two had had MI at birth and therefore required a temporary ileostomy. Their IRT levels were 98.7 ng/mL (DBS samples having been collected in the first week of life) and 88.5 ng/mL (IRT testing having been performed at around the age of one month), the laboratory reference value being 110 ng/mL. The two patients underwent sweat testing and genotyping, a diagnosis of CF being established before neonatal discharge. A third patient underwent newborn screening at the age of 14 days. However, the IRT test for CF was not performed because it was not part of the routine newborn screening panel performed in the state public health system at the time. Because of recurrent respiratory infections requiring hospitalization in the first year of life and because of subnormal weight and height at around the age of six months, the attending physician requested an expanded newborn screening panel including IRT testing, the results of which were reported as normal. Intestinal obstruction requiring laparotomy, together with the aforementioned clinical changes, again raised the suspicion of CF. The patient underwent sweat testing, which confirmed the diagnosis of CF, specialized outpatient treatment being initiated. In addition to having been colonized with *Staphylococcus aureus*, the patient had positive cultures for *Pseudomonas aeruginosa*, which became negative after *P. aeruginosa* eradication therapy. A fourth patient underwent newborn screening at the age of six days, his IRT level being 39.4 ng/mL (laboratory reference value, 110 ng/mL). Because of growth deficit, as well as recurrent steatorrhea, cough, and wheezing, the patient underwent sweat testing (at the age of three years), his sweat chloride level being 118 mmol/L in a 231-g sample.

In countries where CF newborn screening is performed,[7] different protocols are used, all of which have advantages and disadvantages. In Brazil, in the states in which CF newborn screening is routinely performed, a diagnostic test (usually a sweat test) is performed in patients whose IRT levels are measured twice and found to be elevated on both measurements (the IRT/IRT protocol). In the state of Rio Grande do Sul, IRT measurement was officially added to the routine newborn screening panel in June of 2012. In developed countries, CF newborn screening protocols include genetic testing for common mutations in the *CFTR* gene, which encodes the cystic fibrosis transmembrane conductance regulator protein, and *CFTR* gene sequencing (the IRT/DNA protocol).[8] In a study conducted in France, the IRT/DNA protocol and the IRT/pancreatitis-associated protein protocol were compared in terms of their performance in CF newborn screening, the latter being found to be not inferior to the former in screening for CF.[9]

Although the CF newborn screening test for IRT identifies 95-99% of newborns with CF (depending on the screening protocol used), false-negative results can

1. Hospital São Lucas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (RS) Brasil.
2. Programa de Pós-Graduação em Biologia Celular e Molecular, Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.
3. Faculdade de Medicina, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (RS) Brasil.
4. Programa de Pós-Graduação em Pediatria e Saúde da Criança, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre (RS) Brasil.
a. http://orcid.org/0000-0001-9282-272X; b. http://orcid.org/0000-0003-4421-8995; c. http://orcid.org/0000-0001-7120-3022;
d. http://orcid.org/0000-0002-3906-5456

Table 1. Patient characteristics.

Case	IRT level/ RV (ng/ mL)*	Sweat chloride concentration (mmol/L)	Genotype	Meconium ileus	Low weight gain prior to diagnosis	Recurrent respiratory infections	Use of pancreatic enzymes	Airway bacterial colonization
1	98.7/110	85	F508del/ R1162X	X			X	MRSA
2	88.5/110	54	F508del/ F508del	X			X	SA
3	[a]	111	F508del/ R1162X		X	X	X	SA
4	39.4/110	118	F508del/ F508del		X	X	X	SA

IRT: immunoreactive trypsinogen; RV: reference value; MRSA: methicillin-resistant *Staphylococcus aureus*; and SA: *Staphylococcus aureus*. [a]Reported as normal, with no data on the actual level of IRT.

delay diagnosis, particularly when IRT levels are within the normal range and the clinical signs of the disease are overlooked.[10] It should be borne in mind that IRT measurement is not a diagnostic test.[5] Therefore, regardless of serum IRT levels, sweat testing should be performed in all patients clinically suspected of having CF, including those with a negative IRT level and MI, as well as those whose parents have CF-associated mutations.[10] Factors associated with false-negative results were identified in three of the four patients in the present study: two had had MI at birth, which raised the suspicion of CF and led to further diagnostic testing; and one underwent IRT testing at around the age of six months, by which time clinical symptoms had appeared, leading to misinterpretation of the results and delayed diagnosis. In the remaining patient (whose IRT level was 39.4 ng/mL), no false-negative-associated factors were identified.

One group of authors described cases of false-negative newborn screening results despite the use of *CFTR* gene analysis as a screening strategy.[5] In a study evaluating the newborn screening program in the state of Paraná, Brazil, 30 months after its implementation,[11] only one case of a false-negative newborn screening result was found. In a study involving two centers for CF newborn screening in the state of São Paulo, Brazil, no false-negative results were found over a period of nearly two years.[12]

Because CF newborn screening allows timely implementation of therapeutic interventions, a diagnosis of CF should not be based on serum IRT levels alone. Regardless of their serum IRT levels, patients presenting with clinical signs and symptoms suggestive of CF, as well as those with MI at birth and those with CF siblings, should undergo sweat testing, *CFTR* gene sequencing, or both.

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