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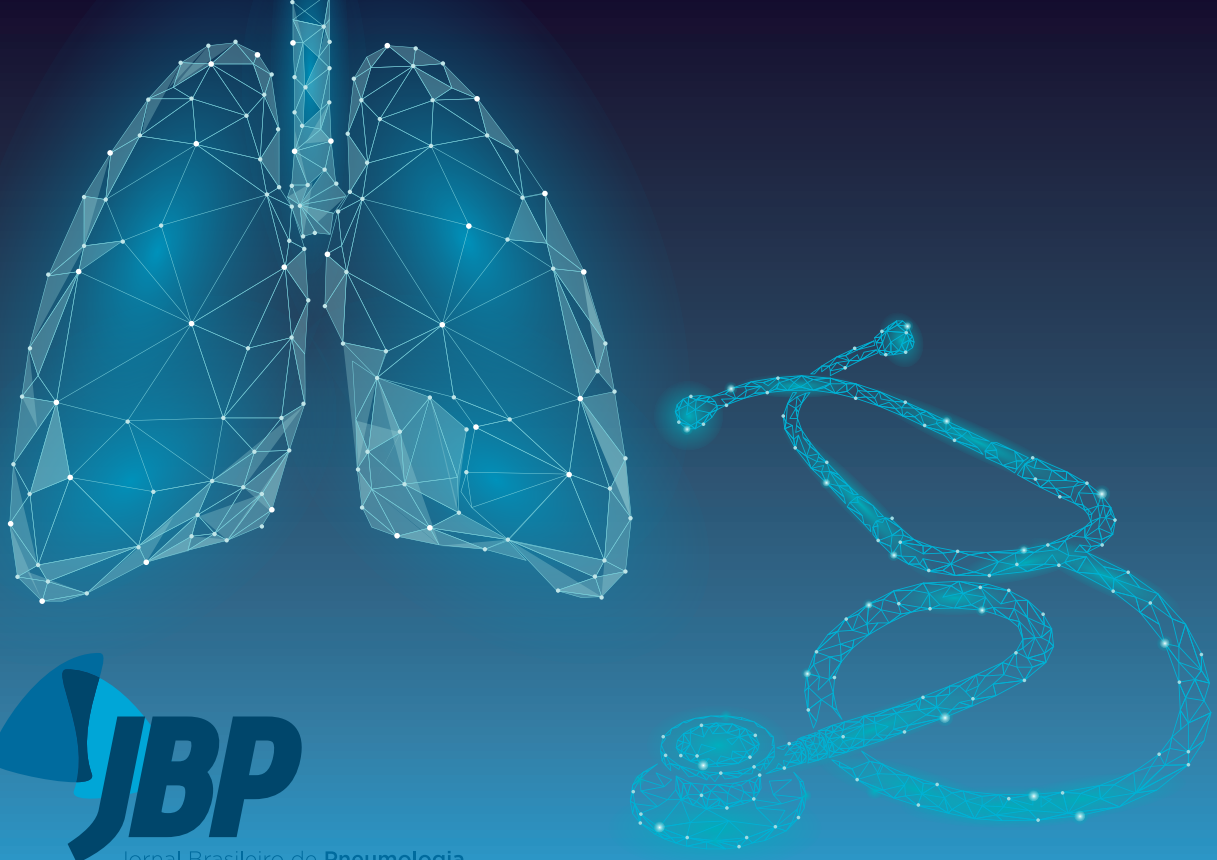
HIGHLIGHT

Pulmonary microbiome

Global TB Network

Patterns of electronic cigarette use



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Epidemiology of asthma: it is necessary to expand our concepts

Maria Alenita de Oliveira[1,2,a]

The knowledge on the epidemiology of diseases, based on data on morbidity and mortality, is important, because it allows to create public health policies for disease prevention and to promote investment in key areas in order to improve health care indicators.

In epidemiological studies that evaluate asthma morbidity and mortality, two large databases, which were brilliantly described in an editorial published in the JBP by Stelmach and Cruz.,[1] have been available for the population in Brazil. One of these databases, from the *Departamento de Informática do Sistema Único de Saúde* (DATASUS, Information Technology Department of the Brazilian Unified Health Care System), allows the correlation of data in order to demonstrate the trends regarding mortality and morbidity in the various regions of the country, specified by the municipalities. The second database is provided by the *Instituto Brasileiro de Geografia e Estatística* (IBGE, Brazilian Institute of Geography and Statistics), which allows the analysis of data in a geographical perspective, according to the regions, allowing the classification of urban and rural populations.

The statistical agencies generally adopt two criteria for the classification of urban and rural areas: the political-administrative criterion, adopted until recently in Brazil, and the demographic index, adopted in other parts of the world, such as Australia and the European Union.[2]

In the present edition of the JBP, the article by Brito et al.[3] analyzed regional data on asthma mortality in the period between 1980 and 2012. For the analysis of the data, urban and rural areas were defined considering population size, population density, and degree of urbanization, as proposed by Veiga et al.[4]; IBGE adopted this definition in 2017, replacing the political-administrative method, which was adopted in previous studies. The authors[3] found a reduction in the mortality rate during the study period, which was similar to the results of previous studies,[4-6] as well as a predominance in females.

In relation to the analysis by urban and rural areas, the study of Brito et al.[3] showed a trend toward a decrease in mortality from asthma in large municipalities; however, there was an increase in asthma mortality in small and medium-sized municipalities. In relation to rural areas, an increase in asthma mortality was found when compared with that in urban areas, in disagreement with the results found in a study by Ponte et al.,[7] in which urbanization was associated with higher asthma morbidity and mortality.

The influence of geographic variations on asthma morbidity is important to improve our understanding of the disease so that interventions to reduce its impact can be developed. Factors, such as indoor and outdoor pollution, exposure to allergens, socioeconomic status, and access to health care services, might interfere with morbidity and mortality.[8,9]

One of the examples reported in the article by Brito et al.[3] is the significant bias caused when we classify a municipality as a participant of a metropolitan area, because it results in a population profile with greater access to health care services. The old classification system, which did not take into account demographic density, could consider some municipalities with small populations but close to large centers as rural municipalities.

Having increased access to health care services is recognized as a factor that reduces morbidity and mortality; in recent years in Brazil, the Family Health Program has provided greater access to health care services for the general population, which might explain the results presented in the study by Brito et al.,[3] since there is greater access to these facilities in areas with higher population density.[10] However, the increase in mortality in rural areas might be associated with factors such as socioeconomic status, limited access to medications, or even a greater number of cases diagnosed with asthma, and, consequently, identifying a greater number of asthma-related deaths due to the amplification of the health care network.

The methodology of epidemiological studies, the need for studies that analyze asthma indicators by differentiating between urban and rural areas, and the mechanisms that influence these indicators should be carefully reflected upon, so that health care policies are stimulated to reduce asthma mortality.

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Getting to know our pneumococcus

Fernando Luiz Cavalcanti Lundgren[1,2,a]

In this issue of the JBP, Dullius et al.[1] present the results of their study involving a population of adult patients with invasive pneumococcal disease (IPD). Although similar studies have been conducted in populations of pediatric patients and immunosuppressed patients, there have been few such studies involving adult populations.

Among pulmonologists, the most well-known pathogenic bacterium is pneumococcus.[2] The species *Streptococcus pneumoniae* causes localized diseases, such as otitis and sinusitis, as well as invasive diseases, such as pneumonia, meningitis, and sepsis. There are more than 90 known serotypes, which differ in terms of their aggressiveness and antibiotic resistance profile.[3]

The occurrence of IPDs among adults in Brazil has not been widely studied. Determining the serotype involved can facilitate the prevention and treatment of the disease in our patients.

Although recommendations for the treatment of pneumonia suggest that the agents involved be investigated in critically ill patients, there is as yet no recommendation for routine pneumococcal serotyping.[3,4]

In Brazil, the antimicrobial resistance of pneumococcus has been shown to be low for penicillins, the group of antibiotics that are most widely used in the treatment of IPDs. Among younger individuals with IPD, the associated hospitalization rate has declined, as has the mortality rate. The higher rates of hospitalization and mortality among older individuals with IPD have been attributed to factors such as immunosenescence and the presence of comorbidities.[5,6]

The study conducted by Dullius et al.,[1] published in this issue of the JBP, brings us the knowledge of pneumococcus in one region of Brazil (the state of Rio Grande do Sul), as well as of the serotyping of isolates from patients with IPD. A series of cultures were collected over an 11-year period, and the pneumococcal serotypes were correlated with the clinical status recorded in medical records and with the mortality rates found. The strength of their study is that it provides us with information about the pneumococcal strains present in adults with IPD, information not generally obtained in studies of patients followed at a general hospital. All of the samples were collected and cultured at the same hospital. Samples for antimicrobial susceptibility testing were sent, via the state epidemiological surveillance program, to a national referral center for pneumococcal serotyping.

Recognizing the most common serotypes and determining which serotypes are present in patients with severe pneumococcal disease could prompt suggestions for adopting new therapies and vaccines. The cases in which the culture was positive for pneumococcus were serotyped and later compared to the data in patient charts, which were not specific to the research, those data then being tabulated.

The study could have been more informative if the data on mortality and bacterial resistance to penicillins had been related to the serotype. The most common IPD cases were those of pneumonia, with comorbidities in 85% of the cases and a mortality rate of 33%. Those are comparable to the values reported in other studies of IPD in patients categorized as being at high risk and reflect the fact that the presence of comorbidities increases the difficulty of treating these patients, even with the correct choice of antimicrobial.[7-9] Some pneumococcal strains are more prone to bacterial resistance and require a differentiated approach.[10,11]

Our knowledge about invasive strains of pneumococci comes from the microbiological surveillance of complex biological samples, such as cerebrospinal fluid and pleural fluid, as well as of blood cultures. A surveillance project on pneumonia and bacterial meningitis at the Adolfo Lutz Institute, in the city of São Paulo,[12] receives such samples from regional surveillance laboratories throughout Brazil, reporting on the behavior and presentation of the most common serotypes, as well as on their bacterial resistance profiles. The series of confirmed strains has shown changes since the introduction of the pneumococcal vaccine into the national pediatric vaccination schedule. The data from this project show that, in Brazil, pneumococcal resistance to beta-lactams is low in samples collected from individuals with respiratory diseases. Data from the Information Technology Department of the Brazilian Unified Health Care System show that there has been a recent increase in the rates of hospitalization and mortality due to pneumonia in elderly patients.[2]

The use of a pneumococcal vaccine has been associated with a reduction in the incidence of IPD. Two types of anti-pneumococcal vaccines are used in adults: the pneumococcal polysaccharide vaccine (PPV) and the pneumococcal conjugate vaccine (PCV). The PPV does not result in changes in the oropharynx and does not produce the herd effect obtained with the PCVs. The PCV currently available for use in adults is the 13-valent PCV13, which covers the 13 most expected, most aggressive serotypes of pneumococci. Various studies have reported that the PPV provides good coverage for IPD in immunocompetent individuals.[13-16] However, there is a failure of that protection in immunocompromised individuals and in

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individuals at high risk for IPD. In the at-risk patients, the PCV13 has shown good protection and reduced the incidence of IPD, as well as the number of pneumonias caused by vaccine strains and by strains not covered by the vaccine.[13-16]

The Brazilian Thoracic Association and the Brazilian Immunization Society have recommended the use of the regimen adopted internationally; that is, vaccinating patients with chronic lung diseases with PCV13 followed by the 23-valent PPV, taking age and other aggregated risk factors into account.[17-19] It has become necessary to monitor changes in the serotypes found in the population in order to modify the vaccine strains and maintain the population coverage for pneumococcus.[20]

In their study, Dullius et al.[1] identified 35 pneumococcal serotypes; the authors theorized that, if the recommended vaccination regimen were used, the coverage would be 50.8%, because the strains not covered by the existing vaccines accounted for 49% of the cases. The Dullius et al.[1] study has the merit of indicating the importance of vaccinating our patients, with a potential reduction in the number of cases of IPD and, consequently, mortality.

There is a need for studies, conducted in settings in which patients are treated, that determine the distribution of serotypes and their sensitivity to antimicrobials, as well as evaluating the prior use of pneumococcal vaccines.

Dullius et al.[1] showed that it is possible to work in collaboration with other institutions, improving treatment and increasing our knowledge of our patients. We hope that the authors will continue to pursue this line of research and will be able to provide us with more answers.

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Electronic cigarettes – the new playbook and revamping of the tobacco industry

Ubiratan Paula Santos[1,a]

The smoking epidemic began in the late nineteenth century, driven by the invention of the cigarette making machine. In the twentieth century, it was driven by the advertising industry, the cinema, and the great wars, as well as by the greater circulation of goods and people. The major health hazards of tobacco use have been consistently demonstrated since 1950.[1] Beginning in the 1990s, efficient anti-smoking policies gained momentum worldwide[2], thus reducing the impact on public health. In Brazil, the implementation of anti-smoking policies, such as a ban on cigarette advertising, warnings on cigarette packs, increased dissemination of information about the harmful effects of tobacco use, a ban on smoking in enclosed spaces, an increase in the price of tobacco products, and the expansion of smoking cessation support services, contributed to a significant reduction in the prevalence of smoking among males and females[3], which fell from 43.3% and 27.0%, respectively, in 1989[4] to 12.6% and 8.2%, respectively, in 2015.[5] However, worldwide and in Brazil, smoking is still the second leading risk factor for mortality, there having been an estimated 7.13 million smoking-related deaths in 2016.[6] In addition, approximately 1.1 billion people ≥ 15 years of age still smoke.[7]

In reaction to the world closing ranks against tobacco use, the recent efforts to ban the use of flavorings, and the imposition of laws to make cigarette packaging more generic, the tobacco industry has devised new strategies. The industry seeks to present itself as a defender of public health, has finally recognized the harmful effects of smoking, and has begun to offer alternatives. It has started to produce products such as electronic cigarettes (e-cigarettes, heating to near 100°C) and vape pens (vaporizers, heating to near 300°C), both of which supply nicotine in a heated form.

The topic of the moment is the controversy among researchers and medical societies about the use of e-cigarettes to reduce harm or as another treatment option for smoking cessation.[8-11] This new strategy of the tobacco industry—investing in e-cigarettes and vaporizers as a way of offering nicotine to current smokers and of encouraging smoking initiation—has been the subject of studies worldwide. By manufacturing vaporizers with attractive designs and adding flavorings to e-liquids, the tobacco industry seeks to attract new users, especially young ones, as a means of maintaining the numbers of individuals who are dependent on nicotine, stimulating dual consumption—the burning of tobacco and the vaporizing of nicotine in electronic devices—and thus retaining its lucrative market.

A growing number of studies provide evidence of increased e-cigarette use by young people and that those individuals are more likely to become regular users of tobacco products, due to the perception of reduced risk, and to become addicted to nicotine.[12] Those factors, together with the risks of e-cigarettes, which contain not only nicotine, an addictive substance that increases the risk cardiovascular disease, but also numerous toxic chemicals[13] and offer a quantity of inhaled particles that far exceeds the recommended limit for environmental exposure to particulate matter,[14] have led international respiratory medical societies[12] to recommend that the devices be classified and regulated as tobacco products, that their sales to minors be prohibited, and that there be a ban on their use in enclosed spaces (i.e., that they be considered to have a negative environmental impact), encouraging further studies on their effects.

In the study conducted by Oliveira et al.,[15] published in this issue of the JBP, the authors evaluated awareness of e-cigarettes and the frequency of experimentation with/use of the devices on the part of university students. They found that 37% were aware of e-cigarettes, 2.7% had it experimented with them, and 0.6% used them regularly. The prevalence of e-cigarette use was associated with being younger, having parents with a higher level of education, and having smokers in the family.[15] Although the prevalence of regular e-cigarette use was lower than that reported for other countries,[16,17] as well as being lower that reported in a study evaluating a sample of individuals over 18 years of age in the Brazilian cities of Rio de Janeiro, São Paulo, and Porto Alegre,[18] the Oliveira et al. study[15] calls attention to the high rate of awareness of e-cigarettes.

A recently published study, conducted in Canada and involving more than 28,000 individuals of both sexes (15-54 years of age), underscores the need for more attention to be given to the subject.[19] That study revealed that 7.7%, 6.0%, and 4.9% of the participants made use of conventional cigarettes only, e-cigarettes + conventional cigarettes, and e-cigarettes only, respectively. The authors found that the level of exposure to environmental tobacco smoke (ETS) was higher among the users of e-cigarettes + conventional cigarettes than among the users of conventional cigarettes only. Although the level of ETS exposure among exclusive users of e-cigarettes was lower than that observed for exclusive users of conventional cigarettes, it was still higher than the level of ETS exposure observed for never-smokers, which provides evidence of the behavioral profile of the e-cigarette user.

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In Brazil, the implementation of the abovementioned anti-smoking policies is responsible for the sharp drop in the prevalence of smoking, which should discourage the adoption of policies allowing the marketing of yet another product by the tobacco industry, whether as a strategy for reducing risks or as a tool for promoting smoking cessation. While we await additional research on the impact of the chronic use of the new devices, there are other measures that can be implemented: banning the use of flavorings in cigarettes; curbing the traffic in contraband cigarettes; eliminating the sale of loose cigarettes at newsstands and other outlets; and expanding smoking cessation support services.

Why should a doctor prescribe a product made by the same industry that, despite having been aware of the disastrous health impacts that its products have, has always been slow to admit that there is such an impact and that nicotine is in fact addictive, steadfastly refusing to pay reparations to its victims, as demonstrated by the exhaustive collection of documents produced by the tobacco industry itself and released to the public in recent decades?[20] On the basis of the current knowledge, there is no reason.

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Global TB Network: working together to eliminate tuberculosis

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In spite of the global efforts of the World Health Organization (WHO) and partners to reduce the incidence of tuberculosis (about 2% per year) and its mortality (about 3% per year), the disease is still a global killer and has primary public health importance, with an estimate of 10.4 million cases in 2016.[1]

The End TB Strategy and its vision (zero tuberculosis-related deaths, cases, and suffering) underlies the possibility to eliminate tuberculosis as a public health priority in countries with a low incidence of tuberculosis.[2-4] Pillar 2 of the End TB Strategy clearly emphasizes the importance of collaboration at different levels. This international collaboration represents the last of the eight core areas to pursue tuberculosis elimination.[4]

Translating that into simple words, international collaboration comprises all of the interactions involving clinicians, laboratory staff, public health officers, national tuberculosis programs, other programs (HIV/AIDS programs and diabetes programs in some countries), Ministries of Health (as well as Ministries of Justice for jails/prisons, Ministries of Interior for the migration-related issues, Ministries of Transportation, etc), private sector, pharmaceutical sector, civil society, representatives of the affected communities, and international organizations, among others.[4,5]

As of today, several TB Networks exist: the Stop TB Partnership[6] has a long tradition in supporting advocacy. In addition, one of the best examples is the Brazilian *Rede TB*, which shows that the best research efforts, even in a large country as is Brazil, can collaborate to solve country-specific priorities and involve national tuberculosis programs and authorities in the plan.[7-11] However, as of today, no global tuberculosis network is operational enough to put together all of the abovementioned actors in order to support the fight against tuberculosis.

To respond to this need, the Global TB Network* (GTN) will be launched at the second Conference of the World Association for Infectious Diseases and Immunological Disorders (WAidid),[12] which will take place in the city of Milan, Italy, from October 18-20 of 2018, involving an international group of experts and covering a wide range of perspectives.

The goal of the GTN is to pursue tuberculosis elimination with a global effort proactively, building on existing collaborations in the area of research, advocacy, and training. Its core objectives are to foster and conduct research on key unmet therapeutic and diagnostic needs in the field of tuberculosis elimination, leveraging on multidisciplinary, multisectoral approaches and supportive interventions (i.e., training and advocacy activities) within the framework of the WHO End TB Strategy. Preliminary plans propose to focus on latent tuberculosis infection, multidrug- and extensively drug-resistant tuberculosis rapid diagnosis, and other neglected areas (pediatric tuberculosis, extrapulmonary tuberculosis, rehabilitation of tuberculosis sequelae, infection control, etc.)

The GTN represents the structured evolution of pre-existing global tuberculosis networks, including the international linezolid, carbapenems and bedaquiline study groups and the International Severe Cases and Rehabilitation Study Group, which produced over 40 articles in the last five years in impact-factor, peer-reviewed journals, as well as various series on tuberculosis in different journals in collaboration with scientific societies, such as the European Respiratory Society, *Sociedade Brasileira de Pneumologia e Tisiologia*, and the *Asociación Latinoamericana del Tórax*.[13-18]

This new global network aims at collaborating with existing organizations, associations, institutions, and partners that are committed to fight against tuberculosis by complementing and boosting (and not duplicating) the existing initiatives.

The GTN is hosted by WAidid,[12] founded in July of 2014 in order to advance the scientific research in the field of infectious diseases and immunology and to disseminate information on the related pathologies. WAidid is the response to the previous lack of a network that links associations and scientific societies focused on infections, vaccines, and immunology. WAidid, whose membership is free of charge, represents the bridge for a global multidisciplinary approach to infections (including tuberculosis) operating across all age groups.

The GTN is composed of three pillars:

1. Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre (RS) Brasil.
2. Centro de Investigación, Prevención y Tratamiento de Infecciones Respiratorias, Hospital Universitario, Universidad de Monterrey, Monterrey, México.
3. Department of Clinical Pharmacy and Pharmacology, University Medical Center Groningen, University of Groningen, Groningen, the Netherlands.
4. Union Headquarters, International Union Against Tuberculosis and Lung Disease, Paris, France.
5. Department of Medicine, Therapeutics, Dermatology and Psychiatry, Kenyatta University, Nairobi, Kenya.
6. Sezione di Epidemiologia Clínica e Statistica Medica, Dipartimento di Scienze Mediche, Chirurgiche e Sperimentali, University degli Studi di Sassari, Sassari, Italia.
7. Sezione di Pediatria generale e specialistica, Dipartimento di Scienze Chirurgiche e Biomediche, Università degli Studi di Perugia, Perugia, Italia.
8. Istituti Clinici Scientifici Maugeri, Istituto di Ricovero e Cura a Carattere Scientifico – IRCCS – Tradate, Italia.
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Pillar 1 includes the Technical Committees covering the main areas relevant to tuberculosis and nontuberculous mycobacteriosis management (Tuberculosis Prevention/Latent Tuberculosis Infection; Tuberculosis Diagnosis; Tuberculosis Treatment; Tuberculosis Pharmacology; Pediatric Tuberculosis; Migrants/Vulnerable Populations; Nontuberculous Mycobacteriosis; Tuberculosis Infection Control; Impact Evaluation, Strategies & Global Health; Clinical Support to Patients (Tuberculosis Consilium); Clinical Trials; Tuberculosis and Surgery; Basic Science; and Epidemiology, Statistics and Methodology).

Pillar 2 includes representatives from each association/organization active in tuberculosis control interested in participating in the GTN.

Pillar 3 includes the private and pharmaceutical sectors.

Several global projects have already started, including an online clinical service aimed at supporting the correct management of difficult-to-treat tuberculosis cases and of individuals with latent tuberculosis infection, as well as the rational introduction of new drugs; a project monitoring adverse events of new antituberculosis drugs; one study on tuberculosis and surgery; and one study on tuberculosis sequelae and rehabilitation.

We hope this initiative will contribute to reaching the ambitious goals of the End TB Strategy.

***Global TB Network provisional Steering Committee:**

Chair: Giovanni Battista Migliori

Secretary General: Denise Rossato Silva

Other Members: Jan-Willem Alffenaar, Jeremiah Muhwa Chakaya, Adrian Rendon, Daniela M. Cirillo, James Chalmers, Sergey Borisov, Erlina Buran, Isabel Saraiva, Masae Kawamura (Observer)

Global TB Network Secretariat

Secretariat in Tradate (Italy): Rosella Centis, Lia D'Ambrosio, Dina Visca, Antonio Spanevello

Secretariat in Milano (Italy): Elisabetta Di Felice

Global TB Network Chairs of Committees

TB Pharmacology Committee: Jan-Willem Alffenaar

Epidemiology, Statistics and Methodology Committee: Giovanni Sotgiu

TB Prevention/LTBI Committee: Delia Goletti

TB Diagnosis Committee: Daniela Maria Cirillo

TB Treatment Committee: Marcela Muñoz Torrico

Paediatric TB Committee: Ben Marais

Migrants/Vulnerable Populations Committee: Claudia Dobler

NTM Committee: James Chalmers

TB Infection Control Committee: Edward Nardell

Impact Evaluation, Strategies & Global Health Committee: Alberto Garcia Basteiro

Clinical Support to Patients (TB Consilium): Marina Tadolini

Clinical Trials Committee: Emanuele Pontali

TB and Surgery Committee: Alessandro Mariani

Basic Science Committee: Tom H.M. Ottenhoff

World Association for Infectious Diseases and Immunological Disorders (WAidid): President: Susanna Esposito

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The importance of strong fundamentals in scientific methodology

Rogério Souza[1,2,a]

In recent decades, there has been considerable growth of the scientific literature. Although that growth has not been uniform across the different fields of science, a persistent trend can be seen in the various databases available. In PubMed for example, there was an annual growth rate of more than 5% between 1997 and 2006.[1] More recently, increases in the number of online journals and the publication of collections of abstracts presented at conferences, as well as expanded access to databases, might have further influenced such growth.

However, such growth is not free from bias—quite the opposite. A few years ago, an article published in *The Economist*, entitled "Unreliable research - Trouble at the Lab,"[2] called attention to numerous problems associated with the state of scientific literature at that time, such as the low reproducibility of published studies and biases related to the exclusive publication of studies showing positive results, which were potentially influenced by funding sources. In addition, within the academic environment, there is increasing pressure to publish, which results in low-quality articles or the "salami slicing" phenomenon, which is characterized primarily by dividing individual research projects into multiple articles, not only reducing their relevance but also increasing redundancy.[3]

One of the mechanisms to minimize or at least to discourage many of the current biases in the scientific literature is to encourage a more solid training of researchers in the fundamentals of scientific research. Information regarding the precepts, not only good practices in clinical research but also the associated technical aspects, should first be offered in undergraduate courses and should be maintained throughout the academic life of the researcher, as continuing education.

In terms of the technical aspects, the entire rationale for the design of a study should be understood, from the development of the main research question[4] to the critical analysis of the methodology used and its limitations, as well as the appropriate use and interpretation of the various statistical tests. Graduate programs tend to focus on those aspects, because their main purpose is to prepare professors and researchers by constructing discipline-specific training centers. However, this initiative seems insufficient, given the extent of the scientific environment and the limited scope of those disciplines.

Scientific journals also play a relevant, albeit less explored, role in this process, not only by creating mechanisms to identify and prevent biases associated with the scientific publishing process but also by disseminating the best practices to be followed. In those two aspects, there is a pressing need to improve the performance of scientific journals. First, they should be able to identify biases. In general, it is well established that the peer review process, despite its various positive qualities, is unable to identify such biases. The lack of alternative models that do not significantly delay the publishing process has perpetuated this limitation of one of the most common editorial processes. One enormous opportunity that has yet to be taken advantage of by scientific journals is the dissemination of methodological concepts. There are few scientific journals in the field of internal medicine that have sections dedicated to the discussion of the fundamentals of scientific research. The potential gains from the dissemination of this type of knowledge are quite significant, not only in terms of improving the training of researchers but also in terms of increasing the overall critical thinking capacity of readers in general, which can, over time, function as a mechanism to improve the quality of the available scientific research.

What the JBP has specifically been doing over the past four years is publishing a series of articles about continuing education in scientific methodology,[5] addressing extremely diverse topics, from how to structure a research project[4,6-8] to the proper interpretation of different types of studies.[9-11] We are now investigating the impact that the publication of that series of articles has had on the JBP readership. However, in general terms, those articles have already been being used as a point of reference for researchers in the area.

The dissemination of methodological concepts addresses only one small aspect of the larger problem. Obviously, continuing education plays an important role, although other initiatives are needed in order to improve the scientific research scenario over the next few years. Funding agencies might have to take more direct action in that sense. The use of audits, making the reporting of formal aspects regarding methodology mandatory, and requiring analysis of the results in a more conclusive fashion are actions that can be implemented and added to the current project review format without significantly increasing the bureaucracy involved in the current submission and review processes.

Any interventions in the scientific publishing process should be agreed upon by consensus among the members of academia, funding agencies, scientific journals, and even readerships. Otherwise, the organic growth in the scientific literature will not be accompanied by a similar growth in quality.

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Paravertebral mass

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

A 59-year-old man presented with anemia and weight loss. He had a history of sickle-cell disease since he was 10 years of age. Chest CT scans revealed bilateral, well-marginated paravertebral masses in the lower half of the thorax, with heterogeneous density (Figure 1). He also presented with a large splenic calcification.

DISCUSSION

The CT features presented by the patient are typical of extramedullary hematopoiesis (EMH), defined as the development and growth of hematopoietic tissue outside the bone marrow. The main considerations in the differential diagnosis of masses in the posterior mediastinum, particularly in the paravertebral region, include neurogenic masses, lymphoma, paravertebral abscess, lateral meningocele, and EMH. EMH is seen in a variety of hematologic disorders, particularly severe hemolytic anemia (thalassemia, sickle-cell anemia, and spherocytosis). Extensive replacement of normal bone marrow occurs when production is insufficient to meet the demands of the body. The most common sites of EMH are the liver, spleen, and lymph nodes, although it can occur in any organ. Thoracic involvement is less frequent, usually manifesting as bilateral lobulated masses in the lower paravertebral areas. The destruction of adjacent ribs and vertebrae is not seen in EMH. The erythropoietic masses are usually asymptomatic, although the presence of EMH within the spinal canal may be associated with spinal cord compression and neurologic deficit related to the level of involvement. The imaging manifestations of thoracic EMH can be unilateral or bilateral, sharply circumscribed, often lobulated, paraspinal soft-tissue masses,[1] most frequently seen in the distal thoracic paraspinal region. CT scans can also be helpful in detecting areas of fat attenuation within these lesions, in depicting bony changes related to hematologic disorders, such as thalassemia and sickle-cell anemia, and in demonstrating splenic infarcts with focal calcifications or a small and dense calcified spleen (autosplenectomy), which can aid the differential diagnosis. The diagnosis of EMH can be established with reasonable certainty on the basis of characteristic radiologic findings in a patient with a predisposing hematologic condition. Invasive diagnostic procedures are potentially hazardous because of the highly vascular nature of the thoracic masses and the hemorrhagic potential of this condition. Microscopic examination shows well-formed hematopoietic tissue. In conclusion, the presence of bilateral, paravertebral masses with associated splenic calcifications are highly suggestive of EMH. The main clinical finding in these patients is anemia.

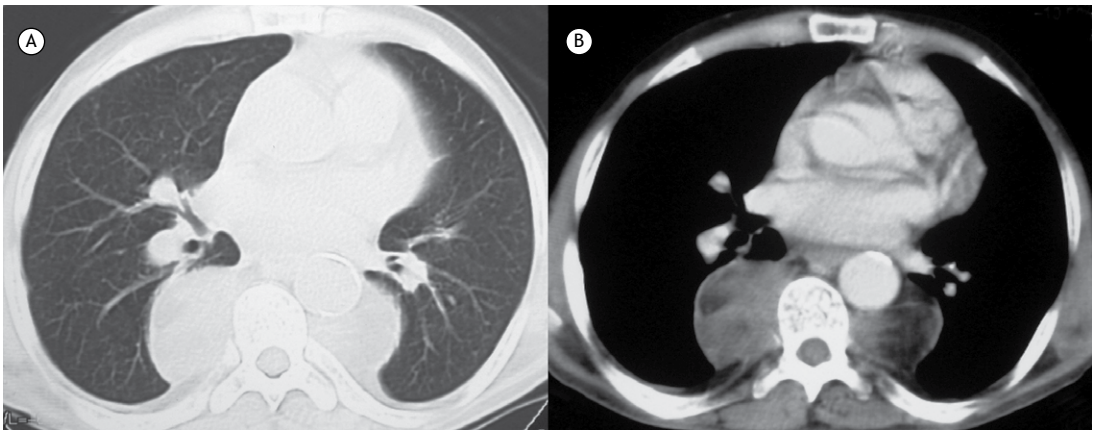

Figure 1. Chest CT scans with lung window (in A) and mediastinal window (in B) settings showing bilateral masses in the inferior paravertebral thoracic regions. The masses were heterogeneous, with low density areas, suggesting a fatty component. The lung parenchyma showed no abnormalities. There is also a large splenic calcification (not shown).

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Meeting the assumptions of statistical tests: an important and often forgotten step to reporting valid results

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

PRACTICAL SCENARIO

A secondary analysis of a randomized controlled trial was conducted to evaluate whether atopic status was associated with asthma severity and asthma control among inner-city adolescents in the USA.[1] To answer this question, the authors evaluated the differences between atopic and non-atopic patients, in terms of asthma control and asthma severity scores, using a t-test, and reported the results as means ± SDs. The results showed that the atopic patients, when compared with non-atopic patients, had similar asthma control scores (18.1 ± 4.2 vs. 18.2 ± 3.7; p = 0.95) but worse asthma severity scores (5.5 ± 2.9 vs. 4.7 ± 2.8; p = 0.04).

BACKGROUND

As part of the process of answering research questions using quantitative methods, investigators select a statistical analytical approach based on various characteristics of the study, such as the nature of the variables collected (e.g., continuous, categorical, time-to-event variables), as well as the study design. Once the analysis is completed, it is expected that investigators take an additional step in the analysis process to make sure that the a priori assumptions of the statistical test selected are met in the dataset assembled for the study.

All statistical tests have underlying assumptions that need to be met so that the test provides results that are valid (*without unacceptable error*) regarding the parameter the test is calculating (e.g., mean, proportion, odds ratio, etc.). In our example, the authors used a t-test to calculate the mean and the standard deviation of asthma control and severity asthma scores in atopic and non-atopic patients using data collected from the study population as a means to represent the truth in similar patients from the source population (adolescents with asthma in the USA). This process, called inference, is only valid if the assumptions of the statistical test are met (Table 1).

It is good practice, as investigators, to acknowledge that the assumptions of the statistical tests used to answer their research question have been evaluated and whether they were met or not. If the assumptions of the tests are met, which should be reported in the results section of the study, this assures the scientific community that the results of the study have met one of the important criteria related to their validity. However, it has been suggested that the assumptions of statistical techniques are often not checked[2] or reported. Reasons for not assessing assumptions include: 1. researchers being unaware of the assumptions of the statistical tests used in the study, such as a t-test, ANOVA, or regression analysis; 2. researchers being unaware of standard approaches used to check assumptions of statistical tests and evaluate if they are violated or not; 3. researchers being unaware of how to remedy violations of the assumptions of a statistical model or how to select a new test when violations cannot be remedied; and 4. researchers being confident in the robustness of the statistical test used and choosing not to check its assumptions.

As educators and investigators, we all need to contribute to the overall goal of reporting high quality research conducted among the populations we serve. Testing the assumptions of statistical tests or models used to answer our research questions is a good start!

Table 1. Example of assumptions of a statistical test.

Statistical test	Assumption	How to corroborate
t-Test	Sampling: The participants in the study are randomly sampled from the source population.	Check the protocol.
	Sample size: The sample size calculated for the study is achieved.	Check sample size calculation in the protocol and check if sample size was reached by the number of participants included in the study.
	Normal distribution: The scale of measurement of the outcome variable is continuous and is normally distributed (or at least symmetric).	Conduct descriptive statistics on the outcome variable and create a graph showing the distribution which should follow a bell curve.
	Homogeneity of variances: The variance (standard deviation) of the data collected on the continuous variable across the two comparison groups is similar.	Use valid statistical methods to test for homogeneity.

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ORIGINAL ARTICLE

Asthma mortality in Brazil, 1980-2012: a regional perspective

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ABSTRACT

Objective: To estimate asthma mortality rates in Brazil for the period 1980-2012. **Methods:** On the basis of data from the Brazilian National Ministry of Health Mortality Database, we estimated mortality rates by calculating moving averages from a municipal perspective that would allow an evaluation differentiating between urban, rural, and intermediate (rurban) Brazil during the period 2002-2012. Trends were assessed using simple linear regression. **Results:** On average, 2,339 asthma-related deaths were reported per year during the study period. Asthma ranged from the 53rd to 95th leading cause of death. There was a decrease in asthma mortality rates in the country, from 1.92/100,000 population in 1980 to 1.21/100,000 population in 2012. From the municipal perspective, rates fell in urban and rurban Brazil, but increased in rural Brazil, except in the 5-34-year age group. Asthma mortality rates fell in the population under 25 years of age and increased among those over 74 years of age. Rates were always higher in females. **Conclusions:** Asthma mortality rates in Brazil have been decreasing slightly, with the decrease being more marked in the decade 2002-2012. Only the northeastern region of Brazil showed the opposite trend. Asthma mortality rates in urban and rurban Brazil showed a downward trend similar to that of the national scenario, whereas rural Brazil showed the opposite behavior. Analysis by age group showed that rates decreased among younger individuals and increased among the elderly aged ≥ 75 years.

Keywords: Asthma/mortality; Brazil; urban population; rural population.

INTRODUCTION

Globally, asthma affects 1-16% of the population in different countries (approximately 300 million people)[1] and causes 346,000 deaths annually.[2] Two epidemics of asthma mortality were described in the 20th century: the first one, in the 1960s, in the United Kingdom, Australia, and New Zealand; and the second one, a decade later, in New Zealand. The first epidemic was attributed to a cardiotoxic effect of high doses of isoprenaline. The second epidemic was associated with the widespread use of fenoterol and other potentially cardiotoxic inhaled bronchodilators.[3-6]

Measuring the impact of asthma mortality not only from a national but also from a regional perspective is particularly important in countries of continental dimensions where there is regional diversity in socioeconomic, development, and infrastructure conditions. Different epidemiological scenarios emerge when intra-urban differentials are included in prevalence studies.

Urbanization, on one hand, can have negative effects on human health that result from aspects of living and housing conditions and air pollution and, on the other, can improve the provision of health services, positively influencing human health.[7] The results obtained will be better if regional differences are taken into account in planning and scheduling control measures, regardless of the regional degree of urbanization.[8-10]

Asthma mortality rates and their trends are presented from a national and macro-regional perspective and from a municipal perspective. From this latter perspective, the *Instituto Brasileiro de Geografia e Estatística* (IBGE, Brazilian Institute of Geography and Statistics) divides the country into two areas: urban and rural. Whereas the first (an urban area) is defined as "an area within the urban perimeter, created by a municipal law, whether for tax or urban planning purposes (Master Plan, zoning, etc.)," the second (a rural area) is defined as "an area that was not included in the urban perimeter by a municipal law."[11] Because this classification can generate bias in the analysis of access to and efficiency of health care when focusing on populations of municipalities considered rural and bordering large urban areas, the municipal analysis performed in the present study was based on the urban and rural area definitions established by José Eli da Veiga,[12] which have been validated by several authors and are described below.

METHODS

The behavior of asthma mortality rates for the period 1980-2012 was analyzed on the basis of official data from the Mortality Database of the *Departamento de Informática do Sistema Único de Saúde* (DATASUS, Information Technology Department of the Brazilian Unified Health Care System), which are available for

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the population. Searching for information on the site followed this sequence: 1) health information and vital statistics; 2) filter: general mortality, in Brazil by region and state/Federal District, and asthma as the cause of death; 3) deaths by occurrence; 4) year of death; 5) gender, age, and municipality of occurrence. For the period 1980-1995, we used the International Classification of Diseases (ICD) 9th revision (ICD-9; code 493: asthma); for the period 1996-2012, we used the ICD 10th revision (ICD-10; code J45: asthma, and code J46: acute severe asthma). Rates were calculated by gender, age group, macro-region, and municipality group. The period chosen was the one for which there was complete information in the DATASUS database.

Analysis of mortality rates by gender, age group, and macro-region was performed by simple linear regression, for the period 1980-2012. Analysis of mortality rates by municipality group was performed only from 2002-2012 and was carried out by calculating moving averages; subsequently, trend averages were evaluated by simple linear regression. We chose to perform the analysis by municipality group only from 2002 onward because, in 2001, 54 new municipalities were created in Brazil, and this would disrupt the analysis, since many municipalities are created within existing ones. Therefore, the analysis was made on the basis of 5,564 municipalities.

Asthma mortality rates were calculated as follows: absolute number of deaths × 100,000/population. In the analysis by gender, we used the male and female populations for each year. In the analysis by age, the age groups were defined according to the classification used by the Pan American Health Organization and the DATASUS.[13] In the analysis of mortality rates by municipality, we used the population in each municipality for each year and we did the same calculation.

Given that studies on asthma mortality usually analyze the 5-34-year age group separately as a marker of global rates, because, in this group, there is less bias of diagnostic confusion with bronchiolitis and especially with COPD,[3,14] all rates calculated were compared with the corresponding ones for this age group.

The classification used by the IBGE defines city as the seat of a municipality. In this classification, every municipality seat (city) and every district seat (village) is considered an urban area. A decree issued in 1938, during the *Estado Novo* regime, transformed all municipal seats into cities, which resulted in the classification used by the IBGE considering any municipal seat to be urban,[11] regardless of its demographic characteristics. This standard may lead to significant biases in the analysis of access to health care by a population residing in a small municipality bordering a large metropolitan center. Even if that municipality has only a minimum health structure, its population has access to health care in the neighboring large metropolitan center. Therefore, if the classification used by the IBGE is applied, the population of that municipality would be considered rural even if, in practice, they have access to health care in the same way as the urban population of the large metropolitan center. Consequently, several authors and institutions have preferred to use another classification of urban or rural population that has been validated and takes other demographic factors into account. This classification, proposed by Veiga,[12] uses a methodology that is more appropriate to local municipal conditions, combining municipality population size (large, medium, or small) with population density and level of urbanization: municipalities within metropolitan areas (MA) and municipalities outside MA.[12] In this methodology, in addition to the concepts of urban—municipalities within MA, by size (large, medium, or small), and large municipalities outside MA—and rural—small municipalities outside MA—there is a new concept: "rurban"—medium municipalities outside MA. This classification eliminates the potential bias mentioned above and was used for the municipal analysis of asthma mortality rates and their trends in Brazil in the present study.

For calculation purposes, for municipalities within MA, we used the sum of the populations of municipalities within MA in Brazil; likewise, the sum of the populations of municipalities outside MA was calculated. The study included 5,564 municipalities. The classification used to categorize them by size was as follows[12]:

- Large: more than 100,000 inhabitants
- Medium: population between 50,000 and 100,000 inhabitants or population density ≥ 80 inhabitants/km^2, even if there were fewer than 50,000 inhabitants
- Small: fewer than 50,000 inhabitants and population density < 80 inhabitants/km^2

Since the analysis also took the level of urbanization into account, all of the 5,564 Brazilian municipalities were further divided into two groups: those within MA and those outside MA.

Statistical analysis was performed with the IBM SPSS Statistics software package, version 24.0 (IBM Corporation, Armonk, NY, USA). Linear regressions were performed with 95% confidence intervals for slope coefficients. In the municipal analysis, moving averages of linear coefficients were used to smooth the time series. The study was approved by the Research Ethics Committee of the Oswaldo Cruz Foundation Fernandes Figueira Institute.

RESULTS

Asthma ranged from the 53rd to 95th leading cause of death in Brazil during the study period. On average, there were 2,339 asthma-related deaths per year in the country between 1980 (n = 2,286) and 2012 (n = 2,354). Figure 1 shows asthma mortality rates per 100,000 population. During the study period as a whole, there is a slight downward overall trend in rates (−0.007), which decreased from 1.92/100,000 population in 1980 to 1.21/100,000 population in 2012. The trend was not uniform, there being alternation between decrease (1980-1991; 1999-2005; 2006-2012)

and increase (1992-1999; 2005-2006), similarly to what occurred in the 5-34-year age group (−0.002 deaths/year), giving consistency to the data.

Figure 2 shows that asthma mortality rates were significantly higher in females, and this difference increased over the years. The highest mortality rates were found in the 75 years and over age group (20.37/100,000 population, on average). The lowest rates were observed in the 15-24-year age group (0.20/100,000 population, on average). A proportional analysis of asthma-related deaths by age group (Figure 3) demonstrated that participation in the proportion of deaths increased over time from age 25 years and over, the increase being marked in the 75 years and over age group (17.6% of deaths in 1980 vs. 40.8% in 2012). Participation in the proportion of deaths decreased among those under 25 years of age, especially in the 0-4-year age group. The 5-14-year age group had the lowest proportion of asthma-related deaths, followed by the 15-24-year age group. From a macro-regional perspective (Figure 4), rates decreased slightly in the North (−0.004 deaths/year), Southeast (−0.023 deaths/year), South (−0.017 deaths/year), and Central-West (−0.007 deaths/year). The only exception was the Northeast, where there was a slight increase in mortality rates (0.025 deaths/year). Mean mortality rates (per 100,000 population) in each region for the period 1980-2012 were as follows: 1.73 (in the South); 1.58 (in the Southeast); 1.48 (in the Northeast); 1.10 (in the Central-West); and 0.79 (in the North).

The temporal evolution of moving averages of asthma mortality rates was analyzed by type of municipality, as defined above. Rates decreased in municipalities within MA and in municipalities outside MA (Figure 5).

Urban Brazil comprises large, medium, and small municipalities within MA and large municipalities outside MA. A detailed analysis of urban Brazil showed a downward trend in the moving averages of asthma mortality rates for large municipalities (both within and outside MA). The same was observed in "rurban" Brazil. However, the opposite was observed in medium and small municipalities within MA, where asthma mortality increased. In rural Brazil, represented by small municipalities outside MA, the moving averages of asthma mortality rates showed an upward trend (0.11 per year; Figure 6).

Analysis of the population aged 5-34 years corroborates the above findings, except for rural Brazil,

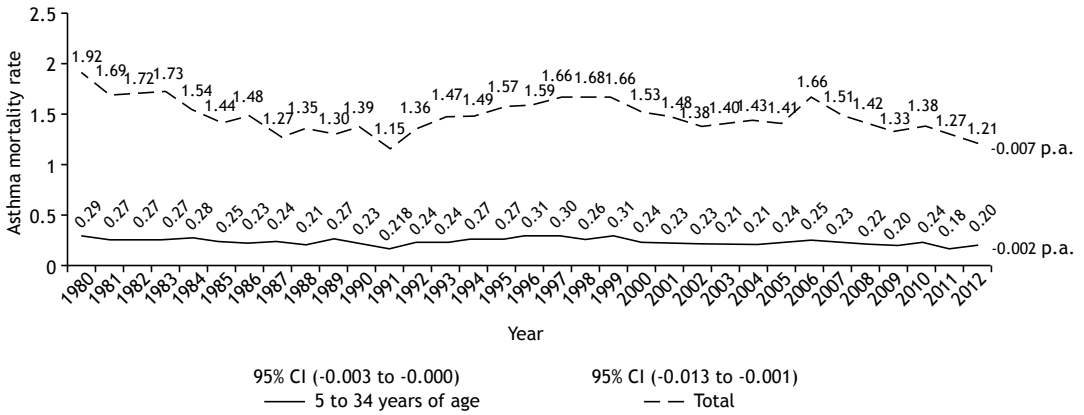

Figure 1. Asthma mortality rates (per 100,000 population) in Brazil; 1980-2012. p.a.: per year.

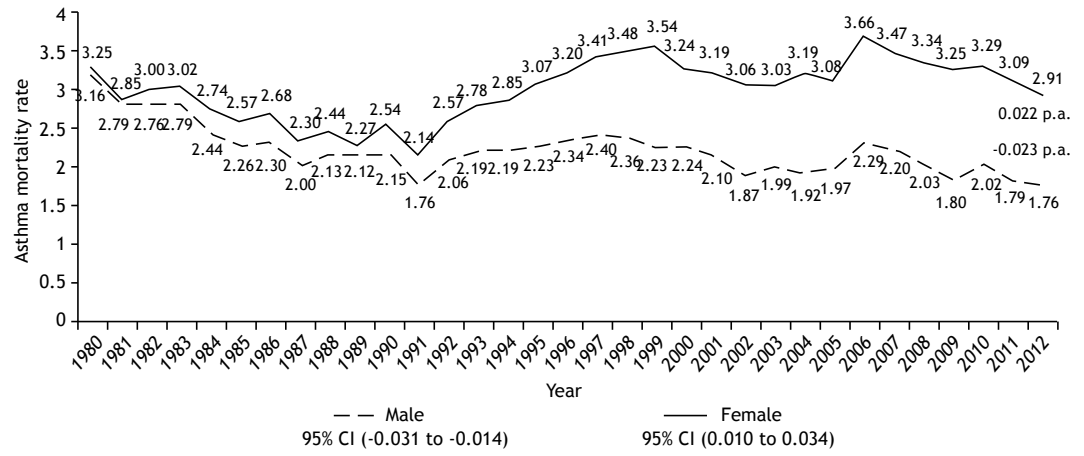

Figure 2. Asthma mortality rates (per 100,000 population) by gender in Brazil; 1980-2012. p.a.: per year.

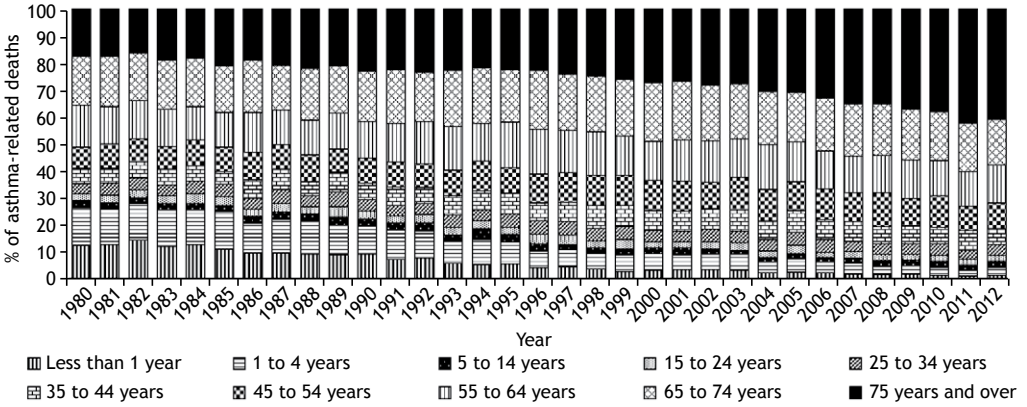

Figure 3. Proportional distribution of asthma-related deaths by age group in Brazil; 1980-2012.

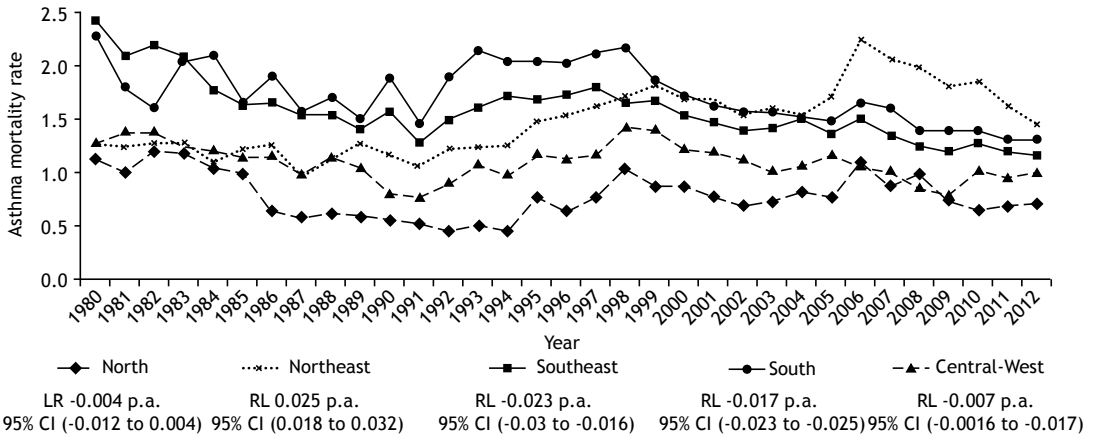

Figure 4. Trends in asthma mortality rates by Brazilian macro-region; 1980-2012. LR: linear regression; and p.a.: per year.

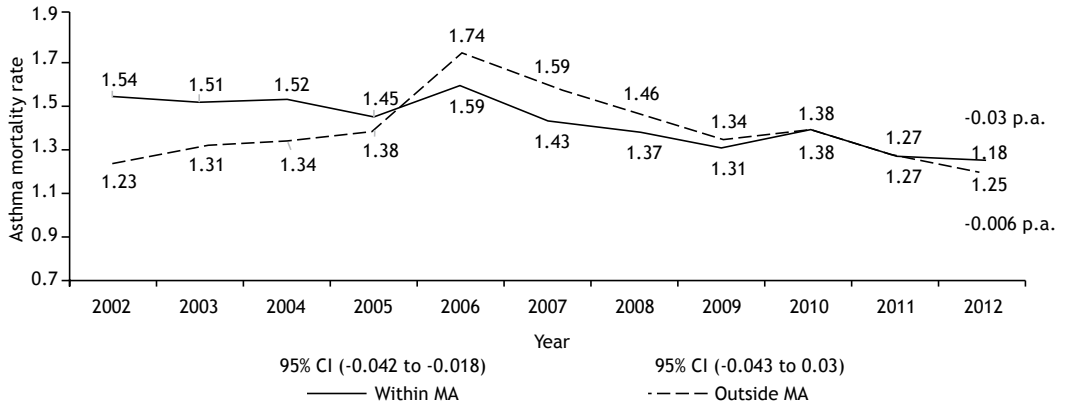

Figure 5. Temporal evolution of asthma mortality rates in municipalities within and outside metropolitan areas in Brazil; 2002-2012. p.a: per year; and MA: metropolitan areas.

because, in small municipalities outside MA, there was a downward trend in the moving averages of asthma mortality rates for this age group.

DISCUSSION

Asthma mortality rates in Brazil are high, despite the fact that they showed a downward trend during the study period, more markedly in the last 3 years. Between 1980 and 2012, there were, on average, 2,339 asthma-related deaths per year. The decrease detected during the last decade of the study (2002-2012) was confirmed by what was observed in the population aged 5-34 years.

Analysis by macro-region showed that, on average, the South had the highest asthma mortality rates per 100,000 population (1.73), followed by the Southeast (1.58), Northeast (1.48), Central-West (1.10), and

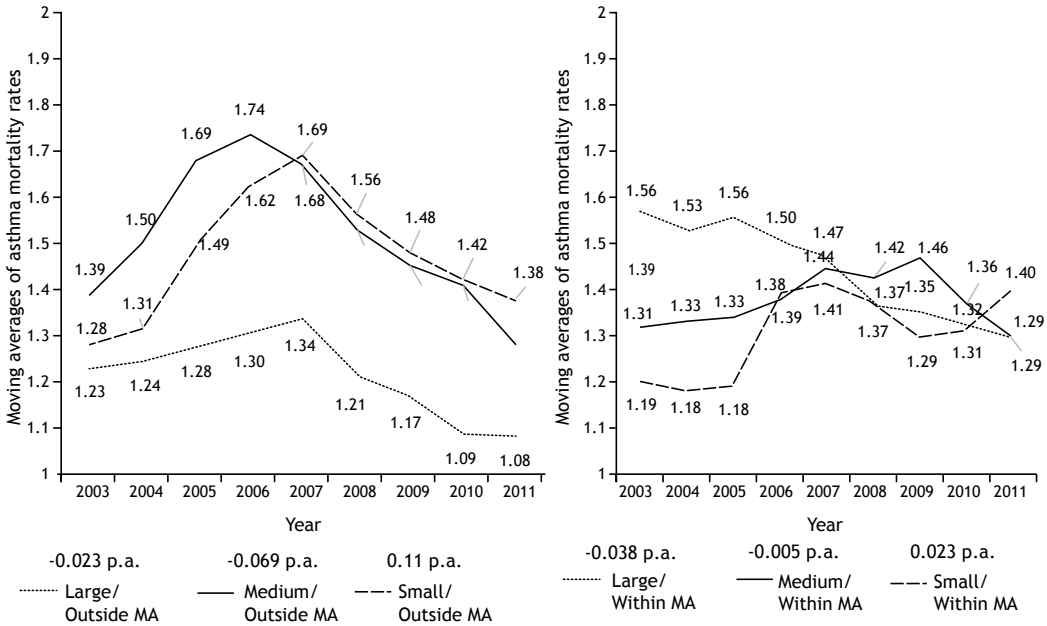

Figure 6. Moving averages of asthma mortality rates (per 100,000 population) in large, medium, and small municipalities, by whether municipalities are within or outside metropolitan areas. General population, Brazil, 2003-2011. p.a: per year; and MA: metropolitan areas.

North (0.79). Asthma mortality rates were equivalent to the national average (1.48) in the Northeast, were higher than this average in the Southeast and South, and were lower than this average in the Central-West and North. Analysis of trends in asthma mortality rates in the different macro-regions revealed a steady downward trend, except for the Northeast. Similar results were reported by Souza-Machado[15] (rates of 1.68 in 1998 and 1.32 in 2009), who also reported an increase in asthma mortality rates only in the Northeast. Regional differences in access to and quality of health care, as well as climatic, environmental, and socioeconomic differences, are possibly involved in the variety observed in asthma mortality rates among the different regions.

The difference in the asthma epidemiological situation between urban and rural environments motivated us to analyze the data collected from a municipal perspective. Consequently, we chose to use a classification that separated municipalities into urban and rural, as well as into large, medium, and small. Among the reasons for this type of data analysis is the fact that positive factors of MA, such as more developed and better equipped health care networks, are counterbalanced by negative factors, such as environmental pollution, causing an impact on mortality rates.[7] Although classifying municipalities as being within MA introduces biases, it results in them having a population profile with greater access to services, such as public transportation, health care facilities, and schools, in comparison with municipalities outside MA. Among the possible biases of this classification is the fact that populations of small municipalities outside MA but bordering large municipalities within MA have almost the same level of access to those services as that observed for the neighboring populations.

The urban environment may be related to an increase in the prevalence of asthma.[16] This hypothesis is consistent with what was observed in a study conducted in Brazil in which the prevalence of asthma was found to be higher in adolescents who lived in urban areas than in those who lived in rural areas.[17] However, the specialized literature reveals disagreements regarding the role of the urban environment in modulating the prevalence of asthma.[7] A study conducted in the United States found no differences in the prevalence of asthma in MA and non-MA (classified as rural) in the state of Montana, being contrary to the argument that urbanization would increase the prevalence of asthma.[18] At the same time, apparently, the greater availability of health care networks has a positive influence on the health of individuals in urbanized areas.[7] A study conducted in Brazil analyzed data on the prevalence of asthma in children and adolescents in the different regions of the country (data obtained from the Brazilian National Household Survey conducted by the IBGE in the years 1998, 2003, and 2008). That study also assessed the prevalence of asthma by place of residence of individuals: urban or rural. The annual increase in the prevalence of asthma was 0.6% in the urban area and 1.8% in the rural area; therefore, the greatest increase in the prevalence of asthma occurred among rural dwellers. Although that study did not assess mortality rates, it showed the impact of asthma in rural areas in Brazil.[19]

The moving averages of asthma mortality rates showed a decrease in large municipalities within and outside MA (a large part of urban Brazil) and also a

decrease in "rurban" Brazil; however, there was an increase in asthma mortality rates in small and medium municipalities within MA (a small part of urban Brazil). In contrast, in rural Brazil (small municipalities outside MA), there was an increase in asthma mortality. This result was confirmed by what was observed in the population aged 5-34 years, except particularly for small municipalities outside MA (rural Brazil). In those small municipalities, unlike what was observed in the general population (an increase in rates), there was a reduction in asthma mortality rates in the population aged 5-34 years. Analysis of asthma mortality data from a regional perspective indicates that asthma control measures should be given priority in part of urban Brazil (in small and medium municipalities within MA, where there is an increase in mortality) and in rural Brazil (for the general population, even if mortality rates decrease in the population aged 5-34 years).

A decline in asthma mortality rates was also observed in other countries in the same period (1980-2012), but on a larger scale. The rate of decline in Brazil (0.67%) is lower than that reported in the United States (33% between 1994 and 2001),[20] where the number of deaths in 2009 was 27% lower than that in 1999[21]; in Brazil, the difference was 20%. In Europe, asthma mortality rates decreased by 80% between 1985 and 2012[21]; in Brazil, they decreased by 16% in the same period. Between 2000 e 2011, there was a decrease of 80% in asthma-related deaths in Costa Rica[22] and a decrease of 17% in Brazil. In Cuba, asthma mortality rates per 100,000 population decreased from 4.5 in 1982 to 2.3 in 2010 (49%),[23] a more substantial reduction than that observed in Brazil: from 1.72 to 1.38, respectively (20%). In Brazil, the Ministry of Health started providing free medication to patients with severe asthma in 2002,[15] and, since 2005, it has also provided free medication to patients with other forms of asthma,[15] which is probably a relevant factor in the reduction of asthma mortality rates.[24,25]

Asthma mortality was lower in the younger age groups, particularly in the 5-14-year age group. Apparently, the older age groups are increasing their participation in the proportion of deaths, and the opposite is occurring among younger individuals. It would be interesting to design a study to determine whether this is due to a bias in death reporting among the elderly or whether asthma lethality has been increasing with age. Asthma mortality rates, although declining in both genders, were consistently higher among females. Higher rates in females were also found in a study conducted in the Brazilian state of Rio Grande do Sul and covering the period 1981-2003.[26] Higher mortality rates in females have also been reported in studies conducted in other countries.[20,22]

A study by Graudenz et al.[27] presented equivalent asthma mortality rates and trends in all age groups, in addition to emphasizing a marked downward trend in rates in the last years of the study (as shown in Figure 1). A study by Campos,[3] which reported that asthma mortality rates in the 5-34-year age group ranged from 0.18/100,000 population to 0.28/100,000 population between 1980 and 1998, as well as a study by Lotufo & Bensenor,[4] which reported that asthma mortality rates in the population aged 5-34 years decreased between 1980 and 1991 but increased between 1992 and 1996, presented equivalent results. However, the study by Lotufo & Bensenor[4] reported a non-significant downward trend between 1997 and 2010, whereas the present study found a slight increase (0.96%).

A study by Ponte et al.,[28] analyzing the 5-24-year age group, reported higher asthma mortality rates among individuals living in urban areas. Our study also showed an increase in asthma mortality rates in part of urban Brazil (small and medium municipalities within MA), although in large municipalities of urban Brazil (both within and outside MA) there was a decrease in the moving averages of asthma mortality rates. This result was found both for the total population and for the 5-34-year age group. The differences between the results found in the present study and those of the study by Ponte et al.[28] are due to the fact that the latter study assessed the population aged 5-24 years and, separately, two 3-year periods (1999-2001 and 2009-2011), whereas our study analyzed 10 consecutive years (2002-2012). In addition, there were differences in the methodologies used; the present study used moving averages and simple linear regression, whereas the study by Ponte et al.[28] used binary logistic regression.

It is of note that the Mortality Database in Brazil uses data from death certificates, which generates potential biases that may underestimate the actual mortality rates and the conclusions of the present study.[29]

In conclusion, there is dissimilarity in the behavior of asthma mortality rates from a regional perspective, with a downward trend in large municipalities (urban Brazil) and an upward trend in small and medium municipalities within MA (also urban Brazil). In rural Brazil, rates increased in the general population and decreased in the 5-34-year age group. Asthma mortality rates were consistently higher in females throughout the study period. Mortality was lowest in the 5-14-year age group, and there was a downward trend among those under 25 years of age. In contrast, rates increased in the 75 years and over age group. This last finding may be due to diagnostic biases in death reporting, but an investigation of factors involved in mortality rate dynamics was not part of the objectives of the present study. By pointing out different regional and age-related impacts, the data presented here may contribute to informing public policies aimed at asthma control.

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ORIGINAL ARTICLE

Theoretical pneumococcal vaccine coverage: analysis of serotypes isolated from inpatients at a tertiary care hospital

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ABSTRACT

Objective: To evaluate *Streptococcus pneumoniae* serotypes isolated from an inpatient population at a tertiary care hospital, in order to determine the theoretical coverage of the 13-valent pneumococcal conjugate vaccine (PCV13) and the 23-valent pneumococcal polysaccharide vaccine (PPV23). **Methods:** This was a cross-sectional study involving 118 inpatients at the *Hospital São Lucas*, in the city of Porto Alegre, Brazil, whose cultures of blood, cerebrospinal fluid, or other sterile body fluid specimens, collected between January 2005 and December 2016, yielded pneumococcal isolates. The theoretical vaccine coverage was studied in relation to the serotypes identified in the sample and their relationship with those contained in the pneumococcal vaccines available in Brazil. **Results:** The majority of the population was male (n = 66; 55.9%), with a median age of 57 years (interquartile range: 33-72 years). The most common manifestation was pneumonia, and the pneumococcus was most commonly isolated from blood cultures. More than one fourth of the study population had some degree of immunosuppression (n = 34; 28.8%). Of the total sample, 39 patients (33.1%) died. There were no significant associations between mortality and comorbidity type, ICU admission, or need for mechanical ventilation. The theoretical vaccine coverage of PPV23 alone and PCV13 plus PPV23 was 31.4% and 50.8%, respectively. **Conclusions:** If the patients in this sample had been previously vaccinated with PCV13 plus PPV23, theoretically, 50.8% of the cases of invasive pneumococcal disease that required hospital admission could potentially have been prevented. Invasive pneumococcal disease should be prevented by vaccination not only of children and the elderly but also of adults in their economically productive years, so as to reduce the socioeconomic costs, morbidity, and mortality still associated with the disease, especially in underdeveloped countries.

Keywords: Pneumococcal infections; Serotyping; Tertiary care centers.

INTRODUCTION

Acute respiratory infections cause approximately four million deaths per year globally, being the leading cause of death in developing countries.[1] *Streptococcus pneumoniae* is the most common etiologic agent of community-acquired bacterial respiratory tract infections. [2] Invasive pneumococcal disease (IPD) accounts for a portion of all *S. pneumoniae* infections, being defined as the contamination of sterile body fluids with this agent, that is, as the isolation of *S. pneumoniae* from cultures of blood, cerebrospinal fluid, pleural fluid, or other normally sterile body sites.

IPD has high socioeconomic costs,[3] with increased (acute and late) morbidity and mortality,[4] especially in susceptible populations (children, the elderly, patients with cardiac comorbidities, patients with pulmonary comorbidities, and immunosuppressed patients in general).[5] IPD continues to be the leading vaccine-preventable cause of death in children under 5 years of age, even with the significant change in the epidemiology of this disease after the implementation of routine vaccination in Australia[6] and in western European countries.[7]

In the USA, after the marketing of the 7-valent pneumococcal conjugate vaccine in the 2000s, there was a significant reduction in the number of cases of IPD in children up to 5 years of age and in adults over 50 years of age because of the herd effect. There was also a replacement of serotypes in the community by others not previously included.[8] With the introduction of the 13-valent pneumococcal conjugate vaccine (PCV13) in that same country, the number of hospitalizations for IPD in children under 5 years of age was further reduced, and there was also some effect on hospitalizations for IPD among some adult age groups.[9]

Therefore, the present study was carried out to evaluate the microbiological characteristics of community-acquired invasive *S. pneumoniae* strains in inpatients at a tertiary care hospital in order to determine the theoretical coverage of the pneumococcal vaccines currently available in Brazil—PCV13 and 23-valent pneumococcal polysaccharide vaccine (PPV23)—as well as to quantify possible prevention. It is important to conduct studies to determine the serotypes

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of IPD and the theoretical coverage of the available vaccines so that new formulations of these vaccines can eventually be developed, including serotypes that have not yet been covered, herd effect can be determined, and the serotypes involved in IPDs in each location can be identified.

The difference between the two vaccines lies in the type of immunity conferred: PPV23 bases its ability to confer immunity on the pneumococcal polysaccharide capsule (B cell-dependent immune response), whereas PCV13 elicits a T cell-dependent immune response (long-term immune memory).[10] Chemical and serological differences between capsules are the basis for grouping pneumococci into different serotypes.[11,12] Each serotype is distinguished by the chemical structure of the capsule, by immune response, that is, by the ability to react with specific antibodies against the capsular antigen, and by other related specific mutations. However, not all of the more than 90 pneumococcal serotypes identified cause disease. Some serotypes are more strongly related to bacterial resistance, and others are more strongly related to deaths and invasive disease.[13] PCV13 includes serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. PPV23 includes all serotypes included in PCV13 except serotype 6A, plus another 11 serotypes: 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F.

In view of these considerations, the present investigation was devised to find some answers on a more local level in an attempt to determine similarities to and differences from what is already known on this subject at the regional and national level.

METHODS

This was a cross-sectional, descriptive, analytical study involving inpatients at a tertiary care hospital in the city of Porto Alegre, Brazil, in whom laboratory testing of sterile body fluids detected *S. pneumoniae*. All specimens were collected between January 2005 and December 2016. Antimicrobial susceptibility was assessed using the Kirby-Bauer method on Mueller-Hinton agar (bioMérieux, Marcy l'Étoile, France) supplemented with blood, and the antimicrobials tested included erythromycin, levofloxacin, oxacillin, sulfamethoxazole/trimethoprim, and vancomycin. All cases in which a halo of inhibition ≥ 20 mm was observed for oxacillin (1 µg) were considered susceptible to penicillin. Those in which a halo of inhibition ≤ 19 mm was observed for oxacillin were submitted to penicillin ETEST® (bioMérieux) to determine the minimum inhibitory concentration. The criteria for determining the antibiotics to be tested, as well as the criteria for interpreting halos of inhibition and susceptibility or resistance to penicillin after ETEST®, followed the recommendations of the Clinical and Laboratory Standards Institute.[14] Clinical and demographic data were collected by review of patient medical records.

Vaccine coverage data were not available from medical records, so we theorized vaccine coverage and correlated it with the serotypes identified in the sample, that is, we sought to relate each identified serotype to the potential coverage of the vaccines available in Brazil. Pneumococcal cultures were sent to the Adolfo Lutz Institute, in the city of São Paulo, Brazil, via the Central Laboratory of the State of Rio Grande do Sul, for serotyping. A decision was made to include patients with immunosuppression in the study sample, because the objective was to determine whether the serotypes identified in the sample were covered by the vaccines available. Categorical variables were expressed as frequencies and proportions, symmetric quantitative variables were expressed as means and standard deviations, and asymmetric quantitative variables were expressed as medians and interquartile ranges (IQRs). Quantitative data were compared by analysis of homogeneity of variance (Cochran's test), whereas nominal data were compared by using McNemar's test. The level of significance was set at $\alpha = 0.05$. Data were analyzed with SPSS Statistics, version 21.0 (IBM Corporation, Armonk, NY, USA). The study was approved by the Scientific Committee of the School of Medicine and the Research Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul (Protocol no. 56187816.5.0000.5336).

RESULTS

A total of 147 pneumococcal strains were initially considered for analysis, but only 118 were analyzed (losses included 15 dead strains, 6 missing samples, 5 contaminated samples, and 3 samples with pending results). The most common serotypes were, in decreasing order, 19A, 3, 12F, 8, 14, and 11A (Figure 1). Figure 2 presents the proportion of serotypes in the study sample.

The patients studied were mostly male (n = 66; 55.9%), with a median age of 57 years (IQR: 33-72 years). The most common manifestation was pneumonia (n = 90; 76.3%), followed by meningitis (n = 12; 10.2%). The most common culture was blood culture (n = 101; 85.6%), followed by cerebrospinal fluid culture (n = 15; 13.7%). Overall mortality was 33.1% (n = 39). Immunosuppressed patients (HIV and/or neoplasm and/or use of corticosteroids and/or use of immunosuppressants) accounted for 28.8% (n = 34) of the sample. In the study population, 19 patients (16.1%) had pulmonary comorbidities, and 58 (49.2%) had previous hospital admissions. ICU admission and mechanical ventilation (MV) were necessary in 41 cases (34.7%) and 28 cases (23.7%), respectively (Table 1).

When comparing the presence/absence of comorbidities as a risk factor for mortality, as well as for ICU admission, MV, and tracheostomy, we found no statistically significant differences (Table 2). There was no significant correlation between mortality and the different serotypes.

In terms of antimicrobial susceptibility, the proportion of samples resistant to sulfamethoxazole/trimethoprim, erythromycin, penicillin, and levofloxacin was 37.3%,

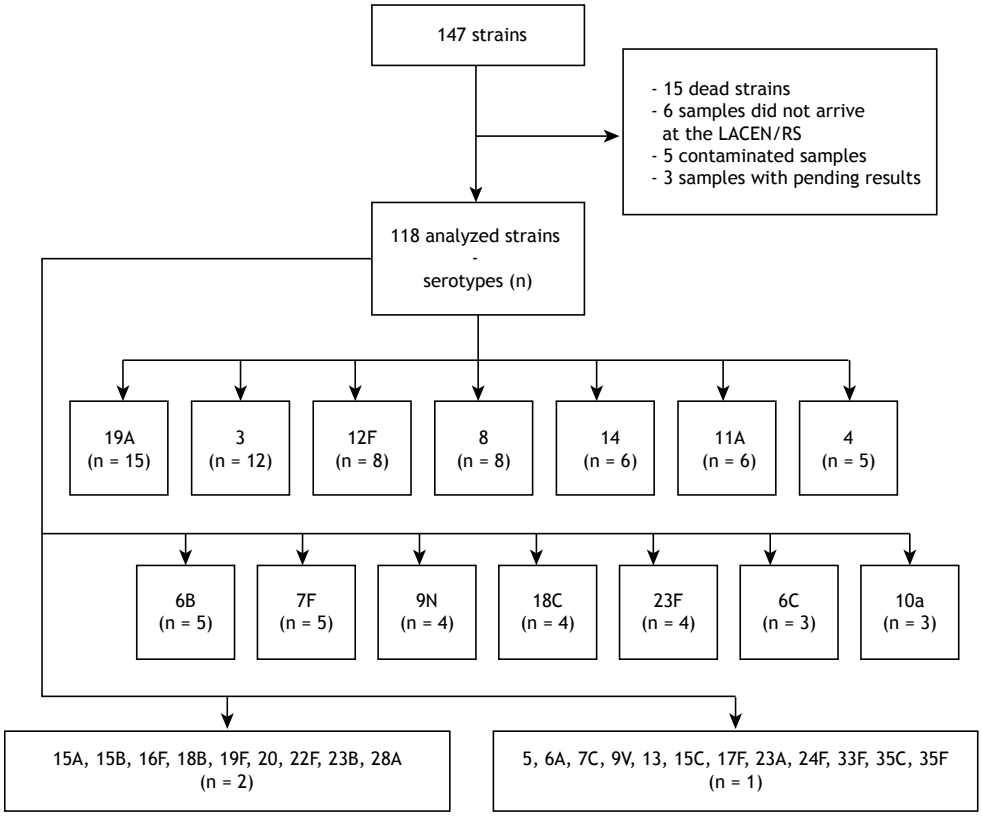

Figure 1. Flowchart of analysis of serotypes in the study population. LACEN/RS: *Laboratório Central do Estado do Rio Grande do Sul* (Central Laboratory of the State of Rio Grande do Sul).

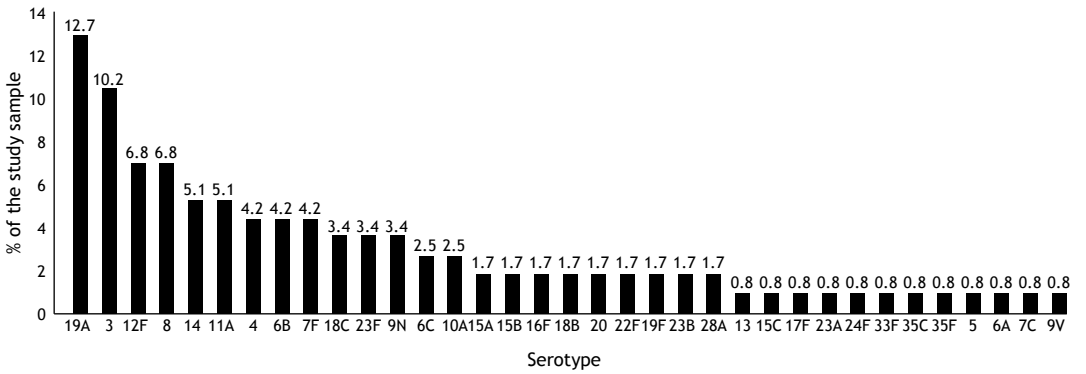

Figure 2. Frequency of serotypes in the study sample.

17.8%, 9.3%, and 1.7%, respectively. There were no cases of resistance to vancomycin. Multiple comparisons with McNemar's test showed that the strains were less susceptible to sulfamethoxazole/trimethoprim and more susceptible to vancomycin and levofloxacin.

Table 3 presents the theoretical coverage of PCV13 and PPV23 alone, as well as in combination, in relation to mortality. No statistically significant differences were detected (p = 0.508).

DISCUSSION

The present study evaluated data on serotyping of pneumococci associated with IPD in inpatients at a hospital that services a considerable portion of the population with IPD in a regional capital city in Brazil. Analysis of pneumococcal serotypes was possible in 118 (80.27%) of the cases in the total sample.

The most common serotypes in our sample were, in decreasing order, 19A, 3, 12F, 8, 14, and 11A, with serotype 19A accounting for 12.7% of the total sample. The literature on Brazilian strains tends to indicate serotype 14 as the most common.[15-19] Usually, serotypes associated with increased mortality include serotypes 3, 6A, 6B, 8, 19F, 23F, and 6C, whereas those associated with decreased mortality include serotypes 23A, 35B, and 35F.[20] In the cases analyzed

here, there were no significant differences between the serotype and the occurrence of death. In Brazil, the frequency of serotype 20B has been increasing,[21] but its detection rate was low in the sample studied here.

Table 1. Characteristics of the sample (N = 118).[a]

Variable	Result
Age, years[b]	57 (33-72)
Male gender	66 (55.9)
Major manifestation	
Pneumonia	90 (76.3)
Meningitis	12 (10.2)
Mastoiditis/abdominal abscess	3 (2.5)
Unknown/other	14 (11.9)
Mortality	39 (33.1)
Culture type	
Blood	101 (85.6)
Cerebrospinal fluid	15 (13.7)
Abdominal fluid/other	4 (3.4)
Clinical comorbidity	
Oncologic	16 (13.6)
Cardiac	48 (40.7)
Pulmonary	19 (16.1)
Gastrointestinal	14 (11.9)
Neurologic	25 (21.2)
Rheumatologic	4 (3.4)
Endocrine	26 (22.0)
Renal	13 (11.0)
Immunosuppression[c]	34 (28.8)
Use of drugs	
Licit	
Smoking	
Yes	23 (19.5)
No	54 (45.8)
Former smoker	4 (3.4)
Unknown	37 (31.4)
Alcoholism	
Yes	5 (4.2)
No	80 (67.8)
Unknown	33 (28.0)
Illicit	2 (1.7)
Previous hospital admissions	58 (49.2)
ICU admission	41 (34.7)
Use of mechanical ventilation	28 (23.7)
Tracheostomy	2 (1.7)
Length of hospital stay, days[b]	10 (5-19)

[a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as median (interquartile range). [c]HIV and/or neoplasm and/or use of corticosteroids and/or use of immunosuppressants.

In the present study, as shown in Table 3, the theoretical coverage of PPV23 alone and of PCV13 plus PPV23 was 31.4% and 50.8%, respectively. This means that, theoretically, patients receiving the vaccine combination would have a reduction in the number of IPD cases associated with the serotypes identified in the present study. Andrade et al.[18] and Mott et al.[16] reported a theoretical coverage of PCV13 of 94.1% and 64.5%, respectively, which is well above our results.

In 1988, the first case of resistance to penicillin was reported in Brazil,[22] and, in 2006, Camargos et al. published that the 7-valent pneumococcal conjugate vaccine covered 89% of penicillin-resistant pneumococci and could then help reduce the spread of these strains, thereby decreasing the need for antibiotics.[23] Regardless of the existence of vaccines, increased pneumococcal resistance to penicillin has become worrisome,[16] and, in the future, therapeutic failure may occur when starting empirical antimicrobial therapy.[24]

As early as 2006, Zettler et al. reported a prevalence of penicillin-resistant strains of 22.8% in cultures of sterile body fluids and sputum.[25] Other Brazilian authors reported finding resistance to penicillin in 13.3% of the strains and resistance to sulfamethoxazole/trimethoprim in 37.7% to 80.0% of the strains.[16,18] In the present study, these resistances were 9.3% and 37.3%, respectively, and there was no resistance to vancomycin. Serotypes 9 and 14 appear to be associated with greater resistance to penicillin,[26] a finding that was not observed in the sample studied here.

As has been shown in other studies,[27,28] the most common manifestation of IPD was community-acquired pneumonia (n = 90; 76.3%). The pneumococcus is most commonly isolated from blood cultures,[18,29] a finding similar to that of our study (n = 101; 85.6%). When studying comorbidities in the present sample in terms of mortality and need for ICU care and/or MV, we detected no significant differences, and overall mortality in our sample was 33.1% (n = 39).

A crucial part in the treatment of (invasive or noninvasive) pneumococcal disease is the use of antimicrobials, which may be decisive in the course and prognosis of the disease.[30,31] Some authors have suggested that surveillance of only cases that required hospitalization probably underestimates the true socioeconomic costs of IPD.[32] The path to reduce the high morbidity, mortality,[4,33,34] and socioeconomic costs[3] of the disease must be through prevention.

Table 2. Selected outcomes by presence of comorbidities.[a]

Outcome	With no comorbidities (n = 24)	With comorbidities (n = 94)	p
Mortality	4 (16.7)	35 (37.2)	0.095
Need for ICU admission	8 (33.3)	33 (35.1)	1.000
Need for mechanical ventilation	5 (20.8)	23 (24.5)	0.917
Tracheostomy	1 (4.2)	1 (1.1)	0.367

[a]Values expressed as n (%).

Table 3. Theoretical vaccine coverage.[a]

Vaccine coverage	Total sample[b] (N = 118)	Death (n = 39)	Non-death (n = 77)	p
PCV13 alone[c]	1 (0.8)	1 (2.6)	0 (0.0)	
PPV23 alone[d]	37 (31.4)	12 (30.8)	25 (32.5)	0.508
PCV13 plus PPV23	60 (50.8)	19 (48.7)	41 (53.2)	
No coverage	20 (16.9)	7 (17.9)	11 (14.3)	

PCV13: 13-valent pneumococcal conjugate vaccine; and PPV23: 23-valent pneumococcal polysaccharide vaccine (PPV23). [a]Values expressed as n (%). [b]Two patients with unknown outcome status (no vaccine coverage). [c]Serotype 6A. [d]Serotypes 3, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, and 33F.

There is as yet no consensus as to whether it would be possible to replace PPV23 with PCV13 in adults, it being considered that, even without having the highest number of serotypes, PCV13 would be better for the adult population because it produces more antibodies in the long term.[35,36] Some authors disagree with this position, emphasizing that cost-effectiveness studies of vaccination of adults with PCV13 (rather than with PPV23) have been influenced by biases. Among these biases are the low herd effect produced by the use of PCV13 in children and the poor prevention against community-acquired pneumonia resulting from the use of PPV23 in adults with any immunosuppression.[35,37]

Another point to be confirmed is the apparent increased frequency of IPD caused by nonvaccine serotypes in some places, including Brazil.[19] This is believed to be due to the increased number of vaccinated children, with the consequent herd effect, and the progressive replacement of vaccine serotypes with nonvaccine serotypes in the etiology of cases.

The present study was aimed at finding some answers on a more local level, that is, it sought to determine whether the serotypes in our region would match those in other parts of Brazil, establishing a connection between these findings and the theoretical coverage of the two currently available pneumococcal vaccines. Our results reinforce the need for an intensive policy of pneumococcal vaccination not only of children but also of adults 18 years of age or older with comorbidities, as well as of the elderly, in order to reduce the still very high morbidity and mortality associated with IPD, especially in developing countries.[2,27,29,38,39]

The importance of the present study lies in it demonstrating that there are several pneumococcal serotypes that caused IPD in our patient sample that are not covered by the current formulations of the available vaccines (in 49.7% of the cases). This theoretical coverage profile reflects our current situation, which should be compared with that observed in similar studies conducted elsewhere.

Our study had some limitations: serotyping was performed at another affiliated institution; and some samples could not be used in this survey. As a result, the number of cases became smaller than we first expected. In addition, vaccine coverage data were missing from the medical records of most of the cases included. Therefore, we had to evaluate theoretical vaccine coverage, that is, we sought to determine whether the identified serotypes were covered by the available vaccines.

Increased antimicrobial resistance and the existence of great serotype diversity are key elements that should be taken into account when addressing such an important issue in clinical practice: the prevention and treatment of pneumococcal diseases.[40]

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ORIGINAL ARTICLE

Electronic cigarette awareness and use among students at the Federal University of Mato Grosso, Brazil

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ABSTRACT

Objective: To analyze the prevalence of electronic cigarette (e-cigarette) awareness and experimentation among university students, as well as the characteristics associated with that awareness. **Methods:** This was a cross-sectional study, conducted in 2015, in which 489 university students at the Federal University of Mato Grosso (Cuiabá campus), Brazil, were interviewed with the use of a specific questionnaire. We estimated the prevalence of e-cigarette awareness and use, as well as analyzing the major characteristics associated with that awareness and use. **Results:** The prevalence of e-cigarette awareness was 37%, and the rate of e-cigarette experimentation was 2.7%. Awareness of e-cigarettes was found to be associated with marital status, work status, the level of parental education, and the presence or absence of smokers in the family. **Conclusions:** A high proportion of university students were aware of e-cigarettes. Although the prevalence of those who had experimented with e-cigarettes was low, there is concern that there could be an increase in the use of these types of device. There is a need for measures targeting university students, in order to build awareness of and prevent e-cigarette use.

Keywords: Electronic Nicotine Delivery Systems; Young adult; Smoking.

INTRODUCTION

Invented in 2003, electronic cigarettes (e-cigarettes), also known as electronic nicotine delivery systems, are devices that produce an aerosol by heating a liquid that contains a solvent (vegetable glycerin, propylene glycol, or a mixture of these), flavorings, and nicotine.[1] Some e-cigarettes have a light-emitting diode at the tip that is activated during use to simulate traditional smoking.[2]

The lack of regulation and quality control policies for e-cigarettes makes it difficult to determine the safety of these devices, and their potential health risks remain unclear.[3] In Brazil, according to Article 1 of Brazilian National Health Oversight Agency Collegiate Board Resolution no. 46/2009,[4] "it is forbidden to market, import, or advertise electronic smoking devices, known as electronic cigarettes, e-cigarettes, e-cigs, e-cigars, vaporizers, etc., especially those claiming to be a substitute for cigarettes, cigarillos, cigars, pipes, and similar products in the smoking habit or aimed at being an aid in smoking cessation treatment."

The use of e-cigarettes has increased exponentially since their invention in 2003. In 2010, 1.8% of U.S. adults reported having used an e-cigarette at some time, a rate that rose to 13% by 2013. The rate of those who reported being current e-cigarette users increased from 0.3% to 6.8% during the same period, and one third of these reported never having used tobacco.[1]

To determine the profile of e-cigarette users in the United States., a study based on data from the U.S. National Center for Health Statistics, published in 2016 by the American Journal of Preventive Medicine, showed that, unlike traditional cigarette smokers (African-Americans and individuals with a low level of education), e-cigarette users tended to be young, White, and single, as well as having a college education level.[5]

Few studies in Brazil have examined e-cigarette awareness and use. A study on awareness, experimentation, and current use of e-cigarettes in 10 countries, which is a result of the International Tobacco Control Project and was published in 2014 in the International Journal of Environmental Research and Public Health, showed that, in Brazil, the prevalence of e-cigarette awareness was 35% and the self-reported rate of e-cigarette experimentation was 3%. Those are comparable to the values reported for Canada and China, but are lower than those found in the United States and Australia, where the prevalence of e-cigarette awareness was 73% and 66%, respectively, and the self-reported rate of e-cigarette experimentation was 15% and 20%, respectively.[6]

Recent studies have suggested that e-cigarette use might be associated with an increased risk of using tobacco products. Enjoyment of the sensations and pharmacological effects of inhaling nicotine via an e-cigarette may increase propensity to use other products

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that also deliver inhaled nicotine, including tobacco products.[7] If e-cigarettes prove to be a means of "transition", leading to an increase in smoking, that will represent a serious public health problem in the fight against tobacco use.[8]

Since that e-cigarette awareness and experimentation have progressively increased, especially among young people, and considering the uncertainties regarding the safety and health risks of e-cigarettes, we designed this study. The objective of this study was to identify the prevalence of e-cigarette awareness and use, as well as to analyze the characteristics associated with that awareness, among students at the Federal University of Mato Grosso (Cuiabá campus), Brazil, in 2015. In addition, we aimed to lay the groundwork future public health initiatives focusing on measures to prevent and build awareness of e-cigarette use.

METHODS

This was an observational cross-sectional study involving undergraduate students at the Federal University of Mato Grosso (Cuiabá campus), Brazil, conducted in 2015.

The sample size was calculated on the basis of the prevalence of smoking among undergraduate health sciences students in the city of Cuiabá, Brazil, in 2009,[9] which was 9%, and the sampling error was set at 0.05. The random sampling was based on data from the 2012 Mandate of the Unified Selection System for the Federal University of Mato Grosso. The courses were grouped by area of knowledge (as defined by the Brazilian National Council for Scientific and Technological Development),[10] and the sample size was calculated proportionally to the number of students in each area. The sample size was increased by 20% to account for losses and refusals. Data were collected with a standardized, pre-codified, multiple-choice self-administered, anonymous questionnaire that was developed by the authors based on the Special Smoking Survey questionnaire (2008 Brazil Report).[11] The questionnaire was previously tested for comprehension on students from a class at the Federal University of Mato Grosso, in order to correct possible problems and standardize the instrument. A total of 524 questionnaires were distributed. Of those, 35 were excluded from the analysis because of inconsistencies in their completion, such as missing data on sociodemographics and smoking status, and, therefore, there were 489 valid questionnaires.

Data analysis was performed using Epi Info, version 3.5.2. In the bivariate analysis, prevalence ratios and their corresponding 95% confidence intervals were used as a measure of association between the dependent variable (e-cigarette awareness) and the other variables studied. At this stage, the chi-square test was used in order to identify statistical differences between proportions.

The study project was approved by the Research Ethics Committee of the Júlio Muller University Hospital (Ruling no. 1,443,745). In addition, permission for data collection was obtained from the Dean's Office of the Federal University of Mato Grosso (Cuiabá campus).

RESULTS

A total of 489 undergraduate students participated in the study. Of those, 258 (52.7%) were male and 231 (47.3%) were female, and the overall mean age was 23.8 years. Among the participants, 28 (5.7%) were smokers, 24 (4.9%) were former smokers, and 437 (89.4%) were nonsmokers.

The prevalence of e-cigarette awareness was 37%, and there was a statistically significant difference between genders, with 106 students (59%) being male and 75 (41%) being female ($p < 0.005$). There was an inversely proportional linear association between e-cigarette awareness and student age, that is, the younger the student, the greater the likelihood that he/she would be aware of e-cigarettes. No course-related difference was noted in e-cigarette awareness. E-cigarette awareness was found to be significantly associated with marital status and work status, 163 students (92.1%) being single and 130 (72.6%) being unemployed ($p < 0.005$). In addition, e-cigarette awareness was associated with a higher level of parental education, given that, in 72 cases (40.4%), the father had a college education level, and, in 86 (47.8%), the mother did it. A positive association was also observed between e-cigarette awareness and the presence of smokers in the family (55.6%; $p = 0.03$).

The rate of current e-cigarette use among the students was 0.61%. In addition, the rate of e-cigarette experimentation among all respondents was 2.7%, and, among those who reported being aware of e-cigarettes, that rate was 7%.

DISCUSSION

The present study is one of the first to examine e-cigarette awareness among university students in Brazil. The results presented here may inform future interventions aimed at promoting healthy lifestyle habits among undergraduate students.

The prevalence of e-cigarette awareness found among university students in this study is higher than that reported by Jeon et al.[12] in a study of university students in Korea in 2016 (21.2%) and that found in the American population in 2010 (32.2%).[13] In addition, it is slightly higher than the national prevalence in Brazil (35%).[6] This may have occurred because of the spread of aggressive advertising campaigns by e-cigarette manufacturers aiming to promote e-cigarette use. The main arguments of the e-cigarette industry include the health benefits of e-cigarettes over traditional cigarettes, the reduction in the consumption of traditional cigarettes, the cessation of smoking, the minimization of passive exposure, and the possibility of using e-cigarettes in places where smoking is forbidden.

Another important piece of information that should be highlighted in this study is the fact that most of the students who were aware of e-cigarettes

were nonsmokers. This datum is different from that reported by some authors,[13] according to whom most of the adults who had heard of e-cigarettes were smokers (49.6%). In addition, we found that the rate of e-cigarette awareness was highest among the youngest students. A study published in 2013[14] also found a similar result among British adults: 41% of the individuals between 19-24 years of age had heard of e-cigarettes. These data can be explained by the fact that this public has greater access to information and is more exposed to e-cigarette industry advertising.

We found that the male gender was more strongly related to e-cigarette awareness, as was also observed in a study published in 2017[5] Our study shows that the students who were aware of e-cigarettes were those whose parents had a higher level of education. Similar data were also reported by a study published in 2005,[15] in which 40% and 44.6% of fathers and mothers, respectively, had a college education level; however, the study focused on traditional smoking rather than e-cigarettes. In contrast, a study published in 2008[16] showed an inverse relationship between the level of parental education and tobacco experimentation.

Although no statistically significant, course-related difference was noted, we found that the rate of e-cigarette experimentation was higher among undergraduates in the human, social, and agricultural sciences. This was also observed among students at the University of Brasília, Brazil, in 2006.[17]

In the present study, there was a statistically significant association between e-cigarette experimentation and having smokers in the family. This was also observed among university students in Korea in 2016.[12] We also found that not having a job was associated with greater e-cigarette awareness.

In our total sample, the rate of e-cigarette experimentation was found to be highest in the group of those who were aware of e-cigarettes (7%), from which it can be inferred that awareness arouses curiosity and leads to experimentation. This percentage is lower than that found in a study published in 2013,[18] in which 16% of those who were aware of e-cigarettes had tried them.

The rate of regular e-cigarette use in this study was 0.61%, a value that is close to that found among university students in Korea: 0.8% of the smokers used only e-cigarettes.[12] A study published in 2013[14] found a 6.9% rate of regular e-cigarette use among British adults; however, it should be considered that e-cigarette use and marketing are allowed and regulated in Great Britain, which explains the higher prevalence of regular users.

Therefore, considering the awareness and availability of e-cigarettes among university students and the general population, it is essential that there are interventions that have the objective of promoting healthy habits among the students and that can discourage the use of these types of device, ultimately preventing the increase in the consumption of other products that also release inhaled nicotine, including tobacco products.

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ORIGINAL ARTICLE

Functional capacity measurement: reference equations for the Glittre Activities of Daily Living test

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ABSTRACT

Objective: To develop reference equations for the Glittre Activities of Daily Living test (Glittre ADL-test) on the basis of anthropometric and demographic variables in apparently healthy individuals. A secondary objective was to determine the reliability of the equations in a sample of COPD patients. **Methods:** This was a cross-sectional study including 190 apparently healthy individuals (95 males; median age, 54.5 years [range, 42-65]; median FEV_1 = 97% [range, 91-105.2]; and median FVC = 96% [range, 88.5-102]) recruited from the general community and 74 COPD patients (55 males; mean age, 65 ± 8 years; body mass index [BMI] = 25.9 ± 4.7 kg/m^2; FEV_1 = 36.1 ± 14.1%; and FVC = 62.7 ± 16.1%) recruited from a pulmonary rehabilitation center. **Results:** The mean time to complete the Glittre ADL-test was 2.84 ± 0.45 min. In the stepwise multiple linear regression analysis, age and height were selected as Glittre ADL-test performance predictors, explaining 32.1% ($p < 0.01$) of the total variance. Equation 1 was as follows: $\text{Glittre ADL-test}_{predicted} = 3.049 + (0.015 \times \text{age}_{years}) + (-0.006 \times \text{height}_{cm})$. Equation 2 included age and BMI and explained 32.3% of the variance in the test, the equation being as follows: $\text{Glittre ADL-test}_{predicted} = 1.558 + (0.018 \times \text{BMI}) + (0.016 \times \text{age}_{years})$. **Conclusions:** The reference equations for the time to complete the Glittre ADL-test were based on age, BMI, and height as independent variables and can be useful for predicting the performance of adult individuals. The predicted values appear to be reliable when applied to COPD patients.

Keywords: Activities of daily living; Exercise test; Reference values.

INTRODUCTION

Reference equations for tests that measure functional capacity are essential for interpreting functional test results, assisting in quantifying the impairment of activities of daily living (ADL) and therapeutic response. Functional status is a multidimensional concept that characterizes the ability that a person has to provide for the necessities of life.[1] Factors such as aging,[2] obesity,[3] and chronic disease,[4] such as COPD, can negatively affect patient functional status. Exercise and ADL limitations are common manifestations in patients with COPD, increasing morbidity and mortality.[5] To evaluate the functional status of COPD patients, Skumlien et al.[6] developed the Glittre ADL-test, which is a standardized set of ADL-like activities known to be difficult for COPD patients. The Glittre ADL-test consists of multiple tasks that require upper and lower limb muscle activity during walking, rising from the seated position, stair climbing/descending, crouching, kneeling, carrying objects, and lifting objects.[6,7]

The Glittre ADL-test is an easily administered, valid, and reliable tool to measure functional status[7] in patients with stable COPD,[6,8,9] exacerbated COPD,[10] heart failure,[11] or community-acquired pneumonia/other respiratory diseases,[10] as well as in obese patients and post-bariatric surgery patients.[12] In addition, the Glittre ADL-test can differentiate between the functional status

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of healthy individuals and that of patients with COPD.[8] Furthermore, the time to complete the Glittre ADL-test has been shown to correlate with walking and sitting time in COPD patients, as well as with other ADL.[13] Similar physiological responses were found between the Glittre ADL-test and the six-minute walk test (6MWT), oxygen uptake being slightly higher during the Glittre ADL-test.[9]

Given that the factors that influence patient performance on the Glittre ADL-test have yet to be determined,[7] there are currently no reference equations to predict patient functional status on the basis of anthropometric and demographic data. Therefore, the objective of the present study was to investigate the influence of the aforementioned variables on patient performance on the Glittre ADL-test in order to generate reference equations for healthy individuals in the 20- to 80-year age bracket. A secondary objective was to determine the reliability of the equations in a sample of patients with COPD.

METHODS

Participants

Apparently healthy individuals were selected from among those living in the city of Florianópolis, Brazil. The inclusion criteria were as follows: being in the 20- to 80-year age bracket and being clinically stable (i.e., having had no severe or unstable disease in the six weeks prior to the study). The exclusion criteria were as follows: post-bronchodilator FEV_1 and FVC < 80% of the predicted value; post-bronchodilator FEV_1/FVC < 0.7; body mass index (BMI) < 18.5 kg/m^2 or > 40 kg/m^2; active smoking in the six months prior to the study; high physical activity level; and inability to understand or perform any of the tested activities. Of the 223 apparently healthy individuals who were selected to participate, 33 were excluded, 190 having therefore remained in the study.

In order to determine the reliability of the reference equations, 74 COPD patients were selected from among those treated at the Santa Catarina State University Treatment, Education, and Research Center for Pulmonary Rehabilitation, located in the city of Florianópolis, Brazil. Patients ≥ 40 years of age with Global Initiative for Chronic Obstructive Lung Disease stage II-IV COPD,[5] a smoking history ≥ 20 pack-years, and clinical stability in the four weeks prior to the study were included in the study. The exclusion criteria were as follows: long-term oxygen therapy, current smoking, lung diseases other than COPD, and comorbidities affecting patient ability to perform any of the tested activities.

Study protocol

Participants were asked questions regarding their medical history, medication use, and smoking history. Patient weight (in kg) and height (in cm) were measured with a digital scale (Tanita Corporation, Tokyo, Japan) and a portable stadiometer (Sanny; American Medical do Brasil Ltda., São Bernardo do Campo, Brazil), respectively, and the BMI was calculated by the formula weight/height^2 (kg/m^2). Lung function was assessed with an EasyOne® spirometer (ndd Medical Technologies, Andover, MA, USA), in accordance with the methods and criteria recommended by the American Thoracic Society and the European Respiratory Society,[14] predicted values being calculated.[15]

Participants performed the Glittre ADL-test twice, with a 30-min interval between tests. The shorter of the two tests was selected for data analysis. In individuals in the 20- to 59-year age bracket and in those in the 60- to 80-year age bracket the level of physical activity was measured by the short form of the International Physical Activity Questionnaire[16] and the version that has been adapted for use in Brazilian elderly individuals,[17] respectively.

The group of COPD patients underwent anthropometric measurements, pulmonary function testing, the Glittre ADL-test, and the 6MWT. The 6MWT was performed indoors along a flat, straight, 20-m corridor, in accordance with the American Thoracic Society guidelines.[18,19] The percent predicted six-minute walk distance (6MWD) was calculated on the basis of an equation developed by Britto et al.[20]

All participants gave written informed consent. The study was approved by the Research Ethics Committee of the Santa Catarina State University (Protocol no. 225/2011).

Glittre ADL-test

The Glittre ADL-test was performed as described by Skumlien et al.[6] and comprises the following tasks: walking along a flat surface; stair climbing and descending; moving objects from one shelf to another (as well as putting them on the floor and back on the shelves); and rising from and sitting in a chair. Female participants carried a backpack weighing 2.5 kg, whereas male participants carried a backpack weighing 5.0 kg.[6] Heart rate, pulse oximetry, dyspnea (modified Borg scale),[21] and blood pressure were measured.

Statistical analysis

Statistical analysis was performed with the IBM SPSS Statistics software package, version 20.0 (IBM Corporation, Armonk, NY, USA) and the GraphPad Prism software, version 5.0 (GraphPad Inc., San Diego, CA, USA). The sample size required to generate a reference equation for healthy individuals in the 20- to 80-year age bracket was calculated by the following formula:

$$N > 50 + 8m$$

where m is the number of independent variables.[22]

For five independent variables based on the theoretical assumptions (gender, age, height, weight, and BMI),[20,23-28] the minimum sample size was calculated to be 90. Given that the study sample comprised individuals in the 20- to 80-year age bracket, it was divided into six age groups (20-29 years; 30-39 years; 40-49 years; 50-59 years; 60-69 years; and 70-80 years),

each of which comprised at least 14 individuals. The sample size required to maintain a balance between the genders was calculated to be 190.

Data were expressed as mean ± standard deviation or median (95% CI). The normality of the data was verified with the Kolmogorov-Smirnov test. Comparisons were performed with the Wilcoxon test, the Mann-Whitney test, or the independent sample t-test.

The association between the time to complete the Glittre ADL-test and the independent variables was tested by simple linear regression analysis. Stepwise multiple linear regression analysis was used in order to evaluate independent variables explaining the variance in the Glittre ADL-test. The normality of the residuals was verified graphically (histogram and Q-Q plot), and multicollinearity was assessed by examining tolerance ($1 - r^2$) and variance inflation factor ($1/1 - r^2$).[29] Reliability was assessed by Spearman's correlation coefficient, linear regression analysis, and the intraclass correlation coefficient (ICC). In addition, a paired-sample t-test or the Wilcoxon test was used in order to compare the actual time to complete the Glittre ADL-test with the predicted value.[20,30] The age-predicted maximal heart rate was calculated by the following formula: $208 - 0.7*age_{years}$.[31] Bland-Altman plots[29,31] were used in order to evaluate the agreement between the actual time to complete the Glittre ADL-test and the predicted value.[30,32] The significance level was set at $p = 0.05$ for all analyses.

RESULTS

Of the 223 apparently healthy individuals who were selected to participate, 190 had normal lung function and completed the study (Table 1). Of those, 95 were male. A total of 33 individuals were excluded, for the following reasons: restrictive or obstructive lung disease, in 17; inability to understand or perform any of the tested activities, in 14; active smoking in the six months prior to the study, in 1; and high self-reported physical activity, in 1. Of the 190 apparently healthy participants, 152 (80%) were classified as physically active. In addition, 51.5% were nonsmokers and 30.5% were former smokers. Self-reported comorbidities included systemic hypertension (in 25%), metabolic disorders (in 14.9%), thyroid disorders (in 8.2%), stable cardiac disease (in 2.6%), and osteoporosis (in 1.3%).

A total of 151 participants (79.5%) performed better on the second Glittre ADL-test than on the first. There was a 0.16 reduction in the mean time to complete the second test in comparison with the first (0.21 min; $p < 0.01$), with a 5.3% learning effect. The best test was completed in 2.84 ± 0.45 min (2 min and 50 s). The shortest and longest tests were 1.92 min (1 min and 55 s) and 4.17 min (4 min and 10 s) in duration, respectively. The median time to complete the first test was 2.95 min (range, 2.95-3.09), i.e., 2 min and 57 s, and the median time to complete the second test was 2.82 min (range, 2.80-2.92), i.e., 2 min and 49 s ($p < 0.01$). With the exception of systolic blood pressure, there were no differences in physiological variables between the first and second tests (Table 2). An ICC of 0.95 (95% CI: 0.93-0.96; $p < 0.01$) was found between the time to complete the first test and the time to complete the second test. There was no significant difference between males and females regarding their performance on the Glittre ADL-test ($p = 0.35$).

The simple linear regression analysis showed that the performance on the Glittre ADL-test was significantly associated with age ($R^2 = 0.30$; $p < 0.01$), height ($R^2 = 0.09$; $p < 0.01$), and BMI ($R^2 = 0.05$; $p = 0.01$) but not with weight or gender. In the stepwise multiple linear regression analysis, age and height were selected as Glittre ADL-test performance predictors, explaining 32.1% ($p < 0.01$) of the total variance. In addition, BMI and age explained 32.3% ($p < 0.01$) of the total variance when only participants with a BMI of < 35 kg/m^2 were taken into account.

The reference equation for the Glittre ADL-test was as follows ($R^2 = 0.321$):

$$\text{Glittre ADL-test}_{predicted} = 3.049 + (0.015 \times age_{years}) + (-0.006 \times height_{cm})$$

A second equation was generated by excluding the participants with a BMI ≥ 35 kg/m^2 ($R^2 = 0.323$):

$$\text{Glittre ADL-test}_{predicted} = 1.558 + (0.018 \times BMI) + (0.016 \times age_{years})$$

No multicollinearity was present (Table 3). The Bland-Altman plots showed good agreement between the actual time to complete the Glittre ADL-test and the predicted values in the group of healthy individuals (Figures 1A and C) but not in the group of patients with COPD (Figures 1B and D).

The reliability of the reference equations for the Glittre ADL-test was tested in 74 COPD patients (Table 1). An ICC of 0.97 (95% CI: 0.95-0.98; $p < 0.01$) was found between the time to complete the first test and the time to complete the second test. A strong correlation was found between the mean time to complete the Glittre ADL-test (4.70 ± 1.9 min; 147.4 ± 57.6% of the predicted value) and the mean 6MWD (435.1 ± 101.1 m; 78.4 ± 17.4% of the predicted value; $r = -0.81$; $p < 0.01$). In addition, a strong correlation was found between percent predicted values for equation 1 ($r = -0.74$; $p < 0.01$) and equation 2 ($r = -0.86$; $p < 0.01$; Figure 2), with a significant association between the two (equation 1: $R^2 = 0.51$; equation 2: $R^2 = 0.65$; $p < 0.01$). Significant differences were found between the actual performance on the Glittre ADL-test and the predicted values for the two equations ($p < 0.05$).

DISCUSSION

To our knowledge, this is the first study to establish reference equations for the Glittre ADL-test on the basis of how healthy individuals perform on the test. The mean time to complete the Glittre ADL-test was 2.84 min (2 min and 50 s). Among the independent

Table 1. Characteristics of the study sample.[a]

Variable	Healthy individuals (n = 190)	Healthy males (n = 95)	Healthy females (n = 95)	COPD patients (n = 74)
Age, years[b]	54.5 (42-65)	54 (43-64)	56 (41-65)	66 (59-73)
Age groups[c]				
20-29	16 (8.4)	7 (7.4)	9 (9.5)	-
30-39	18 (9.5)	8 (8.4)	10 (10.5)	-
40-49	42 (22.1)	25 (26.3)	17 (17.9)	4 (5.4)
50-59	45 (23.7)	24 (25.3)	21 (22.1)	15 (20.3)
60-69	38 (20)	17 (17.9)	21 (22.1)	33 (44.6)
70-80	31 (16.3)	14 (14.7)	17 (17.9)	22 (29.7)
Weight, kg	72.8 (12.8)	78.6 (12.0)	67.1 (10.9)*	72.3 (15.1)
Height, cm[b]	166 (159-174)	174 (169-178)	159 (154-163)*	167 (159-173)
BMI, kg/m^2[b]	25.6 (23.4-28.9)	25.4 (24-27.9)	26.0 (22.7-29.9)	25.3 (22.1-29.6)
BMI categories[c]				
Normal weight	85 (44.7)	44 (46.3)	41 (43.2)	35 (47.3)
Overweight	72 (37.9)	40 (42.1)	32 (33.7)	23 (31.1)
Obesity	33 (17.4)	11 (11.6)	22 (23.2)	16 (21.6)
FEV_1/FVC[b]	0.83 (0.79-0.87)	0.85 (0.79-0.87)	0.82 (0.79-0.87)	0.43 (0.38-0.52)
FEV_1, % predicted[b]	97 (91-105.2)	97 (91-106)	97 (90-105.2)	35.5 (24.7-45.0)
FVC, % predicted[b]	96 (88.5-102)	94 (86-101)	96 (90-102.5)	61 (51-73.2)
Glittre ADL-test, min[b]	2.80 (2.52-3.10)	2.75 (2.43-3.13)	2.85 (2.60-3.08)	4.11 (3.46-5.24)

BMI: body mass index; and ADL: activities of daily living. [a]Data expressed as mean ± SD, except where otherwise indicated. [b]Data expressed as median (interquartile range). [c]Data expressed as frequency (relative frequency). *p < 0.05 vs. males.

Table 2. Differences in physiological parameters between the two Glittre ADL-tests performed by healthy individuals.[a]

Variable	Glittre ADL-test 1	Glittre ADL-test 2	p
Glittre ADL-test, min	2.95 (2.66-3.33)	2.82 (2.52-3.12)	< 0.01
Baseline HR, bpm	77.0 (70.0-86.0)	77.0 (71.0-86.0)	0.91
ΔHR, bpm	39.0 (30.0-46.0)	38.5 (29.0-50.0)	0.64
Age-predicted HR_{max}	69.7 (61.8-78.3)	68.9 (62.2-79.2)	0.99
Initial SBP, mmHg	120.0 (110.0-130.0)	120.0 (110.0-130.0)	0.02
Final SBP, mmHg	140.0 (130.0-160.0)	140.0 (130.0-152.5)	0.13
ΔSBP, mmHg	20.0 (10.0-30.0)	20.0 (20.0-30.0)	0.55
Initial DBP, mmHg	80.0 (70.0-80.0)	80.0 (70.0-80.0)	0.45
Final DBP, mmHg	80.0 (70.0-90.0)	80.0 (70.0-90.0)	0.55
ΔDBP, mmHg	0 (0-10.0)	0 (0-10.0)	0.84
Initial Borg score	0 (0-0)	0 (0-0)	0.55
Final Borg score	0.5 (0-1.0)	0.0 (0-1.0)	0.81
ΔBorg score	0.5 (0.0-1.0)	0.0 (0-1.0)	0.88

ADL: activities of daily living; Δ: variation (final value − baseline value); SBP: systolic blood pressure; DBP: diastolic blood pressure; and Borg: Borg dyspnea scale. [a]Data expressed as median (interquartile range).

variables, age and height were found to be significant independent predictors, explaining 32.1% of the variance in the Glittre ADL-test when individuals with a BMI of 18.5-40 kg/m^2 were taken into account (equation 1). However, when individuals with a BMI ≥ 35 kg/m^2 were excluded (equation 2), age and BMI accounted for 32.3% of the variance in the test.

Of the 190 healthy participants, 79.5% performed better on the second Glittre ADL-test. The learning effect was 5.3%, and the shortest test was 1.92 min (1 min and 55 s) in duration. Skumlien et al.[6] found that the shortest test among healthy individuals was 2 min in duration. However, the demographic and anthropometric characteristics of those individuals were not mentioned. In a previous study, our research group showed that healthy individuals in the 20- to 39-year age bracket completed the Glittre ADL-test in 2.62 ± 0.34 min (2 min and 37 s), the shortest test having lasted 2.03 min (2 min and 2 s), with a 6.3% learning effect.[33] Therefore, it is possible that individuals who complete the test in approximately 2 min have preserved functional capacity.

Anthropometric and demographic variables are usually helpful to determine individual performance on functional tests.[20,23-25] Therefore, we hypothesized that variables such as age, height, BMI, weight, and gender would be predictors of the time to complete the Glittre ADL-test.

Table 3. Model for predicting the time to complete the Glittre ADL-test.

Variable	Unstandardized coefficient (B)	95% CI for B	p	Part correlation	Tolerance	Variance inflation factor
Equation 1						
Constant	3.049	2.095-4.004	< 0.01			
Age, years	0.015	0.011-0.019	< 0.01	0.503	0.902	1.109
Height, cm	−0.006	−0.011 to −0.001	0.02	−0.163	0.902	1.109
Equation 2						
Constant	1.558	1.142-1.974	< 0.01			
BMI	0.018	0.002-0.033	< 0.01	0.165	0.963	1.038
Age, years	0.016	0.012-0.019	< 0.01	0.530	0.963	1.038

ADL: activities of daily living; and BMI: body mass index. Equation 1: Glittre ADL-test$_{predicted}$ = 3.049 + (0.015 × age$_{years}$) + (−0.006 × height$_{cm}$); standard error of the estimate = 0.371. Equation 2: Glittre ADL-test$_{predicted}$ = 1.558 + (0.018 × BMI) + (0.016 × age$_{years}$); standard error of the estimate = 0.373.

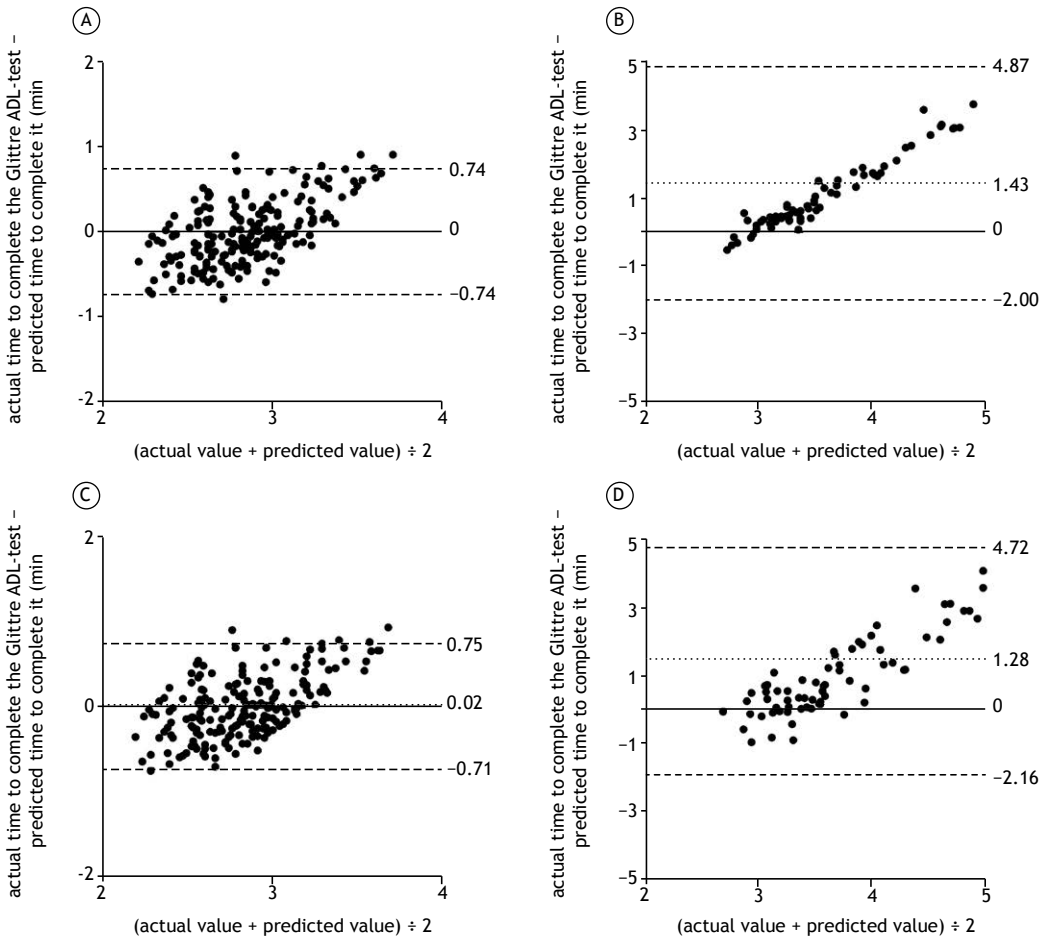

Figure 1. Bland-Altman plots of the difference between the actual time to complete the Glittre ADL-test and the predicted time to complete it (in min), and the mean of the actual and predicted values for healthy individuals (A and C) and COPD patients (B and D). The central dotted line represents the mean difference between the actual and predicted values, whereas the upper and lower dashed lines represent the upper and lower limits of agreement, respectively. ADL: activities of daily living. Equation 1: Glittre ADL-test$_{predicted}$ = 3.049 + (0.015 × age$_{years}$) + (−0.006 × height$_{cm}$). Equation 2: Glittre ADL-test$_{predicted}$ = 1.558 + (0.018 × BMI) + (0.016 × age$_{years}$). Panels A and B refer to equation 1, whereas panels C and D refer to equation 2.

Age was the only independent variable that remained in the two equations. Aging affects muscle mass, strength, resistance, balance, and coordination—all of which are components of functional capacity—leading to a progressive physical decline even in healthy physically active individuals.[34] In the present study, age was related to a longer time to complete the Glittre ADL-test. This might be due to tasks such as

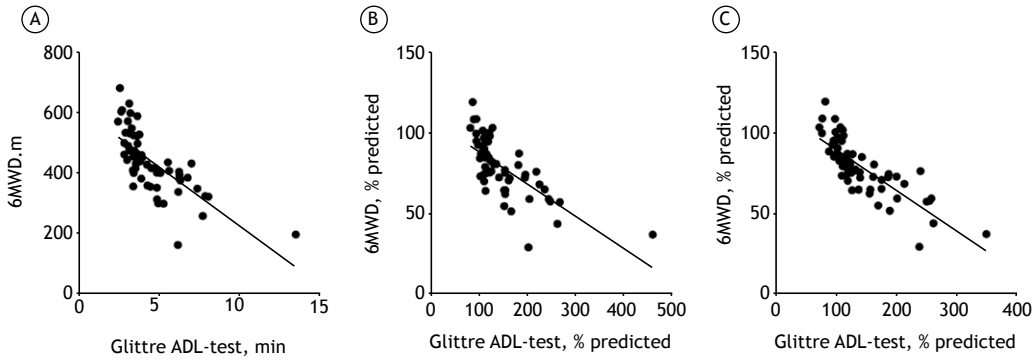

Figure 2. In A, correlation between the time to complete the Glittre ADL-test, in min, and the six-minute walk distance (6MWD), in m ($r = -0.81$; $p < 0.01$). In B, correlation between the percent predicted Glittre ADL-test value (as predicted by equation 1) and the percent predicted 6MWD[20] ($r = -0.74$; $p < 0.01$). In C, correlation between the percent predicted Glittre ADL-test value (as predicted by equation 2) and the percent predicted 6MWD[20] ($r = -0.86$; $p < 0.01$). ADL: activities of daily living. Equation 1: Glittre ADL-test$_{predicted}$ = 3.049 + (0.015 × age$_{years}$) + (−0.006 × height$_{cm}$). Equation 2: Glittre ADL-test$_{predicted}$ = 1.558 + (0.018 × BMI) + (0.016 × age$_{years}$).

sitting in and rising from a chair, walking, crouching/kneeling, and stair climbing/descending. The ability to rise from a chair deteriorates with age; aging is a major source of disability and impaired autonomy, being highly associated with loss of quadriceps strength.[35] Age is also an important predictor of walking speed, being included as a variable in equations for several functional status tests, such as the 6MWT and the incremental shuttle walk test.[23,26,27,36,37] In addition to walking, individuals performing the Glittre ADL-test are required to crouch and kneel, which are activities that a quarter of older adults have difficulty in performing or are unable to perform; this is probably due to ankle plantar flexor and knee weakness, as well as to balance impairment.[38] Stair climbing and descending are also compromised in elderly individuals, because of reduced muscle strength, reduced balance, pain, fear of falling, and impaired sensation.[38]

In the second of the two equations generated in the present study, the BMI was the predictor with the highest coefficient. Previous studies have shown the influence of the BMI on patient performance on functional tests such as the 6MWT[20,27] and the incremental shuttle walk test.[23] In the present study, a higher BMI translated to a worse performance on the Glittre ADL-test. Obesity increases the workload for a given activity,[25] and body composition is significantly associated with walking speed and endurance,[39] as well as with walking performance and the ability to perform the sit-to-stand test without assistance.[35]

Although it could be argued that the inclusion of patients with a BMI ≥ 35 kg/m^2 introduced a bias in the present study because reference values should be derived from normal individuals, a decision was made to include such patients in the model for equation 1 but exclude them from the model for equation 2. In a real-life scenario, health professionals habitually encounter chronic respiratory disease patients with class II or III obesity and should be able to calculate the predicted values for functional capacity tests in such patients. There is controversy in the literature regarding this issue, such patients having been excluded from some studies[24,25,40] but not from others.[20,23,26-28] Given that the two equations were found to have similar coefficients of determination and similar statistical properties, the choice between the two should be made on the basis of the BMI.

We had hypothesized that weight and height were predictors of a longer time to complete the Glittre ADL-test and a shorter time to complete the test, respectively. Overweight influences gait and increases the workload on horizontal and vertical displacements, which occur during the Glittre ADL-test tasks of walking, sitting/standing, stair climbing/descending, and crouching/kneeling.[41] However, weight was not retained in either equation.

Despite its low coefficient, height was considered to be an independent predictor of performance on the Glittre ADL-test, although only for equation 1. This might be due to the fact that the taller the person, the longer his or her legs and, consequently, the longer his or her stride, their walking therefore being more efficient[25] and contributing to a shorter time to complete the Glittre ADL-test.

Gender was found to have no influence on the time to complete the Glittre ADL-test, a finding that is consistent with those of other studies examining patient functional status.[23,24,27,28,33] The lack of association between gender and the time to complete the Glittre ADL-test might be partly explained by the lack of statistical difference between males and females regarding their performance on the test. In addition, it is possible that the heavier backpack carried by males influenced their performance and compensated for potential fitness differences between the genders. Although Skumlien et al.[6] had females carrying a backpack weighing 2.5 kg and males carrying a backpack weighing 5.0 kg, the impact of this additional weight on male performance on the Glittre ADL-test remains unclear.

Although the reliability of a reference equation is usually confirmed in healthy individuals, our equations were tested in COPD patients because the Glittre ADL-test was validated for use in and is primarily administered to such patients. In addition, the significance of the time to complete the Glittre ADL-test has yet to be established. Therefore, the 6MWD and its predicted value were selected to test the reliability of the derived equations, because the 6MWT and the Glittre ADL-test were designed to assess functional capacity and because the latter has been found to correlate strongly with the former in COPD patients.[6,9] In the present study, the predicted Glittre ADL-test value was strongly correlated with the percent predicted 6MWD, which was calculated on the basis of an equation developed by Britto et al.[20] The reference equations for the Glittre ADL-test were found to be reliable because they evidenced the impaired functional status of the patients with COPD, for whom the mean time to complete the test was approximately 45.2% longer than the expected maximum time to complete it. It could be argued that there is poor agreement between actual and predicted values for patients with COPD, especially those with worse functional impairment. However, the proposed equations are expected to underestimate the predicted values because it takes patients with COPD longer to complete the Glittre ADL-test than it does healthy individuals,[6,8,33] COPD patients therefore showing values > 100% of the predicted value. Therefore, the worse the functional impairment, the more the actual performance on the Glittre ADL-test will differ from the predicted value derived from apparently healthy individuals. This reinforces the fact that the Glittre ADL-test is better at differentiating between patients with severe functional limitations[6] and those with preserved functional capacity, given that it takes the latter group of patients almost the same time to complete the test as it does healthy individuals (i.e., approximately 3 min). As expected, poor agreement, moderate correlations, and significant differences were found between the actual performance on the Glittre ADL-test and the predicted value in the group of COPD patients investigated in the present study.

The present study has some limitations that should be noted. Although we calculated the required sample size for the study and attempted to maintain a balance between the genders and the numbers of individuals in each age group, ours was a convenience sample. Given that only approximately 32% of the variance in the time to complete the Glittre ADL-test was explained by the derived equations, it remains to be determined whether other factors, such as balance, peripheral muscle strength, behavior, cognition, and physiological factors, have any influence on the time to complete the test. However, it should be noted that studies aimed at generating reference equations for the 6MWT have shown a variance similar to that observed in the present study, having included independent variables similar to those included in the present study.[24,25,28,40] In order to prioritize clinical practice, we investigated the reliability of our reference equations in a group of COPD patients, in whom impaired functional capacity was evidenced by establishing a relationship between their performance on the Glittre ADL-test and their performance on the 6MWT and by comparing their performance on the Glittre ADL-test with that of healthy individuals. Therefore, future studies should investigate the reliability of the equations in healthy individuals.

In summary, the two equations generated in the present study can explain approximately 32% of the variance in the time to complete the Glittre ADL-test. The choice of equation for use depends on the BMI. The predicted values appear to be reliable when applied to patients with COPD.

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Does methylene blue attenuate inflammation in nonischemic lungs after lung transplantation?

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ABSTRACT

Objective: To evaluate whether methylene blue (MB) could minimize the effects of ischemia-reperfusion injury in the nonischemic lung on a lung transplantation rodent model. **Methods:** Forty female Sprague-Dawley rats were divided into 20 donors and 20 recipients. The 20 recipient rats were divided into two groups (n = 10) according to the treatment (0.9% saline vs. 1% MB solutions). All animals underwent unilateral lung transplantation. Recipients received 2 mL of saline or MB intraperitoneally prior to transplantation. After 2 h of reperfusion, the animals were euthanized and histopathological and immunohistochemical analyses were performed in the nonischemic lung. **Results:** There was a significant decrease in inflammation—neutrophil count and intercellular adhesion molecule-1 (ICAM-1) expression in lung parenchyma were higher in the saline group in comparison with the MB group—and in apoptosis—caspase-3 expression was higher in the saline group and Bcl-2 expression was higher in MB group. **Conclusions:** MB is an effective drug for the protection of nonischemic lungs against inflammation and apoptosis following unilateral lung transplantation in rats.

Keywords: Reperfusion injury; Methylene blue; Lung transplantation; Apoptosis; Inflammation.

INTRODUCTION

The etiology of ischemia-reperfusion injury (IRI) primarily involves the increased formation of reactive oxygen species (ROS).[1,2] A decreased oxygen supply reduces the synthesis and resynthesis of ATP, creating an ionic gradient in the cell membrane due to decreased extracellular active calcium transport. The accumulation of cytoplasmic calcium leads to the activation of a protease that converts xanthine dehydrogenase to xanthine oxidase.[3] Concurrent with these events, there is an accumulation of AMP, which decomposes into substances such as adenosine, inosine, and hypoxanthine. During the reperfusion process, in the presence of oxygen, xanthine oxidase converts hypoxanthine into ROS, such as superoxide, peroxide, and hydroxyl radicals.[3,4] The release of ROS causes cell inflammation and apoptosis of cells as a late phase response of IRI.[5]

Methylene blue (MB) prevents ROS production by acting as an alternative xanthine oxidase electron receptor, competing with molecular oxygen for electron transfer. The electrons are transferred to MB from the iron-sulfur center of xanthine oxidase, thus preventing the conversion of molecular oxygen into superoxide radicals.[4]

We previously demonstrated that MB was able to reduce the effects of IRI when we studied transplanted lungs of rats.[6] Some of the free radicals, proteases, and other mediators produced by ischemia and reperfusion after unilateral lung transplantation are washed out and released into the blood stream, and the mediators reach the contralateral lung. There have been few studies on

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the effect of IRI after single-lung transplantation on the nonischemic lung.[7]

The objective of the present study was to evaluate the effects of MB as an inhibitor of IRI on nonischemic right lungs after left lung transplantation in rats.

METHODS

Forty female Sprague-Dawley rats (300-350 g) were used in the present study (20 donors/20 recipients). Recipient rats were divided into two groups (n = 10) according to treatment with saline solution (SAL) or MB, i.e., SAL group and MB group. The study was approved by our institutional research ethics committee (CAPPesq Protocol no. 3387/09/138) and performed in accordance with the Guide for the Care and Use of Laboratory Animals.[8]

Surgical procedure

Donors

The animals were anesthetized with 5% isoflurane (Isothane; Baxter, Jayuya, PR, USA), orotracheally intubated, and mechanically ventilated (model 683; Harvard Apparatus, Holliston, MA, USA) with a volume of 10 mL/kg and a respiratory rate of 80 breaths/min. General anesthesia was maintained with 2% isoflurane (Isovapor model 1224; Takaoka, São Paulo, Brazil). After median laparotomy, 500 U of heparin were injected into the inferior vena cava. After one minute, a median sternotomy was performed, and pulmonary artery was cannulated for anterograde perfusion with 20 mL of low-potassium dextran (LPD) solution (Perfadex®; Vitrolife, Kungsbacka, Sweden) at 4°C with constant pressure (20 cmH_2O). Prior to perfusion, the inferior vena cava was sectioned to decrease venous return, and the left atrial appendage was amputated to drain the LPD solution. Animals were euthanized by exsanguination in accordance with the American Veterinary Medicine Association.[9]

After perfusion, the trachea of the animals was tied at the end of inspiratory flow, and the cardiopulmonary block was excised and placed into a Petri dish with cold LPD for back table procedure. The left hilum was dissected, and cuffs were applied to the artery, vein, and bronchus, as previously described.[10] Grafts were maintained inflated during the ischemia period (3 h) and were stored in cold LPD until implantation.

Recipients

Recipient animals were anesthetized, intubated, and ventilated as described above. Immediately prior to graft implantation, animals were intraperitoneally injected with 2 mL of either 0.9% SAL or 1% MB solutions. Then, they were placed in right lateral recumbency and subjected to left thoracotomy at the fourth intercostal space. Subsequently, graft implantation was performed using a stereomicroscope (model SZ61; Olympus, Tokyo, Japan) at 8 magnification.[10] In brief, the left hilum was dissected and clamped as proximally as possible. Then, graft implantation was performed by introducing the graft cuffs into a little hole made in the ventral wall of the artery, vein, and bronchus, respectively. After cuff fixation using a 7.0-polypropylene silk suture, the bronchus clamp was slowly opened and air flow was reestablished. In sequence, the vein clamp was removed for retrograde circulation establishment, and, finally, the artery clamp was gently opened, aiming at a soft graft perfusion. The closure of the recipient incision was performed in separate layers using 2.0-monofilament nylon sutures. After surgery completion, animals received analgesia (dipyrone, 400 mg/kg) by gavage and were placed under spontaneous ventilation in individual cages with free access to water and food.

Two hours after graft reperfusion, the animals were once more anesthetized, intubated and placed on mechanical ventilation, according to the previously mentioned parameters. The animals were subjected to exploratory laparotomy and euthanasia by incision of the anterior abdominal aorta, with subsequent removal of the cardiopulmonary block. Next, the blocks were stored in 4% formaldehyde solution over a period of 24 h, and, subsequently, kept in 70% ethyl alcohol solution until the preparation of the slides for histopathological and immunohistochemical analysis.

Histological analysis

Both lungs were fixed by tracheal instillation of 4% formaldehyde solution (20 cmH_2O) and stored for 24 h in the same solution for histological analysis. Paraffin-embedded lung samples were cut into 5-µm sections and stained with H&E. Histomorphometry by point-counting technique was used in order to quantify inflammatory cells in lung parenchyma with a Weibel grid containing 100 points and 50 lines. Ten random and mismatched microscopic fields were examined (magnification, ×400), totaling 1,000 points per slide and covering an area of 62,500 µm per field.[11] The same methodology was used to evaluate the expression of intercellular adhesion molecule-1 (ICAM-1), caspase-3, and Bcl-2, which were evaluated through the analysis of slides prepared with a immunohistochemical method described by Almeida et al.[12]

Statistical analysis

Descriptive analysis was performed for quantitative data with normal distribution, and the results were expressed as mean ± SD. Normality of data distribution and homogeneity of variances were evaluated via the Shapiro-Wilk test and the Levene's test, respectively. The t-test was used for dependent quantitative variables. A type I error of 0.05 (α) was considered for all inferential analyses.

RESULTS

Regarding inflammatory cells in the lung parenchyma, mean neutrophil counts were higher in the SAL group in comparison with the MB group (5.2 ± 2.5% vs.

2.3 ± 0.8%; p = 0.04; Figure 1), as were ICAM-1 expression (4.7 ± 0.8% vs. 2.7 ± 0.7%; p ≤ 0.001; Figure 2) and caspase-3 expression (4.4 ± 1.2% vs. 3.0 ± 1.3%; p ≤ 0.001; Figure 3). However, Bcl-2 expression in lung parenchyma was higher in the MB group in comparison with the SAL group (4.9 ± 1.9% vs. 2.5 ± 0.8%; p ≤ 0.001; Figure 4).

Data on the comparisons between the nonischemic lungs (right lungs) and the grafts (left lungs) of the animals in the SAL and in the MB groups are shown in Tables 1 and 2, respectively.

DISCUSSION

Lung IRI occurs in various cases, such as in cardiopulmonary bypass, lung transplantation, and postenucleation of pulmonary embolism. Recently, much attention has been paid to pulmonary dysfunction resulted from lung IRI.[13]

One-lung IRI can lead to similar, but less severe, injury in the contralateral lung. Because injury in the nonischemic lung develops only after reperfusion of the ischemic one, injury is probably humorally mediated.[14] In our study, we were able to confirm this observation, because the native lungs showed a lower expression of inflammatory and apoptotic markers both among the animals submitted to MB instillation and among the animals in the control group.

Contralateral lung injury induced by unilateral lung ischemia and reperfusion is a distinct and complicated phenomenon, which has yet to be fully understood.[15] Some authors have studied the injury of nonischemic lungs after ischemia and reperfusion of the left lung. Zhu et al.[16] used apocynin, an inhibitor of NADPH oxidase, in rats submitted to 60 min of ischemia by clamping the left pulmonary hilum followed by 30 min of reperfusion. The authors observed that ROS produced by ischemia affected the nonischemic lung. Georgieva et al.[17] concluded that an injured organ affects a remote organ by liberating humoral mediators in an ischemia and reperfusion model similar to that of the study by Zhu et al.[16] To our knowledge, the present study is the first one to assess these effects on nonischemic lungs after pulmonary ischemia and reperfusion induced by unilateral transplantation.

In a previous study,[6] our group evaluated the effects of MB on the lungs of rats submitted to unilateral lung

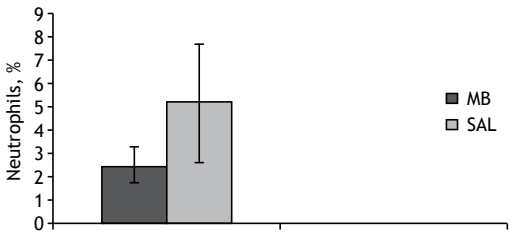

Figure 1. Neutrophil infiltration (%) in the saline group (SAL) and in the methylene blue (MB) group after 3-h cold ischemic time, transplantation, and 2-h reperfusion in nonischemic lungs after unilateral left lung transplantation. p ≤ 0,001.

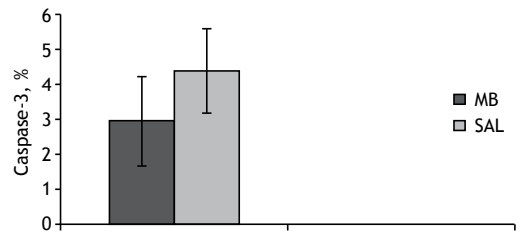

Figure 2. Intercellular adhesion molecule-1 (ICAM-1) activity in the saline group (SAL) and in the methylene blue (MB) group after 3-h cold ischemic time, transplantation, and 2-h reperfusion in nonischemic lungs after unilateral left lung transplantation. p ≤ 0,001.

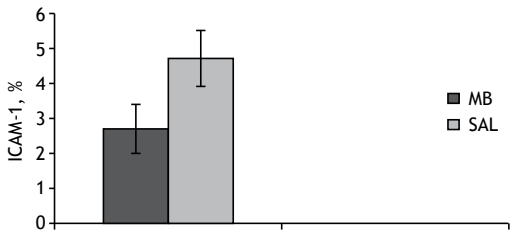

Figure 3. Caspase-3 activity (%) in the saline group (SAL) and in the methylene blue (MB) group after 3-h cold ischemic time, transplantation, and 2-h reperfusion in nonischemic lungs after unilateral left lung transplantation. p ≤ 0,001.

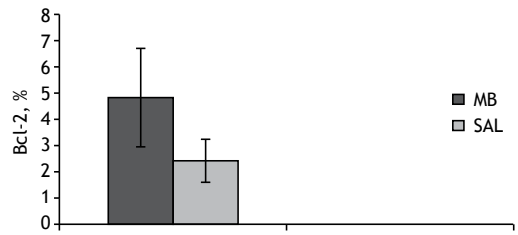

Figure 4. Bcl-2 activity (%) in the saline group (SAL) and in the methylene blue (MB) group after 3-h cold ischemic time, transplantation, and 2-h reperfusion in nonischemic lungs after unilateral left lung transplantation. p ≤ 0,001.

Table 1. Comparison of the degree of inflammation and apoptosis between nonischemic lungs and grafts in the control group.

Variable	Graft (left lung)	Nonischemic lung (right lung)	p
Neutrophils, %	7.9 ± 2.0	5.2 ± 2.5	≤ 0.001
ICAM-1, %	8.1 ± 2.1	4.7 ± 0.8	≤ 0.001
Caspase-3, %	6.3 ± 2.9	4.4 ± 1.2	≤ 0.001
Bcl-2, %	1.2 ± 0.9	2.5 ± 0.8	≤ 0.001

ICAM-1: intercellular adhesion molecule-1.

Table 2. Comparison of the degree of inflammation and apoptosis between nonischemic lungs and grafts in the methylene blue group.

Variable	Graft (left lung)	Nonischemic lung (right lung)	p
Neutrophils, %	4.4 ± 1.4	2.3 ± 0.8	≤ 0.001
ICAM-1, %	5.1 ± 1.1	2.7 ± 0.7	≤ 0.001
Caspase-3, %	5.7 ± 1.8	3.0 ± 1.3	≤ 0.001
Bcl-2, %	2.2 ± 1.1	4.9 ± 1.9	≤ 0.001

ICAM-1: intercellular adhesion molecule-1.

transplantation. As in the present study, MB was able to inhibit neutrophilic infiltration according to the histopathological evaluation. The findings in both studies show that MB, by means of inhibiting the production of ROS, is able to reduce inflammation induced by IRI.

Apoptosis is regulated by a cascade of proteins called caspases, which are activated in ischemia and reperfusion events. Lung ischemia and reperfusion has a direct effect on lung cells, and the increase in caspase-3 activity reflected a larger number of apoptotic cells.[18]

The signaling pathways that lead to apoptosis are maintained by positive and negative regulators. The proteins that promote survival are the antiapoptotic proteins Bcl-2 and Bcl-xL.[19] The release of ROS causes cell apoptosis as a late phase response of IRI. Oxidative stress triggers caspase-3 activation, leading to cell apoptosis. In addition, the balance of anti- and pro-apoptotic proteins responds dramatically to ROS.[5] Decreased Bcl-2 and increased caspase-3 expressions in the present study are similar to those found by Abogresha et al.,[5] who used vitamin C as an antioxidant agent, protecting against the effects of pancreatic injury after renal ischemia. The findings of the present study show the induction of apoptosis as a result of IRI injury after lung transplantation and the ability of MB to inhibit its occurrence.

Endothelial cell adhesion molecules seem to play an important role in IRI by causing adhesion of leukocytes to endothelial cells. ICAM-1 is one of the adhesion molecules that have been shown to be upregulated in response to cytokines. This upregulation leads to leukocyte-endothelial cell adhesion and to neutrophil infiltration in the affected tissue. Meyer et al.[20] performed 45 min of hepatic ischemia followed by 5 h of reperfusion and showed a significant upregulation in ICAM-1 in distant organs, such as the heart, kidney, intestine, and pancreas. In the present study, we were able to identify a higher expression of ICAM-1 in the lungs of rats in the SAL group in comparison with those in the MB group, as well as a higher neutrophil count, both of which represent a less intense inflammatory process in the animals treated with MB.

As a limitation of our study, we can highlight that we used the same dose of MB in all cases. MB has a dose-dependent effect, and a study with different doses could have produced different results than those found in the present study. In addition, the monitoring of hemodynamic parameters, markers of tissue perfusion, and ventilation parameters could be useful for a better understanding of the action of MB.

To our knowledge, the present study demonstrates for the first time that MB is an effective drug for the protection of nonischemic lungs against inflammation and apoptosis following unilateral lung transplantation in rats.

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ORIGINAL ARTICLE

The patient profile of individuals with Alpha-1 antitrypsine gene mutations at a referral center in Brazil

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Study carried out at the Pulmonology Division, Instituto do Coração – InCor – Hospital das Clínicas, School of Medicine, Universidade de São Paulo, São Paulo (SP) Brazil.

ABSTRACT

Objective: The clinical, functional, radiological and genotypic descriptions of patients with an alpha-1 antitrypsin (A1AT) gene mutation in a referral center for COPD in Brazil. **Methods:** A cross-sectional study of patients with an A1AT gene mutation compatible with deficiency. We evaluated the A1AT dosage and genotypic, demographic, clinical, tomographic, and functional characteristics of these patients. **Results:** Among the 43 patients suspected of A1AT deficiency (A1ATD), the disease was confirmed by genotyping in 27 of them. The A1AT median dosage was 45 mg/dL, and 4 patients (15%) had a normal dosage. Median age was 54, 63% of the patients were male, and the respiratory symptoms started at the age of 40. The median FEV1 was 1.37L (43% predicted). Tomographic emphysema was found in 77.8% of the individuals. The emphysema was panlobular in 76% of them and 48% had lower lobe predominance. The frequency of bronchiectasis was 52% and the frequency of bronchial thickening was 81.5%. The most common genotype was Pi*ZZ in 40.7% of participants. The other genotypes found were: Pi*SZ (18.5%), PiM1Z (14.8%), Pi*M1S (7.4%), Pi*M2Z (3.7%), Pi*M1I (3.7%), Pi*ZMnichinan (3.7%), Pi*M3Plowell (3.7%), and Pi*SF (3.7%). We did not find any significant difference in age, smoking load, FEV1, or the presence of bronchiectasis between the groups with a normal and a reduced A1AT dosage, neither for 1 nor 2-allele mutation for A1ATD. **Conclusions:** Our patients presented a high frequency of emphysema, bronchiectasis and bronchial thickening, and early-beginning respiratory symptoms. The most frequent genotype was Pi*ZZ. Heterozygous genotypes and normal levels of A1AT also manifested significant lung disease.

Keywords: Alpha-1 antitrypsin; Emphysema; Alleles.

INTRODUCTION

Alpha-1 antitrypsin deficiency (A1ATD) is a rare genetic disease that is related to the development of early emphysema and liver disease. Epidemiological studies estimate that A1ATD affects 1 in every 2,000 to 5,000 individuals born alive.[1] The only Brazilian study reporting on the prevalence of A1ATD estimates that 2.8% of patients with chronic obstructive pulmonary disease (COPD) have this deficiency.[2] The Platino study showed that, in the city of São Paulo, 15.8% of individuals aged 40 years or older had COPD,[3] which indicates that there is probably a large number of patients with undiagnosed A1ATD.

Alpha-1 antitrypsin (A1AT), a highly pleomorphic glycoprotein, has more than 100 identified alleles, and its main function is to inhibit several proteases.[4,5] Its variants are inherited by codominance, and they are classified according to the protease inhibitor (PI) system.[6,7] The phenotypes that have the highest risk of developing pulmonary emphysema are those associated with low A1AT production, the most common being the Z mutation. However, other mutations, such as S, I, Mmalton, Mnichinan, Plowell and Null, can lead to low dosages of A1AT.[6] The production of a dysfunctional protein can also occur, as in Pittsburgh and F mutations. The most common mutated alleles that occur with normal A1AT serum levels are the M variants, which still do not have a defined clinical significance.[4,6,7]

A1AT is produced primarily in the liver and, through the bloodstream, it reaches the lungs, where it performs its antielastolytic function.[4] When it is deficient, pulmonary emphysema occurs due to an imbalance in the protease-antiprotease ratio, which makes it incapable of protecting the lungs from the elastolytic action of neutrophil elastase,[8] among other aggressions, such as smoking and environmental exposures, leading to accelerated lung damage.

Its diagnosis is made through examining the clinical patterns of the disease and the corresponding laboratory changes. When there is evidence of reduced A1AT serum levels, genotyping should be performed in order to identify their variants.[4,5,9] However, A1AT is an acute phase protein, and its levels may be increased in situations of inflammation, thus a diagnosis of A1ATD cannot be not excluded even with a single normal dosage.[4]

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To date, we do not have a clinical, radiological and functional description of patients with A1ATD in Brazil. Although the A1AT dosage is recommended to be checked routinely in patients with COPD, as suggested by the World Health Organization (WHO), the examination is rarely done due to unawareness, the unavailability of the test, and its high cost to the health system. Knowledge about these characteristics in a Brazilian population of patients can allow for a systematic screening criteria to be designed for individuals with high pretest probability for positive screening,[10] saving costs associated with the generalized screening for all patients with COPD.

The primary objective of this study was the clinical, functional, radiological and genotypic characterization of A1ATD in a referral center specialized in respiratory diseases in Brazil, and to enable the design of a protocol for systematically tracking patients with COPD. We also compared the normal and altered A1AT dosage groups, and the groups with genotypes associated with one and two allele mutations for A1ATD.

METHODS

Study design

A cross-sectional study with patients that have a mutation in the A1AT gene, who were treated at the COPD Outpatient Clinic of the Pulmonary Division of the Hospital das Clínicas at the Faculdade de Medicina of the Universidade de São Paulo (HC-FMUSP), and who were diagnosed until February 28, 2015. It was approved by the Research Ethics Committee of the HC-FMUSP under Resolution Number 1,291,260.

Patients

Clinical and laboratory criteria were established in order to perform the genotyping of A1AT gene mutations in patients treated at the COPD outpatient clinic. These include: a low A1AT serum dosage; the early onset of emphysema (under 45 years of age); emphysema in non-smokers; disproportionate emphysema to smoking load; emphysema in patients with cases of A1ATD in the family; and bronchiectasis of an unknown cause.

All patients older than 18 years of age with an A1T1 gene mutation compatible with A1T1D that was identified by genotyping were included in the study. Patients without a A1T1D diagnosis confirmed by genotyping, those with mutations in the A1AT gene that were not compatible with the deficiency, and those who never performed spirometry with a bronchodilator test and computed tomography (CT) were excluded.

Clinical and demographic data

The data collected were obtained at the time of the medical consultation or by consulting the A1ATD patients' medical records. The data included: age, gender, body mass index (BMI), SpO_2, age of onset of respiratory symptoms, history of alcoholism and smoking, smoking load, comorbidities (described in the patient chart), dyspnea (ranked by the modified Medical Research Council - mMRC), number of exacerbations reported by the patient in the last year (according to GOLD recommendations),[11] current treatment, and evaluation for a lung transplant.

Chest computed tomography and lung function tests

For this study, the following were considered: the most recent chest tomography, spirometry with a bronchodilator test and plethysmography performed by the patient. For the diagnosis of COPD, the GOLD-recommended criteria were used. [11]

The spirometry reference values used were those established by Pereira et al.,[12] where the absolute and percentage of predicted post-bronchodilator (BD) values of forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1), as well as the FEV1/FVC ratio were collected. For the bronchodilator response, criteria described in 2002 by the Guidelines for Pulmonary Function Testing of the Brazilian Society of Pulmonology and Tisiology (FEV1 post-BD ≥ 200 mL of pre-BD and ≥ 7% of predicted and/or post-BD FVC ≥ 350 mL of pre-BD) were used.[13] Pre-BD values of total lung capacity (TLC), residual volume (RV) and pulmonary diffusion (DLCO) were recorded from plethysmography with predicted values from Neder et al.[14]

Liver Disease Evaluation

The patients were considered to have liver impairment, if they presented changes in the evaluation exams at any time during the follow-up consultations, investigated by: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-GT and bilirubin dosages.

ALPHA-1 ANTITRYPSIN DOSAGE AND GENOTYPING

The A1AT dosage was performed by a blood plasma analysis after centrifugation, using an immunoturbidimetric method. Normal A1AT value levels were considered to be ≥ 83 mg/dL, according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines.[4] If the patient had performed more than one test, the lowest value was considered. Genotyping was performed by a polymerase chain reaction (PCR) using a peripheral blood sample analysis that was collected on filter paper. The DNA was extracted from the dried blood and the sample was subjected to the sequencing of exons 2, 3, 4 and 5 of the SERPINA1 gene in order to identify the polymorphisms. Direct sequencing of the PCR products was performed from the BigDye™ Terminator V.3.0 kit (Applied Biosystems, Warrington, England), and the samples were applied to the Genetic Analyzer DNA sequencer (Applied Biosystems, Tokyo, Japan).

Statistical analysis

The collected data were analyzed using the Statistical Package for Social Sciences (SPSS) program, version

21.0, and were reported as absolute numbers, proportions, means and medians, standard deviation and interquartile ranges. The analysis of non-normal distribution numerical variables was compared between two groups by the Mann-Whitney test. For categorical variables, Fisher's exact test was used. Numerical variables of non-normal distribution were correlated through Spearman's Rho test. P-values of <0.05 were considered to be statistically significant.

RESULTS

Using the established clinical criteria, 43 patients with suspected A1ATD were selected from a population of 531 patients undergoing follow-up care at the COPD clinic in 2014. Of the total selected, 1 patient did not undergo genotyping and 15 participants presented a normal A1T1 gene after genotyping and, therefore, were excluded from the study. Thus, a total of 27 patients with A1ATD were included in the study, having a diagnosing accuracy level of 62.8%, after clinical and laboratory suspicion, and a prevalence of 5.1% in our COPD clinic population (Figure 1).

As Table 1 shows, the median A1AT dosage in study participants was 45 mg/dL, and 4 individuals (15%) had normal levels (≥ 83 mg/dL). The median age of the participants was 54 years old. Sixty three percent were male and had a median BMI of 23.7. The median age at the onset of respiratory symptoms was 40 years old. Ten individuals (37%) were non-smokers, 52% were former smokers, and 11% were active smokers. Five individuals did not present comorbidities, and the most prevalent comorbidities were gastroesophageal reflux disease (22%), systemic arterial hypertension and dyslipidemia (both 19%) and rhinitis (22%). The most frequent respiratory diseases were bronchiectasis (52%), asthma (19%) and tuberculosis (15%).

The evaluation of pulmonary function showed an obstructive pattern in the majority of patients, with a reduced median of FEV1 predicted values (43%), FEV1/FVC (0.47) and pulmonary diffusion (59.5% of the predicted value), and important air entrapment seen in the increase in residual volume (169% of the predicted value) (Table 2). In 70% of individuals, FEV1 was less than 60% of the predicted values. A bronchodilator response was present in 12 patients (44.4% of the individuals), and most of it was FVC.

As for the tomographic findings, emphysema was found in 21 individuals (77.8%), 16 were panlobular, and 10 had a lower lobe prominence (respectively: 76.2 and 47.6% of the individuals with emphysema). Of the six participants without emphysema, two individuals had the Pi*ZZ genotype, two individuals had Pi*SZ, and in two individuals the Pi*M1Z genotype was found, with all of them having bronchial thickening, and four of them having bronchiectasis. Bronchiectasis was found in 52% of the participants, bronchial thickening in 81.5% and mosaic perfusion in 44% (Table 3).

Genotypic analysis showed that the most commonly found genotype was Pi*ZZ (40.7% of patients) (Table 4). This genotype shows a median A1AT dosage of 20.0 mg/dL. All of the participants had altered dosages (<83 mg/dL), and in general presented a more severe pulmonary disease with a median FEV1 of 37% of the predicted value. Only 36% were smokers.

Genotypes with a heterozygous Z allele were the most frequent, then Pi*ZZ, with Pi*SZ (18.5% of patients) and Pi*M1Z (14.8% of patients) being the most common. The presence of a history of smoking in the non-Pi*ZZ genotypes was high, reaching 100% in most of them, and there was also a high frequency of bronchiectasis. It was observed that the 4 individuals with a normal A1AT dosage do not have the Z allele (Table 4).

In the study, eight participants had liver impairment (29.6%), and all of them had a homozygous or heterozygous Z allele. In the Pi*ZZ genotype, 45% of the individuals had liver function impairment, and in Pi*M1Z, 50% of the individuals had this alteration (Table 4).

Bronchiectasis was found in 52% of the participants: in 4 (36%) with the Pi*ZZ genotype, but these alterations

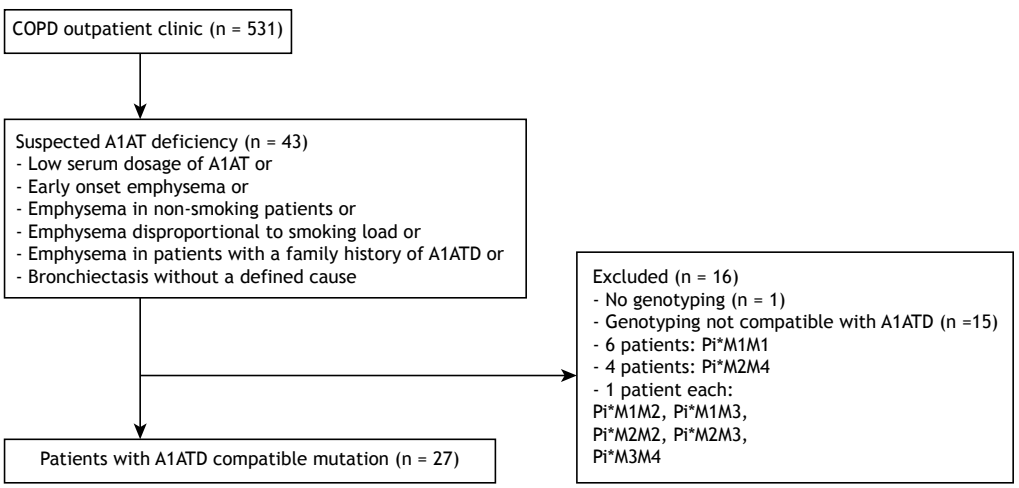

Figure 1. Flowchart of the patients included in the study.

Table 1. Dosage of alfa-1 antitrypsin and demographic characteristics of the patients with a mutation of the alpha-1 antitrypsin gene.

Variables	Population of patients confirmed with the A1AT gene mutation (n = 27)
A1AT serum level- mg/dL Median (interquartile range)	45 (20-81)
A1AT serum level - n (%) Reduced (< 83 mg/dL) Normal (≥ 83 mg/dL)	23 (85) 4 (15)
Age (in years) Median (interquartile range)	54 (42-59)
Age at onset of respiratory symptoms (in years) Median (interquartile range)	40 (25-48)
BMI - kg/m^2 Median (interquartile range)	23.7 (20.6-27.8)
Gender - n (%) Male/female	17 (63)/10 (37)
Alcoholism - n (%) Non-alcoholic Ex-alcoholic Active alcoholic	25 (92) 1 (4) 1 (4)
Smoking - n (%) Non-smoker Ex-smoker Active smoker	10 (37) 14 (52) 3 (11)
Smoking load- packs/year Median (interquartile range)	28.5 (24-37)
Bronchiectasis - n (%) Yes/no	14 (52)/13 (48)
Asthma - n (%) Yes/no	5 (19)/22 (81)
Allergic rhinitis - n (%) Yes/no	6 (22)/21 (78)
Pulmonary tuberculosis - n (%) Yes/no	4 (15)/23 (85)
Systemic arterial hypertension - n (%) Yes/no	5 (19)/22 (81)
Diabetes mellitus - n (%) Yes/no	1 (4)/26 (96)
Dyslipidemia - n (%) Yes/no	5 (19)/22 (81)
Gastroesophageal reflux disease - n (%) Yes/no	6 (22)/21 (78)
Depression - n (%) Yes/no	4 (15)/23 (85)
Osteoporosis - n (%) Yes/no	1 (4)/26 (96)
Past history of cancer - n (%) Yes/no	3 (11)/24 (89)

A1AT: alfa-1 antitrypsin; BMI: body mass index.

were more frequent in the genotypes Pi*SZ (60%) and Pi*M1Z (75%), as well as in all individuals of the Pi*ZMnichinan, Pi*M1S and Pi*SF genotypes (Table 4). Of the individuals with bronchiectasis, four had no emphysema on the tomography, and only one individual had a history of previous pulmonary tuberculosis. The presence of bronchiectasis is not associated with A1AT dosage (p=0.52), age (p=0.79), or smoking (p = 1.00), but it is associated with males (p=0.046).

The four individuals with a normal A1AT dosage had a median dosage of 101.5 mg/dL and had the following genotypes: Pi*M1S, Pi*M1I and Pi*M3Plowell. All of the patients had a diagnosis of COPD, with tomographic emphysema, a history of smoking, a median smoking rate of 28 packs/year, and two patients presented concomitant bronchiectasis. The median FEV1 of these patients was 1.01L (31.5% of the predicted value).

Table 5 shows the characteristics of the group with a normal and altered A1AT dosage, and shows no statistically significance difference between age, smoking load, FEV1 and the presence of bronchiectasis. An analysis of the groups with one A1ATD mutated allele

Table 2. Pulmonary function measurements in patients with a mutation of the alpha-1 antitrypsin gene.

Functional variables	Median (n = 27)	Interquartile range
FEV1 - L	1.37	1.00-2.05
FEV1 - % predicted	43	32-67
FVC - L	3.03	2.52-3.45
FVC - % predicted	71	62-96
FEV1/FVC	0.47	0.39-0.75
TLC - L	6.15	5.3-7.7
TLC - % predicted	118	91-129
RV - L	3.00	2.2-4.8
RV- % predicted	169	128-231
DLCO - % predicted	59.5	31.8-81.5

FEV 1: forced expiratory volume in one second; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; DLCO: pulmonary diffusion.

Table 3. Tomographic characteristics in patients with a mutation of the alpha-1 antitrypsin gene.

Tomographic characteristics	Frequency (%) (n = 27)
Emphysema	21 (77.8%)
Panlobular	16 (59.2%)
Prevalence in upper lobes	4 (14.8%)
Prevalence in lower lobes	10 (37%)
No predominance	7 (25.9%)
Bronchial thickening	22 (81.5%)
Bronchiectasis	14 (52%)
Mosaic perfusion	12 (44%)
Bubbles	7 (26%)
Cysts	1 (4%)

Table 4. Mutations in the alpha-1 antitrypsin gene found with their frequency, clinical, laboratory, and lung function characteristics (n=27).

Genotype	Frequency n (% of the total number of study subjects)	Median A1AT dosage in mg/dL	Number of individuals with A1AT serum level > 83 mg/dL n (% of the same genotype)	FEV1 Median in L (% of predicted)	Current or previous smoking n (% of the same genotype)	Number of patients with BQT n (% of the same genotype)	Number of patients with liver disease n (% of the same genotype)
Pi*ZZ	11 (40.7%)	20.0	0	1.36 (37%)	4 (36%)	4 (36%)	5 (45%)
Pi*SZ	5 (18.5%)	58	0	1.37 (43%)	4 (80%)	3 (60%)	0
Pi*M1Z	4 (14.8%)	79.5	0	1.88 (73%)	2 (50%)	3 (75%)	2 (50%)
Pi*M2Z	1 (3.7%)	76	0	3.02 (103%)	1 (100%)	0	0
Pi*ZMnichinan	1 (3.7%)	27	0	1.28 (43%)	1 (100%)	1 (100%)	1 (100%)
Pi*M1S	2 (7.4%)	106	2 (100%)	1.01 (31.5%)	2 (100%)	2 (100%)	0
Pi*M1I	1 (3.7%)	111	1 (100%)	0.67 (19%)	1 (100%)	0	0
Pi*M3Plowell	1 (3.7%)	88	1 (100%)	1.37 (48%)	1 (100%)	0	0
Pi*SF	1 (3.7%)	81	0	2.05 (58%)	1 (100%)	1 (100%)	0

A1AT: alfa-1 antitrypsin; FEV1: forced expiratory volume in one second; BQT: bronchiectasis.

versus two mutated alleles also showed no statistically significant difference for age, FEV1, smoking load and the presence of bronchiectasis, but the difference between the A1AT dosage, which was higher in the alleles with only one mutation, was statistically significant. This result highlights that, even at normal or near normal dosages, such as those observed in the presence of a single allele for A1ATD, pulmonary disease may be present in a severe form at an early age, despite a similar smoking load.

The clinical evaluation showed that 44.4% of individuals had dyspnea with a mMRC greater than

or equal to 2, more than one exacerbation had manifested in 59.3% of them in the last year, and 18.5% presented SpO2 lower than 92% in ambient air, and are users of home oxygen therapy. There was no statistically significant association between mMRC and normal versus altered A1AT dosage, which reinforces the findings that the group of patients with a normal dosage exhibited a similar disease severity to that of the altered dosage group.

Two individuals with a Pi*ZZ genotype are receiving A1AT replacements. The evaluation for lung transplants was performed in 7 patients (26%), with 4 of them being contraindicated, one of them being followed, one being evaluated, and one being release after an evaluation of the transplant.

DISCUSSION

The present study presented the genotypic analysis and the clinical, radiological and functional evaluations of 27 individuals with a mutation in the A1AT gene in a Brazilian referral center. This study is relevant because of its genotypic analysis of mutations that are not frequently evaluated in other studies, as well as the fact that it used genotyping on individuals with normal serum levels, but who had a high clinical suspicion for A1ATD.

The diagnosis of A1ATD was confirmed in 64.3% of the cases in which it was suspected. We found a prevalence of 5.1% of A1ATD in our COPD outpatient clinic. In a recent study, Russo et al. found that 2.8% of patients with COPD in Brazil had A1ATD,[2] which is an alarming finding considering the low frequency of this diagnosis in clinical practice and the small number of articles published on A1ATD in the Brazilian population.[2,15] Our country has a vast racial diversity, miscegenation and European immigration from countries where the frequency of alleles involved with A1ATD is high.

In our data, we observed that a majority of individuals were male, with a positive history of smoking, and the onset of symptoms present at an early age of 40, which is similar to other studies,[4,16] where 40.7% of their participants had the Pi*ZZ genotype. The individuals of this genotype presented low A1AT dosages, reduced FEV1 values, which connotes advanced lung disease, and a lower percentage of a history of smoking when compared to the other genotypes. Bronchiectasis was found in 36% of the individuals with this genotype, and 2 subjects did not have tomographic emphysema. Other genotypes found in our population, in order of frequency, were: Pi*SZ, Pi*M1Z and Pi*M1S. And the other genotypes had one individual each: Pi*M2Z, Pi*M1I, Pi*ZMnichinan, Pi*M3Plowell and Pi*SF. Many of these genotypes are cited in the literature as not causing clinically significant disease,[4,17,18] especially when there is only one allele with a mutation in the A1AT gene. However, in our study, we found that they had a reduced FEV 1 and a high prevalence of COPD and bronchiectasis. It is probable that the individuals' history of smoking contributed to the onset of lung disease, but the severity of the disease, characterized by low FEV 1 values, may not be justified only by the smoking.

Four individuals had normal A1AT dosages and were heterozygous for A1AT gene mutations. Despite the normal dosage, they had a FEV 1 and frequency of bronchiectasis similar to that of the altered A1AT dosage group. There was also no difference between the amounts of smoking load of these groups, suggesting that smokers with normal A1AT, but with a compatible genotype for an A1AT mutation, are at risk for more rapid loss of lung function. These data demonstrate, for the first time in the national population, the extreme importance of performing A1AT genotyping when there is high clinical suspicion, even when there are normal A1AT dosages.

The presence of bronchiectasis in 52% of our sample was higher than that reported in other studies,[21,22] with 26% being in a study with a greater number of participants.[22] The high frequency of bronchiectasis in our study is not justified by the high incidence of tuberculosis in our country, since only one individual with bronchiectasis presented a history of tuberculosis. Another relevant finding is the high prevalence of bronchial thickening and mosaic perfusion, which brings attention to airway involvement in these patients.[23,24] And the very presence of individuals with bronchiectasis in the absence of emphysematous lesions in four patients should be emphasized, given that it is speculated that bronchiectasis occurs due to a distortion effect of the parenchyma because of

Table 5. Characteristics between the groups with normal and altered alpha-1 antitrypsin dosage, and genotypes with 2 alleles for alpha-1 antitrypsin deficiency and 1 allele for alpha-1 antitrypsin deficiency.

Variable	Normal A1AT dosage (n = 4)	Altered A1AT dosage (n = 23)	p-value	Genotypes with 2 alleles for A1ATD (n = 18)	Genotypes with 1 allele for A1ATD (n = 9)	p-value
A1AT dosage mg/dL (median)	101.5	30.0	< 0.001*	22	81.5	< 0.001*
Age	44	54	0.41*	54	47	0.24*
FEV1 % predicted (median)	31.5	43	0.11*	43	53	0.68*
Smoking load packs/year (median)	28	28.5	0.59*	28.5	29.5	0.80*
Bronchiectasis n (%)	2 (50%)	12 (52%)	1.00$	8 (47%)	6 (60%)	0.70$

A1AT: alfa-1 antitrypsin; A1ATD: alfa-1 antitrypsin deficiency; *Mann-Whitney test; FEV1: forced expiratory volume in one second; $Fisher's exact test.

emphysema, which is not justified in the patients who do not have emphysema. [25]

Our main limitations include the fact that we performed a cross-sectional analysis of a small sample of unicentric medical records. Nevertheless, our sample reflects the rarity of the disease and, because it includes a small population with regular outpatient follow-up, the percentage of missing data was minimal.

The main take away from our study is the characterization of A1ATD in Brazil. By knowing the characteristics of our population, we can systematize a screening process in individuals with a high probability of A1ATD for future studies. Thus, it will be possible to reduce the costs of a generalized screening process for all patients with COPD,[4] in a country with such severe economic limitations.

The characterization of A1ATD in our study showed that the most frequent genotype found was Pi*ZZ. Individuals with a mutation in the A1AT gene in only one allele and a normal A1AT serum dosage also presented significant lung disease. A high frequency of emphysema, bronchiectasis and bronchial thickening, low median values of FEV1 and A1AT, and an early onset of respiratory symptoms were found.

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ORIGINAL ARTICLE

Impact of adherence to long-term oxygen therapy on patients with COPD and exertional hypoxemia followed for one year

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ABSTRACT

Objective: To determine the impact of adherence to long-term oxygen therapy (LTOT) on quality of life, dyspnea, and exercise capacity in patients with COPD and exertional hypoxemia followed for one year. **Methods:** Patients experiencing severe hypoxemia during a six-minute walk test (6MWT) performed while breathing room air but not at rest were included in the study. At baseline and after one year of follow-up, all patients were assessed for comorbidities, body composition, SpO_2, and dyspnea, as well as for anxiety and depression, having also undergone spirometry, arterial blood gas analysis, and the 6MWT with supplemental oxygen. The Saint George's Respiratory Questionnaire (SGRQ) was used in order to assess quality of life, and the Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity (BODE) index was calculated. The frequency of exacerbations and the mortality rate were noted. Treatment nonadherence was defined as LTOT use for < 12 h per day or no LTOT use during exercise. **Results:** A total of 60 patients with COPD and exertional hypoxemia were included in the study. Of those, 10 died and 11 experienced severe hypoxemia during follow-up, 39 patients therefore being included in the final analysis. Of those, only 18 (46.1%) were adherent to LTOT, showing better SGRQ scores, higher SpO_2 values, and lower $PaCO_2$ values than did nonadherent patients. In all patients, SaO_2, the six-minute walk distance, and the BODE index worsened after one year. There were no differences between the proportions of adherence to LTOT at 3 and 12 months of follow-up. **Conclusions:** Quality of life appears to be lower in patients with COPD and exertional hypoxemia who do not adhere to LTOT than in those who do. In addition, LTOT appears to have a beneficial effect on COPD symptoms (as assessed by SGRQ scores).

(Brazilian Registry of Clinical Trials – ReBEC; identification number RBR-9b4v63 [http://www.ensaiosclinicos.gov.br])

Keywords: Respiratory insufficiency; Pulmonary disease, chronic obstructive; Patient compliance; Hypoxia; Oxygen inhalation therapy.

INTRODUCTION

Patients with COPD constitute the largest homogeneous group of patients who have arterial hypoxemia,[1,2] accounting for 67.8-81.6% of all patients on long-term oxygen therapy (LTOT).[3] The use of LTOT improves quality of life and respiratory symptoms, as well as reducing the risk of mortality.[4,5] In some patients, however, hypoxemia occurs only during activities of daily living.[6] The mechanisms involved in exertional hypoxemia are associated with ventilation/perfusion mismatch, decreased diffusion capacity, and increased pulmonary shunt.[6] As a result, exercise tolerance and quality of life are reduced in such patients.[6]

The effectiveness of LTOT in patients with exertional hypoxemia has yet to be established. Although one study has shown that the use of LTOT during pulmonary rehabilitation improves the quality of life of patients with exercise-induced hypoxemia,[7] other studies have shown that the use of LTOT has no beneficial effect on COPD patients with exertional hypoxemia undergoing physical training.[6,8,9] In addition, in patients with moderate resting or exercise-induced hypoxemia, LTOT has been shown to have no beneficial effect on the time to death or first hospitalization.[10] There is no consensus regarding the use of LTOT in such patients.[6,11]

Few studies have examined the impact of adherence to LTOT on clinical outcomes in such patients.[10] Therefore, the objective of the present study was to determine the impact of adherence to LTOT on quality of life, dyspnea, and exercise capacity in patients with COPD and exertional hypoxemia followed for one year.

METHODS

Patients

We evaluated 159 COPD patients referred to the Oxygen Therapy Outpatient Clinic of the São Paulo State University

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Botucatu School of Medicine, in the city of Botucatu, Brazil, in the period between November of 2011 and June of 2012. The inclusion criteria were as follows: having been diagnosed with COPD in accordance with the Global Initiative for Chronic Obstructive Lung Disease criteria[12] and having exertional hypoxemia.

At baseline (and while breathing room air), patients experiencing severe arterial hypoxemia during exercise but not at rest were classified as having exertional hypoxemia. To confirm the presence of a PaO_2 > 59 mmHg[2,13] at rest and on room air, we performed arterial blood gas analysis. All patients performed a six-minute walk test (6MWT) while breathing room air, the presence of exertional hypoxemia being confirmed by an SpO_2 of < 87% during the test. All patients with exertional hypoxemia received a prescription for oxygen supplementation at a flow rate of 0.5 L/min for at least 12 h per day for one year, to be used when performing activities of daily living (including walking) and during sleep.[11] The exclusion criteria were as follows: severe hypoxemia at rest (PaO_2 ≤ 55 mmHg), other respiratory diseases, polycythemia, *cor pulmonale*, cancer, and active smoking. Clinically unstable patients (medication changes, disease exacerbations, or hospital admissions in the preceding 6 weeks) and those who presented with severe arterial hypoxemia at rest (a PaO_2 of < 55 mmHg) during the follow-up period were also excluded. Patients were evaluated at baseline and every 4 months in order to determine the frequency of exacerbations and adherence to LTOT. Exacerbations were confirmed by changes in maintenance therapy or the need to prescribe oral corticosteroids, antibiotics, or both. Cause-of-death data were obtained by reviewing patient medical records. The study was approved by the Research Ethics Committee of the Botucatu School of Medicine University Hospital (Protocol no. 4020-2011), and all participating patients gave written informed consent.

Spirometry, arterial blood gas analysis, and pulse oximetry

Pre- and post-bronchodilator spirometry tests were performed with a Koko spirometer (Ferraris Respiratory, Louisville, CO, USA), in accordance with the American Thoracic Society criteria.[14] Disease severity was determined on the basis of criteria established by the Brazilian Thoracic Association and the Global Initiative for Chronic Obstructive Lung Disease.[2,15] Values of FEV_1 and FVC were expressed as percentages of the predicted values. Radial artery puncture was used in order to collect blood samples for arterial blood gas analysis, PaO_2, $PaCO_2$, and SaO_2 being measured with a blood gas analyzer (Stat Profile 5 Plus; Nova Biomedical, Waltham, MA, USA) and SpO_2 being measured with an Onyx 9500™ pulse oximeter (Nonin Medical, Inc., Plymouth, MN, USA). The aforementioned parameters were assessed with the patients at rest and breathing room air.

Body composition

Body weight and height were measured with participants standing barefoot and wearing light clothing. The body mass index (BMI) was calculated by the formula weight/height^2 (kg/m^2). Body composition was assessed by bioelectrical impedance analysis (BIA 101A; RJL Systems, Inc., Clinton Township, MI, USA). Resistance was measured on the right side of the body in the supine position. Fat-free mass (FFM, in kg) was calculated by a group-specific regression equation developed by Kyle et al.[16] The FFM index (FFMI = FFM/height^2) was also calculated. FFM depletion was defined as an FFMI of < 15 kg/m^2 for women and an FFMI of < 16 kg/m^2 for men.[17]

Exercise capacity

The 6MWT was performed in accordance with the American Thoracic Society guidelines.[18] Patients were instructed to walk, attempting to cover as much ground as possible within 6 min. The walk was timed by a research assistant, and standardized verbal encouragement was given. Following a rest period of at least 30 min, each subject performed a second 6MWT in the same manner as the first. Patient SpO_2 was monitored throughout the test. Before and after the test, data were obtained for SpO_2, heart rate, respiratory rate, and blood pressure. The six-minute walk distance (6MWD) was expressed in meters.

Adherence

Adherence to treatment was assessed by asking questions regarding the oxygen flow rate and the number of hours of LTOT use per day. The information thus obtained was confirmed by comparing it to the prescription data on patient medical records. Treatment nonadherence was defined as LTOT use for < 12 h per day or no LTOT use during exercise. To improve the use of LTOT, adherence education was provided at each medical visit. In addition, nurse home visits were provided in order to reinforce the importance of and assess adherence to LTOT.

Quality of life, the Baseline Dyspnea Index, the Hospital Anxiety and Depression Scale, the Charlson comorbidity index, and the Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity index

The Brazilian Portuguese version of the Saint George's Respiratory Questionnaire (SGRQ)[19] was used in order to evaluate quality of life. Dyspnea was assessed by the Brazilian Portuguese version of the modified Medical Research Council scale, as well as by the Baseline Dyspnea Index (BDI).[20,21] The Hospital Anxiety and Depression Scale was used in order to evaluate anxiety and depression.[22] Comorbidities were quantified by the Charlson comorbidity index.[23] The **B**ody mass index, airflow **O**bstruction, **D**yspnea, and **E**xercise capacity (BODE) index was calculated by using the model of Celli et al.[24] All questionnaires

were administered at baseline and after a one-year follow-up period.

Statistical analysis

For a power of 80% and a type I error of 0.05, the required sample size to detect a mean difference in quality of life (4.0 ± 4.5%) or dyspnea (1.0 ± 2.0) at the end of the follow-up period was calculated to be 34. Data were expressed as mean ± standard deviation or median (interquartile range). The Student's t-test or the Mann-Whitney test was used in order to compare two independent groups according to their distribution. A paired t-test or the Wilcoxon test was used in order to compare repeated measures. The chi-square test was used in order to compare proportions. Multiple linear regression analysis was used in order to evaluate factors associated with the 6MWD and dyspnea after one year. A value of $p < 0.05$ was defined as statistically significant. All analyses were performed with the Stata statistical software package, version 10.0 (StataCorp LP, College Station, TX, USA).

RESULTS

A flow chart of inclusion and exclusion of patients is presented in Figure 1. A total of 60 patients with COPD and exertional hypoxemia were included in the study. Of those, 10 died during the study period. The causes of death were pulmonary complications of COPD, in 6 patients, and cardiovascular disease, in 4. In addition, 11 patients experienced severe hypoxemia during follow-up and were excluded from the analysis, 39 patients therefore being included in the final analysis. The BODE index and the BMI were lower in the patients who died than in those who survived (2.0 [1.0-3.0] vs. 1.0 [0.0-2.0], p = 0.005 and 17.8 [16.4-27.6] vs. 23.7 [19.5-30.0], p = 0.028, respectively), as was adherence to LTOT.

The characteristics of the 39 patients at baseline and after one year of follow-up are presented in Table 1. All 39 were using long-acting beta-adrenoceptor agonists, which were used in combination with long-acting anticholinergics in 15 and in combination with inhaled corticosteroids in 15. In all patients, the 6MWD and the BODE index worsened after one year, whereas SGRQ symptoms domain scores showed significant improvement. The proportion of patients with a lower FFMI at baseline was 12.8%. Adherence to LTOT was low during the study period (46.1%). Nonadherent patients reported using oxygen supplementation for 8.0 ± 1.2 h per day. In comparison with adherent patients, nonadherent patients showed significantly lower resting SpO_2 (91.1 ± 2.5 vs. 93.3 ± 2.3; p = 0.011), higher $PaCO_2$ (39.5 [35.8-47.3] vs. 35.7 [34.2-39.4]; p = 0.044), and worse scores for the SGRQ domains of symptoms (41.5 ± 19.6 vs. 27.6 ± 18.7; p = 0.039), activity (72.9 [66.2-79.7] vs. 60.4 [45.9-70.3]; p = 0.014), and impacts (39.5 ± 15.1 vs. 27.8 ± 18.3; p = 0.047), as well as significantly worse total SGRQ scores (49.9 ± 11.9 vs. 36.4 ± 17.7; p = 0.012), after one year (Table 2).

The scores for the SGRQ domain of symptoms improved significantly after one year of follow-up in the nonadherent group (47.3 ± 18.7 vs. 41.5 ± 19.6;

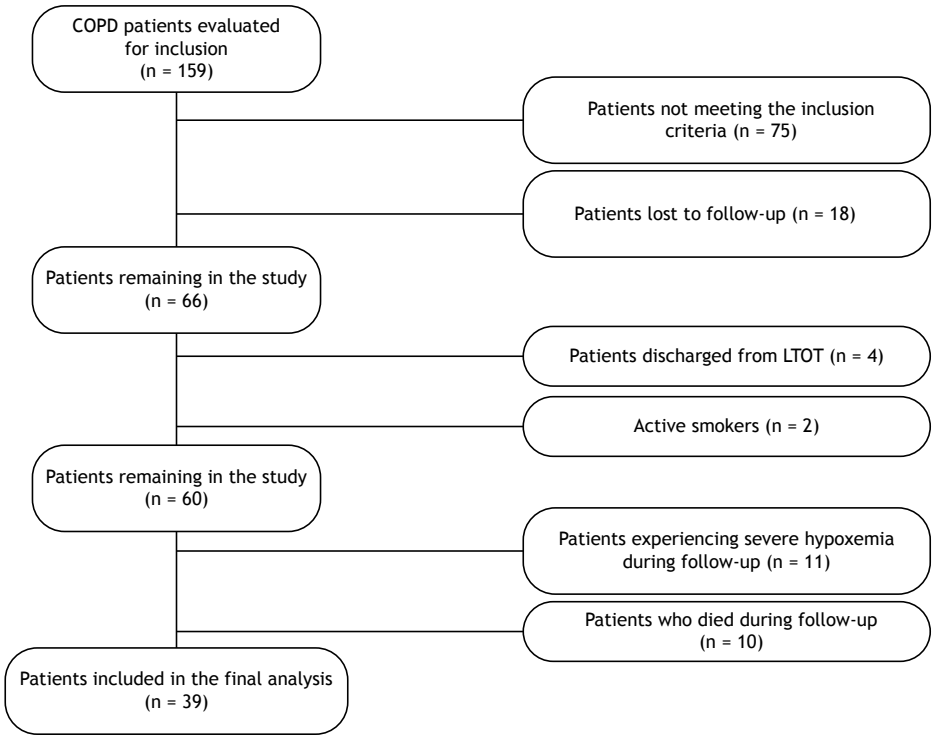

Figure 1. Flow chart of inclusion and exclusion of patients. LTOT: long-term oxygen therapy.

Table 1. General characteristics of patients with COPD and exercise-induced hypoxemia at baseline and after one year of follow-up (N = 39).[a]

Characteristic	Baseline	One year later	p
Men, %	41.1		
Age, years[b]	69.0 (62.0-77)		
BMI, kg/m^2[b]	23.7 (19.5-30.0)	26.5 (21.7-30.8)	0.674
FFM, kg[b]	45.6 (41.1-51.6)	44.3 (38.5-51.8)	0.544
FFMI, kg[b]	18.0 (16.8-20.0)	18.0 (16.3-19.8)	0.642
Charlson comorbidity index	3.2 ± 1.1	3.2 ± 1.1	1.000
BODE index[b]	1.0 (0.0-2.0)	1.5 (0.0-2.0)	0.031
SpO_2, %	92.1 ± 2.5	92.3 ± 2.6	0.885
PaO_2, mmHg	67.0 ± 5.4	63.6 ±7.9	0.128
$PaCO_2$, mmHg	38.1 ± 6.0	38.6 ± 8.5	0.633
FVC, L	2.23 ± 0.78	2.11 ± 0.73	0.177
FEV_1, L	1.13 ± 0.49	1.07 ± 0.40	0.056
FEV_1/FVC, L[b]	0.50 (0.41-0.58)	0.53 (0.41-0.58)	0.754
SGRQ symptoms domain score, %	53.0 ± 19.8	34.5 ± 20.4	< 0.001
SGRQ activity domain score, %	63.6 ± 20.5	65.2 ± 19.4	0.695
SGRQ impacts domain score, %	34.6 ± 18.7	33.5 ± 17.7	0.707
Total SGRQ score, %	46.7 ± 16.5	43.3 ± 16.5	0.232
Anxiety, HADS	4.3 ± 4.4	2.8 ± 2.1	0.065
Depression, HADS	3.3 ± 3.7	4.3 ± 3.8	0.170
mMRC scale[b]	2.0 (1.0-2.5)	2.0 (1.0-2.5)	0.612
BDI	5.5 ± 2.7	5.2 ± 2.7	0.601
6MWD, m[b]	336.0 (264.0-390.0)	306.0 (210.0-355.0)	< 0.001

BMI: body mass index; FFM: fat-free mass; FFMI: fat-free mass index; BODE: Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity; SGRQ: Saint George's Respiratory Questionnaire; HADS: Hospital Anxiety and Depression Scale; mMRC: modified Medical Research Council; BDI: Baseline Dyspnea Index; and 6MWD: six-minute walk distance. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as median (interquartile range). $p < 0.05$ (Student's t-test or Mann-Whitney test).

p = 0.005) and in the adherent group (36.8 ± 16.8 vs. 27.6 ± 18.7; p = 0.001). With regard to the scores for the remaining SGRQ domains and total SGRQ scores, there were no significant differences (Figure 2).

Of the 39 patients in the sample, 16 (41%) were diagnosed with COPD exacerbation, and 26% required hospitalization, the need for hospitalization being unrelated to adherence to LTOT (p = 1.00). The multiple linear regression model showed a negative association between the BDI and SGRQ symptoms domain scores after one year (p = 0.04; Table 3).

DISCUSSION

The objective of the present study was to determine the impact of adherence to LTOT on quality of life, dyspnea, and exercise capacity in patients with COPD and exertional hypoxemia followed for one year. The main finding of our study is that quality of life is worse in patients who do not adhere to LTOT than in those who do. Adherence to LTOT contributes to improving quality of life by improving symptoms. In comparison with the COPD patients who did not adhere to LTOT in the present study, those who did had better quality of life after one year of follow-up. Nevertheless, respiratory symptoms were found to have improved in the group of nonadherent patients, despite the fact that they did not use LTOT for as long as recommended. The mean duration of LTOT use in our study was quite similar to that in a trial conducted by the Long-Term Oxygen Treatment Trial Research Group (i.e., 10.4 h/day). That trial included 148 patients receiving LTOT during exercise and sleep.[10]

In a study analyzing 191 patients with severe hypoxemia, only 52.4% adhered to LTOT as prescribed,[25] a finding that is consistent with those of the present study. The authors of the aforementioned study reported 51 adverse events attributed to the use of oxygen supplementation, 23 patients having reported tripping on the device.[25] Adverse events can contribute to poor adherence to treatment.[10] In a qualitative study of 27 COPD patients receiving LTOT,[26] most (59.2%) reported nonadherence to LTOT, which was reported to be difficult to use during physical activity and was associated with social stigma and fear of side effects.

Previous studies[4,5,27,28] have shown the beneficial effects of LTOT on mortality, quality of life, and respiratory symptoms in COPD patients with severe hypoxemia. The use of LTOT might also be beneficial for patients with exertional hypoxemia, improving their symptoms, dyspnea, functional capacity, and disease progression. Analysis of data from the National Emphysema Treatment Trial, a longitudinal study conducted in the United States, showed that 33.8% of 1,215 patients with emphysema were using LTOT despite having no severe hypoxemia.[6] In comparison

Table 2. Comparison between adherent and nonadherent patients with COPD and exertional hypoxemia.[a]

Variable	Patients who adhered to LTOT (n = 18)	Patients who did not adhere to LTOT (n = 21)	p
Men, %	33.4	28.3	
Age, years	69.1 ± 10.0	71.5 ± 8.7	0.433
BMI, kg/m^2	23.2 ± 8.4	25.5 ± 6.0	0.684
FFM, kg	45.8 ± 9.3	45.0 ± 8.7	0.787
FFMI, kg	17.5 ± 2.1	18.1 ± 2.6	0.459
Borg scale[b]	0.0 (0.0-1.7)	0.0 (0.0-0.0)	0.420
BODE index	1.1 ± 0.9	1.4 ± 1.1	0.081
SpO_2, %	93.3 ± 2.3	91.1 ± 2.5	0.011
PaO_2, mmHg	64.4 ± 6.9	62,7 ± 9.1	0.536
$PaCO_2$, mmHg[b]	35.7 (34.2-39.4)	39.5 (35.8-47.3)	0.044
SaO_2, %[b]	93.3 (90.6-94.3)	91.2 (90.6-94.1)	0.509
FVC, L[b]	1.8 (1.4-2.6)	2.1 (1.5-2.4)	0.609
FEV_1, L[b]	0.9 (0.7-1.0)	1.0 (0.8-1.3)	0.083
FEV_1/FVC, L	0.4 ± 0.1	0.5 ± 0.1	0.072
SGRQ symptoms domain score, %	27.6 ± 18.7	41.5 ± 19.6	0.039
SGRQ activity domain score, %[b]	60.4 (45.9-70.3)	72.9 (66.2-79.7)	0.014
SGRQ impacts domain score, %	39.5 ± 15.1	27.8 ± 18.3	0.047
Total SGRQ score, %	36.4 ± 17.7	49.9 ± 11.9	0.012
Anxiety, HADS	3.1 ± 2.6	2.4 ± 1.7	0.373
Depression, HADS[b]	3.5 (1.0-5.0)	3.0 (0.0-7.0)	0.981
mMRC scale	1.9 ± 0.7	1.6 ±1.2	0.384
BDI[b]	5.0 (4.0-5.0)	6.0 (3.0-8.0)	0.392
6MWD, m	309.6 ± 90.3	266.5 ± 97.1	0.190

BMI: body mass index; FFM: fat-free mass; FFMI: fat-free mass index; BODE: Body mass index, airflow Obstruction, Dyspnea, and Exercise capacity; SGRQ: Saint George's Respiratory Questionnaire; HADS: Hospital Anxiety and Depression Scale; mMRC: modified Medical Research Council; BDI: Baseline Dyspnea Index; and 6MWD: six-minute walk distance. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as median (interquartile range). $p < 0.05$ (Student's t-test or Mann-Whitney test).

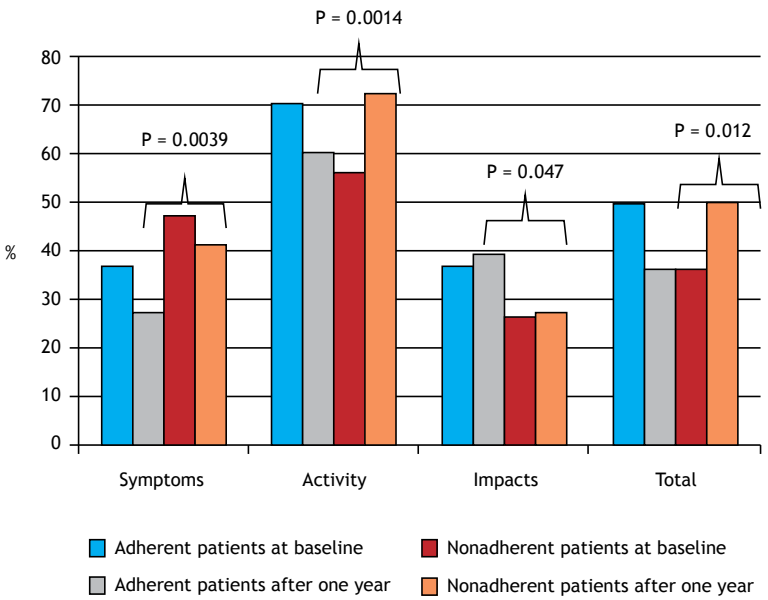

Figure 2. Saint George's Respiratory Questionnaire (SGRQ) domain scores for adherent and nonadherent patients at baseline and after one year of follow-up. $p < 0.05$ (paired t-test or Wilcoxon test).

with the patients who were not using LTOT, those who were had worse dyspnea, lower quality of life, and lower functional capacity, exercise-induced desaturation being more common in those using LTOT.[6]

Table 3. Multiple linear regression analysis of factors associated with the Saint George's Respiratory Questionnaire symptoms domain after one year of follow-up.

Dependent variable	Variable	Coefficient	(95% CI)	p
Symptoms after one year (R^2 = 0.23)	Baseline SpO_2, mmHg	0.01	−2.62 to 2.65	0.99
	BDI	−2.85	−5.59 to −0.107	0.04
	Adherence after one year, yes	−0.86	−18.40 to 16.77	0.91

BDI: Baseline Dyspnea Index.

According to international guidelines, LTOT should not be used in patients with exertional hypoxemia.[6-8,11,19,29,30] In a recently published study,[10] patients with moderate resting or exercise-induced hypoxemia were followed for six years and no differences were found between those using LTOT and those not using it regarding quality of life, lung function, or the 6MWD. In addition, the BODE index was lower in the patients using LTOT than in those not using it (p = 0.007).[10] However, the fact that the study included a heterogeneous sample of patients might have affected the results.

In the present study, quality of life (as assessed by SGRQ symptoms domain scores) was found to have improved after one year (53.0 ± 19.8 vs. 34.5 ± 20.4; p < 0.001). Eaton et al.[7] divided 41 COPD patients with exertional hypoxemia into two groups on the basis of LTOT use during physical exercise performed three times a week for 4 months. The group of patients using LTOT showed significant improvement in quality of life.[7] Garrod et al.[8] reported that patients participating in an 8-week exercise training program and receiving supplemental oxygen showed significant improvement in the 6MWD (169 ± 112 m vs. 269 ± 124 m; p = 0.001) and in Chronic Respiratory Disease Questionnaire scores (24.0 vs. 17.4; p < 0.001) when compared with those who participated in the program but did not receive supplemental oxygen.[8] The finding of a higher mean 6MWD is consistent with those of Morakami et al.,[31] who compared COPD patients in Brazil and Austria and found that mean walking time and movement intensity were greater in the former than in the latter.

Wadell et al.[9] found that the use of LTOT did not improve quality of life or dyspnea in COPD patients with exercise-induced hypoxemia. The authors evaluated 20 patients with COPD and exercise-induced hypoxemia. The participants were randomized to undergo exercise training while breathing room air or receiving supplemental oxygen for 30 min three times per week for 8 weeks.[9] Unlike our study, in which LTOT use was found to improve dyspnea, the study by Wadell et al. included no other periods of LTOT use during the day.[9]

Although our results showed no significant changes in dyspnea scores, multiple linear regression analysis showed that a higher BDI translated to worse SGRQ symptoms domain scores after one year of follow-up. This result is consistent with those obtained by Ferrari et al.,[32] who followed 90 COPD patients for three years and found that dyspnea perception was positively associated with impaired quality of life at the end of the follow-up period.

Reduced functional capacity is a marker of severity and mortality in patients with COPD and exercise-induced hypoxemia.[33] In the present study, the 6MWD decreased significantly after one year of follow-up. Takigawa et al.[34] reported that COPD patients with a ≥ 6% decrease in SpO_2 during the 6MWT were at an increased risk of death. Casanova et al.[35] reported that COPD patients with a ≥ 4% decrease in SpO_2 or an SpO_2 of < 90% during the 6MWT had a relative risk of death of 2.63 when compared with those without those changes.

Exacerbations can potentiate chronic inflammation and, consequently, have a detrimental effect on symptoms, quality of life, and exercise capacity, as well as accelerating the functional deterioration of COPD patients.[35-37] With regard to the frequency of exacerbations in our sample of COPD patients, nearly 50% experienced at least one exacerbation during follow-up. Of those, 26 required hospitalization, the need for which was unrelated to adherence to LTOT. In another study conducted at our institution,[32] 90 COPD patients were followed over a three-year period. Of those, 75% experienced at least one exacerbation, which was classified as severe in 26.6%.[32] In a recent study, no significant differences were found between patients with COPD and moderate hypoxemia using LTOT and patients with COPD and moderate hypoxemia not using LTOT in terms of disease exacerbation and hospitalization.[10] In a study conducted by Marino et al.,[38] 47 COPD patients were followed for 6 months, and 27.6% experienced at least one exacerbation during the study period. In the present study, the mortality rate was 15%. In a study involving 78 COPD patients receiving LTOT for one year, the mortality rate was 15.4%.[39]

Nutritional depletion is a risk factor for clinical worsening in COPD patients receiving LTOT.[37] In the present study, there were no significant changes in the prevalence of nutritional depletion during follow-up. However, the BMI was found to be lower in the patients who experienced worsening hypoxemia or died than in those with transient hypoxemia. In a study evaluating 128 COPD patients using LTOT and followed for three years, the number of deaths was higher among those with a BMI of < 25 kg/m^2 and a higher number of comorbidities.[39]

Our study has some limitations. First, given that patient adherence to LTOT was self-reported, it is impossible to determine actual adherence. Nevertheless, in a recent study conducted by the Long-Term Oxygen Treatment Trial Research Group,[10] adherence to LTOT was also self-reported, and adherence education was

provided at each medical visit in order to improve LTOT use, as was done in the present study. Unfortunately, there is currently no other method for accurately determining patient adherence to LTOT. Second, there was no true control group (i.e., a group of patients receiving no supplemental oxygen). Second, by including a group without oxygen (true control group) will probably add more significant alterations on several parameters of disease progression and quality of life. If there had been one, we might have observed significant changes in several disease progression and quality of life parameters. However, although the changes observed in the group of patients who did not adhere to LTOT were not as marked as they might have been in a true control group, quality of life was found to have improved in the group of patients who adhered to LTOT. Therefore, our findings are relevant.

In conclusion, quality of life appears to be lower in patients with COPD and exercise-induced hypoxemia who do not adhere to LTOT than in those who do. In addition, LTOT appears to have a beneficial effect on COPD symptoms (as assessed by SGRQ scores) in patients with COPD and exercise-induced hypoxemia. Further studies investigating LTOT use during exercise are needed in order to provide a better understanding of patient clinical response to LTOT.

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ORIGINAL ARTICLE

Noncompliance with the law prohibiting the sale of cigarettes to minors in Brazil: an inconvenient truth

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ABSTRACT

Objective: To draw up an up-to-date scenario of compliance with the law prohibiting the sale of cigarettes to minors. **Methods:** We used data about youth access to cigarette purchase that were obtained through a nationwide survey conducted in 2015 among students aged 13-17 years. We estimated simple proportions of attempts to buy cigarettes, success of attempts, purchase of cigarettes on a regular basis, and purchase of cigarettes on a regular basis in a store or bar. All estimates were stratified by gender, age group, and Brazilian macro-region. Crude absolute difference and adjusted absolute difference in the proportion of smokers in each category by variable of interest were analyzed by a generalized linear model with binomial distribution and identity link function. **Results:** Approximately 7 in every 10 adolescent smokers attempted to buy cigarettes at least once in the 30 days prior to the survey. Of those, approximately 9 in every 10 were successful, and individuals aged 16-17 years (vs. those aged 13-15 years) were less often prevented from buying cigarettes (adjusted absolute difference, 8.1%; $p \leq 0.05$). Approximately 45% of all smokers aged 13-17 years in Brazil reported buying their own cigarettes on a regular basis without being prevented from doing so, and, of those, 80% reported buying them in a store or bar (vs. from a street vendor). **Conclusions:** Our findings raise an important public health concern and may contribute to supporting educational and surveillance measures to enforce compliance with existing anti-tobacco laws in Brazil, which have been disregarded.

Keywords: Smoking/epidemiology; Smoking/legislation & jurisprudence; Adolescent behavior; Public health.

INTRODUCTION

Brazil has achieved great advances in the fight against the tobacco epidemic in recent years because of the implementation of a series of legislative and educational measures based on the World Health Organization Framework Convention on Tobacco Control.[1-4]

The reduction in the proportion of smokers reflects not only increased smoking cessation but also a likely decrease in smoking initiation among adolescents and young adults.[4] In fact, Brazilian national data from recent household surveys have shown a reduction in the proportion of smokers aged 18 to 24 years, which decreased from 13.6% to 10.6% from 2008 to 2013, as well as a reduction in the proportion of cigarette use among adolescents aged 14 to 17 years, which decreased from 6.2% to 3.4% from 2006 to 2012.[4,5]

Given that the mean age at initiation of regular cigarette smoking in Brazil is around 16 years,[6] it is of fundamental importance that compliance with the law prohibiting the sale of cigarettes to minors be periodically assessed.[7,8] It has been observed, for example, that, despite the reduction in the proportion of adolescent smokers that occurred in Brazil between 2006 and 2012,[5] surveys conducted among students aged 13 to 15 years in several Brazilian cities between 2002 and 2009 indicated that compliance with the law prohibiting the sale of cigarettes to children and adolescents was pretty far from ideal.[9] In fact, among the adolescent smokers who had tried to buy cigarettes in the 30 days prior to the surveys, the proportion of those who reported that they had not been prevented from buying cigarettes ranged from 51.0% to 91.6%. This suggests that the proportions of adolescent smokers in Brazil could have been further reduced. In addition, in 2012 and 2015, comparative national data on the proportion of cigarette consumption among students aged 13 to 15 years signaled a reversal of the decrease in smoking initiation among youth (5.1% vs. 5.6%).[10,11]

The objective of the present study was, therefore, to draw up an up-to-date scenario of compliance/noncompliance with the law prohibiting the sale of cigarettes to minors. To that end, we used data about youth access to cigarette purchase that were obtained through a nationwide survey conducted in 2015 among students aged 13 to 17 years.[11] This study may contribute not only to the understanding of the course of the smoking epidemic in Brazil but may also provide grounds for action, if necessary, toward the effective implementation of this law.

METHODS

We used data from the *Pesquisa Nacional de Saúde do Escolar* (PeNSE, Brazilian National School-Based Adolescent Health Survey), conducted in 2015, to

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assess compliance with the law prohibiting the sale of cigarettes to minors in Brazil.[11]

The PeNSE is a survey of students that was first conducted in 2009 and occurs every 3 years in Brazil. Important innovations were introduced into the 2015 edition of the PeNSE.One of the most significant was that data were derived from a sample that included sixth to ninth graders and tenth to twelfth graders at public and private schools nationwide. Details regarding the cluster sampling procedure can be obtained elsewhere.[11] The indicators used in the present study concern the 13- to 17-year age group, totaling 10,926 questionnaires.

Although the cigarette consumption-related themes investigated in 2015 are the same as those of the previous editions of the survey, in that year, two new questions regarding youth access to cigarettes were also included: "In the past 30 days, did anybody refuse to sell you cigarettes (at any time) because of your age?"—the response choices were as follows: "I did not try to buy cigarettes in the past 30 days" OR "Yes, someone refused to sell me cigarettes because of my age" OR "No, my age did not keep me from buying cigarettes"; and "In the past 30 days, how did you usually get your own cigarettes?"—the response choices were as follows: "I bought them in a store or bar" OR "I bought them from a street vendor" OR "I gave someone else money to buy them for me" OR "I borrowed them from someone else" OR "An older person gave them to me" OR "I got them some other way".

Data analysis

The analysis of the variables regarding youth access to cigarettes was restricted to the adolescents who reported having smoked cigarettes in the past 30 days (n = 688).The adolescent smokers were separated into two age groups (13- to 15-year olds vs. 16- to 17-year olds) in order to assess the impact that the individual's physical aspect, related to growth and hormonal maturity, has on the attempts to buy cigarettes and, subsequently, on the success (or failure) of those attempts.

We estimated simple proportions of the following variables: "attempts to buy"; "success of attempts"; "purchase on a regular basis"; and "purchase on a regular basis in a store or bar." All estimates were stratified by gender, age group, and Brazilian macro-region. To that end, we created a dichotomous variable called "attempts to buy", classified as follows: (1) a combination of individuals who were prevented from buying cigarettes sometime in the 30 days prior to the completion of the questionnaire and those who were not prevented from doing so; and (0) individuals who did not attempt to buy cigarettes during that period.

We also created another dichotomous variable called "success of attempts", which was classified as follows: (1) individuals who were not prevented from buying cigarettes in the 30 days prior to the survey; and (0) individuals who were prevented from buying cigarettes during that period.

In addition, for the adolescent smokers who reported having been able to buy cigarettes sometime in the 30 days prior to the survey, we created a dichotomous variable called "purchase on a regular basis", described as follows: (1) individuals who reported having bought cigarettes usually from a store, bar, or street vendor during that period; and (0) individuals who reported having gotten cigarettes through means other than a purchase during that period.

Finally, for the adolescent smokers who were not prevented from buying cigarettes in the 30 days prior to the survey, we created a dichotomous variable called "purchase on a regular basis in a store or bar", as follows: (1) individuals who bought cigarettes in a store or bar during that period; and (0) individuals who bought cigarettes on a regular basis from a street vendor during that period.

Crude absolute difference and adjusted absolute difference in the proportion of smokers in each category by gender, age group, and Brazilian macro-region were analyzed by a generalized linear model with binomial distribution and identity link function.[12] In this model, "attempts to buy" (OR "success of attempts" OR "purchase on a regular basis" or "purchase in a store or bar") was used as a dependent variable, whereas gender, age group, and Brazilian macro-region were used as independent variables. Confidence intervals for adjusted absolute differences obtained from the regression model were calculated on the basis of a type I error of 5%. Additive interactions among independent variables were assessed by inclusion of corresponding interaction terms. The choice of an additive model, including for assessing interactions, was based on the importance of the results from the standpoint of prevention of youth access to cigarettes.[13]

Variables were processed and data were analyzed using STATA 12.0 (StataCorp LP, College Station, TX, USA).[14] The STATA *svy* command was used in order to handle cluster sampling appropriately and allow introduction of expansion fractions in the analyses.

The 2015 PeNSE[11] was approved by the Brazilian National Health Council-*Comissão Nacional de Ética em Pesquisa* (CONEP, Brazilian National Research Ethics Committee; no. 1.006.467 of 03/30/2015).

RESULTS

Among the approximately 810,000 adolescent smokers who reported having smoked cigarettes in the past 30 days prior to the survey, there were higher proportions of 13- to 15-year-olds (vs. 16- to 17-year-olds), males, and Southeastern students (Table 1).In 2015, the proportion of smokers among students aged 13 to 17 years in Brazil was estimated to be 6.6% (the proportions of smokers among 13- to 15-year-olds and among 16- to 17-year-olds were estimated to be 5.4% and 8.4%, respectively).

Approximately 7 in every 10 smokers aged 13 to 17 years tried to buy cigarettes at least once in the 30 days prior to the survey; this proportion was significantly lower among girls (adjusted absolute difference, −9.5%; p ≤ 0.05; Table 2).

Of the adolescent smokers who tried to buy cigarettes sometime in the past 30 days, approximately 9 in every 10 were successful (Table 3).We also found that the adolescents aged 16 to 17 years were less often prevented from buying cigarettes than were the younger ones (adjusted absolute difference, 8.1%; p ≤ 0.05).In addition, when compared with the adolescent smokers attending schools in the Southeast, South, or Central-West, those attending schools in the Northeast reported a higher proportion of success in purchasing cigarettes.

Of the adolescent smokers who reported having been able to buy cigarettes at least once in the 30 days prior to the survey, approximately 7 in every 10 said that they did it regularly, that is, they usually got their own cigarettes by buying them directly from a store, bar, or street vendor. We found that the proportion of this behavior was considerably higher among the smokers aged 16 to 17 years than among those aged 13 to 15 years (adjusted absolute difference, 24.5%; p ≤ 0.05).In addition, the adolescent smokers attending schools in the Northeast apparently had the lowest proportion of "purchase on a regular basis" (Table 4).

Among the adolescents whose most common mode of cigarette acquisition in the 30 days prior to the survey was direct purchase, approximately 8 in every 10 reported having bought cigarettes in a store or bar (vs. from a street vendor).The smokers aged 16 to 17 years (in comparison with those aged 13 to 15 years) and the adolescent smokers attending schools in the Southeast, South, or Central-West of Brazil (in comparison with those attending schools in the Northeast) reported having bought cigarettes on a regular basis and more often in a store or bar than from a street vendor (Table 5).

Table 1. Distribution of the individuals who reported having smoked in the 30 days prior to the survey, by age group, gender, and Brazilian macro-region.

Characteristic	Result		
	n[a]	n[b]	%[c]
Total	688	807,676	-
Age group, years			
13-15	379	414,032	51.3
16-17	309	393,644	48.7
Gender			
Male	373	432,003	54.4
Female	315	375,673	45.6
Region			
North	104	73,376	8.9
Northeast	105	169,823	22.3
Southeast	151	354,090	43.2
South	168	135,461	16.6
Central-West	160	74,926	8.9

Brazilian Institute of Geography and Statistics.[11] [a]Only 4 individuals did not answer whether they had smoked in the 30 days prior to the survey and were excluded from the analysis. [b]Number of smokers taking sample weight into account. [c]Proportion of smokers taking sample weight into account.

DISCUSSION

The results presented here paint a dismal picture of the effectiveness of the implementation of the law prohibiting the sale of tobacco products to minors in Brazil. Approximately 7 in every 10 adolescent smokers

Table 2. Crude absolute difference (AD) and adjusted AD in the proportion of smokers who tried to buy cigarettes sometime in the 30 days prior to the survey, by age group, gender, and Brazilian macro-region.

Characteristic	Smokers[a]		
	% (95% CI)	Crude AD	Adjusted AD*
Total	72.3 (67.5-76.6)	-	-
Age group, years			
13-15	71.0 (64.3-76.8)	Ref.	Ref.
16-17	73.6 (66.4-79.7)	2.6	2.2
Gender			
Male	76.5 (72.1-79.8)	Ref.	Ref.
Female	67.4 (62.0-72.0)	−9.1**	−9.5**
Region			
North	77.3 (66.5-85.5)	2.9	2.3
Northeast	74.4 (63.9-82.7)	Ref.	Ref.
Southeast	68.8 (59.7-76.6)	−5.6	−6.8
South	75.3 (67.9-81.7)	0.8	1.0
Central-West	73.3 (65.1-80.2)	−1.1	−1.6

Brazilian Institute of Geography and Statistics.[11] [a]Only 3 individuals did not answer whether they had tried to buy cigarettes sometime in the 30 days prior to the survey and were excluded from the analysis. Adolescent smokers who reported having gotten cigarettes usually by buying them from a store, bar, or street vendor in those 30 days and who also reported that they did not try to buy cigarettes (at any time) in the past 30 days were reclassified and combined with those who tried to buy cigarettes sometime in the past 30 days (n = 20). *Age-, gender-, and region-adjusted generalized linear model, as appropriate, with binomial distribution and identity link function. No interaction term was statistically significant. **p ≤ 0.05.

Table 3. Crude absolute difference (AD) and adjusted AD in the proportion of smokers who were not prevented from buying cigarettes at any time in the 30 days prior to the survey, by age group, gender, and Brazilian macro-region, among the smokers who tried to buy cigarettes sometime in the 30 days prior to the survey.

Characteristic	Smokers[a]		
	% (95% CI)	Crude AD	Adjusted AD*
Total	86.1 (81.5-89.7)	-	-
Age group, years			
13-15	82.3 (79.4-85.6)	Ref.	Ref.
16-17	89.9 (85.9-93.9)	7.6**	8.1**
Gender			
Male	85.7 (78.8-90.6)	Ref.	Ref.
Female	86.6 (80.1-91.2)	0.9	1.2
Region			
North	85.6 (74.5-92.4)	−8.3	−8.0
Northeast	93.9 (88.5-95.0)	Ref.	Ref.
Southeast	83.9 (75.8-88.0)	−10.0**	−10.3**
South	82.8 (74.3-88.3)	−11.1**	−11.8**
Central-West	84.4 (77.5-88.5)	−9.5**	−9.6**

Brazilian Institute of Geography and Statistics.[11] [a]Only 3 individuals did not answer whether they had tried to buy cigarettes sometime in the 30 days prior to the survey and were excluded from the analysis. Adolescent smokers who reported having bought cigarettes usually from a store, bar, or street vendor in the past 30 days and who also reported that they were prevented from buying them (sometime) in the past 30 days were reclassified and combined with those who were not prevented from buying cigarettes in the past 30 days because of their age (n = 73). *Age-, gender-, and region-adjusted generalized linear model, as appropriate, with binomial distribution and identity link function. No interaction term was statistically significant. **p ≤ 0.05.

Table 4. Crude absolute difference (AD) and adjusted AD in the proportion of smokers who usually bought cigarettes from a store, bar, or street vendor in the 30 days prior to the survey,[a] by age group, gender, and Brazilian macro-region, among the smokers who were not prevented from buying cigarettes at any time in the 30 days prior to the survey.

Characteristic	Smokers[b]		
	% (95% CI)[b]	Crude AD	Adjusted AD*
Total	69.5 (63.1-75.2)	-	-
Age group, years			
13-15	56.7 (47.4-65.7)	Ref.	Ref.
16-17	81.2 (73.1-87.2)	24.5**	24.4**
Gender			
Male	74.7 (68.8-79.0)	Ref.	Ref.
Female	62.9 (55.8-68.7)	−11.8**	−10.1
Region			
North	79.6 (68.8-85.1)	20.3**	24.6**
Northeast	59.3 (48.0-68.5)	Ref.	Ref.
Southeast	71.6 (59.7-81.1)	12.3	11.7
South	72.5 (62.1-80.3)	13.2	13.8
Central-West	70.0 (58.2-79.6)	10.7	12.0

Brazilian Institute of Geography and Statistics.[11]. [a]Versus other acquisition options as follows: "I gave someone else money to buy cigarettes for me" OR "I borrowed them from someone else" OR "I took it without permission" OR "An older person gave them to me" OR "I got them some other way". [b]Only 3 individuals did not answer whether they had tried to buy cigarettes sometime in the 30 days prior to the survey and were excluded from the analysis. *Age-, gender-, and region-adjusted generalized linear model, as appropriate, with binomial distribution and identity link function. No interaction term was statistically significant. **p ≤ 0.05.

felt motivated to try at least once to break this law. Even worse, the vast majority of those who chose to venture into this illegal behavior were rewarded by encountering no great resistance from retailers and/or street vendors to them purchasing cigarettes; to make matters further worse, a substantial proportion of those same adolescents reported buying cigarettes on a regular basis. As expected, the closer adolescents were to adulthood (age 16-17 years), the greater the likelihood of them not being prevented from buying cigarettes because of their age and, consequently, of them doing so more regularly and in licensed places, such as stores or bars. When we applied specific proportions of each of these factors listed above, we found that approximately 45% of all adolescent smokers aged 13 to 17 years in Brazil reported buying their own cigarettes on a regular basis without being prevented from doing so.

Table 5. Crude absolute difference (AD) and adjusted AD in the proportion of smokers who usually bought cigarettes from a store or bar in the 30 days prior to the survey,[a] by age group, gender, and Brazilian macro-region, among the smokers who were not prevented from buying cigarettes at any time in the 30 days prior to the survey and who purchased cigarettes on a regular basis in those 30 days.

Characteristic	Smokers[b]		
	% (95% CI)	Crude AD	Adjusted AD*
Total	81.1 (74.2-86.5)	-	-
Age group, years			
13-15	73.7 (64.2-79.6)	Ref.	Ref.
16-17	85.9 (79.7-89.8)	12.2**	11.3**
Gender			
Male	83.7 (75.1-89.7)	Ref.	Ref.
Female	77.3 (65.0-86.2)	−6.4	−5.4
Region			
North	75.2 (50.5-90.0)	10.0	12.3
Northeast	65.2 (52.0-75.4)	Ref.	Ref.
Southeast	85.1 (75.5-95.0)	19.9**	19.4**
South	90.6 (80.2-95.8)	25.4**	25.7**
Central-West	87.2 (75.8-94.9)	22.0**	22.6**

Brazilian Institute of Geography and Statistics.[11] [a]Versus the smokers who usually bought cigarettes from a street vendor. [b]Only 3 individuals did not answer whether they had tried to buy cigarettes sometime in the 30 days prior to the survey and were excluded from the analysis. *Age-, gender-, and region-adjusted generalized linear model, as appropriate, with binomial distribution and identity link function. No interaction term was statistically significant. **$p \leq 0.05$.

Recent comparative data from a study of Brazilian students aged 12 to 17 years conducted between 2013 and 2014[15] and from the 2015 PeNSE[11] suggest a trend toward an increase in the proportion of adolescent smokers (5.7% and 6.6%, respectively). It is worth noting that the proportion of adolescent smokers reflects the sum of the effectiveness of a series of tobacco control policies aimed at reducing smoking initiation that are currently in effect in Brazil. For example, it is likely that the new structure of tobacco product taxation implemented in 2012 has contributed enormously to discouraging adolescents from starting smoking.[16,17] In addition, the regulation that prohibited smoking in enclosed collective areas as of late 2014[18] may have contributed to a further reduction in the social acceptability of smoking in bars and nightclubs, which are places where many youth start smoking. However, low compliance with the law prohibiting youth access to tobacco products may be undermining the effects of the Brazilian national tobacco control policy measures on the prevention of smoking initiation by adolescents.

Although cigarette packs contain a warning about legislation prohibiting the sale of cigarettes to minors, data from the PeNSE[11] indicate that there is a great deal of irresponsibility on the part of retailers and that compliance with the law is poorly enforced by the responsible agencies. This situation is aggravated by the widespread availability of points of sale,[19] by the tobacco industry point-of-sale marketing strategies for placement of cigarette packs always next to candy, and by the suspension of the Brazilian National Health Oversight Agency resolution that, in 2012, prohibited the addition of sweet flavors to cigarettes.[20] In addition, there has been an exponential growth in the availability of contraband tobacco products, which are sold at very low prices in stores, in bars, and mainly by street vendors.[21]

It is of note that the PeNSE[11] questions related to youth access to cigarettes also address another practice that is in violation of current laws, that is, the sale of single cigarettes. In fact, Law no. 7212/2010 establishes that, in Brazil, cigarettes must be sold, as well as displayed for sale, only in packs of 20 units.[22] Buying a single cigarette, even if at a higher per-unit price, but still at a lower price than that of a whole pack, with no health warning attached,[23] facilitates smoking initiation and cigarette use on a regular basis. Surveys among ninth graders (aged 13-15 years) conducted in several Brazilian cities between 2002 and 2009 indicated that, in fact, buying single cigarettes is a widespread practice in Brazil, with rates reaching above 90% in some cities.[9]

Because the effect of tobacco use is cumulative, our findings raise an important public health concern, since smoking is a risk factor that has a major impact on the burden of chronic non-communicable diseases.[24] In fact, a recent study indicated that, in 2015, approximately 156,000 people died from diseases directly related to smoking in Brazil.[25] Another nationwide study found that, in 2013, there were approximately 280,000 "all-cause deaths" directly or indirectly attributable to smoking in Brazil[26]; in addition, that study showed that the cumulative risk of mortality from COPD or lung cancer in Brazil, both for men and women, is more than 20 times higher in smokers than in nonsmokers.[26]

The limitations of the present study lie in the fact that the cross-sectional nature of the PeNSE[11] prevents us from establishing any temporal or causal relationship between findings. However, even if cigarette experimentation was not stimulated by violation of

the law prohibiting the sale of cigarettes to minors, the data suggest that noncompliance with this law is important for the maintenance of this health-harmful behavior in nearly half of the adolescent smokers in Brazil. In addition, the study is subject to information bias because all information was self-reported by the participants. However, given that questionnaires were self-administered and anonymity was ensured,[11] at least the influence of parents and/or interviewers on the responses may have been minimized.

The two major questions analyzed in our study were related only to the sale of cigarettes to minors. It is known, however, that there are other tobacco products, such as water pipes, that are very commonly used among adolescents,[27] all of which should be subject to the same restrictions as cigarettes.[7,8] According to data from the PeNSE,[11] the proportions of youth aged 13 to 17 years who reported being smokers and using tobacco products other than cigarettes concomitantly or exclusively were 3% and 4%, respectively. For those youth, unfortunately, we have no information about attempts to buy these other tobacco products nor about the success of attempts and means of acquisition.

Regarding generalization of the findings to all adolescents in Brazil, it should be borne in mind that the PeNSE[11] was conducted on individuals attending school and that elementary and middle schooling is widely available in the country, thereby reducing potential losses.[28] However, the fact that adolescent smokers are likely to have higher rates of school absenteeism than do adolescent nonsmokers cannot be left out.[29]

Our findings may contribute to supporting compliance with existing laws in Brazil that are aimed at reducing smoking initiation and therefore, in the future, may also contribute to reducing cigarette smoking-related morbidity and mortality. The scenario described in the present study indicates the need to stimulate federal, state, and municipal powers to take educational and surveillance measures, including through joint efforts with retail trade bodies and with unions representing the newsstand sector and other commercial sectors. Therefore, it would also be important to motivate agencies such as the Public Prosecutor's Office to negotiate a conduct adjustment term with the tobacco companies supplying the vast network of retailers nationwide,[19] in which the companies agree to take shared responsibility for enforcing the law prohibiting the sale of tobacco products to minors.[7,8] At the same time, it would also be important to mobilize members of the House of Representatives and the Senate to propose and pass a federal law restricting the sale of tobacco products to licensed tobacco stores.

The information presented in this study may also help leverage the implementation of other measures to combat smoking, such as a ban on cigarettes with additives[20] and approval of the protocol to eliminate illicit trade in tobacco products[30]; such measures would prevent cigarettes from being available to adolescents in stores and through street vendors

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2018 recommendations for the management of community acquired pneumonia

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ABSTRACT

Community-acquired pneumonia (CAP) is the leading cause of death worldwide. Despite the vast diversity of respiratory microbiota, *Streptococcus pneumoniae* remains the most prevalent pathogen among etiologic agents. Despite the significant decrease in the mortality rates for lower respiratory tract infections in recent decades, CAP ranks third as a cause of death in Brazil. Since the latest Guidelines on CAP from the *Sociedade Brasileira de Pneumologia e Tisiologia* (SBPT, Brazilian Thoracic Association) were published (2009), there have been major advances in the application of imaging tests, in etiologic investigation, in risk stratification at admission and prognostic score stratification, in the use of biomarkers, and in the recommendations for antibiotic therapy (and its duration) and prevention through vaccination. To review these topics, the SBPT Committee on Respiratory Infections summoned 13 members with recognized experience in CAP in Brazil who identified issues relevant to clinical practice that require updates given the publication of new epidemiological and scientific evidence. Twelve topics concerning diagnostic, prognostic, therapeutic, and preventive issues were developed. The topics were divided among the authors, who conducted a nonsystematic review of the literature, but giving priority to major publications in the specific areas, including original articles, review articles, and systematic reviews. All authors had the opportunity to review and comment on all questions, producing a single final document that was approved by consensus.

Keywords: Pneumonia/diagnosis; Pneumonia/prevention & control; Pneumonia/therapy; Pneumonia/drug therapy.

INTRODUCTION

Community-acquired pneumonia (CAP) is the leading cause of death worldwide, with a significant impact on morbidity rates.[1] Despite the vast diversity of respiratory microbiota, the widespread dissemination of potentially pathogenic agents, the phenomenon of globalization, and the occurrence of viral epidemics, *Streptococcus pneumoniae* remains the most prevalent pathogen among the etiologic agents of CAP.[2]

In Brazil, as well as in other countries, there has been a significant decrease in the mortality rates for respiratory tract infections, although the magnitude of this decrease has lessened in recent decades. Among pneumonias, CAP remains the one with the greatest impact and is the third leading cause of mortality in Brazil. Although the absolute number of deaths in Brazil has increased because of population growth and aging, when the mortality rate for CAP is standardized by age, a 25.5% decrease is observed between 1990 and 2015.[3] An improved socioeconomic situation, greater access to health care, national availability of antibiotics, and vaccination policies partially explain the decrease in mortality rates in Brazil.[4]

Since the latest Guidelines on CAP from the *Sociedade Brasileira de Pneumologia e Tisiologia* (SBPT, Brazilian Thoracic Association) were published,[5] several topics have been reviewed, such as advances in the application of imaging tests; advances in and impact of etiologic investigation, particularly investigation of viral etiology and atypical pathogens in subgroups of patients; risk stratification at admission; prognostic score stratification; the role of biomarkers in therapeutic management;

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recommendations for antibiotic therapy and its duration; and recommendations regarding influenza and pneumococcal vaccination.

METHODS

The authors consensually determined specific topics to be addressed, on the basis of relevant publications in the literature on CAP with regard to imaging tests, etiologic investigation, risk stratification at admission and prognostic score stratification, use of biomarkers, recommendations for antibiotic therapy and its duration, and prevention through vaccination. To review these topics, the SBPT Committee on Respiratory Infections summoned 13 members with recognized experience in CAP in Brazil who developed 12 questions concerning the previously determined topics. The questions were divided among the authors, who conducted a nonsystematic review of the literature, but giving priority to major publications in the specific areas, including original articles, review articles, and systematic reviews. All participants had the opportunity to review and comment on all questions, producing a document that was approved by consensus at the end of the process.

RECOMMENDATIONS FOR IMAGING METHODS IN CAP

Chest X-ray

Chest X-ray, in combination with anamnesis and physical examination, is part of the classic diagnostic triad for CAP; it is recommended that, when available, posteroanterior and lateral chest X-rays should be routinely performed. In addition to contributing to diagnosis, chest X-ray allows us to assess the extent of the lesions and detect complications, as well as facilitating differential diagnosis.[6]

Despite the existence of numerous guidelines, there is no consensus regarding recommendations for the management of CAP in primary care, especially in terms of ancillary tests, which are often not readily available. At this level of care, when the clinician is sure of the diagnosis, chest X-ray is not required for treatment initiation, and antimicrobials can be prescribed appropriately. However, fewer than 40% of physicians are able to diagnose pneumonias solely on the basis of physical examination. In this context, chest X-ray should be mandatory for patients with suspected CAP.[7] Chest X-ray is also recommended if there is doubt about the diagnosis or differential diagnosis from lung cancer is required and if, during treatment follow-up, clinical response is unsatisfactory. Chest X-ray is recommended for all patients admitted to the hospital.[8,9]

Chest ultrasound

Chest ultrasound (CUS) has greater sensitivity and accuracy in detecting parenchymal changes than does chest X-ray. Major ultrasound findings in CAP include consolidations, a focal interstitial pattern, subpleural lesions, and pleural line abnormalities. The specificity of CUS for consolidations is 100%, whereas chest X-ray reaches a sensitivity of only 94% for this type of change.[10]

Bedside ultrasound performed by clinicians in the emergency department has a sensitivity of 95% and a negative predictive value of 67% in the diagnosis of CAP, compared with 60% and 25%, respectively, for chest X-ray. Specificity is similar for both diagnostic methods.[11,12]

When conducted by ultrasound specialists, ultrasound reaches a sensitivity of 94% and a specificity of 96%. However, the yield of ultrasound conducted by clinicians in the emergency department has yet to be further evaluated, and more robust evidence is needed. It is important to bear in mind the usefulness of U/S in pregnant women and bedridden individuals, in whom X-ray quality is lower than desired. In addition, CUS has a high yield in detecting complications such as pleural effusion, as well as permitting visualization of loculations in the cavity. Referral for aspiration of pleural effusion (whether loculated or not) is one of the indications for CUS.[13-16] Therefore, the need for specific training in ultrasound and the unavailability of the method in primary care and in many health care facilities in Brazil currently restrict the use of ultrasound to advanced care centers.

Chest CT

Chest CT is the most sensitive method for identifying infectious involvement of the lung parenchyma, despite its high cost and the high level of radiation exposure.[17]

Chest CT is especially useful in cases in which the accuracy of chest X-ray and chest U/S is low, such as in obese patients, immunosuppressed patients, and individuals with previous abnormal radiological findings. In addition, chest CT is indicated in suspected fungal infections and for assisting the exclusion of other diagnoses in selected cases. In one study, the use of chest CT in patients with suspected CAP in the emergency department resulted in 16% of the patients having alternative diagnoses or findings, such as pulmonary thromboembolism and neoplasia, and, of those, 8% were diagnosed with pulmonary tuberculosis.[18] More recently, other authors have demonstrated that the use of chest CT increases the rate of diagnosis in patients with CAP and normal chest X-rays, but it may also not confirm the disease in patients with opacities on chest X-rays, which would allow the discontinuation of antibiotics in a significant proportion of cases.[19,20]

Because of the high radiation exposure from CT, some authors have suggested the use of chest U/S as an intermediate ancillary test before the use of CT in the diagnosis of difficult-to-diagnose cases.[21]

In addition, the importance of chest CT in the assessment of CAP-related complications, such as lung abscess and loculated pleural effusion, and in the investigation of reasons for the lack of clinical response to treatment has been emphasized.[22,23]

ETIOLOGIC INVESTIGATION OF OUTPATIENT AND INPATIENT CAP: WHAT ARE THE RECOMMENDATIONS?

Although there may be inadequate response to empiric treatment, etiologic testing is not necessary in patients with non-severe CAP receiving outpatient treatment. Therefore, the recommendations that etiologic testing be performed only in patients with severe CAP or CAP unresponsive to the initial empiric treatment regimen, as well as in ICU patients, remain valid.

In selecting tests to be performed, one should take into account patient age, presence of comorbidities, disease severity, and prior anti-infective therapy.[24]

The development of new methods for microbiological identification in general, and for microbiological identification of CAP in particular, has increased the chances of adequately choosing the spectrum of the antibiotic to be used in the treatment of pneumonia. Of note are radiological methods, such as chest U/S, and microbiological methods, namely Multiplex PCR[25] and matrix-assisted laser desorption ionization-time of flight mass spectrometry, a promising method for rapid identification of pathogens,[26]

With regard to microbiological studies, direct examination and culture of sputum samples (or of nasotracheal aspirates for patients who cannot expectorate) should meet sample quality criteria, that is, fewer than 10 epithelial cells and more than 25 leukocytes per field examined. In addition, technical norms for collection, transport, and analysis of biological samples should be adhered to.[27]

In an observational study of 670 hospitalized patients with CAP, 478 good quality sputum samples were obtained of a total of 591 samples. Specificity was much higher than sensitivity (*S. pneumoniae*: 91.5% vs. 62.5%), very similar to those of other bacterial agents identified. It is of note that the treatment of the cases in which the pathogen was identified was similar to the treatment started empirically.[28]

Molecular tests have been shown to be more effective in detecting atypical agents. Film array respiratory panel is a rapid (1 hour), multiplex molecular test that detects 20 respiratory pathogens (17 viruses and three bacteria: *Mycoplasma pneumoniae, Chlamydophila pneumoniae*, and *Bordetella pertussis*). Another test (Nxtag respiratory pathogen panel) can identify 18 viruses, *M. pneumoniae*, and *C. pneumoniae*.[29] The current recommendations for the use of molecular tests include: (1) highly accurate rapid testing for influenza; (2) rapid molecular testing for *M. tuberculosis* (feasible in a few hours); (3) rapid testing for respiratory viruses that can cause CAP or lower respiratory tract infection; and (4) rapid testing for detecting atypical pathogens (*M. pneumoniae, C. pneumoniae, Legionella* sp., and *B. Pertussis*).[30]

Patients with severe CAP should be etiologically investigated with the basic tests available: sputum smear microscopy and sputum culture; blood culture; urinary antigen testing for *S. pneumoniae* and *Legionella* sp.; serological tests; and, eventually, culture for atypical pathogens. In selected cases and in an appropriate clinical context, special cultures and galactomannan and (1-3)-β-D-glucan tests for fungi, as well as the latest antigen or molecular biology tests for viruses and atypical pathogens, may be performed, but are not indicated in the routine management of CAP.

In patients on mechanical ventilation, in nonresponders to the initial empiric therapy, and in those in whom less common etiologic agents are suspected, as well as in cases in which differential diagnosis from noninfectious lung diseases, such as tumors, vasculitis, or interstitial lung disease, is required, it may be necessary to collect samples invasively via bronchoscopy, endotracheal aspiration, bronchoalveolar lavage, or thoracentesis, in cases of ipsilateral pleural effusion.[5]

ROLE OF VIRUSES AND RECOMMENDATIONS FOR THEIR INVESTIGATION IN CAP

The advent of the use of molecular tests in clinical practice has signaled that viruses play a more relevant role as possible etiologic agents of CAP. Studies including PCR as a diagnostic tool in their scope have detected viruses in approximately one third of CAP cases in adults,[20,21] with influenza being the most commonly isolated virus. In addition of influenza, other viral agents, such as rhinovirus, respiratory syncytial virus, parainfluenza virus, adenovirus, and metapneumovirus, are considered possible etiologic agents of CAP.[31] Musher et al. evaluated 259 patients hospitalized for CAP, in order to identify the etiologic agents. Forty-four viruses were identified in 42 patients: rhinovirus, in 26; coronavirus, in 7; parainfluenza, in 4; respiratory syncytial virus, in 3; metapneumovirus, in 1; and influenza, in 1. Viruses were the only pathogens detected in 30 of the patients. The authors found strong evidence of the activity of viruses as causative agents of pneumonia in 28 of the 42 patients.[32]

However, uncertainty remains as to the true role of viruses in CAP because of the difficulty in determining whether viruses act as co-pathogens or as colonizers. One example of this is in a study by Jartti et al., which showed the presence of viruses in nasopharyngeal swabs in approximately 30% of healthy adults. However, isolation of influenza, respiratory syncytial virus, and metapneumovirus is rare in asymptomatic adults.[33]

Another possible activity of viruses in CAP would be impairment of the defense mechanisms of the upper airways, facilitating the establishment of another microorganism in the lower airways; this seems to be the role of rhinovirus and coronavirus.[34,35] Interaction between viruses and bacteria seems to be associated with a more severe clinical profile of CAP. Johansson et al. demonstrated that viral-bacterial coinfection occurred in 20% of the cases, being responsible for more severe pneumonia requiring longer hospitalization than does CAP caused by a bacterial agent alone.[34]

The evidence from those studies support that ancillary tests, particularly molecular tests, such as PCR, are indicated for the diagnosis of viruses especially in cases of severe CAP.[36]

CURRENT STATUS OF SCORING SYSTEMS FOR THE ASSESSMENT OF CAP SEVERITY AT ADMISSION AND SCORING SYSTEMS FOR EARLY IDENTIFICATION OF RISK FOR THE NEED VENTILATORY AND/OR VASOPRESSOR SUPPORT TO PREVENT THE DEVELOPMENT OF SEVERE SEPSIS OR TREATMENT FAILURE. WHAT ARE THE RECOMMENDATIONS?

Patients with a diagnosis of CAP should always be assessed for disease severity, a precaution that has a direct positive impact on mortality.[37-40] Currently available prognostic scoring systems measure severity and help predict prognosis in CAP, informing the decision regarding site of care (outpatient, inpatient, or ICU), the need for etiologic investigation, and the choice of antibiotics and their route of administration.[5,37]

Validated instruments include the Pneumonia Severity Index (PSI); mental **C**onfusion, **U**rea, **R**espiratory rate, **B**lood pressure, and age ≥ **65** years (CURB-65); CRB-65 (no measurement of urea); the 2007 American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) guidelines; **S**ystolic blood pressure, **M**ultilobar involvement, **A**lbumin, **R**espiratory rate, **T**achycardia, **C**onfusion, **O**xygenation, and **p**H (SMART-COP); and Severe Community-Acquired Pneumonia (SCAP)—the last three being related to severe pneumonia and ICU admission.[41-46]

It is important to stress that disease severity as determined by scoring systems is a major factor in the decision regarding hospital admission; however, other factors, such as the possibility of using oral drugs, comorbidities, psychosocial factors and socioeconomic characteristics that indicate vulnerability of the individual, should be taken into account.[5,22,44] Ideally, SpO_2 should always be monitored: SpO_2 values below 92% should be an indication for hospital admission.[22,47]

PSI

The PSI comprises 20 items including demographic characteristics, comorbidities, abnormal laboratory test results, abnormal radiological findings, and physical examination findings.[41] The PSI classifies patients into five categories, estimating 30-day mortality and suggesting the site of care (Charts 1 and 2). However, the PSI may underestimate CAP severity in young patients without concomitant diseases because its scoring system gives too much weight to age and presence of comorbidities.[22,39]

Another negative point is the use of many variables, which makes calculation complex; however, this calculation can be facilitated by using calculators available online, such as the PSI/Pneumonia Patient Outcomes Research Team (PORT) Score: PSI for CAP and PSI Calculator.[1]

CURB-65 and CRB-65

CURB-65 is an acronym for the variables it assesses: mental **C**onfusion (an Abbreviated Mental Test score ≤ 8)[48]; **U**rea > 50 mg/dL; **R**espiratory rate > 30 breaths/min; **B**lood pressure (systolic < 90 mmHg or diastolic < 60 mmHg; and age ≥ **65** years (Figure 1).[42] CRB-65 (no measurement of urea), which is a simplified version of CURB-65, is useful in settings in which laboratory tests are not available, such as in primary care (Figure 2).[43]

The major limitation of CURB-65 and CRB-65 is the exclusion of comorbidities that may increase the risk of complications in CAP, such as alcoholism, heart or liver failure, and neoplasia, which results in their negative predictive value for mortality being slightly lower than that of the PSI.[5,40] However, CURB-65 and CRB-65 are qualified by their simplicity, immediate applicability, and ease of use, whether in the hospital setting or elsewhere.

2007 ATS/IDSA guidelines

The severity criteria proposed in the ATS/IDSA consensus guidelines[44] and their simplified version[49] are classified as major or minor (Chart 3). The presence of one of the major criteria (septic shock or need for mechanical ventilation) is an indication for ICU admission. The presence of three or more minor criteria is also an indication for intensive care. These criteria, however, do not lend themselves to the assessment of outpatients, which is why the guidelines themselves recommend the use of the PSI or CURB-65 to inform decision-making about outpatients.

SCAP and SMART COP

Other tools for predicting the occurrence of severe CAP have been developed to assess outcomes other than the generic risk of death or ICU admission. These outcomes include, in addition to the need for ICU admission, the development of severe sepsis, the need for mechanical ventilation, and the risk of treatment failure, for SCAP, and outcomes more specifically associated with the need for the use of invasive or noninvasive mechanical ventilatory support or the use of vasopressors for circulatory support, for SMART-COP.[45,46]

These outcomes have been considered more objective markers of CAP severity, given the heterogeneity of indications and protocols for ICU admission across different institutions and health care systems.

SCAP

The major criteria are pH < 7.30 (13 points) and systolic blood pressure < 90 mmHg (11 points). The

1 https://www.mdcalc.com/psi-port-score-pneumonia-severity-index-cap
https://www.thecalculator.co/health/Pneumonia-Severity-Index-(PSI)-Calculator-977.html

Chart 1. Pneumonia Severity Index scoring.

Demographic factors	Score	Laboratory and radiological findings	Score
Age, years		pH < 7.35	+30
Men	n	Urea > 65 mg/L	+20
Women	n – 10	Sodium < 130 mEq/L	+20
Nursing home residents	+10	Glucose > 250 mg/L	+10
		Hematocrit < 30%	+10
		PO_2 < 60 mmHg	+10
		Pleural effusion	+10
Comorbidities		**Physical examination**	
Neoplasia	+30	Altered mental status	+20
Liver disease	+20	RR > 30 breaths/min	+20
CHF	+10	SBP < 90 mmHg	+20
Cerebrovascular disease	+10	Temperature < 35° or > 40°C	+15
Kidney disease	+10	HR ≥ 125 bpm	+10

Adapted from Corrêa et al.[5] CHF: congestive heart failure; and SBP: systolic blood pressure.

Chart 2. Risk stratification by the Pneumonia Severity Index.

Class	Points	Mortality, %	Suggested site of care
I	-	0.1	Outpatient
II	≤ 70	0.6	Outpatient
III	71-90	2.8	Outpatient or brief inpatient
IV	91-130	8.2	Inpatient
V	> 130	29.2	Inpatient

Adapted from Corrêa et al.[5]

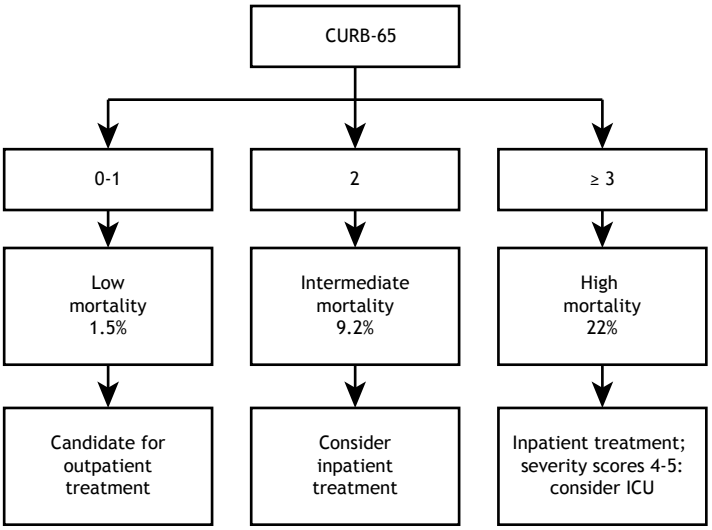

Figure 1. CURB-65 score and suggested site of care for patients with community-acquired pneumonia. Adapted from Corrêa et al.[5] CURB-65: mental **C**onfusion; **U**rea > 50 mg/dL; **R**espiratory rate > 30 breaths/min; **B**lood pressure (systolic < 90 mmHg or diastolic < 60 mmHg); and age ≥ **65** years; and CAP: community-acquired pneumonia.

minor criteria are RR > 30 breaths/min (9 points); PaO_2/FiO_2 < 250 (6 points); urea > 30 mg/dL (5 points); altered level of consciousness (5 points); age ≥ 80 years (5 points); and radiological findings of multilobar or bilateral infiltrate (5 points).[46]

A score ≥ 10 predicts an increased risk for the use of mechanical ventilation and the need for vasoactive drugs.[46]

SMART-COP

The SMART-COP scoring system is as follows: systolic blood pressure < 90 mmHg (2 points); multilobar involvement (1 point); albumin < 3.5 g/dL (1 point); RR ≥ 25 breaths/min (1 point); HR > 125 bpm (1 point); mental confusion (1 point); SpO_2 < 93% or PaO_2 < 70 mmHg (2 points); and pH < 7.30 (2 points).[45] A score greater than 3 identified 92% of the patients

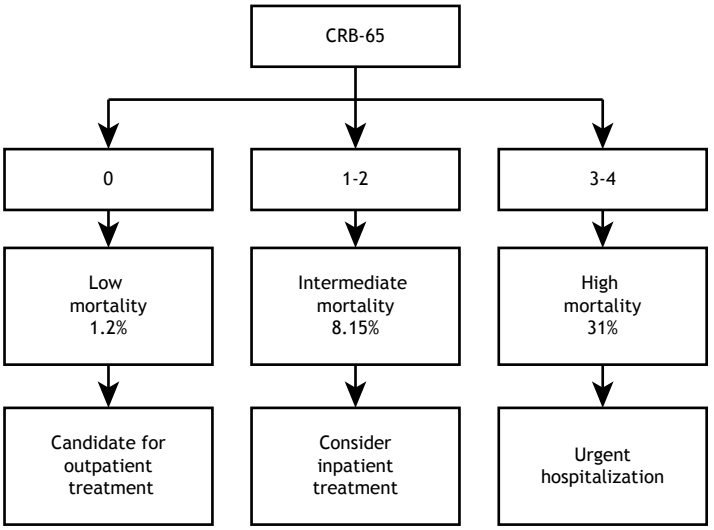

Figure 2. CRB-65 score and suggested site of care for patients with community-acquired pneumonia. Adapted from Corrêa et al.[5] CRB-65: mental **C**onfusion; **R**espiratory rate > 30 breaths/min; **B**lood pressure (systolic < 90 mmHg or diastolic < 60 mmHg); and age ≥ **65** years.

Chart 3. Risk stratification based on a simplified version of the American Thoracic Society/Infectious Diseases Society of America consensus guidelines criteria.

Major criteria	
Septic shock	Need for mechanical ventilation
Minor criteria	
RR > 30 breaths/min	Mental confusion
PaO_2/FiO_2 < 250	Urea ≥ 20 mg/dL
Multilobar infiltrates	SBP < 90 mmHg

SBP: systolic blood pressure.

who required mechanical ventilation or vasoactive drugs during the course of CAP.

Therefore, it is recommended that patients with CAP should be objectively evaluated in the emergency room for initial disease severity and for early identification of risk of developing severe outcomes, such as the need for ICU admission, the development of severe sepsis, the need for invasive or noninvasive ventilatory support, the need for inotropic support, or the risk of treatment failure (SCAP, SMART-COP, or the simplified version of the ATS/ISDA criteria, although further external validation is still required). In the absence of severe CAP, socioeconomic indications for hospital admission, concomitant decompensated diseases, and hypoxemia, and when oral intake of medications is possible and there is a score of 0-1 on CURB-65 (or a score of 0 on CRB-65 or a score of 70 or less on the PSI), the attending physician should consider outpatient treatment for patients with CAP.

RECOMMENDATIONS FOR THE USE OF BIOMARKERS IN THE MANAGEMENT OF CAP

A biomarker is defined as any measurable molecule that can help diagnose or estimate prognosis of patients with a clinical condition. Since CAP is a condition with intense inflammatory activity, several studies have evaluated various biomarkers (C-reactive protein, procalcitonin, proadrenomedullin, lactate, natriuretic atrial peptide, D-dimers, cortisol, etc.) in recent years, with C-reactive protein and procalcitonin being the most commonly studied. Procalcitonin is produced in large quantities by parenchymal cells in response to bacterial toxins and proinflammatory cytokines, but its production is minimized in the presence of viral infections. Procalcitonin levels increase within 2 h after bacterial stimulation, more rapidly than do C-reactive protein levels, and are even more specific for bacterial infections, given that C-reactive protein levels increase in any inflammatory process.[50,51]

C-reactive protein is secreted by hepatic cells in response to an increase in interleukin-6, interleukin-1β, and TNF-α levels. Other recognized sources of C-reactive protein are lymphocytes, monocytes, neurons, and atherosclerotic plaques. C-reactive protein levels peak approximately 48 h after an injurious stimulus, and the plasma half-life of C-reactive protein is approximately 19 h both in health and in disease. Müller et al.[52] demonstrated a significant improvement in diagnostic accuracy when they combined the determination of procalcitonin and C-reactive protein levels with clinical signs and symptoms in patients with suspected CAP who were treated in primary care and emergency settings. These biomarkers outperformed increased leukocyte counts and body temperature, and helped differentiate between patients with bacteria and those without. The area under the curve for clinical signs and symptoms alone was 0.79 (95% CI: 0.75-0.83), whereas, for clinical signs and symptoms combined with procalcitonin and ultra-sensitive C-reactive protein levels, it was 0.92 (95% CI: 0.89-0.94; p < 0.001). A

recent study investigated the value of four biomarkers and three severity scales in predicting 28-day mortality in patients with CAP who were treated in emergency settings.[53] The results showed that procalcitonin was the best single biomarker for predicting mortality. The models combining procalcitonin and/or C-reactive protein with the PSI showed better results than did the PSI alone.[53] A recent study demonstrated that, if procalcitonin levels do not decrease by 50% within 3 days of treatment and remains above 75 mg/L, the risk of 30-day mortality is increased.[54] A study of 191 patients with CAP admitted to the ICU showed that mortality was 4.8% among those in whom procalcitonin levels decreased rapidly (n = 66), 17.3% among those in whom procalcitonin levels decreased slowly (n = 81), and 36.4% among those in whom procalcitonin levels did not decrease (n = 44).[55] Therefore, on the basis of the findings of those studies, procalcitonin can be used as an aid in the diagnosis of CAP, and procalcitonin and/or C-reactive protein can be used in the assessment of treatment response. It is important to emphasize that biomarkers should be used in complement to clinical evaluation rather than as a single criterion to determine or change the therapeutic approach (Chart 4 and Figure 3).

A recently updated meta-analysis of 50 clinical trials, including data from 12 countries, demonstrated that the use of procalcitonin as a guide for initiation and duration of antibiotic therapy resulted in a reduced risk of mortality, reduced antibiotic use, and a reduced risk of antibiotic-related side effects.[56] The results were similar for any type of lower respiratory tract infection. It is important to emphasize that treatment failure was similar between cases in which antibiotic discontinuation was guided by a decrease in procalcitonin levels and those cases in which procalcitonin was not used to guide antibiotic discontinuation.[56,57]

ANTIBIOTIC THERAPY IN CAP: RECOMMENDATIONS FOR THE USE OF MONOTHERAPY AND COMBINATION THERAPY

Treatment of outpatients

The initial antibiotic regimen is determined empirically because it is impossible to obtain microbiological results, which would enable the choice of antibiotics directed at specific agents, immediately after the diagnosis of CAP. The choice of an antibiotic should take the following into account: 1) the most likely pathogen in the site of disease acquisition; 2) individual risk factors; 3) presence of concomitant diseases; and 4) epidemiologic factors, such as recent trips, allergies, and cost-effectiveness ratio.

Antibiotic coverage for atypical pathogens in cases of less severe CAP remains controversial, and several studies have shown no advantages with the use of this approach. A crossover study comparing β-lactams vs. β-lactams plus macrolides vs. new fluoroquinolones against respiratory pathogens (levofloxacin, moxifloxacin, or gemifloxacin) demonstrated that β-lactams alone were not inferior to the other antibiotic regimens in non-severe CAP in terms of 90-day mortality.[58]

American, European, British, and Latin-American guidelines differ with regard to the treatment of outpatients. British and European guidelines, as well guidelines by the *Asociación Latinoamericana del Tórax*, place less importance on atypical pathogens for less severe cases and do not recommend initial coverage for these pathogens. British and European guidelines recommend amoxicillin as the treatment of choice, reserving macrolides as alternatives.[59-62]

The 2007 ATS/IDSA guidelines advocate treatment of atypical pathogens and pneumococci and suggest macrolides or doxycycline if no antibiotic resistance

Chart 4. Advantages and disadvantages of using biomarkers in infectious diseases.

Advantages
Provide information that is specific to infections requiring antibiotics
High levels in bacterial infections and low levels in viral infections
Levels increase rapidly in bacterial infections
Response does not depend on the organism
Levels may be altered at disease onset, before clinical and radiological abnormalities
May help define prognosis
Improve the yield of severity scores
Help monitor therapeutic response
May be more specific than clinical manifestations
May help reduce antibiotic use without adverse consequences
Disadvantages
Results may conflict with careful clinical assessment
Previous use of antibiotics may rapidly reduce levels and lead to false-negative findings
May not differentiate between pneumonia caused by atypical pathogens and viral pneumonia
Do not always recognize influenza complicated by bacterial infection
Do not distinguish between chemical aspiration pneumonia and secondary bacterial aspiration pneumonia

Adapted from Müller et al.[52]

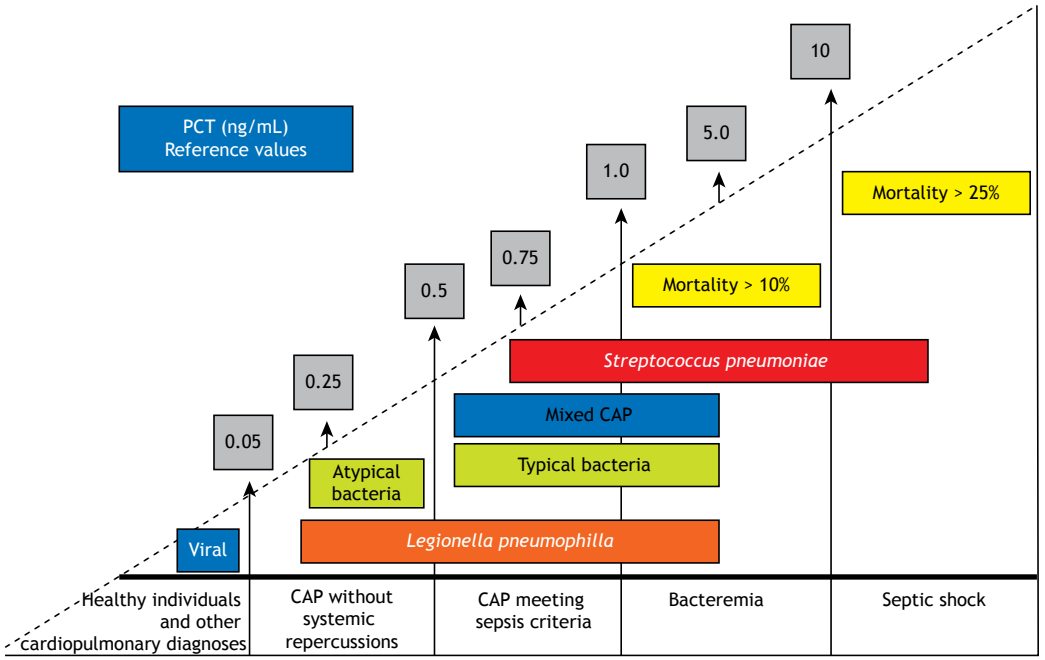

Figure 3. Serum procalcitonin (PCT) levels in community-acquired pneumonia (CAP). Adapted from Julián-Jiménez et al.[57]

is suspected.[44] A retrospective cohort study of outpatients with CAP who received monotherapy, conducted between 2011 and 2015, showed that 22.1% of the patients required additional treatment.[63] This occurred in older patients, women, and patients with comorbidities. The drugs most associated with treatment failure were β-lactams (in 25.7%), followed by macrolides (in 22.9%), tetracyclines (in 22.5%), and new fluoroquinolones (in 20.8%).[63] In Brazil, the most recent data indicate that pneumococcal resistance to penicillin should not be a concern for less severe cases of CAP.[64]

The proposal by the executive group responsible for the present recommendations is the use of monotherapy with a β-lactam or macrolides for outpatients with no comorbidities, no recent use of antibiotics, no risk factors for resistance, and no contraindication or history of allergy to these drugs (Chart 5).

For such cases, it is suggested that fluoroquinolone use be avoided because of the recent warning from the U.S. Food and Drug Administration regarding the potential risk of severe side effects.[65] Fluoroquinolones should be reserved for patients with risk factors and more severe disease or if there is no other treatment option, situations in which the benefits would outweigh the potential risks. Regarding macrolides, azithromycin is more effective in vitro against most strains of *Haemophilus influenzae* than is clarithromycin and should therefore be preferred in patients with COPD.[44,66]

The risk of infection with resistant pathogens and the risk of treatment failure are higher when patients have used an antibiotic within the previous three months, when patients come from regions where the local rate of resistance to macrolides is greater than 25%—which occurs, for instance, in the United States and some other countries—and when patients have concomitant diseases (COPD, liver or kidney disease, cancer, diabetes, congestive heart failure, alcoholism, or immunosuppression). For these specific cases, combination therapy with a macrolide and a β-lactam or monotherapy with a respiratory fluoroquinolone for at least 5 days is recommended for the outpatient treatment of CAP.

Treatment of ward patients

Monotherapy with a respiratory fluoroquinolone (levofloxacin, moxifloxacin, or gemifloxacin) or combination therapy with a β-lactam and a macrolide has been guideline recommended for the treatment of ward patients with CAP because these regimens provide good coverage and produce good results in infections caused by *S. pneumoniae, M. pneumoniae, C. pneumoniae, H. influenzae*, or *Legionella* sp.[29,51,54] Respiratory fluoroquinolones provide wide microbiological coverage, have a convenient dosing schedule, and have the ability to switch from parenteral to oral therapy. However, excessive use of respiratory fluoroquinolones can induce subsequent emergence of multidrug-resistant organisms among treated patients, as has also been observed with β-lactams.[67] It is of note that ciprofloxacin, despite being a second-generation fluoroquinolone, is not recommended for the treatment of CAP caused by community pathogens because it lacks activity against the pneumococcus and other gram-positive organisms. Monotherapy with a macrolide is not indicated in Brazil for use in such cases because of the high prevalence of *S. pneumoniae* resistance to this class of antibiotics. According to data from a 2014 survey, in the 5-49-year

Chart 5. Empiric antibiotic therapy for community-acquired pneumonia.

Treatment of outpatients	
With no comorbidities, no recent use of antibiotics, no risk factors for resistance, no contraindications or history of allergy to these drugs	
Amoxicillin or amoxicillin + clavulanic acid or macrolides: azithromycin or clarithromycin	7 3-5 7
With risk factors, more severe disease, recent use of antibiotics	
ß-lactam + macrolide	5-7
If allergic to ß-lactams/macrolides	
Moxifloxacin or levofloxacin or gemifloxacin	5-7
Treatment of ward patients	
Third-generation cephalosporins (ceftriaxone or cefotaxime) or amoxicillin + clavulanic acid + a macrolide (azithromycin or clarithromycin) or	7-10
Third-generation cephalosporins (ceftriaxone or cefotaxime) or amoxicillin + clavulanic acid or macrolides: azithromycin or	7-10
Levofloxacin or moxifloxacin or gemifloxacin as monotherapy	5-7
Treatment of ICU patients	
Third-generation cephalosporins (ceftriaxone or cefotaxime) or ampicillin/sulbactam + a macrolide (azithromycin or clarithromycin) or	7-14
Third-generation cephalosporins (ceftriaxone or cefotaxime) + respiratory quinolone	
Target-specific therapy	
Penicillin-resistant pneumococcus	
Not severe: high-dose ß-lactam (amoxicillin 3 g/day or amoxicillin + clavulanic acid 4 g/day; alternatives: ceftriaxone, cefotaxime, cefepime, or ceftaroline) + macrolide or respiratory fluoroquinolone	5-7
Severe: ceftriaxone, cefotaxime, cefepime, or ceftaroline	7-10
Methicillin-resistant *Staphylococcus aureus*: community-acquired	
Clindamycin or linezolid or vancomycin	7-21
Methicillin-resistant *S. aureus*	
Linezolid or vancomycin	7-21
Extended-spectrum ß-lactamase-producing enterobacteriaceae	
Ertapenem	7-14
Pseudomonas spp.	
Antipseudomonal fluoroquinolones, piperacillin/tazobactam, meropenem, polymyxin B (monotherapy or combined therapy)	10-14
Patients with suspected aspiration pneumonia	
Aspiration pneumonia: quinolones or third-generation cephalosporins Aspiration of gastric contents, necrotizing pneumonia, lung abscess, or severe periodontal disease: ß-lactam + ß-lactamase inhibitor, piperacillin/tazobactam, clindamycin, or moxifloxacin	7-10 7-21

age group, pneumococcal resistance to erythromycin was found in 16.9% of a total of 425 samples and sensitive strains were found in 83.1%. Among patients over 50 years of age, resistance was found in 13.6% of a total of 418 samples. For the total of 986 samples, including all age groups (from under 12 months to over 60 years of age), the rate of *S. pneumoniae* resistance to erythromycin was 17.2%.[64]

The actual need for specific coverage for atypical pathogens has been debated in the current literature. Studies investigating this issue have demonstrated that, because the incidence of *Legionella* sp. was low in non-severe CAP, monotherapy with a β-lactam was not inferior to combination therapy with a β-lactam and a macrolide or monotherapy with a fluoroquinolone.[68,69] The result of the investigation was that dose adjustment occurred only if *Legionella* sp. was found.[68,69] Studies comparing combination therapy with a β-lactam and a macrolide with monotherapy with a fluoroquinolone have shown no differences in 90-day mortality, length of hospital stay, or prescription of an oral antibiotic.[67,69,70]

The current recommendation is to use a β-lactam plus a macrolide or a respiratory fluoroquinolone alone. A β-lactam alone can be used if *Legionella* sp. is positively excluded (Chart 5).

Treatment of ICU patients

In severe CAP, studies evaluating combination therapy have shown favorable results regarding various clinical outcomes. A large observational study of patients with severe CAP (N = 956) compared monotherapy with combination therapy (two antibiotics) in terms of early mortality (60 days). In multivariate analysis, 60-day mortality was not significantly different between dual therapy and monotherapy (hazard ratio [HR]: 1.14; 95% CI: 0.86-1.50; p = 0.37).[71] In contrast, combination

therapy increased the likelihood of adequate initial therapy, defined as one or more antibiotics with in vitro activity against the microorganisms identified or, in the absence of such identification, treatment started at ICU admission and requiring no adjustment 48 h later. Adequate initial therapy was independently associated with better survival in the general cohort (HR: 0.63; 95% CI: 0.42-0.94; p = 0.02).[71] An observational study[72] compared the impact on mortality of combination therapy with at least two antimicrobials with different mechanisms of action with that of monotherapy and other antimicrobial combinations in ICU patients with severe sepsis or septic shock. Among 1,022 patients with community-acquired infection, 362 had CAP. The mortality rate was significantly lower in patients receiving combination therapy with different classes of antibiotics than in those receiving monotherapy or other antimicrobial combinations (34% vs. 40%; p = 0.042).[72] In a case-control study, a change in antibiotic therapy prescription and administration practices in favor of combination therapy (a macrolide plus a β-lactam) and, at the same time, early administration, was associated with a 15% reduction in mortality from pneumococcal pneumonia in ICU patients.[73] A similar result was observed in a study using a similar methodology and involving ICU patients with CAP caused by various etiologic agents, excluding pneumococci.[74]

A prospective observational study[75] including 218 intubated patients with CAP (75.7% of whom were in septic shock or had severe sepsis) found, after a severity-adjusted statistical analysis, that macrolide use was associated with lower ICU mortality (HR: 0.48; 95% CI: 0.23-0.97; p = 0.04) when compared with fluoroquinolone use. A separate analysis of patients with severe sepsis and septic shock (n = 92) revealed similar results (HR: 0.44; 95% CI: 0.20-0.95; p = 0.03).[75] In a systematic review with meta-analysis involving almost 10,000 patients with severe CAP, macrolide use was associated with an 18% relative reduction and a 3% absolute reduction in mortality compared with nonmacrolide therapies.[76] Dual antibiotic therapy with a β-lactam and a macrolide was superior to combination therapy with a β-lactam and a quinolone in a systematic review with meta-analysis, but randomized studies are needed to confirm these results because of the high risk of methodological bias across the studies analyzed.[77]

Therefore, combination therapy should be recommended for patients with severe CAP and an indication for ICU admission, because it reduces mortality. Antibiotics should be administered as early as possible, and antibiotic regimens should preferably include a macrolide and a β-lactam, both administered intravenously.

Except for clinical settings in which there is a great likelihood that specific pathogens are the causal agents (see Antibiotic therapy in CAP: recommendations for the use of monotherapy and combination therapy), the suggestions for initial antibiotic therapy in severe CAP are described in Charts 5 and 6.

RECOMMENDATIONS FOR PATHOGEN-SPECIFIC, TARGETED THERAPY IN PATIENTS AT RISK FOR INFECTION WITH GRAM-NEGATIVE ROD BACTERIA, *STAPHYLOCOCCUS AUREUS*, AND OTHER POTENTIALLY DRUG-RESISTANT PATHOGENS IN THE COMMUNITY

The recognition of risk factors for the leading etiologic agents of CAP helps determine optimal therapy, especially in an age of dissemination of drug-resistant bacteria in the community. Currently, we can classify bacterial etiologic agents into standard pathogens—*S. pneumoniae*, *H. influenzae, S. aureus, M. pneumoniae,* group A *Streptococcus* sp., *Legionella* sp., *Chlamydophila* sp., and *Moraxella catarrhalis*[78,79]—and multidrug-resistant pathogens—community-acquired methicillin-resistant S. aureus (CA-MRSA) and penicillin-resistant pneumococcus.[80,81]

Pneumonias caused by standard pathogens have age, occupational exposure, and presence of comorbidities as risk factors, as occurs in invasive pneumococcal disease of the lung, common in patients with chronic respiratory disease, diabetes, heart disease, or immunosuppression.[82] Pneumonias caused by multidrug-resistant pathogens are mainly dependent on local epidemiology. In addition, rapidly progressive necrotizing pneumonia is a typical presentation of CA-MRSA, which can be associated with skin lesions or with group sports participation in healthy individuals.[81]

Recently, a new group of multidrug-resistant bacteria has been associated with CAP in patients with previous contact with a health care service, such as home care services, dialysis services, outpatient services for chronic wound care, and nursing homes. In these patients, MRSA, extended-spectrum β-lactamase-producing Enterobacteriaceae, and multidrug-resistant *Pseudomonas* sp. have been common agents of pneumonia, even without recent hospitalization, simply because patients remain colonized.[83] The following are risk factors for infection with these bacteria: hospitalization within 90 days before the episode of pneumonia; antibiotic use within the previous 90 days; immunosuppression; use of gastric acid-suppressive agents; enteral feeding; hemodialysis; and previous intestinal colonization by multidrug-resistant bacteria or nasal MRSA.[84]

Unlike in first-line therapy for CAP, which is based on regional factors, such as the local incidence of standard pathogens and a patient's severity factors,[69,85] in specific targeted therapy, the risk factors for and the local prevalence of drug-resistant microorganisms are assessed with a view to guiding therapy. In Brazil, there have been few publications on the epidemiology of multidrug-resistant bacteria in the respiratory tract. Data from a regional report revealed a mean penicillin sensitivity of 93% for respiratory isolates, with an observed increase in the circulation of serotype 19A in adults, which had a penicillin sensitivity of only 50%.[64] The same report described a mean ceftriaxone sensitivity of 95%, a mean erythromycin sensitivity

of 83%, a mean trimethoprim/sulfamethoxazole sensitivity of 66%, and a mean chloramphenicol sensitivity of 99%.[64]

For CA-MRSA, national data are scarce, and risk factors should be taken into account, as occurs for multidrug-resistant pathogens associated with health care services. The drugs of choice for the treatment of CA-MRSA infection are those that inhibit toxin production: clindamycin, linezolid, or vancomycin, which can be used as monotherapy, as combination therapy with each other (linezolid plus clindamycin or vancomycin plus clindamycin), or as combination therapy with rifampin in cases of drug-resistant strains or difficulty in penetrating necrotic tissue.[86,87]

Penicillin-resistant pneumococcal infection is treated with cephalosporins, including ceftriaxone, cefotaxime, and cefepime.[63] Recently, a study of a new cephalosporin, ceftaroline, demonstrated the superiority of ceftriaxone over ceftriaxone for the treatment of pneumococcal pneumonia.[88] In cases of non-severe infection, in which oral monotherapy is a choice, cefuroxime and ampicillin-sulbactam have been safe options in regions with low resistance to β-lactams, as have fluoroquinolones, since pneumococci are rarely resistant.[89] In cases of CA-MRSA infection, the objective is to suppress toxin production, and the treatment of choice is clindamycin, trimethoprim/sulfamethoxazole, or linezolid. The potential for inducible clindamycin resistance in high-inoculum infections via efflux or ribosomal alterations should be taken into account.[90] An antibiotic disc diffusion assay (D-test) identified inducible clindamycin resistance in erythromycin-resistant, clindamycin-susceptible *S. aureus* isolates.[91] Linezolid has been shown to be superior to vancomycin in the treatment of severe MRSA infections, especially in ICU patients. Infection with extended-spectrum β-lactamase-producing Enterobacteriaceae can be treated on an outpatient basis with ertapenem, because of its dosing schedule of a single intramuscular or intravenous daily dose, which allows it to be administered on a day-hospital basis. Infections with drug-resistant strains of *Pseudomonas* sp. have been treated with fluoroquinolones, piperacillin/tazobactam, meropenem, or polymyxin B, as monotherapy or combination therapy (Chart 6).[92,93]

DURATION OF ANTIBIOTIC THERAPY FOR OUTPATIENTS AND INPATIENTS WITH CAP

The optimal duration of antibiotic therapy for the treatment of CAP has yet to be definitively established. Short-term antibiotic therapy seems to be the most appropriate, given that it provides less patient exposure to the effects of antibiotics, reduces the occurrence of adverse effects, reduces the development of drug resistance by microorganisms, improves patient adherence, and can minimize length of hospital stay and financial costs.[94] In addition, very long-term treatments favor the development of bacterial resistance and the occurrence of potentially severe adverse effects, such as infections with *Clostridium difficile*.[95] However, short-term treatment should be as effective as longer-term treatments in terms of rates of mortality, complications, and disease recurrence.

Recommendations regarding the optimal duration of antibiotic therapy have changed over time, and there are discrepancies on this issue across guidelines (Table 1).

> Treatment duration sufficient to ensure CAP treatment success (considering mortality as the primary outcome, but also considering adverse effects and treatment failure) may vary based on CAP severity as defined by currently available severity scores. Treatments lasting 5 to 7 days seem to be sufficient in most cases, especially in non-severe infections.

According to a meta-analysis evaluating the efficacy of short-term (less than 7 days) regimens in adult patients with mild to moderate CAP and involving 2,796 patients in 15 selected studies, shorter-term treatments did not underperform relative to traditional regimens.[95] Another meta-analysis investigated the efficacy and safety of short-term (equal to or less than 7 days) treatments vs. long-term (greater than or equal to 2 days' difference) treatments for CAP with the same antibiotics and the same dosing schedules.[94] Five randomized controlled trials involving adult patients of mild to moderate severity were included. No differences were found between short-term (3 to 7 days) treatments and long-term (7 to 10 days) treatments regarding clinical success (N = 1,095 patients; OR = 0.89; 95% CI: 0.74-1.07), microbiological improvement, recurrence and mortality rates, or adverse effects.[94]

A document by the U.K. National Institute for Health and Care Excellence, published in 2014, recommends that the duration of treatment should be determined by the severity of pneumonia rather than by the etiologic agents or the antibiotic chosen.[60] Therefore, for mild CAP, monotherapy for 5 days seems to be sufficient; extending treatment should be considered if symptoms do not improve after 3 days. For moderate to severe CAP, the document recommends that treatment for 7 to 10 days should be sufficient, according to the working group's consensus opinion, given that the available evidence comes from the analysis of a subgroup of patients from only one study.[96]

Strategies and procedures aimed at shortening the duration of antibiotic therapy have been tested by comparing short- and long-term treatments in terms of efficacy. Murray et al.[97] evaluated the impact of a multidisciplinary intervention intended to reduce the duration of antibiotic therapy: stop dates of antibiotic therapy were determined on the basis of severity of disease as assessed by the CURB-65 score. On those dates, clinicians received a reminder from the clinical pharmacy department, after which the attending physicians decided, on the basis of data regarding the patient's clinical course, whether or not to continue treatment. The intervention resulted in an 18% reduction in the duration of antibiotic therapy and a 39% reduction in the rate of antibiotic-related adverse

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

Drug	Route	Dose	Interval, h
Amoxicillin/clavulanic acid	Oral	875/125 mg	8
Amoxicillin/clavulanic acid	Oral	2,000/135 mg	12
Amoxicillin/clavulanic acid	Intravenous	1,000-2,000/200 mg	8-12
Ampicillin/sulbactam	Intravenous	1.5/3.0 g	6-8
Azithromycin	Oral-Intravenous	500 mg	24
Cefepime	Intravenous	2 g	12
Cefotaxime	Intravenous	1-2 g	8
Ceftaroline	Intravenous	600 mg	12
Ceftriaxone	Intravenous	1 g	12
Ciprofloxacin	Oral	500-750 mg	12
Ciprofloxacin	Intravenous	400 mg	8-12
Clarithromycin	Oral	500 mg	12
Extended-release clarithromycin	Oral	1,000 mg	24
Clarithromycin	Intravenous	500 mg	12
Clindamycin	Oral	600 mg	12
Clindamycin	Intravenous	600 mg	8
Ertapenem	Intravenous	1 g	24
Imipenem	Intravenous	1 g	8
Levofloxacin	Oral	500-750 mg	24
Levofloxacin	Intravenous	750 mg	24
Linezolid	Oral-Intravenous	600 mg	12
Meropenem	Intravenous	1 g	8
Moxifloxacin	Oral	400 mg	24
Piperacillin/tazobactam	Intravenous	4 g/0.5 g	6-8
Vancomycin	Intravenous	500 mg/1,000 mg	6/12

Note: If the infection is caused by a microorganism requiring a minimal inhibitory concentration > 0.5 mg/L, the antimicrobial should be administered every 8 h to prevent the selection of resistant strains.

effects. There was no reduction in mortality or length of hospital stay.[97] Other authors evaluated the use of a three-step systematized pathway to transition from intravenous to oral antibiotic therapy and thereby reduce length of hospital stay. Those authors demonstrated that using objective criteria for switching to oral antibiotic therapy and deciding on hospital discharge results in a reduction in length of hospital stay and duration of intravenous antibiotic therapy, without any adverse consequences.[98] In addition, biomarkers (especially C-reactive protein and procalcitonin) have been widely studied to help in the clinical monitoring of patients with CAP, as a method to help decide whether to change or discontinue treatment.

It is recommended that, for mild CAP treated on an outpatient basis, treatment should be 5-day monotherapy. Moderate to severe CAP should be treated with the antibiotic regimens discussed above, for periods of 7 to 10 days. Treatment can be extended up to 14 days at the discretion of the attending physicians.

RECOMMENDATIONS FOR CORTICOSTEROID USE AS ADJUVANT TREATMENT IN CAP

During an infectious course, an adequate balance between activation of the immune response and control of inflammation is key to fighting the infection without adjacent tissue injury. Activation of the hypothalamic-pituitary-adrenal axis is responsible for the production of cortisol, an endogenous corticosteroid, which, during an pneumonic course, induces the expression of anti-inflammatory proteins and the inhibition of pro-inflammatory molecules.[101]

In recent years, randomized clinical trials and meta-analyses evaluating the role of corticosteroids in CAP have been published, but some gaps still have to be filled. Moderate- to high-quality evidence suggests that, when combined with antibiotics and usual therapy, corticosteroids improve the course of treated patients with CAP. The benefits include a reduction in length of hospital stay and time to clinical stability, as well as a reduction in the rate of mechanical ventilation and progression to acute ARDS.[102-106]

Most of those studies evaluated the role of corticosteroids in severe CAP requiring hospitalization. With regard to mortality, the role of corticosteroids in preventing CAP-related deaths has yet to be well defined,[103] although data regarding individuals with a severe presentation suggest benefits of this therapy in this subgroup.[102,104,107] Another important aspect to take into account is the fact that the treatment regimens used in clinical trials are not standardized.

Table 1. Guideline recommendations on duration of antibiotic therapy for community-acquired pneumonia.[a]

Authors (reference)	Recommended duration	Level of evidence
Mandel et al.[99]	- At least 5 days (level 1), no fever for 48-72 h and no signs of clinical instability before discharge (level 2) - Longer duration: if the initial therapy is inactive against a previously identified pathogen or if there are extrapulmonary complications, such as meningitis or endocarditis (level 3)	Level 1: high (RCT) Level 2: moderate (nonrandomized controlled studies, cohort studies, series of patients, case-control studies) Level 3: low (case studies and expert opinion)
Lim et al.[59]	- Outpatient and non-severe inpatient CAP and uncomplicated CAP: 7 days of antibiotic therapy - Severe CAP caused by an unidentified agent: 7-10 days - 14-21 days if there is suspicion or confirmation of *Staphylococcus aureus* or gram-negative enteric bacilli (C)	C: formal combination of expert opinions
Corrêa et al.[5]	- Mild to moderate CAP: up to 7 days - Recommendation valid for the classes of antibiotics then recommended	A: randomized controlled trials and/or rich database
Torres et al.[100]	- Duration should not exceed 8 days in patients responsive to treatment (C2)	C2: insufficient evidence, from one or more randomized controlled trials, but without systematic review or meta-analysis
Eccles et al.[60]	- Mild CAP: 5 days of antibiotic therapy - Consider extending if there is no clinical improvement within 3 days - Patients and caregivers: if there is no improvement of symptoms (or there is worsening) within 3 days, seek medical attention again	- Low and moderate quality evidence; heterogeneous studies, but with consistency in demonstrating equivalence in efficacy between short- and long-term treatments
	- Moderate to extremely severe CAP: 7-10 days of treatment	- Low quality evidence; recommendation based on the consensus of the members of the working group

RCT: randomized clinical trials; and CAP: community-acquired pneumonia.

Table 2 shows the main corticosteroid treatment regimens used for the treatment of CAP.[107-113]

In 2015, two important randomized clinical trials were published. Blum et al.[108] evaluated the use of prednisone (50 mg/day for 7 days) in 785 patients. Patients in the corticosteroid-treated group had shorter time to clinical stability than did those in the control group (3.0 days vs. 4.4 days; $p < 0.0001$). Clinical stability was defined as a return to normal levels of temperature, HR, RR, SpO_2, mental status, systolic blood pressure, and ability to tolerate oral food intake.[108] Torres et al.[109] tested the effects of the use of methylprednisolone (0.5 mg/kg every 12 h for 5 days) in individuals with severe CAP, as defined by ATS criteria or high PSI risk class, and with high inflammatory response, characterized as a serum C-reactive protein level > 150 mg/L. Patients who received methylprednisolone had a lower risk of treatment failure compared with those in the control group (OR = 0.34; 95% CI: 0.14-0.87; $p = 0.02$). In addition, the study showed that the radiological course was better in the group of patients who received methylprednisolone. A distinguishing positive aspect of the study, compared with previous research, is that the sample was more homogeneous, including a phenotype of individuals with increased inflammatory expression (high C-reactive protein levels).[109]

With regard to safety outcomes, corticosteroid use resulted in good tolerance without increasing the incidence of adverse effects, except for hyperglycemia, which was more commonly reported in the group receiving corticosteroid therapy. However, the rates of other complications usually attributed to corticosteroid use, such as gastrointestinal bleeding, neuropsychiatric complications, and hospital readmission, were similar in the corticosteroid and control groups.[102-104]

In conclusion, corticosteroid use in severe CAP has proved to be both safe and beneficial in several important clinical outcomes. However, further studies are needed to confirm the impact of corticosteroid therapy on CAP-related mortality, although meta-analyses have suggested a reduction in this rate, especially in the subgroup of patients with a more severe presentation.

On the other hand, it should be emphasized how important it is to avoid the indiscriminate use of corticosteroid therapy, prioritizing its use in individuals who are most likely to benefit clinically from it, such as those with a higher level of systemic inflammation. In this context, C-reactive protein can be considered a useful biomarker, identifying patients who are at higher risk of CAP-related complications and who, consequently, may benefit from adjuvant corticosteroid therapy.

These recommendations should not be extrapolated to patients with less severe CAP who are treated on an outpatient basis.

CURRENT RECOMMENDATIONS FOR VACCINATION IN ADULTS: INFLUENZA AND PNEUMOCOCCAL VACCINES

Influenza vaccine

Influenza is a viral infection with systemic manifestations, caused by viruses of the family *Orthomyxoviridae*, which are classified as antigenic types A, B, or C. Influenza type A infection is associated with pandemics and with disease of greater severity; influenza type B infection is associated with regional epidemics; and influenza type C infection is associated with small isolated outbreaks, which have little clinical relevance in humans.

The flu, caused by influenza types A and B viruses, is associated with increased morbidity and mortality in patients with chronic diseases.[114,115] There is a strong relationship between influenza infections and secondary bacterial pneumonias following viral infections.[116] Vaccination reduces the intensity of symptoms, the need for hospitalization, and mortality.[117,118]

The influenza virus has high mutation rates, and annual (seasonal) epidemics are due to new subtypes arising from small antigenic drifts that occur during viral replication. The occurrence of these mutations in the viral structure contributes to an increase in the seasonal incidence of the disease and justifies the need for annual influenza vaccination, given that the vaccine's protection is temporary.[115] The composition of the influenza vaccine is determined by the World Health Organization on the basis of information from referral laboratories regarding the prevalence of circulating strains. The World Health Organization usually makes annual recommendations on the composition of the vaccine in the second semester so that the next year's vaccine can be developed to cover the influenza strains most likely to be circulating that subsequent year.[119]

In Brazil, the available influenza vaccines are made up of inactivated fragmented viruses (therefore, carrying no risk of infecting patients), which are obtained from cultures derived from embryonated chicken eggs. Inactivated vaccines reduce the magnitude of the respiratory symptoms when the circulating virus strain is similar to the vaccine strains, leading to a greater than 60% decrease in the incidence of the disease.[120] There are two types of influenza vaccine that are approved by the Brazilian National Health Oversight Agency for use in the country:

- Trivalent influenza vaccine (influenza A/H1N1, influenza A/H3N2, and influenza B): available for specific indications, through the Brazilian Unified Health Care System, in primary health care clinics during vaccination campaigns (and subsequently until there are no more doses available)
- Tetravalent—or quadrivalent—influenza vaccine (influenza A/H1N1, influenza A/H3N2, and two strains of influenza B): available in private clinics and administered for the same indications

Although the influenza vaccine can be used from the age of 6 months onward, the vaccine has been prioritized for high-risk groups by the vaccination schedule of the Brazilian National Ministry of Health.[5,121-123]

Priority (non-exclusive) indications

- Adults aged 60 years or older
- Patients with chronic pulmonary, cardiovascular (except systemic arterial hypertension), renal, hepatic, hematologic, or metabolic disorders
- Adults who are immunosuppressed
- Individuals with neuromuscular disorders, pulmonary function impairment, and difficulty in clearing secretions
- Women who are, or are planning to become, pregnant and women who are breastfeeding
- Residents of nursing homes
- Potential transmitters of the virus to individuals at higher risk
- Health professionals
- Home caregivers of children (under 5 years of age) and of adults (over 50 years of age)
- Indigenous people and people deprived of their liberty

Individuals who should not be vaccinated

- People with severe allergy (anaphylaxis) to chicken eggs, to any component of the vaccine, or to a previous dose of the vaccine
- Children under 6 months of age
- People with a history of Guillain-Barré syndrome, especially if the syndrome developed after influenza vaccination

Notes

- People with a history of severe allergy to chicken eggs, with signs of anaphylaxis, should receive the vaccine in a setting in which anaphylactic reactions can be treated and should remain under observation for at least 30 minutes
- In cases of fever, vaccination should be postponed until remission occurs
- In cases of a history of Guillain-Barré syndrome occurring within 6 weeks after a previous dose of the vaccine, careful medical evaluation of the risk-benefit ratio is recommended before administration of another dose
- Except for the aforementioned cases, no precautions are needed before vaccination
- Cold compresses can relieve reactions at the vaccine injection site, and, for more severe cases, medically prescribed pain medication can be used
- Any severe and/or unexpected symptom after vaccination should be reported to the facility where vaccination was performed
- Persistent symptoms or adverse events lasting more than 72 h (depending on the symptom) should be investigated for other causes

Pneumococcal vaccine

Two types of pneumococcal vaccine are currently available: a 23-valent pneumococcal polysaccharide

Table 2. Main corticosteroid treatment regimens used for the treatment of community-acquired pneumonia.

Study (reference)	Country	Treatment regimen
Torres et al.[109]	Spain	Methylprednisolone 0.5 mg/kg every 12 h for 5 days
Fernandez-Serrano et al.[107]	Spain	Methylprednisolone 20 mg every 6 h for 3 days, 20 mg every 12 h for 3 days, 20 mg/day for 3 days
Blum et al.[108]	Switzerland	Prednisone 50 mg/day for 7 days
Snijders et al.[110]	The Netherlands	Prednisolone 40 mg/day for 7 days
Confalonieri et al.[111]	Italy	Hydrocortisone 200 mg/day for 7 days
Sabry et al.[112]	Egypt	Hydrocortisone 300 mg/day for 7 days
Li et al.[113]	China	Methylprednisolone 80 mg/day for 7 days

vaccine (PPSV23), not conjugated to a carrier protein, containing the capsular polysaccharide antigens of 23 pneumococcal serotypes; and a pneumococcal conjugate vaccine (PCV) composed of capsular polysaccharide antigens conjugated to a carrier protein. This latter formulation increases immunogenicity and, because it stimulates immune memory by T cells, provides longer-lasting protection. Two new conjugated vaccine formulations containing the capsular polysaccharide antigens of 10 (PCV10) and 13 (PCV13) pneumococcal serotypes are available in Brazil. PCV10 is approved for preventing invasive pneumococcal disease in children aged 2 years or younger, whereas PCV13 is approved for children aged 6 weeks or older and for adults. Pneumococcal serotypes are associated with disease severity, and, therefore, the clinical impact of vaccination is dependent on serotype coverage.[124]

PCV13 should be administered as a single dose to adults aged 50 years or older, including those previously vaccinated with the pneumococcal polysaccharide vaccine. The need for revaccination with a subsequent dose of PCV13 has not been established.

Routine sequential administration of PCV13 and PPSV23 is recommended by the Brazilian Immunization Society for individuals aged 60 years or older.[125] For individuals with comorbidities, sequential administration of PCV13 and PPSV23 is recommended. A dose of PCV13 should be given first, followed by a dose of PPSV23 6-12 months later and a second dose of PPSV23 5 years after the first one. For people who have received a dose of PPSV23, a 1-year interval is recommended, that is, PCV13 should be given 1 year after PPSV23. The second dose of PPSV23 should be given 5 years after the first one and 6-12 months after PCV13. For those who have received two doses of PPSV23, it is recommended that a dose of PCV13 be given at least 1 year after the most recent dose of PPSV23. If the second dose of PPSV23 was given before age 65 years, it is recommended that a third dose be given after this age, at least 5 years after the most recent dose. According to this vaccination schedule, PCV13 can be administered to adults aged 50-59 years, at the discretion of the attending physician. Pneumococcal polysaccharide vaccines result in a reduction in the occurrence of invasive pneumococcal disease in the adult population and are less effective in preventing CAP in patients with reduced immunity. The pneumococcal conjugate vaccine results in a 45.6% reduction in cases of vaccine-serotype CAP, a 45% reduction in cases of bacterial pneumonia, and a 75% reduction in cases of invasive pneumococcal disease.[126] The vaccine is indicated for individuals at increased risk of CAP.[82,115,126-129]

Indications for the vaccine

- Adults aged 60 years or older
- Individuals between 2 and 59 years of age with chronic heart disease, chronic lung disease, sickle cell disease, diabetes, alcoholism, liver cirrhosis, cerebrospinal fluid fistulas, or cochlear implants
- Individuals between 2 and 59 years of age with an immunosuppressive disease or condition, such as Hodgkin disease, lymphoma, or leukemia; kidney failure; multiple myeloma; nephrotic syndrome; HIV infection or AIDS; damaged spleen or no spleen, or organ transplant
- Individuals between 2 and 59 years of age who are receiving immunosuppressive drugs, such as long-term corticosteroids or drugs used to treat cancer, or who have undergone radiotherapy
- Adults between 19 and 59 years of age who smoke or have asthma
- Residents of nursing homes or long-term care facilities

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The pulmonary microbiome: challenges of a new paradigm

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ABSTRACT

The study of the human microbiome—and, more recently, that of the respiratory system—by means of sophisticated molecular biology techniques, has revealed the immense diversity of microbial colonization in humans, in human health, and in various diseases. Apparently, contrary to what has been believed, there can be nonpathogenic colonization of the lungs by microorganisms such as bacteria, fungi, and viruses. Although this physiological lung microbiome presents low colony density, it presents high diversity. However, some pathological conditions lead to a loss of that diversity, with increasing concentrations of some bacterial genera, to the detriment of others. Although we possess qualitative knowledge of the bacteria present in the lungs in different states of health or disease, that knowledge has advanced to an understanding of the interaction of this microbiota with the local and systemic immune systems, through which it modulates the immune response. Given this intrinsic relationship between the microbiota and the lungs, studies have put forth new concepts about the pathophysiological mechanisms of homeostasis in the respiratory system and the potential dysbiosis in some diseases, such as cystic fibrosis, COPD, asthma, and interstitial lung disease. This departure from the paradigm regarding knowledge of the lung microbiota has made it imperative to improve understanding of the role of the microbiome, in order to identify possible therapeutic targets and to develop innovative clinical approaches. Through this new leap of knowledge, the results of preliminary studies could translate to benefits for our patients.

Keywords: Microbiota; Microbiology; Immune system.

INTRODUCTION

"The lungs of healthy humans are sterile sites, unlike the upper airways where there are commensal microorganisms—they live in homeostasis with the human body".[1] Although the respiratory system has a surface area greater than 70 m^2—which is the size of a tennis court—and is in direct contact with the environment, the concept above pervaded knowledge of the respiratory system until the early 21st century, when the first studies based on molecular techniques for the identification of bacterial DNA revealed the presence of genetic material from microorganisms in the lower respiratory tract.[1,2] Much of this delay in knowledge of the lung microbiota is due to the difficulty in characterizing the human lung environment by means of conventional culture techniques based on bacterial growth in material collected by bronchoalveolar lavage.[2,3] This occurs because the bacterial load of the lungs is lower than that of other sites in the human body, such as the gastrointestinal and genitourinary tracts. In addition, there has always been intense debate over possible contamination of material collected from the lower airways with microorganisms from the upper airways, which resulted in exclusion of the lungs from early studies mapping the human microbiome.[2,4-7]

After the overcoming of these initial obstacles in the study of the lung microbiome, science has advanced to an understanding of the interaction of this microbiota with the local and systemic immune systems, through which it modulates the immune response in the context of health and of various respiratory diseases. The characterization of the lung microbiome therefore has the potential to provide new concepts about the pathophysiological mechanisms of homeostasis in the respiratory system and the loss of this balance, known as dysbiosis, in some diseases such as cystic fibrosis (CF), COPD, asthma, and interstitial lung diseases.[8-11]

It is highly likely that the microbiome and its changes have a direct influence on the natural history of respiratory diseases, as well as there is certainly a change in the microbiota resulting from antibiotic treatment of infectious respiratory tract diseases. In addition, increasing knowledge of the lung microbiome has brought about a discussion of a possible distinction between those bacterial species that are pathogens and those that behave as commensals in the composition of our physiological microbiome.[12-15]

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To better tread this new path that opens up to pulmonology, some concepts are important. Microbiota, microbiome, metagenome, and 16S rRNA are terms that pervade studies in this field, and mastery of these terms facilitates the understanding of this new dimension of knowledge (Chart 1). With regard to technique, analysis of the bacterial microbiome is based on the identification and sequencing of variable regions of the *16S* gene encoding bacterial rRNA. Given that this gene is not present in mammals, the confounding bias with human DNA is nonexistant.[1,4,8] Finally, the 16S DNA sequence contains nine variable regions that can be identified by various techniques, the most commonly used being pyrosequencing, phylogenetic microarrays, and terminal restriction fragment length polymorphism.[1,3,4] Figure 1 summarizes the sequence of events leading to the recognition of the microbiome at a given site.

Although the results of published studies differ a little, Proteobacteria, Firmicutes, and Bacteroidetes are the most commonly identified bacterial phyla in healthy individuals. With regard to genus, *Streptococcus*, *Prevotella*, *Fusobacteria*, and *Veillonella* predominate, with potential pathogens, such as *Haemophilus* and *Neisseria*, contributing a small fraction. However, those studies are based on case series involving a small number of healthy subjects and few centers around the world.[2,3,16]

Being as important as qualitative knowledge of the bacteria present in the lungs, describing the richness of organisms and the coexistence of various species is essential. Although the healthy lung microbiome presents low colony density, it presents high diversity; however, some pathological conditions lead to a loss of that diversity, with increasing concentrations of some bacterial genera, to the detriment of others.[15,17,18] This could imply the development of specific therapies, to the detriment of broad-spectrum antibiotic therapies, with great potential to cause more imbalance in an already dysbiotic microbiome.[19] Following this line of reasoning, the anti-inflammatory effects of macrolides, which are considered to be immunomodulators and are used for prolonged periods in diseases such as bronchiectasis, bronchiolitis obliterans, and COPD, have been revisited, since these anti-inflammatory properties appear to be related to changes in the lung microbiota and in the microbial metabolites, with subsequent downregulation of alveolar macrophage function.[13]

Finally, as far as the microbial population in the respiratory system is concerned, early studies failed to address two important components of the lung ecosystem: viruses and fungi. The lung mycobiome and virome, especially in some conditions, such as CF and lung transplantation, appear to have particular importance.[20] Viral and fungal identification uses the same technique as that used in bacterial microbiota analysis, but with detection of 18S rRNA in the case

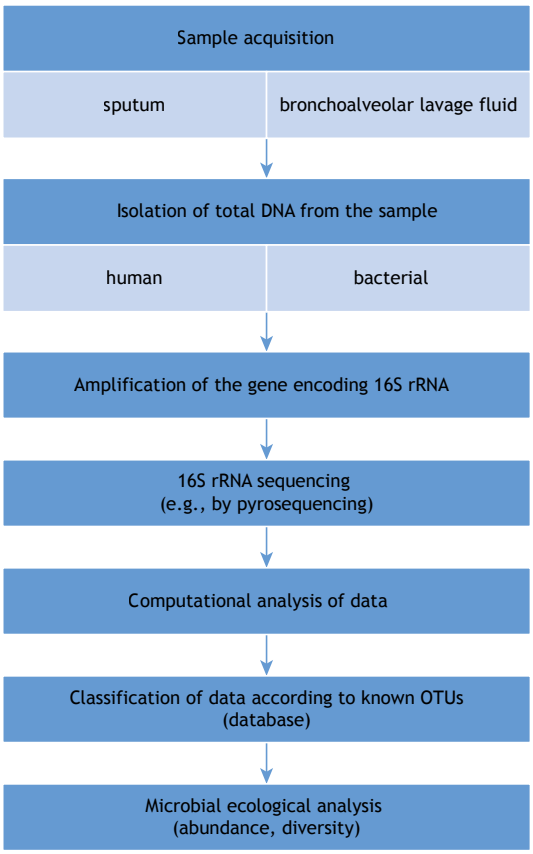

Figure 1. Sequence of events leading to the recognition of the microbiome at a given site. OTU: operational taxonomic unit.

Chart 1. Glossary of nomenclature and definitions used in the routine evaluation of the human microbiome.

Microbiota	All of the microorganisms of a given region or habitat
Microbiome	The collection of microorganisms, their genes, and their environmental interactions
Metagenome	Genetic information of the microbiota, obtained from genetic sequencing that is analyzed, organized, and identified through computational tools, using databases of previously known sequences
16S rRNA	Component of the prokaryotic 30S subunit. It encodes DNA 16S genes, used to obtain phylogenetic data
Operational taxonomic unit	Operational definition of a species or group of species, used when only DNA sequence data are available
Dysbiosis	An imbalance in the composition of the microbiota of a given niche, related to changes in local conditions

of fungi and with nucleic acid sequencing and PCR in the case of viruses.[21]

THE LUNG MICROBIOME AND ITS RELATIONSHIP WITH THE GASTROINTESTINAL TRACT

The theory that the facial sinuses were the major determinants of the microbiological changes found in the lower respiratory tract persisted for a long time. The glottis was then considered to be an effective structure in protecting the lungs from the gastrointestinal tract. However, the microbiome of the lower respiratory tract is now recognized to be similar to that of the oropharynx, leading to the concept that microbial migration from this region is the major determinant of the lung microbiome in healthy individuals.[22] Microaspiration appears to play a key role in shaping the lung microbiome, although other bacteria that are present in the lower respiratory tract, such as those of the genera *Prevotella*, *Veillonella*, and *Streptococcus*, have their origin attributable to inhalation through the upper airways.[23,24] The interrelationship of these systems and the local determinants of the lung microbiome are presented in Figures 2 and 3.

THE MICROBIOTA AND IMMUNE MODULATION

Commensal interactions between microorganisms and humans throughout evolution, as well as the relevance of the luminal ecosystem (genitourinary and gastrointestinal tracts) in immune modulation, have emerged as a new paradigm. This ecosystem is separated from the interior of the host by a thin layer of epithelial cells that acts as an interface between the host and the environment, and this epithelium is equipped with cilia, microvilli, mucus-producing cells, and intercellular junctions that allow physiological functions while in contact with the microbiota.[25] Studies of sterile (germ-free) mice have shown that resident bacteria directly influence epithelial metabolism, proliferation, turnover, and barrier function.[25] In the respiratory system, members of the microbiota, in association with environmental non-viable particulate antigens, are continuously presented to the mucosa and processed by dendritic cells and macrophages, with subsequent formation of memory or activation of T and B effector cells.[25] In addition, studies of the gastrointestinal tract have demonstrated the ability of the immune system to discriminate between pathogenic and commensal bacteria, through toll-like receptors present in T lymphocytes, in a process that would allow symbiotic colonization, that is, a kind of "peace agreement" between the resident microbiota and the respiratory mucosa,[25,26] as exemplified in Figure 4.

Evidence, however, shows that abnormal regulation of this host-microbiota relationship plays an important role in the pathophysiology of several lung inflammatory disorders. Therefore, characterizing the composition of the airway microbiota as a prognostic marker or as a guide to drug therapy is of interest in several chronic lung diseases,[27] as described below.

THE MICROBIOTA IN VARIOUS LUNG DISEASES

Asthma

Asthma is a complex, heterogeneous disease associated with allergic phenomena that has increased in prevalence in recent decades. The hygiene hypothesis is one of the major theories explaining this finding.[28] Low-level exposure to bacterial infections during childhood may be responsible for modulation of the immune response with strong emphasis on the Th2-allergic pathway. Therefore, there has been increasing interest in the role of both the lung and gastrointestinal microbiome. An experimental study of bacteria-free mice demonstrated that those animals showed an exaggerated Th2 response when stimulated with ovalbumin, developing increased eosinophilia in the airways, hyperresponsiveness, and mucus hypersecretion. When those animals were placed to grow alongside mice with the usual bacterial microbiota, both groups showed the same Th2 response intensity, which indicates that the usual microbiome functions as a protective factor against allergic diseases.[29]

Given that bacterial colonization of mucous membranes is related to the development and orchestration of the immune response of healthy individuals, changes in this interrelationship in early stages of life may contribute to the development of allergic diseases in adulthood.[30] In a study comparing two agricultural communities with similar habits, but with a distinct prevalence of asthma and allergic sensitization, the presence of a microbial composition with increased endotoxin production was related to a lower prevalence of allergic disorders.[31] In addition, a nasal microbiota with decreased diversity of species, especially when accompanied by the presence of *Moraxella* spp., has also been associated with a higher prevalence of asthma.[32]

Among adults, patients with asthma have been shown to have a higher prevalence of organisms of the phylum Proteobacteria, such as *Haemophilus influenzae*, compared with healthy controls.[33,34] Studies in this area are scarce and involve a small number of patients, leading to heterogeneity of findings. Nevertheless, all indicate the presence of lung-microbiota-related dysbiosis in asthma patients, which can be influenced both by disease severity and by inhaled or systemic corticosteroid use.[35,36]

COPD

Studies comparing the microbiome of smokers, former smokers, and healthy individuals are scarce and report some conflicting results regarding the long-term effects of tobacco exposure.[37,38] Nevertheless, there are indications that dysbiosis occurs in smokers, with an increase in the prevalence of the phylum Firmicutes and of *Neisseria* spp., associated with a relative

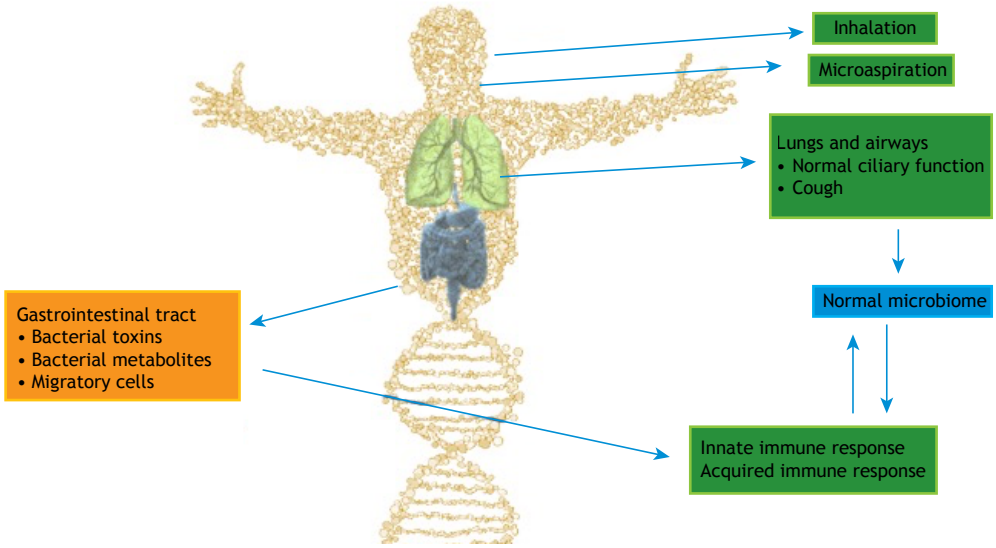

Figure 2. Determinants of the lung microbiome and the intestine-lung axis. The composition of the human microbiota is determined by the association of environmental factors, the host immune response, and genetic characteristics. The intestine microbiota, which is incomparably greater in size than the lung microbiota, can influence the lower respiratory tract both directly, through microaspiration, and indirectly, through modulation of the immune response as a result of the production of bacterial metabolites and their interaction with the host inflammatory cells. Inhalation of external agents is also a pathway to lung colonization and will depend, as will intestinal tract colonization, on local factors, such as oxygen tension, tissue pH, blood perfusion, nutrient concentration, proper mucociliary transport, and disruption of the lung architecture.

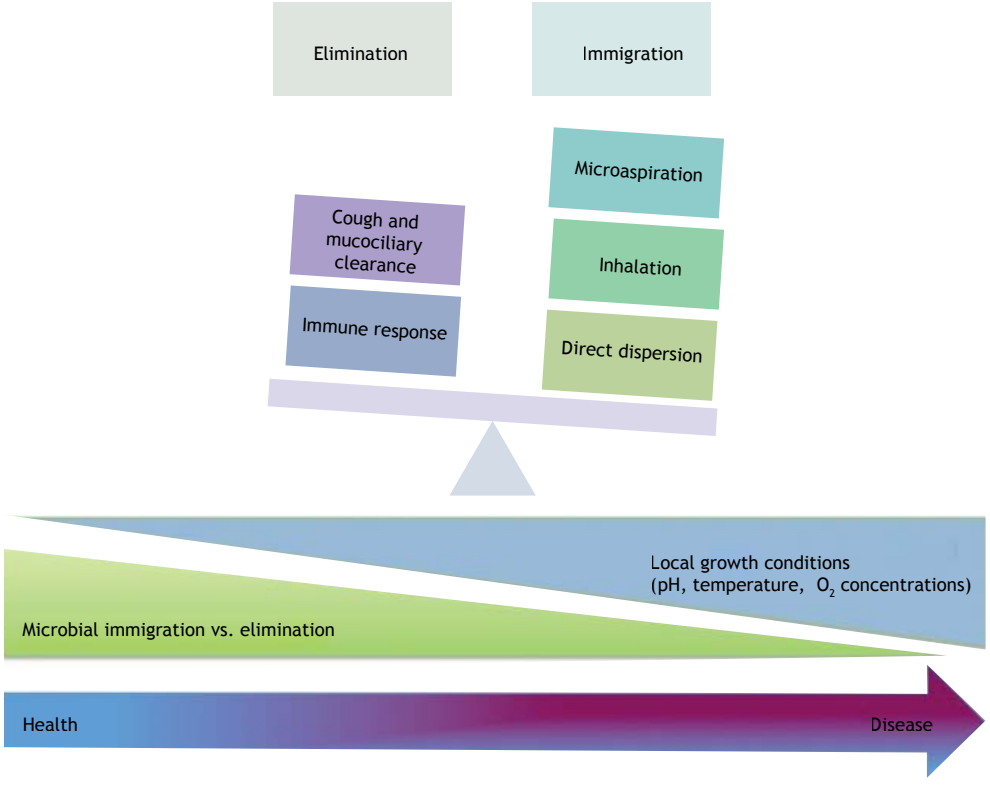

Figure 3. Determinants of the microbiome of the respiratory system: microbial immigration, elimination, and proliferation. In healthy individuals, the microbiome is determined primarily by immigration and elimination. In severe lung disease, local growth conditions are determinants of the composition of the microbiome.

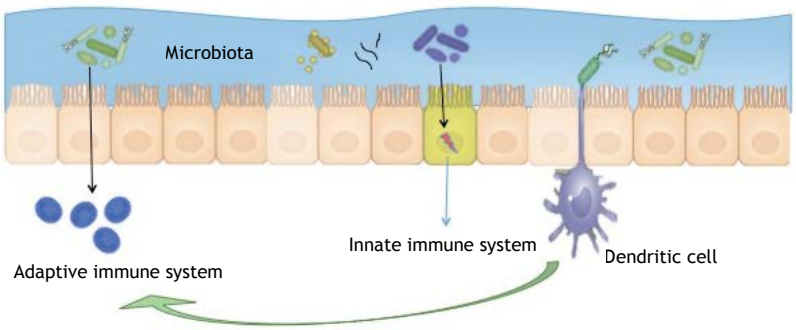

Figure 4. Microbiota interface and interaction with local immunity. Members of the microbiota, in association with environmental non-viable particulate antigens, are continuously sampled by the mucosa and processed by dendritic cells and macrophages, with subsequent formation of memory or activation of T and B effector cells. Therefore, various commensal microorganisms influence the innate immunity and the adaptive immunity.

decrease in the abundance of Proteobacteria.[37] In contrast, several studies of patients with COPD have revealed that their lung microbiome is clearly different from that of healthy controls.[39-42] In addition, among patients with COPD, depending on the site from which the material is collected, differences are also found in the composition of the microbiome, such as when, for instance, sputum and bronchoalveolar lavage fluid are compared.[42]

With regard to COPD exacerbations, numerous studies have also demonstrated that there is a relative increase in the abundance of a given genus, to the detriment of others.[43-45] This change is related to a pro-inflammatory state and can be triggered, among other causes, by viral infections[43] and by bacterial-fungal interactions in the airway.[46] These findings further question the role of antibiotics in COPD exacerbation, given that antibiotics can play a deleterious role in the lung microbiome because they reduce bacterial abundance. However, systemic corticosteroid use does not significantly change microbiological diversity and, in parallel, can increase the abundance of certain genera that are considered as normal flora.[44]

Bronchiectasis and CF

Airway colonization in suppurative lung diseases—CF and non-CF bronchiectasis—plays a key role in the progression of their clinical and radiological manifestations, and understanding the role of the microbiota is key to understanding the pathophysiology of these manifestations. While traditional culture-based knowledge shows the importance of well-known pathogens, such as *Haemophilus influenzae*, *Pseudomonas aeruginosa*, and *Moraxella catarrhalis* in non-CF bronchiectasis, as well as *Staphylococcus aureus* and *Burkholderia cepacia* complex in CF, molecular studies have shown that previously unrecognized organisms are abundantly present in some patients with suppurative disease.[27] Examples of this colonization include the presence of *Stenotrophomonas maltophilia* and *Achromobacter* spp., as well as reports of *Mycobacterium abscessus* and *Aspergillus fumigatus*.[10]

Studies of the microbiome in patients with CF have demonstrated that samples from younger, healthier patients usually exhibit bacterial communities that are more diverse, whereas lung explants from patients with end-stage lung disease show extremely low diversity, with only one or two detectable pathogenic bacteria, such as *P. aeruginosa* and *S. maltophilia*.[47] This microbiological change, during the lifetime of a patient with CF, is also accompanied by increased abundance and greater phylogenetic similarity among the colonies of each species.[48,49]

In non-CF bronchiectasis, the simultaneous competition for survival between pathogenic and commensal bacteria elicits an innate immune response from the host, with polarization of T-cell subtype response, activating or perpetuating the inflammatory process in the terminal airways, similarly to what occurs in chronic inflammatory bowel diseases, such as ulcerative colitis. In the context of inflammatory bowel diseases, it is of note that there is an association between the development of bronchiectasis and colectomy for advanced ulcerative colitis, raising the possibility that the intestinal microbiota influences the lung microbiota through systemic immunoregulation after excluding in intestinal barrier.[12]

Finally, in suppurative diseases, an understanding of the pathogenic and commensal microbiota is crucial for differentiating between infection and colonization, that is, balance/health vs. dysbiosis/disease. It should also be borne in mind that, for this group of patients, the study of the fungal and viral microbiome (microbiome and virome) is essential, and there have been few studies addressing these issues to date. The literature still lacks controlled clinical trials; most studies are descriptive studies or reviews.

Pulmonary tuberculosis

The microbiome in the context of tuberculosis remains a poorly studied area, despite the high global burden of tuberculosis.[50] Many existing studies have focused on the microbiota outside the respiratory system,

including reports of an increased presence of *Candida* spp. and a loss of diversity in the intestinal microbiota as a result of tuberculosis treatment.[51,52] In addition, there is little agreement between those studies and analyses of the lung microbiome in tuberculosis. Cui et al.[53] reported that healthy lungs and those infected with *Mycobacterium tuberculosis* had many microorganisms in common, including those of the phyla Bacteroidetes, Proteobacteria, and Actinobacteria, with a predominance of Firmicutes and Bacteroidetes. In contrast, Wu et al.[54] found a very different list of microorganisms associated with tuberculosis, including those of the genera *Streptococcus*, *Granulicatella*, and *Pseudomonas*. An interesting aspect of that study is the comparison among the microbiota of patients with newly diagnosed tuberculosis, that of recurrence cases, and that of treatment failure cases. The *Pseudomonas*-to-*Mycobacterium* ratio was higher in recurrence cases than in newly diagnosed cases, whereas the *Treponema*-to-*Mycobacterium* ratio was lower in recurrence cases than in newly diagnosed cases, indicating that disruption of these bacteria may be a risk factor for recurrence of tuberculosis.[54] These data suggest that the presence of certain bacteria and lung dysbiosis may be associated not only with the development of tuberculosis but also with recurrence of tuberculosis and treatment failure, indicating a possible role of the microbiota in the pathogenesis of tuberculosis and in tuberculosis treatment outcomes.

INTERSTITIAL LUNG DISEASES

In 2008, Varney et al.[55] published a clinical trial evaluating the use of trimethoprim-sulfamethoxazole in patients with idiopathic pulmonary fibrosis (IPF). They demonstrated that the group receiving the antibiotic therapy showed clinical and functional improvement,[55] and hypothesized a potential effect on the lung microbiota. More recently, data from a cohort study assessing 55 patients with IPF demonstrated that there is a relationship between the predominance of specific bacteria of the genera *Staphylococcus* and *Streptococcus* and interstitial lung disease exacerbation.[56] Also in 2014, Molyneaux et al.[57] observed that bronchoalveolar lavage fluid from patients with IPF exhibited an increased quantity of bacteria compared with that from healthy controls, as well as observing differences in the composition and diversity of this microbiota, and linked this dysbiosis to parenchymal disease progression. Later, genetic analyses of patients with IPF showed increase and maintenance in the expression of genes related to the host immune response, which acts as a continuous stimulus damaging to the alveolar epithelium, as well as being related to local fibroblast activation,[58,59] subsequently suggesting a relationship between the microbiome and fibrosis progression. Attempts at reversal of dysbiosis and, ultimately, the cessation of tissue damage have been extensively investigated in the context of interstitial fibrosing diseases; however, it is too early to say that the microbiota is directly related to disease progression.[27]

OTHER CLINICAL SETTINGS IN PULMONOLOGY

After initial advances in the understanding of the microbiome of the respiratory system in the context of the most prevalent diseases, it is expected that this understanding will revolutionize the concepts of pathogenesis in several clinical settings. In the context of mechanical ventilation and ventilator-associated pneumonia (VAP), that is not different. A recent study of 35 patients suggested that mechanical ventilation per se is more strongly associated with a change in the lung microbiota than is the use of systemic antibiotics, and that respiratory tract dysbiosis is more intense in patients who developed VAP than in those who did not.[60] Still in the context of VAP, microbiome analysis may also aid in etiologic diagnosis and in differentiating between pneumonia and colonization with a potential pathogen.[61]

Lung transplantation is another area that is in a state of flux. The respiratory system of transplant recipients is a point of special interest, given the wide use of prophylactic antibiotics and immunosuppressive drugs in this population. The microbiota of the transplanted lung appears to be different from that of healthy lungs, mainly because of the presence of the family Burkholderiaceae.[62] In addition, the change in the microbiota appears to influence the development of chronic graft dysfunction.[63] A study of 203 bronchoalveolar lavage fluid samples from 112 transplant recipients revealed that some bacteria played a pro-inflammatory role (genera *Staphylococcus* and *Pseudomonas*) and some played a role of lower stimulation of the immune system (genera *Prevotella* and *Streptococcus*).[64] A dysbiosis in those individuals appears to be associated with various profiles of inflammation and elaboration by lung macrophages, contributing to the genesis of chronic dysfunction. This interaction between bacterial communities and innate immune response offers new intervention pathways to preventing chronic graft dysfunction.

Clinical oncology has also advanced in the understanding of the correlations between microorganisms and lung neoplasms. After the identification of molecular markers, such as EGFR, programmed cell death protein-1, and anaplastic lymphoma kinase, which have customized the therapeutic approach, it is now the microbiome that presents itself as a possible marker of disease activity and perhaps a therapeutic target. Some microorganisms have shown a direct relationship with neoplasms in other organs, such as *Helicobacter pylori* in stomach cancer and HPV in uterine cervical cancer. Periodontal disease can be associated with lung cancer, suggesting an association between the oral microbiome and the risk of lung carcinoma.[65] Corroborating these hypotheses, Vogtmann et al. reported that cases of lung cancer among nonsmoking females showed a

decreased relative abundance of organisms of the phyla Spirochaetes and Bacteroidetes and an increased relative abundance of those of the phylum Firmicutes in analyses of the oral microbiota.[66] Finally, a study comparing bronchoalveolar lavage fluid from patients with neoplasms with that from patients with benign tumors found the genera *Veillonella* and *Megasphaera* to be predictors of lung cancer, suggesting an association between an altered lung microbiota and the presence of neoplasm.[67]

SKEPTICISM IN THE ANALYSIS

Albeit exciting, the study of the microbiome should be viewed with some caution, and it has been said that its greatest risk is that of drowning in its own tsunami of information.[68] There is a risk of a series of speculative associations being made between the microbiota and states of health and disease, and of these connections being shown to be spurious or much more complex than shown by early evaluations.

Would the microbial communities be altered in the lungs because of respiratory diseases or are the lungs diseased because of the dysbiosis of these microorganisms? The immune system and the microbiome are so closely intertwined that this differentiation is extremely difficult. Most studies on this subject have been descriptive and, despite being replete with provocative correlations, have failed to elucidate causality between modulation of respiratory tract diseases and the resident microbiota, and to determine what temporally comes first: dysbiosis or lung disease.[69] These are some of the issues that are pressing when it comes to designing new studies.

FINAL CONSIDERATIONS

We are entering a new era in the understanding of lung diseases from the standpoint of the interaction of bacterial communities and metabolites with the immunological and functional mechanisms of various respiratory tract diseases. In this context, given the departure from the paradigm regarding knowledge of the lung microbiota, it is imperative to improve understanding of the interaction between the microbiota and the host so that we can advance our understanding of the pathophysiological processes of respiratory diseases, as well as identifying possible therapeutic targets and developing innovative clinical approaches. The translation of this information into better patient care will undoubtedly be the greatest challenge in the study of the microbiome and its potentialities.

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Hamman's syndrome

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Hamman's syndrome is characterized by the sudden occurrence of spontaneous pneumomediastinum related to high intensity physical exercise, severe cough, or drug inhalation.[1]

With an incidence of about 1 per 30,000 emergency patients,[2] Hamman's syndrome mainly affects males in the second decade of life, several of whom having asthma. The most common signs and symptoms are sudden chest pain and dyspnea, followed by stridor, dysphagia, or dysphonia.[3]

A 25-year-old male, former smoker, had an episode of intense cough during a football match, developing sudden oppressive intense precordial chest pain, dyspnea, dysphonia, and odynophagia. No history of trauma, surgery/other invasive procedures, drug inhalation, or vomiting was reported. Admitted to the ER, the patient showed an exuberant cervical swelling and crackles during palpation. CT scanning showed massive pneumomediastinum and cervical subcutaneous emphysema (Figure 1). After exhaustive investigation, we found that the abrupt increase in intrathoracic pressure was the consequence of an episode of forceful cough during an intense physical activity, characterizing Hamman's syndrome. Spontaneous pneumomediastinum was totally resolved without the use of invasive procedures.

We intend to alert that Hamman's syndrome should be included in the differential diagnosis of young patients with sudden cervicothoracic complaints, because it is potentially fatal if it is not rapidly diagnosed.

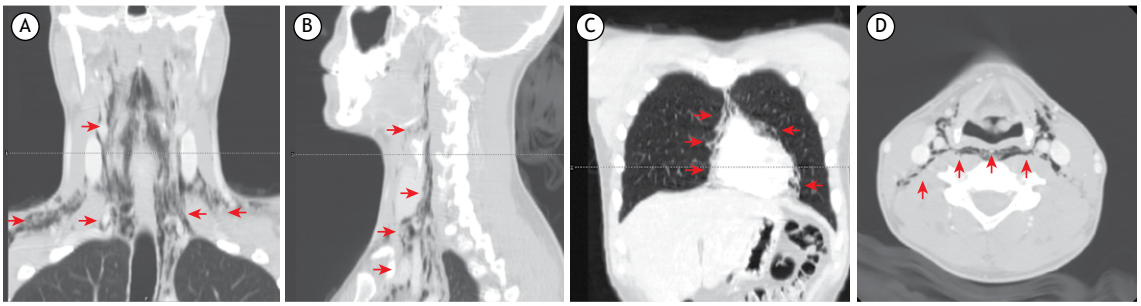

Figure 1. CT scans showing massive spontaneous pneumomediastinum: coronal view (in A) and sagittal view (in B) of subcutaneous emphysema dissecting through the cervical fascia; massive pneumomediastinum (in C); and subcutaneous emphysema reaching the base of the skull (in D).

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Clinical aspects of the Mycobacterium abscessus complex

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DEAR EDITOR:

The study by Monteiro et al.[1] on clinical aspects in patients with pulmonary infection caused by mycobacteria of the *Mycobacterium abscessus* complex (MABSC) in the Brazilian Amazon is very interesting. The authors concluded that the "treatment response of pulmonary disease caused by MABSC was less favorable than that of pulmonary disease caused by other nontuberculous mycobacteria." We would like to share ideas on this observation. In our setting, Indochina, the similar high prevalence of mycobacterium pulmonary infection is also observed. MABSC complex has become a new interesting emerging infection. The poor response to the standard anti-mycobacterial therapy is also observable.[2,3] The failure of treatment is usually related to late diagnosis and previous antibiotic treatment due to the lack of standard microbiological testing to confirm the specificity of the pathogen. This situation seems to be similar to that reported by Monteiro et al.[1]; most of the patients receive antibiotic treatment before having the final diagnosis of MABSC. In addition, the availability of drugs of choice against MABSC (such as imipenem) is limited in large tertiary hospitals that cannot correspond to the increased incidence of the problem in a community hospital. The possible new paradigm against the emergence of MABSC might be the early diagnosis by specific confirmation of mycobacterial isolates and financial support for the availability of highly effective antibiotic treatment in community hospitals.

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Authors' reply

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Initially, on behalf of our team, I would like to thank the authors for their correspondence, aimed at the exchange of ideas about a complex clinical condition. The challenge of treating respiratory infection with mycobacteria of the *Mycobacterium abscessus* complex is established from the outset, either by the difficulty of isolating and identifying the bacteria, or by the fact that the patients are critically ill, typically developing structural lung changes prior to becoming ill.[1]

Respiratory infections caused by nontuberculous mycobacteria (NTM) represent an emerging public health problem. In a survey conducted in Germany in 2017 and involving patients with health insurance, the rate of hospitalization was three times higher among those infected with NTM than among controls matched for age, gender, and the Charlson comorbidity index, such hospitalizations accounting for 63% of total costs.[2]

In Brazil, access to centers that perform genotype identification remains limited, as does access to sensitivity testing, constituting an impasse in the clinical approach to patients with respiratory infection caused by NTM. According to the 2017 guidelines of the British Thoracic Society, when *M. abscessus* is isolated, sensitivity tests should be performed, those tests including at least three antibiotics (clarithromycin, cefoxitin, and amikacin), as well as (ideally) tigecycline, imipenem, minocycline, doxycycline, moxifloxacin, linezolid, cotrimoxazole, and clofazimine.[3]

The abovementioned wide variety of drugs compose the therapeutic arsenal available for use, which is nevertheless of limited efficacy because of the bacterial resistance of the *M. abscessus* complex, mainly to macrolides and aminoglycosides. This excessive number of drugs creates barriers to a satisfactory clinical outcome,[1] the main barriers being the prolonged duration of treatment, which makes adherence difficult; the high incidence of adverse effects; the long hospital stay (due to parenteral administration of drugs); and the high economic cost.

Considering that respiratory infections caused by the *M. abscessus* complex are far from being under control in many countries, the exchange of information is always of great value, increasing knowledge and building a body of scientific evidence regarding such infections.

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Smoking cessation before initiation of chemotherapy in metastatic non-small cell lung cancer: influence on prognosis

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TO THE EDITOR:

Cigarette smoking is the most established risk factor for lung cancer (LC), and approximately 70% of LC-related deaths are attributable to tobacco.[1] Carcinogens in tobacco smoke may not only act as genetic inducers but also act as promoters of disease progression.[2] In addition, smoking has various other negative effects, such as decreased quality of life[3] and worsening of performance status[4] in patients that continue to smoke after LC diagnosis. Previous data have shown that continued smoking after a diagnosis of early-stage LC is associated with higher risk of LC recurrence, second primary tumor, and all-cause mortality.[5] The impact of smoking cessation during treatment on outcomes in patients with metastatic disease is not well defined. Herein, our objective was to evaluate the impact of smoking cessation prior to the initiation of chemotherapy on overall survival (OS) on patients with advanced non-small cell lung cancer (NSCLC).

Between January of 2011 and December of 2015, patients referred to our center and diagnosed with metastatic adenocarcinoma or squamous cell carcinoma (SCC) were retrospectively studied. Patients with active smoking habits and treated with at least one cycle of chemotherapy were included; patients treated with tyrosine kinase inhibitors were excluded. The systemic therapy was never delayed regardless of the smoking status of the patients. All patients included in the study were submitted to a brief intervention for smoking cessation and were invited to participate in a specialized consultation. Smoking cessation was confirmed by exhaled CO measurements. We compared the clinical characteristics of the patients who achieved smoking cessation with those who did not. These two groups were further subdivided according to histological results in order to investigate OS, which was defined as the time interval between the pathological diagnosis and death or last follow-up evaluation. Survival estimates were obtained using the Kaplan-Meier method. Cox regression was used to test the impact of multiple variables on OS.

The study comprised a total of 97 patients (mean age = 57 ± 10 years), 89 of whom were male (91.8%). The main histological type was adenocarcinoma, in 74 patients (76.3%); 52 patients (53.6%) were classified as having an Eastern Cooperative Oncology Group performance status scale[6] score of 1; and 55 (56.7%) showed no weight loss at diagnosis. The most prevalent comorbidities were arterial hypertension, in 18 patients (18.6%); and diabetes mellitus (DM), in 7 (7.2%). Of the 97 patients, 79 (81.4%) had a smoking history > 30 pack-years. The chemotherapy regimens used were platinum combined with pemetrexed, in 67 patients (39.1%); platinum combined with gemcitabine, in 17 (17.5%); and monotherapy with oral vinorelbine, in 13 (13.4%). Smoking cessation occurred in 50 patients (51.5%), but it only occurred after the initiation of chemotherapy in 47 (48.5%), and only 11 (22%) participated in a specialized consultation. The median time of smoking cessation was 4 months (interquartile range: 12.2). The comparison of these two subgroups regarding the characteristics studied showed no significant differences except for gender (Table 1). The subgroup of patients who quit smoking prior to chemotherapy initiation, when compared with those who continued to smoke during chemotherapy, showed a higher median OS in general. However, this difference was significant in those diagnosed with SCC (7.0 months vs. 2.5 months; p = 0.010), but not in those with adenocarcinoma (10 months vs. 9 months; p = 0.754; Figure 1). The multivariate analysis showed that smoking cessation prior to chemotherapy was the only factor associated to longer OS—hazard ratio (HR) = 0.19; p = 0.004; 95% CI: 0.06-0.59—in SCC patients. In patients with adenocarcinoma, the multivariate analysis showed a poorer prognosis in those treated with carboplatin plus pemetrexed (HR = 2.29; p = 0.003; 95% CI: 1.32-3.40) or monotherapy with oral vinorelbine (HR = 3.46; p = 0.002; 95% CI: 1.57-7.63) when compared with patients treated with cisplatin plus pemetrexed. The presence of DM was associated with a protective effect (HR = 0.27; p = 0.029; 95% CI: 0.08-0.87), as well as the total time of smoking cessation, with a decrease of approximately 8% in the risk of death for each month of smoking cessation (HR = 0.92; p < 0.001; 95% CI: 0.90-0.95).

Smoking has been described as an independent prognostic factor for poor survival in patients with advanced NSCLC.[7] However, the impact of smoking cessation on metastatic LC prognosis prior to the initiation of chemotherapy was not evaluated. Our retrospective review of a five-year experience in managing the two most common types of NSCLC has shown that continued tobacco use by SCC patients during chemotherapy is associated with decreased survival. We also found a similar tendency in patients with adenocarcinoma. The difference regarding statistical significance between SCC and adenocarcinoma subgroups could be explained by the greater proportion of patients classified as in M1a staging[8] in the SCC

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Table 1. Comparison of the characteristics of the sample by smoking status at chemotherapy initiation.[a]

Characteristic	Smoking cessation prior to chemotherapy		p
	No (n = 47; 48.5%)	Yes (n = 50; 51.5%)	
Gender			
Male	47 (100)	42 (84)	0.006
Female	0 (0)	8 (16)	
Age, years	59 ± 10	60 ± 10	0.324
Histological classification			
Adenocarcinoma	37 (78.7)	37 (74.0)	0.585
Squamous cell carcinoma	10 (21.3)	13 (26.0)	
Staging[b]			
M1a	17 (36.2)	18 (36.0)	0.653
M1b	26 (55.3)	29 (58.0)	0.876
M1c	4 (8.5)	3 (6.0)	0.365
Comorbidities			
Cardiovascular disease	0 (0)	2 (4)	0.495
Diabetes mellitus	5 (10.6)	2 (4)	0.259
Hypertension	9 (19.1)	9 (18)	0.884
Smoking history > 30 pack-years			
No	11 (23.4)	7 (14.0)	0.234
Yes	36 (76.6)	43 (86.0)	
Smoking history, pack-years	51 ± 22	51 ± 21	0.800
Performance status[c]			
0	20 (42.6)	22 (44)	0.800
1	26 (55.3)	26 (52)	
2	0 (0)	1 (2)	
3	1 (2.1)	1 (2)	
Weight loss[d]			
0%	25 (53.2)	30 (60)	0.662
> 5%	16 (34)	15 (30)	
> 10%	6 (12.8)	5 (10)	
Chemotherapy regimen			
Cisplatin plus pemetrexed	20 (42.6)	15 (30)	0.751
Carboplatin plus pemetrexed	13 (27.7)	19 (38)	
Cisplatin plus gemcitabine	3 (6.4)	3 (6)	
Carboplatin plus gemcitabine	5 (10.6)	6 (12)	
Vinorelbine monotherapy	6 (12.8)	4 (14)	

[a]Values expressed as n (%) or mean ± SD. [b]Brierley et al.[7]. [c]Eastern Cooperative Oncology Group performance status scale. [d]Proportion of weight loss within a six-month period.

subgroup than in the adenocarcinoma subgroup. However, the multivariate analysis did not show any influence of metastasis staging on survival in those subgroups. Previous data showed that nicotine inhibits apoptosis induced by systemic therapies in patients with metastatic disease and, consequently, increases resistance to treatment.[9] In addition, nicotine increases tumor growth and neovascularization.[5] Therefore, both exposition to tobacco prior to starting treatment and the interaction of nicotine with chemotherapy might provide possible explanations for smokers having worse prognoses. In our study, the multivariable analysis showed a negative impact of some types of chemotherapy, such as carboplatin plus pemetrexed or monotherapy with oral vinorelbine, on the survival of patients with adenocarcinoma. One possible explanation could be the worse performance status of the patients not treated with cisplatin. In contrast, a previous study showed that the survival of smokers with advanced NSCLC was significantly shorter than that of never smokers, even after adjustment for sensitivity to a specific type of chemotherapy.[10] The multivariate analysis also showed a positive prognostic influence of DM in patients with adenocarcinoma (however, the number of DM patients was low). The effect of DM on patients with NSCLC prognosis remains uncertain, but previous data showed increased survival in patients with DM.[11] We found that a large number of patients in our sample achieved smoking cessation, but only a small proportion of those sought any intensive medical help. The impact of medical advice on smoking behavior might be particularly compelling during cancer treatment, when patients heavily rely on clinicians for support and are generally more motivated to

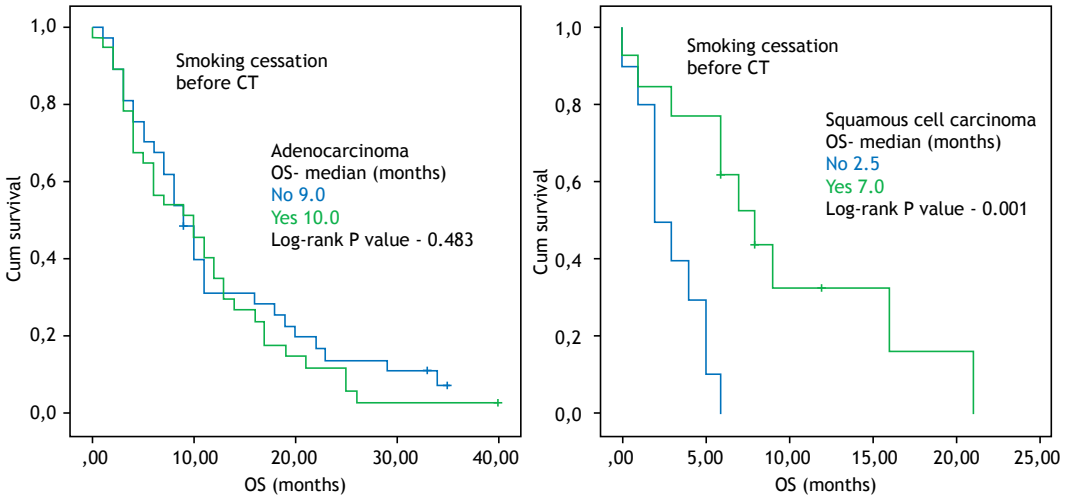

Figure 1. Overall survival (OS) in patients with adenocarcinoma and squamous cell carcinoma according to their smoking status at chemotherapy (CT) initiation. Cum: cumulative.

quit smoking. In a previous study, 65% of smoking patients being treated for lung or head-and-neck cancer reported that they were offered smoking cessation assistance by a medical professional; half of the smokers reported being interested in smoking cessation programs.[12] Physician-based interventions might need to be combined with higher-intensity behavioral and pharmacological interventions to increase long-term smoking cessation among LC patients. The conclusions of our study are tempered by the acknowledgment of the limitations inherent to any retrospective study and by the small sample size. In addition, other factors related to smoking status, such as anxiety levels and quality of life, were not evaluated.

In our sample, smoking cessation was an independent prognostic factor in advanced SCC patients, suggesting that efforts to encourage those patients to quit smoking might be beneficial. Prospective assessments of the determinants of continued smoking in this population is needed to guide effective interventions.

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Omalizumab as add-on therapy in patients with asthma and allergic bronchopulmonary aspergillosis

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TO THE EDITOR:

Allergic bronchopulmonary aspergillosis (ABPA) is characterized by a pulmonary hypersensitivity reaction to *Aspergillus* spp. ABPA primarily affects immunocompetent patients with atopic asthma and patients with cystic fibrosis.[1] *A. fumigatus* is the most common etiologic agent of ABPA. The prevalence of ABPA in patients with asthma ranges from 1.0% to 3.5%. In patients with corticosteroid-dependent asthma, the prevalence of ABPA is 7-28%. At our facility, the prevalence of ABPA is 19%.[2]

Omalizumab is a humanized monoclonal antibody approved for use in Brazil for the treatment of difficult-to-treat allergic asthma and refractory chronic spontaneous urticaria. Omalizumab has also been used off label for the treatment of diseases in which IgE has a relevant pathophysiological role, including ABPA and anaphylaxis.

In patients with ABPA, omalizumab appears to reduce the numbers of exacerbations and hospitalizations, as well as the need for systemic and inhaled corticosteroids, thus improving quality of life.[3,4] However, only a few studies have examined the use of omalizumab in asthma patients with ABPA without cystic fibrosis.[3,5,6] Controversies regarding the role of omalizumab in patients with ABPA prompted us to report the following case.

In 1994, a 53-year-old female patient who had had asthma since childhood was admitted to the Immunology Department of the Federal University of Rio de Janeiro Clementino Fraga Filho University Hospital, located in the city of Rio de Janeiro, Brazil. Although she had been under treatment with budesonide (800 µg/day) and formoterol (24 µg/day), she presented with uncontrolled asthma. She often needed systemic corticosteroid therapy and antibiotic therapy for asthma exacerbations and respiratory infections.

Laboratory tests showed a total IgE of 780 IU/mL (reference value, < 100 IU/mL), a peripheral eosinophilia of 5% (305 cells/mm^3; reference value: 100-300 cells/mm^3), and positive *A. fumigatus* precipitins. Skin prick testing was positive to *A. fumigatus*, with a wheal of 4 mm in diameter, positive and negative controls being 8 mm and 2 mm, respectively. Intradermal testing to *A. fumigatus* was positive (8 mm).

A chest X-ray showed bibasilar "tramline" infiltrates. A noncontrast HRCT scan of the chest showed diffuse pulmonary hyperinflation, peribronchial thickening, and pleural thickening, with no signs of bronchiectasis. Spirometry showed moderate obstructive lung disease with reduced FVC, as well as positive bronchodilator test results, but no normalization of FVC.

In 1996, the patient presented with difficult-to-treat asthma, a positive skin prick test to *A. fumigatus*, high total IgE levels, and positive *A. fumigatus* precipitins, but without bronchiectasis, being diagnosed with allergic asthma and serologic ABPA.

The patient had been receiving continuous systemic corticosteroid therapy for 18 years (mean dose, 40 mg/day). Attempts to reduce the dose to ≤ 20 mg/day were unsuccessful. During that period, the patient had infectious complications, hypertension, osteoporosis, and Cushing's syndrome.

Approximately 17 years after being diagnosed with ABPA, the patient presented with a total IgE of 450 IU/mL and an eosinophil count of 5% (245 cells/mm^3). Spirometry revealed very severe obstructive lung disease (FEV_1, 24% of predicted; FVC, 53% of predicted; and FEV_1/FVC, 45%), with negative bronchodilator test results. Static lung volume measurements showed significant air trapping that persisted after bronchodilator administration. A noncontrast HRCT scan of the chest showed an increased anteroposterior chest diameter, scattered air trapping, pleural thickening, peripheral reticular opacities in the upper lobes, ectatic bronchi with unevenly thickened walls, centrilobular nodules, and bronchiolar filling with a tree-in-bud pattern, as well as opacities in the lower lobes, suggesting mucoid impaction and ABPA with bronchiectasis (Figure 1).

In December of 2012, despite treatment, the patient remained classified as being a stage IV (corticosteroid-dependent) ABPA patient. Because of refractoriness to standard treatment, she was started on omalizumab (at a dose of 300 mg every 15 days). Thereafter, she presented with controlled asthma and stage II ABPA (i.e., disease remission), and systemic corticosteroid therapy was discontinued.

At this writing, the patient was receiving treatment with omalizumab (300 mg every 15 days) in combination with budesonide (800 µg/day), formoterol (24 µg/day), and beclomethasone (500 µg/day). Emergency department visits were no longer needed. There was a significant improvement in quality of life. However, acute exacerbations of chronic rhinosinusitis remain common.

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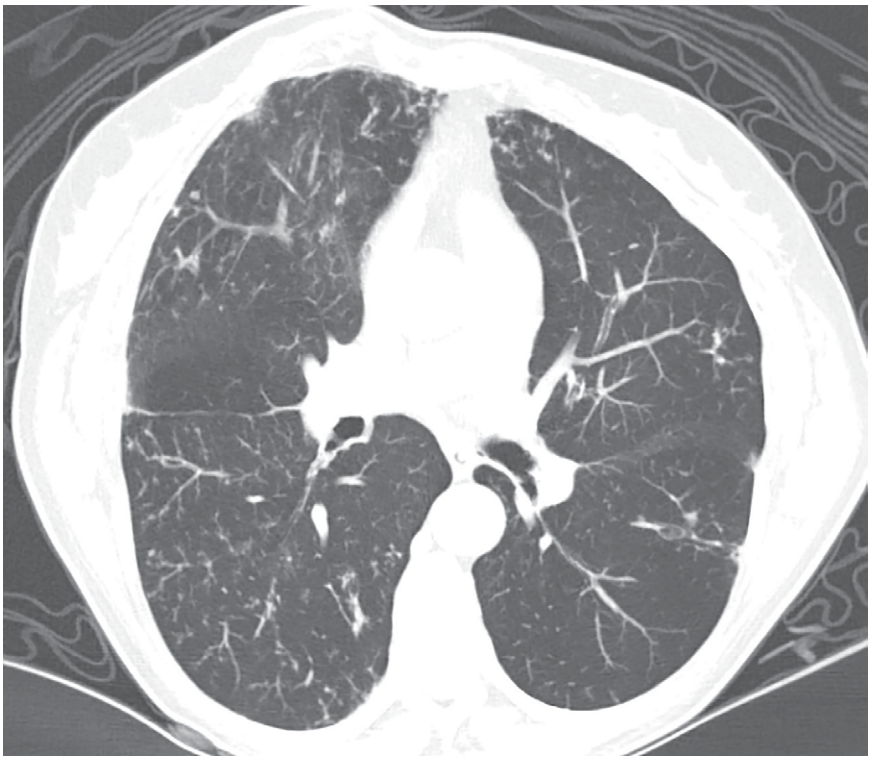

Figure 1. HRCT scan of the chest showing an increased anteroposterior chest diameter, scattered air trapping, pleural thickening, and peripheral reticular opacities in the upper lobes. Note ectatic bronchi with unevenly thickened walls, centrilobular nodules, and bronchiolar filling with a tree-in-bud pattern, as well as opacities in the lower lobes, suggesting mucoid impaction and allergic bronchopulmonary aspergillosis with bronchiectasis.

Table 1. Spirometric parameters.

Year	1994					2012					2014				
	Pre-BD		Post-BD		%	Pre-BD		Post-BD		%	Pre-BD		Post-BD		%
Parameter	n	% p	n	% p	variation	n	% p	n	% p	variation	n	% p	n	% p	variation
FVC, L	1.64	44.0	2.07	56.0	26.0	1.40	53.4	1.40	53.4	0.0	1.87	72.6	1.92	74.3	2.4
FEV_1, L	0.73	23.0	1.08	34.0	47.0	0.53	24.0	0.60	27.3	13.6	0.77	35.6	0.80	37.0	4.0
FEV_1/FVC	0.45	53.0	0.52	61.0	16.0	0.37	45.0	0.40	48.3	7.5	0.41	49.1	0.42	49.8	1.6
$FEF_{25-75\%}$, L/min	0.34	9.0	0.55	15.0	62.0	0.19	7.5	0.24	9.6	29.0	0.23	9.5	0.27	11.2	18.7

BD: bronchodilator; n: absolute value; and % p: percentage of predicted value.

A comparison between data collected in 2012 and 2014 (i.e., before and after treatment with omalizumab) showed improved lung function (FEV_1 and FVC; Table 1). However, improved lung function following treatment with omalizumab is uncommon in the literature. Pérez-de-Llano et al.[5] followed 18 adult patients with ABPA (16 patients with asthma and 2 patients with cystic fibrosis) for a mean period of 16 weeks and found improvement in daily asthma symptoms and FEV_1, as well as a reduction in systemic corticosteroid use. Tillie-Leblond et al.[6] followed 16 patients with asthma and ABPA for 12 months and found a reduction in the number of exacerbations and in the dose of systemic corticosteroids, with no significant changes in lung function.

Before treatment with omalizumab, total IgE levels and peripheral eosinophil levels were 511 IU/mL and 7% (266 cells/mm^3), respectively. After one year of treatment with omalizumab, total IgE levels and peripheral eosinophil levels decreased to 477 IU/mL and 2% (108 cells/mm^3), respectively.

Total IgE levels were found to have decreased after initiation of treatment with omalizumab. This finding is inconsistent with the literature, given that total IgE levels are expected to remain high throughout the treatment period because of formation and prolonged clearance of immune complexes. However, the fact that ABPA includes stages of remission and exacerbation might explain the variation in total IgE levels.

Eosinophils also decreased. Given that the patient had been receiving long-term systemic corticosteroid therapy, it is possible that eosinophils were underestimated before treatment with omalizumab. Therefore, the fact that eosinophil levels decreased

after initiation of treatment with omalizumab is even more significant.

Improved clinical status and laboratory tests provide further evidence that omalizumab is beneficial in patients with ABPA. Omalizumab had a corticosteroid-sparing effect and improved asthma symptoms and asthma control, allowing discontinuation of systemic corticosteroid. Other studies have shown the positive effects of omalizumab as add-on therapy in patients with refractory ABPA.[3,5-7]

Although omalizumab can be used as add-on therapy in such patients, further studies involving a larger sample size are needed in order to determine the actual role of omalizumab in such patients, as well as its effective dose and duration of treatment.

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Lung transplantation with extracorporeal membrane oxygenation as intraoperative support

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TO THE EDITOR:

Improvements in sequential bilateral lung transplantation techniques have made it possible to perform lung transplantation without the need for extracorporeal circulation (ECC). The use of ECC continues to be restricted to certain situations, such as hemodynamic instability, intolerance to single-lung ventilation, and pulmonary hypertension. ECC is associated with primary graft dysfunction and excessive bleeding due to the use of coagulation factors. Support devices, such as extracorporeal membrane oxygenation (ECMO), have been used not only in the treatment of primary graft dysfunction but also as a bridge to transplantation and, more recently, for intraoperative support.

We report the case of a 23-year-old male patient diagnosed with pulmonary fibrosis caused by chronic fibrosing interstitial pneumonia and secondary pulmonary hypertension, with a mean pulmonary artery pressure of 45 mmHg and resistance of 6.52 WU. The patient had grade 3-4 dyspnea, as determined with the modified Medical Research Council scale, under continuous oxygen therapy. He also presented leg edema, controlled after optimization of diuretic usage. The brain natriuretic peptide level was 538 pg/ml. His echocardiogram showed marked enlargement of the right heart chambers, right ventricular dysfunction, marked tricuspid insufficiency, and a pulmonary artery systolic pressure of 65 mmHg. He was on the waiting list for transplantation, presenting relevant increase in the cardiac silhouette and signs of right heart failure. Therefore, we recommended bilateral transplantation involving circulatory support with ECMO.

The patient was submitted to bilateral anterior transsternal thoracotomy (clamshell incision), together with opening of the pleural and pericardial cavities. We observed an increase in the cardiac silhouette, due to right heart chamber enlargement and marked dilatation of the right ventricle. We performed central cannulation, cannulating the aorta with a 20 Fr cannula and the right atrium with a two-stage 28 Fr cannula (Medtronic-Synectics, Skovlunde, Denmark), after which we set up an ECMO system (ECMO PLS System®; Maquet, Rastatt, Germany), maintaining a flow of 3 L and 2,630 pm. The patient had firm pleuropulmonary adhesions, mainly on the right, requiring decortication, which caused significant bleeding. We performed the conventional sequential transplantation technique, initially on the right, with an ischemia time of 6 h on the right side, compared with 7 h and 40 min on the left. We also used a continuous autotransfusion system (C.A.T.S.®; Fresenius Medical Care, Bad Homburg, Germany), with recovery of 986 mL of packed red cells. Due to the coagulation disorder and right ventricular dysfunction, we opted to delay the closure, externalizing the cannulas and maintaining the central ECMO. An echocardiogram obtained on postoperative day (POD) 1 confirmed relevant dyskinesia of the right ventricle. The patient underwent a second operation, due to bleeding, on POD 2. On the basis of the activated coagulation time or the activated partial thromboplastin time, anticoagulation with unfractionated heparin was maintained. On POD 5, the ECMO flow was reduced to 1.5 L/30 min, with good hemodynamic impact, and thoracic closure. During the cardiopulmonary support the patient was maintained under sedation (with midazolam and fentanyl) and paralysis (with cisatracurium). On POD 10, an echocardiogram showed normal systolic function of the right ventricle. On POD 13, he was tracheostomized after two extubation failures, remaining in the ICU for 26 days, being decannulated and discharged on POD 48. During hospitalization, he presented no organ dysfunction other than the cardiac dysfunction, which prompted the maintenance of the ECMO. Immunosuppression followed institutional guidelines: use of basiliximab in anesthetic induction and on POD 4, along with corticosteroids, cyclosporine, and mycophenolate. The patient did not present any noteworthy pulmonary infection; during hospitalization, the most significant clinical complication was perforated acute abdomen on POD 20, leading to right colectomy and ileostomy, due to Ogilvie's syndrome. After the end of the ECMO, anticoagulation was maintained at a prophylactic dose. Figure 1 shows chest X-rays obtained preoperatively and at month 6 after transplantation, demonstrating significant cardiac remodeling.

In order to avoid system coagulation, the use of extracorporeal circulation requires total heparinization, with a higher usage of coagulation factors and leads to an inflammatory process due to the contact of the blood with the air and with the extracorporeal circuit. These factors increase the risk of bleeding and, consequently, the need for transfusion of blood products, which causes systemic inflammation. This inflammatory process is associated with the development of pulmonary edema and primary graft dysfunction.[1]

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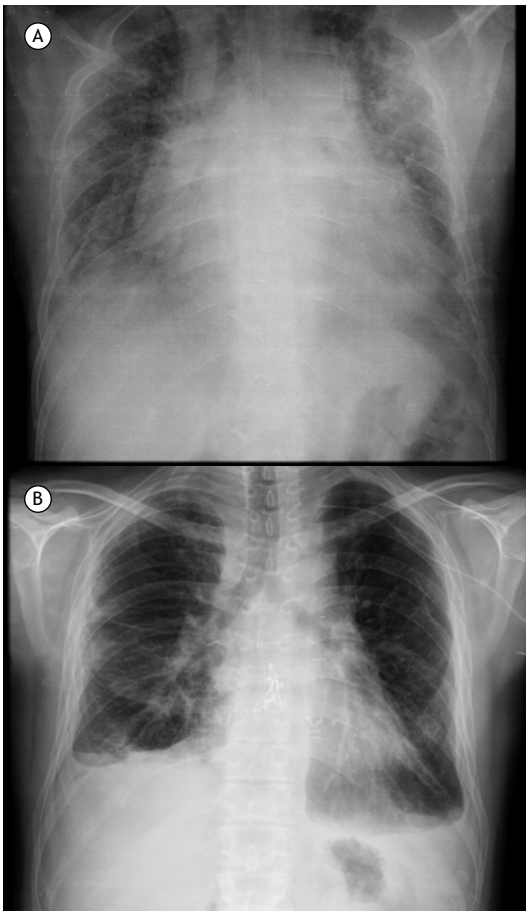

Figure 1. Chest X-rays: in the preoperative period (A); and six months after transplantation (B), showing significant cardiac remodeling.

Conventional polypropylene membrane oxygenators maintain good function for periods shorter than 3-4 hours; after which they start to show a loss of exchange function and plasma leakage, further impairing coagulation and inflammation. Bilateral transplantation can easily take longer than that, mainly in cases with firm pleuropulmonary adhesions. In such cases, the risk of bleeding due to pulmonary decortication increases the risk of the development of primary graft dysfunction and of postoperative mortality.[2]

The development of polymethylpentene membrane oxygenators radically changed the circulatory support setting, allowing the maintenance of the system for days, rather than hours. There is no doubt that the development of this polymer was the biggest change in this technique in recent years.[3] In conjunction with this technological development, the H1N1 influenza epidemic widened the use of ECMO as respiratory support in severe cases.

In lung transplantation, ECMO can be associated with either primary graft dysfunction treatment or as bridge to treatment in the pretransplantation period. Its use as circulatory support during surgical procedures was initially described for patients with peripheral pulmonary arterial hypertension, which might or might not persist after surgery. Peripheral cannulation was initially preferred because it provides a tube-free surgical field and facilitates exposure for the transplantation, with minor incisions.[4] In lung transplantation, ECC with cardiotomy reservoir has the advantage of allowing faster management of the volume and blood aspiration of the cavity and its reinfusion, although its mandatory use is restricted to cases in which there is a need for the correction of heart defects or for myocardial revascularization. Because these concomitant procedures are rare, it is possible to replace ECC with ECMO in most of the cases in which support is required. Heparin-coated circuits and the possibility of controlling the reperfusion of a newly transplanted lung make the use of ECMO feasible and advantageous as intraoperative support.[5]

Recent studies comparing intraoperative circulatory support with ECMO and conventional ECC have shown that the former presents advantages, such as less need for transfusion of blood products, shorter time on mechanical ventilation, shorter ICU stays, and less primary graft dysfunction.[5-9] Only Bittner et al.,[4] in their comparative study, showed disadvantages of the use of ECMO, with higher mortality and more need for transfusion. In the only meta-analysis on this topic, Hoechter et al.[10] did not note a decrease in the use of blood products, although there was a decrease in the length of ICU stays. However, the authors concluded that more studies on the subject were required.

To our knowledge, this is the first report of a case in which ECMO was used for intraoperative support during lung transplantation in Brazil, the ECMO being maintained in order to avoid primary graft dysfunction and to wait for myocardial remodeling. Although this was an isolated case, recently studies have shown advantages of using ECMO as support, mainly shorter ICU stays, lower rates of reoperation, less bleeding, and less need for blood transfusion. Therefore, the use of ECMO should be considered when there is a need for circulatory support during lung transplantation.

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Data: 27, 28 e 29 de junho de 2019
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Informações: (31)3213-3197
smpct@smpct.org.br | www.smpct.org.br

XII Congresso Brasileiro de Asma
VIII Congressos Brasileiros de DPOC e Tabagismo
Congresso Norte e Nordeste
Data: 14 a 16 de agosto de 2019
Local: Centro de Convenções de João Pessoa, João Pessoa/PB
Informações: 0800616218
eventos@sbpt.org.br

XVII Congresso de Pneumologia e Tisiologia do Estado do Rio de Janeiro
Data:12 a 14 de setembro de 2019
Local: Centro de Convenções SulAmérica
Rio de Janeiro/RJ
Informações: 21 2548-5141
pneumo2019@metodorio.com.br

10º Congresso do Centro-Oeste de Pneumologia e Tisiologia
Data: 25 a 27 de outubro 2019
Local: Associação Médica do Mato Grosso do Sul (AMMS)
Av. Des. Leão Neto do Carmo, 155 - Jardim Veraneio, Campo Grande - MS
Informações: (67) 3327-4110 (Luciane)
especialidades@amms.com.br
(67)98162-8382 (Henrique Brito)
hfbrito@icloud.com

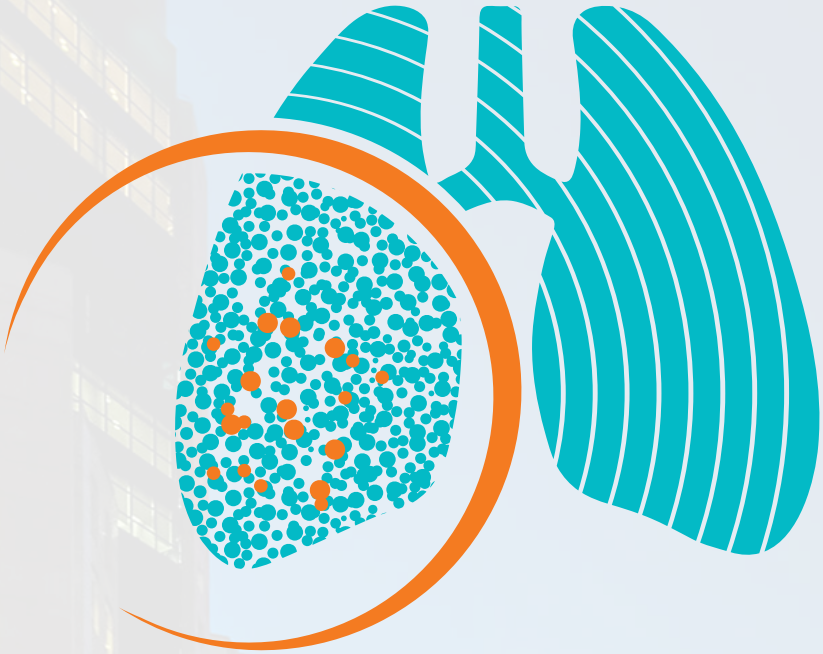
18º Congresso Paulista de Pneumologia e Tisiologia
Data: 20 e 23 de novembro de 2019
Local: Centro de Convenções Rebouças
Informações: 0800161718
www.sppt.org.br

INTERNACIONAIS

ATS 2019
Data: 17 a 22 de maio de 2019
Local: Dallas, Texas/USA
Informações: www.thoracic.org

ERS 2019
Data: 29 de setembro a 02 de outubro de 2019
Local: Madrid/Espanha
Informações: www.ersnet.org

CHEST 2019
Data: 19 a 23 de outubro 2019
Local: New Orleans/EUA
Informações: www.chestnet.org



XIII CURSO DE DOENÇAS INTERSTICIAIS

22 E 23/março 2019

GRANDE AUDITÓRIO DO CENTRO DE CONVENÇÕES REBOUÇAS, SÃO PAULO/SP

As inscrições para o DIP 2019 já estão abertas!

Acesse o site e garanta sua vaga: https://sbpt.org.br/dip2019/

XII CONGRESSO BRASILEIRO DE ASMA

VIII CONGRESSO BRASILEIRO DE DPOC E TABAGISMO

XVIII CONGRESSO NORTE E NORDESTE DE PNEUMOLOGIA E TISIOLOGIA

14 A 16 DE AGOSTO DE 2019

CENTRO DE CONVENÇÕES DE JOÃO PESSOA/PB