ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Published once every two months J Bras Pneumol. v.40, number 3, p. 207-324 May/June 2014

PUBLICAÇÃO OFICIAL DA SOCIEDADE BRASILEIRA DE PNEUMOLOGIA E TISIOLOGIA

COPD

Oxygen desaturation during the six-minute walk test in COPD patients

Hospitalized patients with COPD: analysis of prior treatment

EXPERIMENTAL

Effects of acute and chronic administration of methylprednisolone on oxidative stress in rat lungs

PULMONARY FUNCTION

Pulmonary function in advanced uncomplicated singleton and twin pregnancy

DIAGNOSTIC METHODS

Reliability of a rapid hematology stain for sputum cytology

POLLUTION

Indoor air quality and health in schools

SMOKING

Nicotine dependence and smoking habits in patients with head and neck cancer

COUGH

Leicester Cough Questionnaire: translation to Portuguese and cross-cultural adaptation for use in Brazil

TUBERCULOSIS

Association between serum selenium level and conversion of bacteriological tests during antituberculosis treatment

Tuberculose em pacientes hospitalizados: características clínicas dos pacientes que iniciaram tratamento nas primeiras 24 h de permanência hospitalar

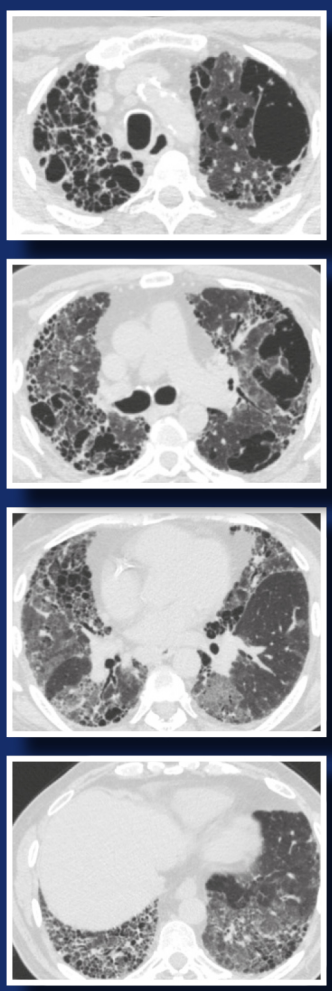
ICU

Performance of ICU ventilators during noninvasive ventilation with large leaks in a total face mask: a bench study

HIGHLIGHT

Questionnaire about chronic cough
Editorial: Catherine Acquadro, Ana Bayles, Elizabeth Juniper

Review Article:
Combined emphysema and pulmonary fibrosis



Jornal Brasileiro de Pneumologia

May/June 2014 volume 40, number 3

p.207-324

Free Full Text in English

www.jornaldepneumologia.com.br

ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Published once every two months J Bras Pneumol. v.40, number 3, p. 207-324 May/June 2014

ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Expediente

BRAZILIAN THORACIC SOCIETY

Office: SCS Quadra 01, Bloco K, Asa Sul, salas 203/204. Edifício Denasa, CEP 70398-900, Brasília, DF, Brazil. Tel. +55 61 3245-1030/+55 0800 616218. Website: www.sbpt.org.br. E-mail: sbpt@sbpt.org.br

ADMINISTRATIVE SECRETARIAT OF THE BRAZILIAN JOURNAL OF PULMONOLOGY
Address: SCS Quadra 01, Bloco K, Asa Sul, salas 203/204. Edifício Denasa, CEP 70398-900, Brasília, DF, Brazil. Tel. +55 61 3245-1030/+55 0800 616218.
Assistant Managing Editor: Luana Maria Bernardes Campos. E-mail: jpneumo@jornaldepneumologia.com.br
Circulation: 1,100 copies
Distribution: Free to members of the BTS and libraries
Printed on acid-free paper

SUPPORT:
Ministério da Educação
Ministério da Ciência, Tecnologia e Inovação
GOVERNO FEDERAL BRASIL PAÍS RICO É PAÍS SEM POBREZA

ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Published once every two months J Bras Pneumol. v.40, number 3, p. 207-324 May/June 2014

Sumário/Contents

EDITORIAL

207 - Fighting respiratory diseases: divided efforts lead to weakness
Combate a doenças respiratórias: esforços divididos levam ao enfraquecimento
Rogelio Pérez-Padilla, Rafael Stelmach, Manuel Soto-Quiroz, Álvaro Augusto Cruz

211 - Translating patient-reported outcome measures: a multi-step process is essential
Tradução de medidas de resultados relatados pelo paciente: um processo composto de várias etapas é essencial
Catherine Acquadro, Ana Bayles, Elizabeth Juniper

ARTIGOS ORIGINAIS / ORIGINAL ARTICLES

213 - Leicester Cough Questionnaire: translation to Portuguese and cross-cultural adaptation for use in Brazil
Questionário de Leicester sobre tosse crônica: tradução e adaptação cultural para a língua portuguesa falada no Brasil
Manuela Brisot Felisbino, Leila John Marques Steidle, Michelle Gonçalves-Tavares, Marcia Margaret Menezes Pizzichini, Emilio Pizzichini

222 - Oxygen desaturation during the six-minute walk test in COPD patients
Análise da dessaturação de oxigênio durante o teste de caminhada de seis minutos em pacientes com DPOC
Maria Ângela Fontoura Moreira, Gabriel Arriola de Medeiros, Francesco Pinto Boeno, Paulo Roberto Stefani Sanches, Danton Pereira da Silva Júnior, André Frotta Müller

229 - Hospitalized patients with COPD: analysis of prior treatment
Pacientes portadores de DPOC hospitalizados: análise do tratamento prévio
Irai Luis Giacomelli, Leila John Marques Steidle, Frederico Fernandes Moreira, Igor Varela Meyer, Ricardo Goetten Souza, Mariângela Pimentel Pincelli

238 - Effects of acute and chronic administration of methylprednisolone on oxidative stress in rat lungs
Efeitos da administração aguda e crônica no estresse oxidativo em pulmões de ratos
Ronaldo Lopes Torres, Iraci Lucena da Silva Torres, Gabriela Laste, Maria Beatriz Cardoso Ferreira, Paulo Francisco Guerreiro Cardoso, Adriane Belló-Klein

244 - Pulmonary function in advanced uncomplicated singleton and twin pregnancy
Função pulmonar em mulheres com gestação únicaou gemelar avançada e sem complicações
Anwar Hasan Siddiqui, Nazia Tauheed, Aquil Ahmad, Zehra Mohsin

250 - Reliability of a rapid hematology stain for sputum cytology
Confiabilidade da coloração hematológica rápida para citologia de escarro
Jéssica Gonçalves, Emilio Pizzichini, Marcia Margaret Menezes Pizzichini, Leila John Marques Steidle, Cristiane Cinara Rocha, Samira Cardoso Ferreira, Célia Tânia Zimmermann

259 - Indoor air quality and health in schools
Qualidade do ar interno e saúde em escolas
Ana Maria da Conceição Ferreira, Massano Cardoso

ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Published once every two months J Bras Pneumol. v.40, number 3, p. 207-324 May/June 2014

Sumário / Contents

269 - Association between serum selenium level and conversion of bacteriological tests during antituberculosis treatment
Associações entre níveis de selênio sérico e conversão de testes bacteriológicos durante o tratamento antituberculose
Milena Lima de Moraes, Daniela Maria de Paula Ramalho, Karina Neves Delogo, Pryscila Fernandes Campino Miranda, Eliene Denites Duarte Mesquita, Hedi Marinho de Melo Guedes de Oliveira, Antônio Ruffino-Netto, Paulo César de Almeida, Rachel Ann Hauser-Davis, Reinaldo Calixto Campos, Afrânio Lineu Kritski, Martha Maria de Oliveira

279 - Tuberculosis in hospitalized patients: clinical characteristics of patients receiving treatment within the first 24 h after admission
Tuberculose em pacientes hospitalizados: características clínicas dos pacientes que iniciaram tratamento nas primeiras 24 h de permanência hospitalar
Denise Rossato Silva, Larissa Pozzebon da Silva, Paulo de Tarso Roth Dalcin

286 - Nicotine dependence and smoking habits in patients with head and neck cancer
Dependência nicotínica e perfil tabágico em pacientes com câncer de cabeça e pescoço
Adriana Ávila de Almeida, Celso Muller Bandeira, Antônio José Gonçalves, Alberto José Araújo

294 - Performance of ICU ventilators during noninvasive ventilation with large leaks in a total face mask: a bench study
Desempenho de ventiladores de UTI durante ventilação não invasiva com grandes vazamentos em máscara facial total: estudo em simulador mecânico
Maria Aparecida Miyuki Nakamura, Eduardo Leite Vieira Costa, Carlos Roberto Ribeiro Carvalho, Mauro Roberto Tucci

ARTIGO DE REVISÃO / REVIEW ARTICLE

304 - Combined pulmonary fibrosis and emphysema: an increasingly recognized condition
Combinação de fibrose pulmonar e enfisema: uma doença cada vez mais reconhecida
Olívia Meira Dias, Bruno Guedes Baldi, André Nathan Costa, Carlos Roberto Ribeiro Carvalho

RELATO DE CASO / CASE REPORT

313 - Simultaneous interstitial pneumonitis and cardiomyopathy induced by venlafaxine
Pneumonite intersticial e miocardiopatia simultâneas induzidas por venlafaxina
Pedro Gonçalo Ferreira, Susana Costa, Nuno Dias, António Jorge Ferreira, Fátima Franco

CARTAS AO EDITOR / LETTER TO THE EDITOR

319 - Lung cancer and schwannoma — the pitfalls of positron emission tomography
Câncer de pulmão e schwannoma — as armadilhas da tomografia por emissão de prótons
Fernando Luiz Westphal, Luiz Carlos de Lima, José Correa Lima-Netto, Michel de Araújo Tavares, Felipe de Siqueira Moreira Gil

322 - Unilateral pulmonary agenesis
Agenesia pulmonar unilateral
Nulma Souto Jentzsch

Fighting respiratory diseases: divided efforts lead to weakness

Combate a doenças respiratórias: esforços divididos levam ao enfraquecimento

Rogelio Pérez-Padilla, Rafael Stelmach, Manuel Soto-Quiroz, Álvaro Augusto Cruz

Various respiratory diseases have been leading causes of death and morbidity over time, and that could be expected considering the huge interface between the respiratory system and the often hostile environment. The respiratory system filters almost 100,000 liters of air in an adult every day. The relevance of the burden of respiratory diseases has been recently emphasized by publications of the major respiratory societies in the world.[1-4]

Tuberculosis, the white plague and the origin of pulmonology, was an epidemic and still causes a great number of deaths and morbidity, due to public health neglect and the AIDS epidemic. In addition, inefficacious treatment is an increasing problem, with the presence of mycobacteria that are resistant to all existing drugs. Acute respiratory infections (ARIs), especially pneumonia, cause deaths in all ages, being especially relevant in children in developing countries. ARIs are the most common cause of outpatient consultation in the majority of countries. Tuberculosis and ARIs will most likely remain as leading health problems in the near future.[5,6] More recently, we have had to face chronic noncommunicable diseases in the rise, which was highlighted by the World Health Organization (WHO) in 2005.[7] Bronchial asthma affects approximately 10% of the world population, with great variations among the countries according to the International Study of Asthma and Allergy in Childhood,[8-16] causing morbidity, impairment, poor quality of life, and substantial health expenditures, being on the rise in various countries. Although deaths are uncommon in asthma, virtually all ARIs can be considered preventable, and they decrease progressively with the proper treatment of patients. In addition, COPD affects from 8-20% of the adult populations in five cities of Latin America[17] and is the third leading cause of death in the world. The disease, which ranks third or fourth among the top illnesses in various countries, has high morbidity and generates remarkably high health expenditures. Lung cancer is also on the rise, being among the ten leading causes of death in 2010. Recently, a successful detection program in high-risk subjects based on chest CT scanning has been described.[18]

These "big five" respiratory diseases (ARI, tuberculosis, asthma, COPD, and lung cancer), due to their extremely high combined burden, should receive more attention by health and economy authorities in developing countries, in accordance with the recommendations of a recent report by the Forum of the International Respiratory Societies.[4] In fact, if we group the codes of all respiratory diseases in the International Classification of Diseases, 10th revision (ICD-10) together, their burden and mortality are quite similar to those of cardiovascular diseases and cancer, and much higher than those of diabetes, which usually receives abundant funds for health care, research, and health promotion activities. Except for some priority regarding tuberculosis, respiratory diseases are often neglected in developing countries. In 2005 and 2008, respectively, 14.7% and 13.4% of all deaths had acute or chronic respiratory origin in Mexico,[19] which was close to the proportion of all cancer and cardiovascular-related deaths. This happens frequently in other countries.[19]

The results of various studies have markedly increased the knowledge of the natural history of asthma and showed the relationship between persistent asthma during childhood and the development of chronic lung disease. It is relevant that prematurity is associated with significant decreases in lung function early in life, which influences the development of chronic lung disease later.

It is relevant to analyze why this is happening. One factor that contributes to the problem is the underestimation of the burden of respiratory diseases, and this is related to the imperfections of the ICD-10, which is based on different perspectives: by organs or systems (respiratory diseases for example, the J codes), by mechanisms of disease (infections, in which ARIs and tuberculosis are inserted), but also by the period of life of the patients (neonatal diseases, infectious or respiratory, are in a separate

section, as well as obstetrical problems). Various diseases overlap organs or systems and have to be classified in one of the major codes. For example, pulmonary thromboembolism, which is one of the major causes of mortality in pulmonary medicine, is classified in the cardiovascular disease group. Cancer is a very heterogeneous group, and lung cancer is classified in the C34 codes, although it is more closely related to COPD, due to smoking—a shared risk factor, than to other neoplasias.

The formation of the ICD-10 groups also depends on the emphasis and priorities identified by the WHO and other health care organizations, which explains the existence of a group for neonatal and obstetrical diseases, regardless of the organ affected or the mechanism of disease.

Obstructive sleep apnea syndrome (OSAS) is also extremely common, since it affects 2-4% of the population.[20,21] In childhood, it appears because of tonsil and adenoid hypertrophy; in adulthood and in the elderly, obesity is the leading risk factor.[22] OSAS requires permanent treatment with continuous positive airway pressure, increases the risk of accidents and learning difficulties, reduces quality of life, and leads to metabolic and cardiovascular complications.[23] Hypoxemia is relevant in cities at moderate or high altitude, which are common in Latin America. In Mexico City (2,240 m above sea level), 6% of individuals aged 40 years or older present with $SaO2 \leq 88\%$[24]; however, less than 8% of these individuals have been prescribed oxygen therapy.

Official Ministries and Departments of Health in various countries, as well as the WHO, tend to follow the ICD-10 compartmentalization. For example, there are different departments or divisions for tuberculosis, ARIs, and chronic respiratory diseases. This segregation conflicts with the everyday reality in primary health care,[25] which is relevant and shall be integrated and multifunctional.[26] Integrality and multifunctionality are difficult to achieve with separate programs for common respiratory diseases. In addition, patients with acute or chronic lung diseases seek primary care because of a limited variety of respiratory symptoms. Guidelines for the treatment of respiratory diseases are varied, and before using them, health professionals have to decide what is appropriate for the patient, knowing that overlapping of acute and chronic diseases is common, such as asthma or COPD and acute respiratory infection, or acute respiratory infection and lung cancer.

The training of health care personnel, both in primary care and in specialties, disregards the ICD-10 classification and the WHO recommendations and includes acute, chronic, communicable, and noncommunicable diseases all together, as it happens in the real world.

Cumulative exposure to tobacco (by active or second-hand smoking), occupational fumes, and indoor and outdoor air pollution are known risks for various respiratory diseases. With aging, these risks are increased by higher prevalences of obesity and diabetes, lack of vaccination for preventable infections, and other traditional causes of death, such as tuberculosis.

National respiratory programs are uncommon when compared with programs for other diseases, despite their relevance. When they exist, they are separated in accordance with the ICD codes or the WHO departments; however, we need them to be integrated in primary health care. A patient with chronic cough and phlegm requires an investigation for tuberculosis; nevertheless, most of them do not have tuberculosis and are finally sent somewhere else. More importantly, that patient has a health problem, which requires evaluation and treatment, regardless of whether it is an acute, subacute, or chronic condition. This should be the role of an integrated program, including medical attention for infections, cancer, asthma, and COPD, regardless of their ICD-10 codes or the organization of departments in Ministries of Health or the WHO. The compartmentalization of public heath care initiatives against respiratory conditions in Latin America and in other countries might result in the weakness of the whole system. Just as an example, COPD causes more deaths than AIDS, breast cancer, cervical cancer, and prostate cancer together in Mexico and in Latin America. There are national cancer programs in many countries, but no COPD programs that cover all levels of health care.

The Practical Approach to Lung Health (PAL), a WHO program,[27-31] proposes integration in primary health care by using as its first step the syndromic diagnosis, starting in the tuberculosis clinics that are present everywhere, and taking care of all of the individuals screened for tuberculosis with negative tests. This is a sound program, since it expands with funding and training from existing assets and reinforces the tuberculosis

 http://dx.doi.org/10.1590/S1806-37132014000300001

program, which is abandoned or underfunded in various places. An integrated program not only deals with neglected diseases, such as COPD, asthma, and lung cancer, but also reinforces existing programs, such as tuberculosis programs and, in some countries, acute respiratory disease programs. Programs similar to PAL have been able to reduce the use of antibiotics and symptomatic medications, as well as to resolve increasingly health problems in primary health care, reinforcing the fight against tuberculosis. Single disease programs have shown that asthma and COPD patients receive better health care, impairment decreases, and fewer hospitalizations occur, provided that access to drugs is secured.[32-34] In addition, this type of programs reduces deaths and health expenditures in asthma and COPD,[35] which would be included in integrated programs.

Prevention is definitely the key, and anti-tobacco regulations considerably help. The health care costs of tobacco-related diseases are considerably higher than are tobacco product taxation. Campaigns against other respiratory risk factors—outdoor and indoor air pollution, occupational risks, obesity, among others—are required but uncommon. Anti-tobacco advice and medications, which are part of the effective recommendations by the WHO, should be included in an integrated respiratory program.

One-disease national programs have various potential problems emphasized by the WHO: difficulties in long-term sustainability and in the transition to multifunctional integrated programs; duplicity of supervision and training; and possible discrimination against patients outside the program. Integrated programs similar to PAL require adaptations to the necessities of the area or the country in which they are implemented. For example, in South Africa, an integrated program includes the diagnosis and treatment of HIV and AIDS, which are leading local health problems.

In summary, we would do a great service to patients with respiratory diseases if we offer them integrated primary health care programs similar to what PAL proposes. We would have better grounds to compete for funding if the proposal is part of a successful strategy that includes integration. The way things are at the moment—the fragmented, underfunded organization of health care, regardless of its origins—might be an obstacle rather than an asset in order to improve respiratory health.

Rogelio Pérez-Padilla
Investigator, National Institute of Respiratory Diseases, Mexico City, Mexico

Rafael Stelmach
Associate Professor, Pulmonary Division, Heart Institute, University of São Paulo Hospital das Clínicas, São Paulo, Brazil

Manuel Soto-Quiroz
Chief of Pulmonary Medicine, Hospital Nacional de Niños, San José, Costa Rica

Álvaro Augusto Cruz
Head, Center of Excellence in Asthma, Federal University of Bahia, Salvador, Brazil

References

1. Ferkol T, Schraufnagel D. The global burden of respiratory disease. Ann Am Thorac Soc. 2014;11(3):404-6. http://dx.doi.org/10.1513/AnnalsATS.201311-405PS
2. Schluger NW, Koppaka R. Lung disease in a global context. A call for public health action. Ann Am Thorac Soc. 2014;11(3):407-16 http://dx.doi.org/10.1513/AnnalsATS.201312-420PS
3. Zar HJ, Ferkol TW. The global burden of respiratory disease-impact on child health. Pediatr Pulmonol. 2014;49(5):430-4 http://dx.doi.org/10.1002/ppul.23030
4. American Thoracic Society [homepage on the Internet]. New York: the Society. [cited 2014 Apr 11]. Respiratory diseases in the world: realities of today–opportunities for tomorrow. An advocacy statement of the Forum of International Respiratory Societies (FIRS). Available from: http://www.thoracic.org/newsroom/firs.php
5. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet. 1997;349(9064):1498-504. http://dx.doi.org/10.1016/S0140-6736(96)07492-2
6. Lopez AD, Mathers CD. Measuring the global burden of disease and epidemiological transitions: 2002-2030. Ann Trop Med Parasitol. 2006;100(5-6):481-99. http://dx.doi.org/10.1179/136485906X97417
7. World Health Organization. Preventing chronic diseases: a vital investment. Geneva: World Health Organization; 2005.
8. Del-Rio-Navarro B, Del Rio-Chivardi JM, Berber A, Sienra-Monge JJ, Rosas-Vargas MA, Baeza-Bacab M. Asthma prevalence in children living in north Mexico City and a comparison with other Latin American cities and world regions. Allergy Asthma Proc. 2006;27(4):334-40. http://dx.doi.org/10.2500/aap.2006.27.2880
9. Del-Rio-Navarro B, Berber A, Blandón-Vijil V, Ramirez-Aguilar M, Romieu I, Ramírez-Chanona N, et al. Identification of asthma risk factors in Mexico City in an International Study of Asthma and Allergy in Childhood

survey. Allergy Asthma Proc. 2006;27(4):325-33. http://dx.doi.org/10.2500/aap.2006.27.2874
10. Morfin-Maciel B, Barragán-Meijueiro Mde L, Nava-Ocampo AA. Individual and family household smoking habits as risk factors for wheezing among adolescents. Prev Med. 2006;43(2):98-100. http://dx.doi.org/10.1016/j.ypmed.2006.04.002
11. Violante R, del Río Navarro BE, Berber A, Ramírez Chanona N, Baeza Bacab M, Sienra Monge JJ. Obesity risk factors in the ISAAC (International Study of Asthma and Allergies in Childhood) in Mexico City. Rev Alerg Mex. 2005;52(4):141-5.
12. Del Río-Navarro BE, Hernández-Román MP, Espinola Reyna G, Berber A, Escalante-Domínguez AJ, González-Reyes M, et al. A comparative study of bronchodilator reversibility with albuterol, between asthma symptomatic and asymptomatic children according to ISAAC questionnaire in Mexico City. Allergol Immunopathol (Madr). 2004;32(6):334-9. http://dx.doi.org/10.1016/S0301-0546(04)79265-4
13. Barraza-Villarreal A, Sanín-Aguirre LH, Téllez-Rojo MM, Lacasa-a-Navarro M, Romieu I. Prevalence of asthma and other allergic diseases in school children from Juarez City, Chihuahua [Article in Spanish]. Salud Publica Mex. 2001;43(5):433-43. http://dx.doi.org/10.1590/S0036-36342001000500007
14. Mendoza-Mendoza A, Romero-Cancio JA, Pe-a-Rios HD, Vargas MH. Prevalence of asthma in schoolchildren from the Mexican city Hermosillo [Article in Spanish]. Gac Med Mex. 2001;137(5):397-401.
15. Rojas Molina N, Legorreta Soberanis J, Olvera Guerra F. Prevalence and asthma risk factors in municipalities of the State of Guerrero, Mexico [Article in Spanish]. Rev Alerg Mex. 2001;48(4):115-8.
16. Tatto-Cano MI, Sanin-Aguirre LH, González V, Ruiz-Velasco S, Romieu I. Prevalence of asthma, rhinitis and eczema in school children in the city of Cuernavaca, Mexico [Article in Spanish]. Salud Publica Mex. 1997;39(6):497-506.
17. Menezes AM, Perez-Padilla R, Jardim JR, Mui-o A, Lopez MV, Valdivia G, et al. Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study. Lancet. 2005;366(9500):1875-81. http://dx.doi.org/10.1016/S0140-6736(05)67632-5
18. International Early Lung Cancer Action Program Investigators, Henschke CI, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, et al. Survival of patients with stage I lung cancer detected on CT screening. N Engl J Med. 2006;355(17):1763-71. http://dx.doi.org/10.1056/NEJMoa060476
19. Perez-Padilla R. Hidden respiratory disease-associated deaths. Int J Tuberc Lung Dis. 2008;12(4):458-64.
20. Torre-Bouscoulet L, Chávez E, Meza MS, Vázquez JC, Franco F, Muino A, et al. Snoring and sleep-related symptoms in three Latin-American cities. Proc Am Thorac Soc. 2005;2:A767.
21. Torre MA, Vazquez JC, Mui-o A, Marquez M, Lopez MV, Lisboa C, et al. Síntomas asociados al dormir en 4 ciudades de Latinoamérica: estudio con base poblacional. 100° Reunión Reglamentaria de la Asociación de Investigación Pediátrica. 2005 Jun 3-4; Hacienda Vista Hermosa, Morelos, México; 2005.
22. Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T. Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study. Arch Intern Med. 2005;165(20):2408-13. http://dx.doi.org/10.1001/archinte.165.20.2408
23. Torre Bouscoulet L, López Escárcega E, Castorena Maldonado A, Vázquez García JC, Meza Vargas MS, Pérez-Padilla R. Continuous positive airway pressure used by adults with obstructive sleep apneas after prescription in a public referral hospital in Mexico City [Article in Spanish]. Arch Bronconeumol. 2007;43(1):16-21. http://dx.doi.org/10.1157/13096996
24. Perez-Padilla R, Torre-Bouscoulet L, Mui-o A, Marquez MN, Lopez MV, de Oca MM, et al. Prevalence of oxygen desaturation and use of oxygen at home in adults at sea level and at moderate altitude. Eur Respir J. 2006;27(3):594-9. http://dx.doi.org/10.1183/09031936.06.00075005
25. International Conference on Primary Health Care. Declaration of alma-ata. WHO Chron. 1978;32(11):428-30.
26. Organización Mundial de la Salud. La atención primaria de salud. Ginebra: Organización Mundial de la Salud; 2008.
27. Bateman E, Feldman C, Mash R, Fairall L, English R, Jithoo A. Systems for the management of respiratory disease in primary care--an international series: South Africa. Prim Care Respir J. 2009;18(2):69-75.
28. Bheekie A, Buskens I, Allen S, English R, Mayers P, Fairall L, et al. The Practical Approach to Lung Health in South Africa (PALSA) intervention: respiratory guideline implementation for nurse trainers. Int Nurs Rev. 2006;53(4):261-8. http://dx.doi.org/10.1111/j.1466-7657.2006.00520.x
29. English RG, Bateman ED, Zwarenstein MF, Fairall LR, Bheekie A, Bachmann MO, et al. Development of a South African integrated syndromic respiratory disease guideline for primary care. Prim Care Respir J. 2008;17(3):156-63. http://dx.doi.org/10.3132/pcrj.2008.00044
30. Camacho M, Nogales M, Manjon R, Del Granado M, Pio A, Ottmani S. Results of PAL feasibility test in primary health care facilities in four regions of Bolivia. Int J Tuberc Lung Dis. 2007;11(11):1246-52.
31. Murray JF, Pio A, Ottmani S. PAL: a new and practical approach to lung health. Int J Tuberc Lung Dis. 2006;10(11):1188-91.
32. Haahtela T, Tuomisto LE, Pietinalho A, Klaukka T, Erhola M, Kaila M, et al. A 10 year asthma programme in Finland: major change for the better. Thorax. 2006;61(8):663-70. http://dx.doi.org/10.1136/thx.2005.055699
33. Tuomisto L, Erhola M, Kaila M, Brander PE, Puolijoki H, Kauppinen R, et al. Asthma Programme in Finland: high consensus between general practitioners and pulmonologists on the contents of an asthma referral letter. Prim Care Respir J. 2004;13(4):205-10. http://dx.doi.org/10.1016/j.pcrj.2004.04.001
34. Tuomisto LE, Erhola M, Kaila M, Brander PE, Kauppinen R, Puolijoki H, et al. The Finnish national asthma programme: communication in asthma care--quality assessment of asthma referral letters. J Eval Clin Pract. 2007;13(1):50-4. http://dx.doi.org/10.1111/j.1365-2753.2006.00645.x
35. Pietinalho A, Kinnula VL, Sovijärvi AR, Vilkman S, Säynäjäkangas O, Liippo K, et al. Chronic bronchitis and chronic obstructive pulmonary disease. The Finnish Action Programme, interim report. Respir Med. 2007;101(7):1419-25. http://dx.doi.org/10.1016/j.rmed.2007.01.022

Translating patient-reported outcome measures: a multi-step process is essential

Tradução de medidas de resultados relatados pelo paciente: um processo composto de várias etapas é essencial

Catherine Acquadro, Ana Bayles, Elizabeth Juniper

What does "translating" mean? Whilst theories are discussed elsewhere,[1] the definition given by Umberto Eco[2] seems refreshing and sensible: translating means "saying *almost* the same thing." What, however, is the extent of "almost" and how do you evaluate it? According to Eco, being faithful to a source document is not performing a "word for word" translation but a "world for world" translation and negotiating with the requirements of the source world becomes the key issue. In other words, the elasticity of *almost* depends on criteria that should be discussed and defined before embarking on the translation as such and in collaboration with the author of the original text.

When preparing patient-reported outcome (PRO) instruments for use internationally, it is helpful to remember Umberto Eco's observation. Regulators have focused their interest on the validity of the translations and their ability to express and investigate equivalent concepts across all language versions. With the question: "Are health-related quality of life (HRQoL) instruments internationally validated?,"[3] the European Medicines Agency clearly made the aspect of equivalence one of the key issues of HRQoL evaluation. The US Food and Drug Administration shares this view in its guidance, section III.G.3., and provides some recommendations.[4] The guidance states: *"Regardless of whether the instrument was developed concurrently in multiple cultures or languages or whether a fully developed instrument was adapted or translated to new cultures or languages, we recommend that sponsors provide evidence that the content validity and other measurement properties are adequately similar between all versions used in the clinical trial. We will review the process used to translate and culturally adapt the instrument for populations that will use them in the trial."*

Several reviews[5,6] suggest that using a rigorous and multi-step process with centralized review procedures may lead to better translations of PRO measures and can meet the regulators' requirements stated above. Usually, the process of translating PRO measures involves the following steps: translation of the original instrument into the target language by two independent translators and reconciliation into one version (forward step); translation of the reconciled version back into the language of the original instrument (backward step); review of the reconciled version with the participation of the developer of the original instrument; test on a panel of patients living in the target country (cognitive interview step); review of the test by a panel of experts; and finalization of the translated version of the instrument. Of course, all of those steps should always be preceded by one crucial step: one should always request permission to translate from the developer of the original instrument, to prevent any misuse or modifications that would impair the right of the original developer to the integrity of the instrument.[7]

As previously mentioned, equivalence in content validity between the original and the translated versions is crucial. Validating the content of a PRO measure requires providing evidence that the questionnaire contains all the problems that are most important to the patients who are going to complete the questionnaire. Cultural and environmental (i.e., climate) issues have to be taken into consideration (e.g., going to market/doing one's shopping may be a problem in many countries but not so much in the United States, where most people drive; in tropical countries, asthma patients do not have to contend with snow and icy winds). Therefore the cognitive interviews with the patients must cover not only issues of comprehension but also cultural and environmental issues. Relevance of the questions to respondents should be checked and those that are obviously lacking from the perspective of content validity should be changed. For example, in the Pediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ), which

was developed in Canada, caregivers reported that they were "angry" because their child had asthma. Therefore, this concept was included in the original PACQLQ. However, in every other country in the world, that is not an emotion that is frequently experienced by caregivers—instead, they are "sad". Translated versions of the PACQLQ include this concept of sadness.

In their paper entitled *"Leicester Cough Questionnaire: translation to Portuguese and cross-cultural adaptation for use in Brazil"*, Felisbino et al.[8] describe such a multi-step process and provide evidence that the Brazilian version of the Leicester Cough Questionnaire (LCQ) measures the same concepts as the original English version and can be widely used to assess the quality of life of patients with chronic cough in Brazil. They describe how the Brazilian version of the LCQ was created in collaboration with the developer of the original questionnaire to ensure that the intent of the original items was appropriately captured in the translation. They report that there were no difficulties in translating words referring to symptoms, physical activities, or activities of daily living. However, some English-language idioms and phrases, such as "fed up" and "overall enjoyment", were the objects of review and discussion. In addition, there was a need to adjust the verb tense so that the addressed situation made sense in Portuguese. Felisbino et al. also show that testing the translation on a panel of Brazilian patients was a crucial step in developing the final translated version of the LCQ. The questionnaire was administered to ten participants with chronic cough in order to determine its acceptability, clarity, and understandability. Although the participants had varied educational levels, no significant difficulties that would prevent them from understanding the questionnaire were identified. This indicates that the measure produced can be administered to individuals from various socioeconomic classes and cultural backgrounds. The analysis of the responses given during the cognitive debriefing process showed that few items needed to be revised because of problems related to understandability. This finding is of great relevance because it shows the robustness of the process of translation and cross-cultural adaptation. The next steps will involve clinical studies in patients with chronic cough to evaluate the psychometric properties (i.e., validity, reliability and responsiveness) of the Brazilian LCQ, with the objective of achieving properties similar to those of the original.

Catherine Acquadro
Scientific Advisor, Mapi, Lyon, France

Ana Bayles
Head of Linguistic Validation, Mapi, Lyon, France

Elizabeth Juniper
Professor Emeritus, Department of Clinical Epidemiology and Biostatistics, McMaster University, Canada

References

1. Acquadro C, Jambon B, Ellis D, Marquis P. Language and translation issues. In: Spilker B, editor. Quality of life and pharmacoeconomics in clinical trials. 2nd ed. Philadelphia: Lippincott-Raven; 1996. p. 575-85.
2. Eco U. Experiences in Translations. Toronto: University of Toronto Press; 2008.
3. Chassany O, Sagnier P, Marquis P, Fulleton S, Aaronson N. Patient reported outcomes and regulatory issues: the example of health-related quality of life - A European guidance document for the improved integration of HRQL assessment in the drug regulatory process. Drug Inf Assoc J. 2002;36(1):209-38. http://dx.doi.org/10.1177/009286150203600127
4. U.S. Food and Drug Administration [homepage on the Internet]. Silver Spring, MD: the Administration [cited 2014 Jan 1]. Guidance for Industry. Patient-reported outcome measures: use in medical product development to support labeling claims. [Adobe Acrobat document, 39p.]. Available from: http://www.fda.gov/downloads/Drugs/Guidances/UCM193282.pdf
5. Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A, et al. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. Value Health. 2005;8(2):94-104. http://dx.doi.org/10.1111/j.1524-4733.2005.04054.x
6. Acquadro C, Conway K, Hareendran A, Aaronson N; European Regulatory Issues and Quality of Life Assessment (ERIQA) Group. Literature review of methods to translate health-related quality of life questionnaires for use in multinational clinical trials. Value Health. 2008;11(3):509-21. http://dx.doi.org/10.1111/j.1524-4733.2007.00292.x
7. World Intellectual Property Organization [homepage on the Internet]. Geneva: the Organization [cited 2014 Apr 24]. Berne Convention for the Protection of Literary and Artistic Works. [about 45 screens]. Available from http://www.wipo.int/treaties/en/ip/berne/trtdocs_wo001.html. Accessed 24 April 2014.
8. Felisbino MB, Steidle LJ, Gonçalves-Tavares M, Pizzichini MM, Pizzichini E. Leicester Cough Questionnaire: translation to Portuguese and cross-cultural adaptation for use in Brazil. J Bras Pneumol. 2014;40(3):213-221.

 http://dx.doi.org/10.1590/S1806-37132014000300002

Leicester Cough Questionnaire: translation to Portuguese and cross-cultural adaptation for use in Brazil*

Questionário de Leicester sobre tosse crônica: tradução e adaptação cultural para a língua portuguesa falada no Brasil

Manuela Brisot Felisbino, Leila John Marques Steidle, Michelle Gonçalves-Tavares, Marcia Margaret Menezes Pizzichini, Emilio Pizzichini

Abstract

Objective: To translate the Leicester Cough Questionnaire (LCQ) to Portuguese and adapt it for use in Brazil. **Methods:** Cross-cultural adaptation of a quality of life questionnaire requires a translated version that is conceptually equivalent to the original version and culturally acceptable in the target country. The protocol used consisted of the translation of the LCQ to Portuguese by three Brazilian translators who were fluent in English and its back-translation to English by another translator who was a native speaker of English and fluent in Portuguese. The back-translated version was evaluated by one of the authors of the original questionnaire in order to verify its equivalence. Later in the process, a provisional Portuguese-language version was thoroughly reviewed by an expert committee. In 10 patients with chronic cough, cognitive debriefing was carried out in order to test the understandability, clarity, and acceptability of the translated questionnaire in the target population. On that basis, the final Portuguese-language version of the LCQ was produced and approved by the committee. **Results:** Few items were questioned by the source author and revised by the committee of experts. During the cognitive debriefing phase, the Portuguese-language version of the LCQ proved to be well accepted and understood by all of the respondents, which demonstrates the robustness of the process of translation and cross-cultural adaptation. **Conclusions:** The final version of the LCQ adapted for use in Brazil was found to be easy to understand and easily applied.

Keywords: Quality of life; Translations; Questionnaires; Cough.

Resumo

Objetivo: Traduzir e adaptar culturalmente o *Leicester Cough Questionnaire* (LCQ) para a língua portuguesa falada no Brasil. **Métodos:** A adaptação cultural de um questionário de qualidade de vida envolve a tradução conceitualmente equivalente à versão original e culturalmente aceitável ao país em que será utilizado. O protocolo aplicado consistiu na tradução do LCQ para a língua portuguesa por três tradutores brasileiros com fluência na língua inglesa e sua retradução para a língua original por um tradutor nascido em um país de língua inglesa e com fluência na língua portuguesa. A versão retraduzida foi avaliada por um dos autores do questionário original para assegurar sua equivalência e, posteriormente, o questionário foi revisado por um comitê de especialistas que realizou ampla revisão do instrumento. O desdobramento cognitivo consistiu em testar a compreensão, clareza e aceitabilidade do questionário traduzido na população alvo, aplicando-o em dez pacientes portadores de tosse crônica. Com base nisso, foi realizada a formulação da versão brasileira final do LCQ após sua aprovação pelo comitê. **Resultados:** Poucos itens foram questionados pelo autor da versão original e revistos pelo comitê de especialistas. A versão portuguesa do LCQ apresentou boa aceitabilidade e compreensão por todos os entrevistados no desdobramento cognitivo, demonstrando a robustez do processo de tradução e adaptação cultural. **Conclusões:** A versão final traduzida e adaptada para uso no Brasil mostrou ser de fácil compreensão e aplicação.

Descritores: Qualidade de vida; Traduções; Questionários; Tosse.

*Study carried out at the Polydoro Ernani de São Thiago University Hospital, *Universidade Federal de Santa Catarina* – UFSC, Federal University of Santa Catarina – Florianópolis, Brazil.
Correspondence to: Emílio Pizzichini. Núcleo de Pesquisa em Asma e Inflamação das Vias Aéreas, Hospital Universitário da UFSC, Campus Universitário, Trindade, CEP 88040-970, Florianópolis, SC, Brasil.
Tel/Fax: 55 48 3234-7711. E-mail: pizzichi@matrix.com.br
Financial support: None.
Submitted: 27 August 2013. Accepted, after review: 5 December 2013.

Introduction

Cough is one of the most common symptoms in clinical practice. Typically, cough is acute and self-limiting; however, in a significant proportion of patients, cough can present as an isolated chronic symptom.[1] Such patients suffer considerable physical and psychological morbidity.[2] Chronic cough is defined as any cough lasting more than eight weeks, with no concomitant clinical findings, and remaining without a definitive diagnosis after the initial clinical evaluation.[3] Chief among the most common causes of cough are postnasal drip syndrome, cough variant asthma, gastroesophageal reflux disease, and eosinophilic bronchitis.[4,5]

The impact of symptoms over a given period of time can be quantified and standardized by means of generic quality-of-life questionnaires,[6] or, more recently, by means of disease-specific questionnaires[7,8] or questionnaires designed to assess a specific problem, such as chronic cough.[9,10] Currently, there are two established questionnaires that assess quality of life in patients with cough: the Cough Quality-of-Life Questionnaire,[9] developed by French et al.; and the Leicester Cough Questionnaire (LCQ),[10] developed and validated by Birring et al. with the purpose of assessing this symptom and its impact on the health status of patients with chronic cough in a simple objective way. The LCQ can also be used to assess the temporal course of cough and monitor the response to treatment. The LCQ is self-administered and requires less than five minutes for completion. It comprises 19 items divided into three domains: physical (questions 1, 2, 3, 9, 10, 11, 14, and 15); psychological (questions 4, 5, 6, 12, 13, 16, and 17); and social (questions 7, 8, 18, and 19). Responses are given on a Likert-type scale ranging from 1 to 7 points. To calculate the LCQ score, the points assigned to each question in each domain must be aggregated and divided by the number of questions in each respective domain. The total score is the sum of each domain score and ranges from 3 to 21, with scores closer to 21 indicating better health status or a weaker influence of cough on patient quality of life.

Because the LCQ is a measure originally developed in the English language, it should be translated to the target language and adapted to the social and cultural circumstances of the target country; otherwise, another such measure should be developed.[11] Therefore, cross-cultural adaptation of a psychometric measure is a complex process that requires a translated version that is conceptually equivalent to the original version and culturally acceptable in the target country.[12] Technical and semantic equivalence should be sought between the source and target versions in order to avoid misinterpretation of data in the future. Cross-cultural adaptation of a measure will be complete when the psychometric properties of the translated version have been evaluated.[13]

To date, no health-related quality-of-life measure for patients with chronic cough has been developed or validated for use in Brazil. Therefore, the purpose of the present study was to translate the LCQ[10] to Portuguese and adapt it for use in Brazil.

Methods

This was a methodological study involving the translation to Portuguese of a specific health-related quality-of-life measure for patients with chronic cough, the LCQ,[10] and its cross-cultural adaptation for use in Brazil. The study was approved by the Human Research Ethics Committee of the *Universidade Federal de Santa Catarina* (UFSC, Federal University of Santa Catarina). The process of translation and cross-cultural adaptation of the LCQ was performed as described by Guillemin et al.[14] and Wild et al.[15] In Brazil, Tavares et al. used this methodology to translate an asthma control questionnaire to Portuguese and adapt it for use in Brazil.[16] Figure 1 illustrates each phase of the study.

The study sample intentionally consisted of 10 male and female patients over 18 years of age who were literate, had chronic cough, and were receiving no specific treatment. Those patients, recruited from the Pulmonology Outpatient Clinic of the UFSC University Hospital and from a private practice in respiratory medicine in the city of Florianópolis, Brazil, were invited to participate in the cognitive debriefing phase of the process of cross-cultural adaptation of the LCQ. This phase was used to assess the acceptability, clarity, and understandability of the translated adapted version.

For the present study, chronic cough was defined as cough lasting more than eight weeks and remaining without a definitive diagnosis after the initial clinical evaluation, which included chest X-ray as well as complete spirometry and

 http://dx.doi.org/10.1590/S1806-37132014000300002

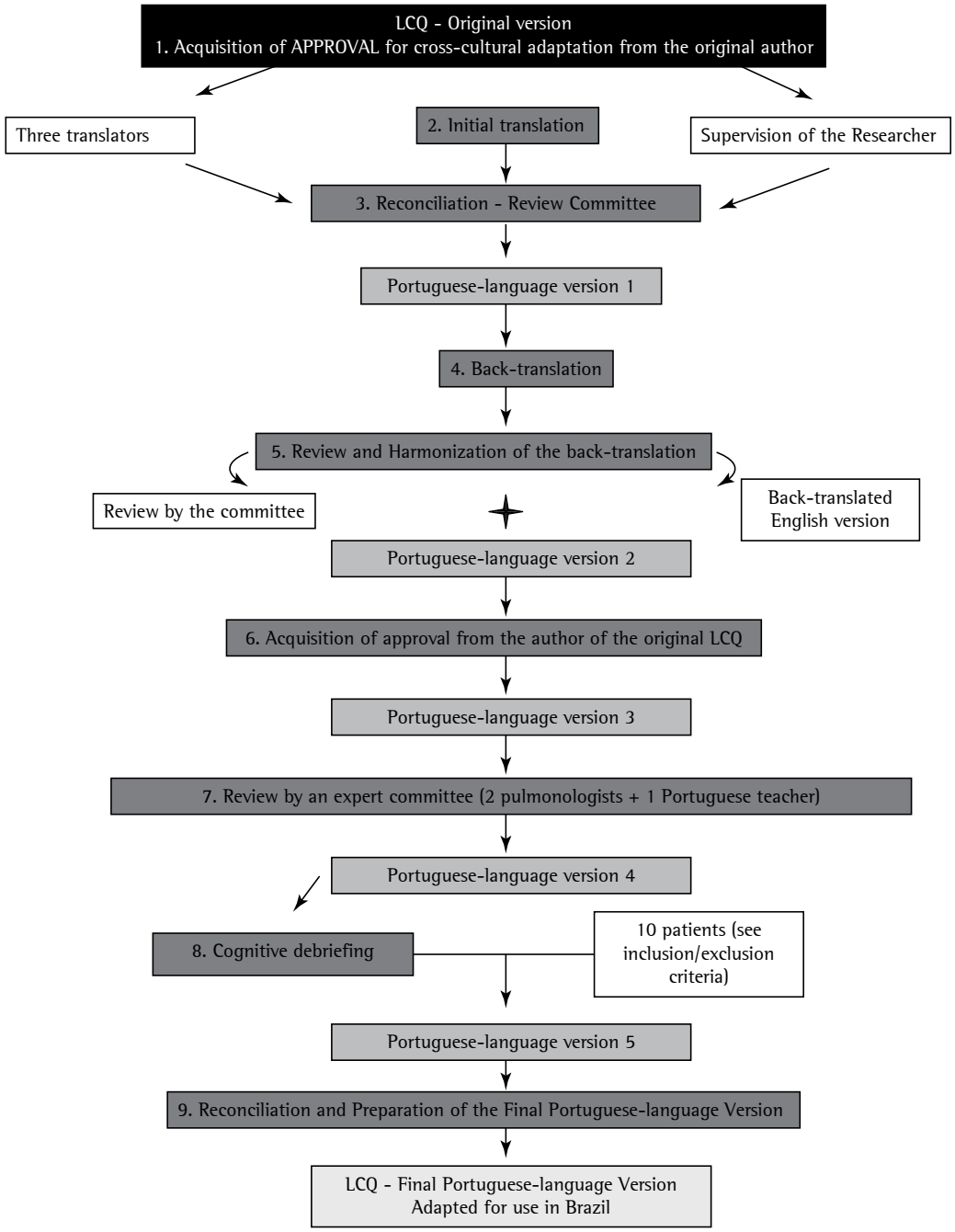


Figure 1 - Summary of the process of translation and cross-cultural adaptation of the Leicester Cough Questionnaire (LCQ) for use in Brazil.

bronchodilator response testing. We excluded smokers, former smokers, patients with other lung diseases (cystic fibrosis, COPD, pneumonia, etc.), patients with severe diseases of other body systems, and patients on medications that could confound the results. Since the present study does not permit a statistical analysis, the data are reported as absolute numbers and proportions, as means and standard deviations, or as medians and interquartile ranges.

The phases of the cross-cultural adaptation process were performed strictly in accordance with internationally accepted guidelines[14]: acquisition of permission for cross-cultural adaptation and of the rights of use of the LCQ from the developer of the questionnaire; translation of the LCQ from

English to Portuguese; reconciliation; back-translation; review and harmonization of the back-translation; acquisition of approval from the developer of the LCQ; review of the Portuguese-language version of the LCQ by experts; cognitive debriefing; and reconciliation and preparation of the final version.

In the English-to-Portuguese translation phase, three Brazilian translators who were fluent in English independently translated the LCQ. Subsequently, a review committee met to produce a first Portuguese-language version. This first version was back-translated to English by another translator who was a native speaker of English and fluent in Portuguese. The back-translation was then reviewed by the committee, which produced a back-translated English version and a matching Portuguese-language version of the LCQ. The back-translated version was sent to the author of the original LCQ for evaluation, and, once approved, its matching version was used to produce a third Portuguese-language version of the LCQ. This third version was reviewed by an expert committee, which consisted of two bilingual pulmonologists and a Portuguese teacher, and, subsequently, a fourth Portuguese-language version of the LCQ was produced. This fourth version was used in the cognitive debriefing phase, at the end of which a fifth version was produced. After reconciliation, the final Portuguese-language version of the LCQ was produced (Figure 1).

The purpose of cognitive debriefing was to identify problematic questions on the questionnaire and offer solutions to make such questions easier to understand. To that end, ten participants with chronic cough who showed good comprehension and language skills were interviewed. The cognitive debriefing process consisted of testing the understandability, clarity, and acceptability of the translated questionnaire in the target population. In this phase, individuals who met the inclusion criteria were consecutively scheduled for a single visit to the study site. During this visit, the study was explained in detail, and individuals who agreed to participate gave written informed consent. In addition, we collected demographic data and specific data on current and previous history of cough, duration and characteristics of cough, associated symptoms, final diagnosis (if defined), smoking history, and comorbidities. The questionnaire was administered to each participant by the principal investigator. Individuals were informed that they should not worry about the accuracy of their responses, but rather just report what they understood, the difficulty of each question or statement on the questionnaire, and their level of acceptance of the questionnaire. At the end, individuals were asked to make a general open comment about the questionnaire so that its overall acceptability, understandability, and clarity could be assessed. All comments were recorded on a specific form.

Finally, in the reconciliation phase, the review committee and the expert committee met to produce the final Portuguese-language version of the LCQ. To that end, the latest provisional version of the measure was analyzed item by item. The cognitive debriefing findings were discussed, and the relevant changes were made. Therefore, the final Portuguese-language version of the measure was produced.

Results

Of the ten patients interviewed in the cognitive debriefing phase, seven were female. All were White, were nonsmokers, and resided in the greater metropolitan area of Florianópolis, Brazil. Patient age ranged from 23 to 72 years, and patient educational level ranged from elementary school to college. Most patients had dry cough, which was associated with other symptoms, such as nasal obstruction, sneezing, and odynophagia, in 40% of the cases. Only two patients had no comorbidities, and the most common comorbidities were systemic arterial hypertension, type 2 diabetes mellitus, dyslipidemia, hypothyroidism, allergic rhinitis, and depression (Table 1).

Half of the patients interviewed were still undergoing diagnostic evaluation. For the remaining patients, one or more causes of cough had been found (Table 2).

In the phases of translation and back-translation, no questions or corrections were raised. However, in the phase of acquisition of approval from the author of the original LCQ, some items on the back-translated version were in part questioned by him because they showed a slight difference in wording. However, since the concept was preserved, no changes were made. The following items were questioned: "by sputum (phlegm) production when you cough?", which was back-translated as "by any phlegm you've coughed up?"; and "with the overall enjoyment

 http://dx.doi.org/10.1590/S1806-37132014000300002

of my life", which was back-translated as "with the enjoyment of my life".

The review performed by the expert committee indicated some grammatical errors and offered conceptual suggestions, all of which are described in Table 3. In addition, the questionnaire formatting was modified: the Likert-type scale with response choices arranged in horizontal sequence was placed within a single-row, seven-column table (Appendix 1; available in the online version of the Brazilian Journal of Pulmonology; http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_anexo.pdf).

Table 1 - Distribution of patients by demographic and disease-specific characteristics.[a]

Characteristic	Result
Age, years[b]	52.1 ± 14.6
Female gender	7 (70)
High school diploma or less	5 (50)
Duration of cough, months[c]	90 (10-198)
Dry cough	7 (70)
Presence of associated symptoms[d]	4 (40)
Presence of comorbidities[e]	8 (80)

[a]Values expressed as n (%), except where otherwise indicated. [b]Value expressed as mean ± SD. [c]Value expressed as median (interquartile range). [d]Odynophagia (in 10%); nasal obstruction (in 20%); and sneezing (in 10%). [e]Allergic rhinitis (in 40%); systemic arterial hypertension (in 30%); dyslipidemia (in 30%); depression (in 20%); diabetes mellitus (in 10%); and hypothyroidism (in 10%).

Table 2 - Distribution of patients by final diagnosis.[a]

Diagnosis	Result
Under investigation	5 (50)
Chronic sinusitis	3 (30)
Eosinophilic bronchitis	3 (30)
Gastroesophageal reflux disease	1 (10)
Cough variant asthma	1 (10)

[a]Values expressed as n (%). Note: Any given patient may have more than one diagnosis.

In the cognitive debriefing phase, three questions produced understandability difficulties. In addition, the title of the questionnaire was a source of difficulty for nearly half of the respondents. Therefore, in the final reconciliation phase, in which the review committee and the expert committee met, it was unanimously agreed that changes should be made to the title and to two of the questions. Table 4 shows the changes made after cognitive debriefing. The final version of the document incorporated those changes, as shown in Appendix 1.

Discussion

In the present study, a health-related quality-of-life measure for patients with chronic cough was translated to Portuguese and adapted for use in Brazil. The original version of the LCQ was developed primarily to assess patients in English, and, to date, only a Dutch-language version has been produced and validated.[17] Cross-cultural adaptation is relevant because, currently, there is no other quality-of-life measure for patients with chronic cough in Brazil. The decision to culturally adapt the LCQ, rather than to develop a new measure, was based on the fact that the adaptation of a previously described and validated measure, which has been translated and validated to other languages, makes it possible to compare results across studies conducted in different countries. In addition, this is a current trend that aims to facilitate the use of such a measure in

Table 3 - Changes made after the review by the expert committee.

LCQ – Portuguese-language version 3	LCQ – Portuguese-language version 4
"Elaborado"	"Desenvolvido"
"Responda circulando a resposta"	"Circule o número da resposta"
"O mais honestamente possível"	"Da maneira mais honesta possível"
"Como consequência"	"Em consequência"
"Esteve incomodado"	"Se incomodou"
"Esteve cansado"	"Se cansou"
"Me fez sentir ansioso"	"Me deixou ansioso"
"No aproveitamento da minha vida"	"No prazer de aproveitar minha vida"
"Saturado"	"Farto"
"Ficou preocupado"	"Se preocupou"
"Incomodou"	"Aborreceu"
"Responder este questionário"	"Responder a este questionário"

LCQ: Leicester Cough Questionnaire.

Table 4 - Changes made after the cognitive debriefing process.

LCQ – Portuguese-language version 4	LCQ – Portuguese-language version 5
"Questionário de Tosse Leicester"	"Questionário de Leicester sobre Tosse"
"Nas últimas 2 semanas, minha tosse me fez sentir farto (cheio)."	"Nas últimas 2 semanas, minha tosse me fez sentir de "saco cheio"
"Nas últimas 2 semanas, você teve muita energia?"	"Nas últimas 2 semanas, mesmo com sua tosse, você teve muita energia?"

LCQ: Leicester Cough Questionnaire.

international multicenter studies and has boosted the translation and cross-cultural adaptation of several generic and specific instruments to several languages.[18,19] Furthermore, the development of a new questionnaire would be a more laborious, time-consuming, and costly process.

Kalpaklioglu et al.[20] compared the LCQ with the Cough Quality-of-Life Questionnaire and showed that there is a significant correlation between the measurements of the two questionnaires. The present study aimed to translate and culturally adapt the LCQ because it is a careful questionnaire, which consists of well-formulated questions and is structured by domains. The methodology used in the development of the LCQ[10] ensures proper validation of content. In addition, the LCQ is valid and reproducible,[10] as well as being discriminative[21] and responsive to longitudinal changes.[10] Several studies have successfully used the LCQ to assess the response to several therapies for cough, as has been shown by Ryan et al.[22] for gabapentin therapy for refractory chronic cough and by Patel et al.[23] for cough-suppression physiotherapy. Therefore, guidelines on the management of chronic cough describe the LCQ as an important tool for quantification of cough and assessment of patient quality of life,[24-26] since there are few objective and well-validated instruments for quantification of cough. In more recent studies, the LCQ has been validated for assessment of chronic cough in the context of specific diseases[27,28] and for use in acute cough.[29]

One factor that ensures the applicability of the LCQ in Brazil is the methodology used in the process of translation and cross-cultural adaptation of the questionnaire, which has been shown to preserve the sensitivity of the measure,[14] as well as promoting an appropriate level of equivalence between the versions. In addition, it is known that the internal structure, semantics, and psychometric characteristics of a measure may change when this measure is translated to another language. This is more common if the process of cross-cultural equivalence is not performed correctly. The need to take into account cultural influences on health and disease is increasingly being recognized in multicenter and multinational studies. The purpose of adapting a quality-of-life measure is to obtain health measurements that are appropriate and valid in different cultural groups. This means developing a measure that is conceptually equivalent in different cultures.[30]

In the present study, the difficulties encountered in the translation phase resulted from the need to produce a conceptual translation. There were no difficulties in translating words referring to symptoms, physical activities, or activities of daily living. However, some English-language idioms and phrases, such as "fed up" and "overall enjoyment", were a matter of review and discussion. In addition, there was a need to adjust the verb tense so that the addressed situation made sense in Portuguese. In the phase of acquisition of approval from the original author, only two items were questioned by him as to differences in the literal translation. However, since, according to the original author himself, conceptual equivalence was preserved, no changes were needed. Once the back-translated version was approved, an expert committee met to evaluate its matching Portuguese-language version in order to detect errors, make suggestions, and analyze content and structure. In this phase, it is of particular value that the expert committee include bilingual members.[14]

The first modification was to the questionnaire formatting. The original version uses a Likert-type scale with response choices arranged in horizontal sequence. In the Portuguese-language version, the same Likert-type scale was placed within a single-row, seven-column table. The modification made it easier to visualize all response choices. In order to achieve semantic, conceptual, and idiomatic equivalence, some expressions, words, prepositions, and verb tenses were changed. The difficulty lies in the fact that some English-language expressions have no literal equivalent

 http://dx.doi.org/10.1590/S1806-37132014000300002

in Portuguese, and, in such cases, conceptual equivalence is sought. Corrections of grammatical errors were made by the Portuguese-language expert, and the questionnaire version intended for use in the cognitive debriefing phase was then produced.

Cognitive debriefing is an essential phase in the cross-cultural adaptation process, because even a detailed methodological process does not ensure equivalence between target and source versions.[14] The questionnaire was administered to ten participants in order to determine its acceptability, clarity, and understandability. Although the participants had varied educational levels, no significant difficulties that would prevent them from understanding the questionnaire were identified. This demonstrates that the measure produced can be administered to individuals from various socio-cultural classes. To ensure that the entire translation was easy to understand, cognitive debriefing involved an item-by-item review, rather than a random sample review. An analysis of the responses given during the cognitive debriefing process showed that few items needed to be revised because of understandability difficulties. This finding is of great relevance because it shows the robustness of the process of translation and cross-cultural adaptation. Therefore, the final version was produced after changes, which were unanimously agreed by the review committee and the expert committee, were made to three items, among which was the title of the questionnaire.

The respondents' comments on the questionnaire were very positive. All stated that, in general, the questionnaire was clear, easy to understand, and easy to answer, with simple and quick-to-follow instructions. In addition, the questionnaire was considered to be significantly relevant in the evaluation of chronic cough, being well adapted to that condition and covering its various aspects in detail.

In conclusion, the LCQ has been translated to Portuguese and adapted for use in Brazil. The final Portuguese-language version of the questionnaire, designated *Questionário de Leicester sobre Tosse Crônica*, was found to be easy to understand and easily applied, as well as being a single measure of health-related quality-of-life variables in patients with chronic cough.

Acknowledgments

We would like to thank the developer of the LCQ, Prof. Ian D Pavord, for his attention and cooperation in all phases of the process of translation and cross-cultural adaptation, as well as the research team of the *Núcleo de Pesquisa em Asma e Inflamação das Vias Aéreas* (NUPAIVA, Center for Research on Asthma and Airway Inflammation) and the UFSC.

References

1. Irwin RS, Madison JM. The diagnosis and treatment of cough. N Engl J Med. 2000; 343(23):1715-21. PMid:11106722 http://dx.doi.org/10.1056/NEJM200012073432308
2. French CL, Irwin RS, Curley FJ, Krikorian CJ. Impact of chronic cough on quality of life. Arch Intern Med. 1998;158(15):1657-61. PMid:9701100 http://dx.doi.org/10.1001/archinte.158.15.1657
3. Canning BJ. Anatomy and neurophysiology of the cough reflex: ACCP evidence-based clinical practice guidelines. Chest. 2006;129(1 Suppl):33S-47S. PMid:16428690 http://dx.doi.org/10.1378/chest.129.1_suppl.33S
4. Irwin RS, Baumann MH, Bolser DC, Boulet LP, Braman SS, Brightling CE, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. Chest. 2006; 129(1 Suppl):1S-23S. PMid:16428686 PMCid:PMC3345522 http://dx.doi.org/10.1378/chest.129.1.1
5. II Brazilian guidelines for the management of chronic cough [Article in Portuguese]. J Bras Pneumol. 2006;32 Suppl 6:S403-46. PMid:17420904 http://dx.doi.org/10.1590/S1806-37132006001000002
6. Mahler DA, Mackowiak JI. Evaluation of the short-form 36-item questionnaire to measure health-related quality of life in patients with COPD. Chest. 1995;107(6):1585-89. http://dx.doi.org/10.1378/chest.107.6.1585
7. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airway limitation. The St Georges's respiratory questionnaire. Am Rev Respir Dis. 1992;145(6):1321-7. PMid:1595997 http://dx.doi.org/10.1164/ajrccm/145.6.1321
8. Juniper EF, Guyatt GH, Ferrie PJ, Griffith LE. Measuring quality of life in asthma. Am Rer Respir Dis. 1993;147(4):832-8. PMid:8466117 http://dx.doi.org/10.1164/ajrccm/147.4.832
9. French CT, Irwin RS, Fletcher KE, Adams TM. Evaluation of a cough specific quality-of-life questionnaire. Chest. 2002;121(4):1123-31. PMid:11948042 http://dx.doi.org/10.1378/chest.121.4.1123
10. Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). Thorax. 2003;58(4):339-43. PMid:12668799 PMCid:PMC1746649 http://dx.doi.org/10.1136/thorax.58.4.339
11. Leplege A, Hunt S. The problem of quality of life in medicine. JAMA. 1997;278(1):47-50. http://dx.doi.org/10.1001/jama.1997.03550010061041

12. Bryant-Comstock L, Conway K, Mear I, Cramer J. The process of translation and cross-cultural adaptation of the quality of life epilepsy inventory (QOLIE-31). Annual meeting of the American Epilepsy Society. San Francisco, California, December 7-10, 1996. Abstracts. Epilepsia. 1996;37 Suppl 5:24.
13. Acquadro C, Janbom B, Ellis D, Marquis P. Language and translation issues. In: Spilker B, editor. Quality of life and pharmacoeconomics in clinical trials. 2nd ed. Philadelphia: Lippincott-Raven; 1996. p. 575-85.
14. Guillemin F, Bombardier C, Beaton D. Cross-cultural adaptation of health-related quality of life measures: literature review and proposed guidelines. J Clin Epidemiol. 1993;46(12):1417-32. http://dx.doi.org/10.1016/0895-4356(93)90142-N
15. Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A, et al. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: report of the ISPOR Task Force for Translation and Cultural Adaptation. Value Health. 2005;8(2):94-104. PMid:15804318 http://dx.doi.org/10.1111/j.1524-4733.2005.04054.x
16. Tavares MG, Pizzichini MM, Steidle LJ, Nazário NO, Rocha CC, Perraro MC, et al. The Asthma Control Scoring System: translation and cross-cultural adaptation for use in Brazil. J Bras Pneumol. 2010;36(6):683-92. PMid:21225170
17. Huisman AN, Wu MZ, Uil SM, van den Berg JW. Reliability and validity of a Dutch version of the Leicester Cough Questionnaire. Cough. 2007;3:3. PMid:17313670 PMCid:PMC1804278 http://dx.doi.org/10.1186/1745-9974-3-3
18. Cramer JA, Perrine K, Devinsky O, Bryant-Comstock L, Meador K, Hermann B. Development and cross-cultural translations of a 31-item quality of life in epilepsy inventory. Epilepsia. 1998;39(1):81-8. http://dx.doi.org/10.1111/j.1528-1157.1998.tb01278.x PMid:9578017
19. Güell R, Casan P, Sangenís M, Morante F, Belda J, Guyatt GH. Quality of life in patients with chronic respiratory disease: the Spanish version of the Chronic Respiratory Questionnaire (CRQ). Eur Respir J. 1998;11(1):55-60. PMid:9543270 http://dx.doi.org/10.1183/09031936.98.11010055
20. Kalpaklioglu AF, Kara T, Kurtipek E, Kocyigit P, Ekici A, Ekici M. Evaluation and impact of chronic cough: comparison of specific vs generic quality-of-life questionnaire. Ann Allergy Asthma Immunol. 2005;94(5):581-5. http://dx.doi.org/10.1016/S1081-1206(10)61137-4
21. Birring SS, Matos S, Patel RB, Prudon B, Evans DH, Pavord ID. Cough frequency, cough sensitivity and health status in patients with chronic cough. Respir Med. 2006;100(6):1105-9. PMid:16266801 http://dx.doi.org/10.1016/j.rmed.2005.09.023
22. Ryan NM, Birring SS, Gibson PG. Gabapentin for refractory chronic cough: a randomized, double-blind, placebo-controlled trial. Lancet. 2012;380(9853):1583-9. http://dx.doi.org/10.1016/S0140-6736(12)60776-4
23. Patel AS, Watkin G, Willig B, Mutalithas K, Bellas H, Garrod R, et al. Improvement in health status following cough suppression physiotherapy for patients with chronic cough. Chron Respir Dis. 2011;8(4):253-8 PMid:21990570 http://dx.doi.org/10.1177/1479972311422547
24. Morice AH, Fontana GA, Sovijarvi AR, Pistolesi M, Chung KF, Widdicombe J, et al. The diagnosis and management of chronic cough. Eur Respir J. 2004;24(3):481-92. PMid:15358710 http://dx.doi.org/10.1183/09031936.04.00027804
25. Morice AH, McGarvey L, Pavord I; British Thoracic Society Cough Guideline Group. Recommendations for the management of cough in adults. Thorax. 2006;61 Suppl 1:i1-24. PMid:16936230 PMCid:PMC2080754 http://dx.doi.org/10.1136/thx.2006.065144
26. Irwin RS. Assessing cough severity and efficacy of therapy in clinical research: ACCP evidence-based clinical practice guidelines. Chest. 2006; 129(1 Suppl):232S-237S. PMid:16428716 http://dx.doi.org/10.1378/chest.129.1_suppl.232S
27. Berkhof FF, Boom LN, ten Hertog NE, Uil SM, Kerstjens HA, van den Berg JW. The validity and precision of the Leicester Cough Questionnaire in COPD patients with chronic cough. Health Qual Life Outcomes. 2012;10:4. PMid:22230731 PMCid:PMC3311606 http://dx.doi.org/10.1186/1477-7525-10-4
28. Murray MP, Turnbull K, MacQuarrie S, Pentland JL, Hill AT. Validation of the Leicester Cough Questionnaire in non-cystic fibrosis bronchiectasis. Eur Respir J. 2009;34(1):125-31. PMid:19196812 http://dx.doi.org/10.1183/09031936.00160508
29. Yousaf N, Lee KK, Jayaraman B, Pavord ID, Birring SS. The assessment of quality of life in acute cough with the Leicester Cough Questionnaire (LCQ-acute). Cough. 2011;7(1):4. PMid:21767404 PMCid:PMC3169450 http://dx.doi.org/10.1186/1745-9974-7-4
30. Guyatt GH. The philosophy of health-related quality of life translation. Qual Life Res. 1993;2(6):461-5. PMid:8161980 http://dx.doi.org/10.1007/BF00422220

About the authors

Manuela Brisot Felisbino
Resident in Pulmonology. *Universidade de São Paulo* – USP, University of São Paulo – School of Medicine, São Paulo, Brazil.

Leila John Marques Steidle
Adjunct Professor. Department of Clinical Medicine, *Universidade Federal de Santa Catarina* – UFSC, Federal University of Santa Catarina – Florianópols, Brazil.

Michelle Gonçalves-Tavares
Professor. *Universidade do Sul de Santa Catarina* – UNISUL, University of Southern Santa Catarina – Tubarão, Brazil.

Marcia Margaret Menezes Pizzichini
Associate Professor. Department of Clinical Medicine, *Universidade Federal de Santa Catarina* – UFSC, Federal University of Santa Catarina – Florianópols, Brazil.

Emilio Pizzichini
Professor. . Department of Clinical Medicine, *Universidade Federal de Santa Catarina* – UFSC, Federal University of Santa Catarina – Florianópols, Brazil.

Oxygen desaturation during the six-minute walk test in COPD patients*

Análise da dessaturação de oxigênio durante o teste de caminhada de seis minutos em pacientes com DPOC

Maria Ângela Fontoura Moreira, Gabriel Arriola de Medeiros, Francesco Pinto Boeno, Paulo Roberto Stefani Sanches, Danton Pereira da Silva Júnior, André Frotta Müller

Abstract

Objective: To evaluate the behavior of oxygen saturation curves throughout the six-minute walk test (6MWT) in patients with COPD. **Methods:** We included 85 patients, all of whom underwent spirometry and were classified as having moderate COPD (modCOPD, n = 30) or severe COPD (sevCOPD, n = 55). All of the patients performed a 6MWT, in a 27-m corridor with continuous SpO_2 and HR monitoring by telemetry. We studied the SpO_2 curves in order to determine the time to a 4% decrease in SpO_2, the time to the minimum SpO_2 (Tmin), and the post-6MWT time to return to the initial SpO_2, the last designated recovery time (RT). For each of those curves, we calculated the slope. **Results:** The mean age in the modCOPD and sevCOPD groups was 66 ± 10 years and 62 ± 11 years, respectively. At baseline, SpO_2 was > 94% in all of the patients; none received supplemental oxygen during the 6MWT; and none of the tests were interrupted. The six-minute walk distance did not differ significantly between the groups. The SpO_2 values were lowest in the sevCOPD group. There was no difference between the groups regarding RT. In 71% and 63% of the sevCOPD and modCOPD group patients, respectively, a ≥ 4% decrease in SpO_2 occurred within the first minute. We found that FEV_1% correlated significantly with the ΔSpO_2 ($r = -0.398$; $p < 0.001$), Tmin ($r = -0.449$; $p < 0.001$), and minimum SpO_2 ($r = 0.356$; $p < 0.005$). **Conclusions:** In the sevCOPD group, in comparison with the modCOPD group, SpO_2 was lower and the Tmin was greater, suggesting a worse prognosis in the former.

Keywords: Pulmonary disease, chronic obstructive; Exercise test; Blood gas monitoring, transcutaneous.

Resumo

Objetivo: Avaliar o comportamento da curva de saturação de oxigênio durante o teste de caminhada de seis minutos (TC6) em pacientes com DPOC. **Métodos:** Incluímos 85 pacientes e todos realizaram espirometria, sendo classificados como portadores de DPOC moderada (DPOCm, n = 30) ou grave (DPOCg, n = 55). Todos os pacientes realizaram TC6 em um corredor de 27 m com monitoramento contínuo da SpO_2 e FC por telemetria. A partir das curvas de SpO_2, foram analisados os tempos para atingir a queda de 4% da SpO_2, para atingir a SpO_2 mínima (Tmin) e para a recuperação da SpO_2 após o TC6 (TR). Foram calculadas as inclinações dessas curvas. **Resultados:** A média de idade nos grupos DPOCm e DPOCg foi de 62 ± 11 anos e 66 ± 10 anos, respectivamente. Todos os pacientes iniciaram o teste com SpO_2 > 94%, nenhum recebeu suplementação de oxigênio durante o TC6, e não houve interrupções. A distância percorrida no TC6 não apresentou diferença significativa entre os grupos. Os menores valores da SpO_2 ocorreram no grupo DPOCg. Não houve diferença no TR entre os grupos, e 71% e 63% dos pacientes nos grupos DPOCg e DPOCm, respectivamente, apresentaram queda de SpO_2 ≥ 4% até o primeiro minuto. O VEF_1% apresentou correlações significativas com ΔSpO_2 ($r = -0,398$; $p < 0,001$), Tmin ($r = -0,449$; $p < 0,001$) e SpO_2 mínima ($r = 0,356$; $p < 0,005$). **Conclusões:** As curvas dos pacientes do grupo DPOCg em relação às do grupo DPOCm apresentaram valores menores de SpO_2 e maior Tmin, sugerindo um pior prognóstico nos primeiros.

Descritores: Doença pulmonar obstrutiva crônica; Teste de esforço; Monitorização transcutânea dos gases sanguíneos.

*Study carried out in the Pulmonary Physiology Clinic, Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Correspondence to: Maria Ângela Fontoura Moreira. Avenida Neusa Goulart Brizola, 550/605, Petrópolis, CEP 90460-230, Porto Alegre, RS, Brasil.
Tel. 55 51 3359-8198. E-mail: maanmo@terra.com.br

Financial support: None.
Submitted: 21 October 2013. Accepted, after review: 5 April 2014.

 http://dx.doi.org/10.1590/S1806-37132014000300004

Introduction

Advances in research on and in the treatment and diagnosis of lung diseases have shown the importance of including the six-minute walk test (6MWT) in the functional assessment of lung disease patients, more specifically in the detection of exercise-induced hypoxemia, which is considered an important marker of respiratory disease severity. The acquisition of reproducible measurements is necessary for this assessment.[1-5]

The 6MWT is widely requested since, in addition to being easy to administer, inexpensive, and well-tolerated by the patient, it is the mode of submaximal exercise that most closely approximates activities of daily living. It is attractive because it combines ease of performance and operational simplicity. Therefore, it is usually used as an adjunctive tool in the assessment of COPD, cystic fibrosis, heart disease, peripheral vascular disease, etc.[2-4,6]

The American Thoracic Society guidelines recommend that the 6MWT be performed indoors, along a flat, straight, 30-m track, on which the patient should walk for six minutes, with the aim of covering the greatest distance possible.[7]

The 6MWT is among the most commonly used tests to assess exercise tolerance in individuals with chronic obstructive disease and individuals with interstitial disease. Such patients may experience a significant decrease in SpO_2 during submaximal exercise or even desaturation at rest. Exertional hypoxemia can be explained by pathophysiological factors, such as airflow limitation, imbalance between oxygen supply and consumption, systemic inflammation, and oxidative stress, affecting peripheral muscle oxygenation. The significant decrease in the levels of circulating oxygen, resulting from the increased demand caused by the effort put forth, can lead to increased blood pressure, increased dyspnea, and increased muscle fatigue, thereby reducing submaximal exercise tolerance.[8]

Patients with COPD do not show the same limitation during exercise or activities of daily living. Exercise performance and exercise maintenance depend primarily on flawless interaction among the systems that control ventilation, gas exchange, blood flow, hemoglobin, oxygen/carbon dioxide transport, oxygen use, and carbon dioxide production.[8]

In patients with COPD, one of the most important adverse events during the 6MWT is oxygen desaturation, which can be more accurately assessed if there is continuous monitoring throughout the test. Therefore, the objective of the present study was to evaluate the behavior of oxygen saturation curves throughout the 6MWT in patients with COPD.

Methods

The data were collected between January and December of 2012 in the Pulmonary Physiology Clinic of the Department of Pulmonology of the *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre *Hospital de Clínicas*), located in the city of Porto Alegre, Brazil. This study was analyzed and approved by the HCPA Health Research Ethics Committee (Project no. 09-549), and the patients invited to participate in the study gave written informed consent before performing the 6MWT.

We included male and female patients who had been diagnosed with COPD,[9] were stable, and had spirometry results indicative of moderate COPD (modCOPD) or severe COPD (sevCOPD), as classified by the 2002 Brazilian Thoracic Association Guidelines for Pulmonary Function Tests.[10] Spirometry was performed by spirometry technicians certified by the Brazilian Thoracic Association. A spirometer (Jaeger, Würtzburg, Germany) was used, and the predicted values of Crapo were employed.[11] Spirometry was performed 1 h before the 6MWT on the same day. Values of FEV_1 and VC were obtained from the flow-volume curves.

The 6MWT was conducted in a 27-m corridor in accordance with the America Thoracic Society guidelines.[7] At the HCPA, it is possible to monitor HR and SpO_2 continuously by telemetry throughout the 6MWD with the use of a digital oximetry module and of a software program developed by the Biomedical Engineering team at HCPA. This system allows the simultaneous transfer of HR and SpO_2 data to the computer, making it possible to monitor the degree of oxygen desaturation in real time, which thereby allows a better assessment of the degree of disease severity.[5,7] Figure 1 shows a recorded curve. All of the included patients completed the 6MWT without interruption and had a baseline SpO_2 > 94%. None of the patients received supplemental oxygen during the test. We excluded from the sample those with orthopedic impairments, interstitial diseases, or pulmonary arterial hypertension, or with any condition that would compromise

their ability to perform the 6MWT. The curves showing a ≥ 4% decrease in SpO_2 were analyzed.

We studied the SpO_2 curves in order to determine the time to a 4% decrease in SpO_2 and the time to the minimum SpO_2, as well as the post-6MWT time to return to the initial SpO_2, designated recovery time. We calculated the slope of each of those curves with the following formula: (final SpO_2 – initial SpO_2) ÷ Δtime between those points

The slopes were compared to determine changes in them because of the severity of airway obstruction. Figure 2 shows an example of the slopes calculated.

The statistical analysis of the collected data was performed with the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). Data were analyzed for normality and homogeneity of variance. The independent sample t-test was used for the comparison between the two groups. Pearson's correlation test was used for analysis of correlations. For all analyses, the level of significance was set at $p < 0.05$. Values are expressed as means and standard deviations.

Results

The study sample consisted of 85 patients: 55 with sevCOPD (mean age of 62 ± 11.3 years and mean body mass index [BMI] of 22.5 ± 3.3 kg/m²); and 30 with modCOPD (mean age of 66.0 ± 10.1 years and BMI of 25.1 ± 2.8 kg/m²). Table 1 shows the variables assessed in the two groups.

The groups were found to be homogeneous with respect to age and pre-6MWT SpO_2. In neither of the groups did the BMI exceed 30 kg/m², a value above which spirometry results are affected.[10] The six-minute walk distance did not differ significantly between the groups. The minimum SpO_2 was significantly lower in the sevCOPD group ($p < 0.014$). A 4% decrease in SpO_2 occurred within the first minute in 63% and 71% of the modCOPD and sevCOPD group patients, respectively. The time to desaturation of 4% and the recovery time did not differ significantly between the groups; however, the time to the minimum SpO_2 was greater in the sevCOPD group than in the modCOPD group ($p < 0.001$). The slopes of the SpO_2 curves for

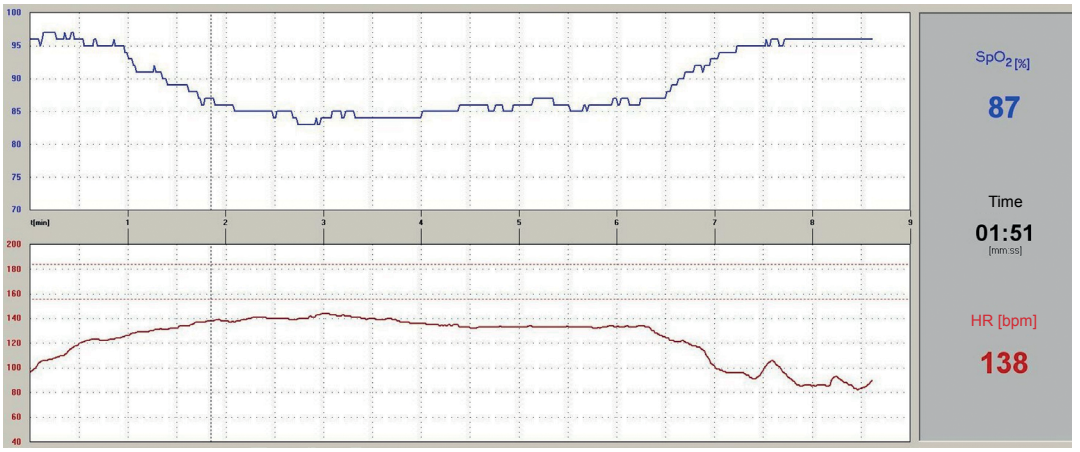


Figure 1 - Monitoring of HR and SpO_2.

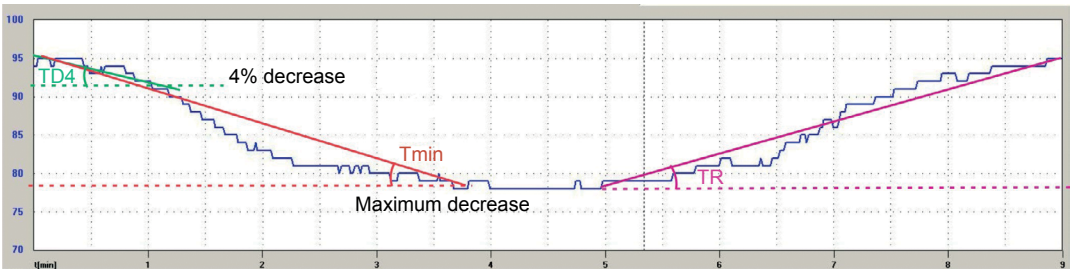


Figure 2 - Example of slopes of the curves. TD4: time to desaturation of 4%. Tmin: time to the minimum SpO_2; and RT: recovery time (i.e., time to return to the initial SpO2).

 http://dx.doi.org/10.1590/S1806-37132014000300004

Table 1 - General characteristics of the study population.

Variable	modCOPD (n = 30)	sevCOPD (n = 55)	p
BMI, kg/m^2	25.1 ± 2.8	22.5 ± 3.3	0.01*
Age, years	66 ± 10	62 ± 11	0.11
Pre-6MWT SpO_2, %	95.0 ± 1.9	95.0 ± 2.1	0.85
Minimum SpO_2, %	87.3 ± 3.4	85.0 ± 4.3	0.01*
TD4, s	64 ± 36	66 ± 41	0.81
Tmin, s	109 ± 55	168 ± 67	0.01*
RT, s	112.6 ± 28.8	120.2 ± 31.5	0.28
FEV_1%	53.1 ± 9.6	28.0 ± 6.2	0.01*
6MWD, m	451 ± 73	435 ± 74	0.35
Slope of the line for a ≥ 4% decrease in SpO_2	0.08 ± 0.04	0.08 ± 0.04	0.89
Slope of the line for maximum decrease in SpO_2	0.08 ± 0.03	0.07 ± 0.05	0.27
Slope of the line for return to the initial SpO_2	0.07 ± 0.02	0.09 ± 0.04	0.05

modCOPD: moderate COPD; sevCOPD: severe COPD; BMI: body mass index; 6MWT: six-minute walk test; TD4: time to desaturation of ≥ 4%; Tmin: time to the minimum SpO_2; RT: recovery time (i.e., time to return to the initial SpO_2); and 6MWD: six-minute walk distance.

desaturation of 4%, maximum decrease, and recovery were not found to differ significantly between the groups. The change in SpO_2 (ΔSpO_2) between the baseline value and the maximum decrease was statistically different between the two groups (p = 0.005).

We found that FEV_1% showed a moderate positive correlation with the minimum SpO_2 (r = 0.356; p < 0.005), a moderate negative correlation with the ΔSpO_2 (r = −0.398; p < 0.001), and a moderate negative correlation with the time to the minimum SpO_2 (r = −0.449; p < 0.001).

The slope of the maximum decrease in SpO_2 showed a moderate negative correlation with the time to the minimum SpO_2 (r = −0,467; p < 0,001), time to desaturation of 4% (r = −0.437; p < 0.001), and minimum SpO_2 (r = −0.393; p < 0.001). The six-minute walk distance (6MWD) showed no significant correlations with SpO_2 or its variations or with FEV_1%.

Discussion

Exercise-induced desaturation can be measured in the 6MWT and is an index that has prognostic value in interstitial diseases and COPD. A ≥ 4% decrease in SpO_2 suggests significant desaturation and is used for assessing the need for oxygen supplementation in patients with chronic lung disease.[12] Another index of functional capacity is the 6MWD, which has prognostic value in COPD.[13]

However, hypoxemia is a major problem in respiratory medicine, since it is very common in

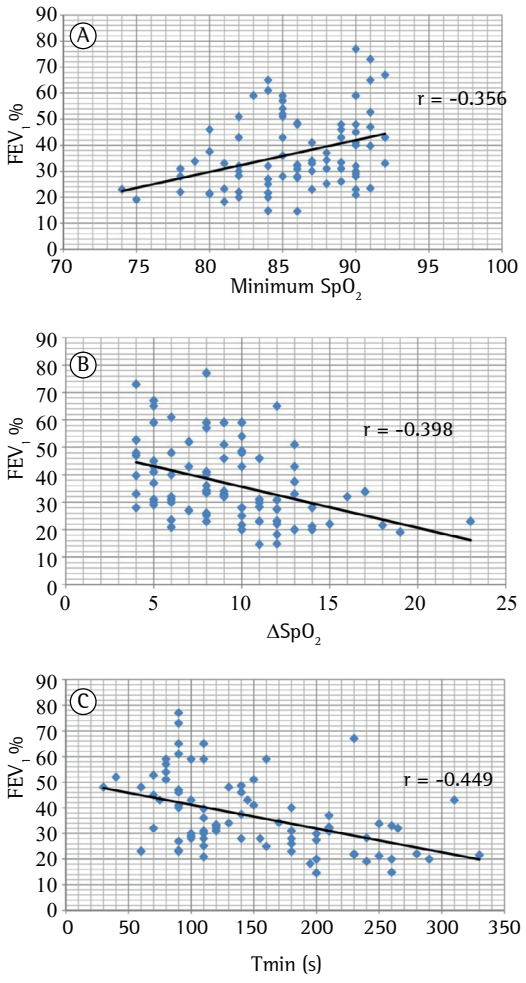


Figure 3 - Correlations of changes in FEV_1% with the minimum SpO_2 (in A), ΔSpO_2 (in B), and time to the minimum SpO_2 (Tmin; in C).

patients with lung disease and must be rapidly assessed and treated to prevent irreversible organ damage.

Exercise-induced desaturation is commonly observed in patients with COPD; however, clinical parameters cannot identify such change. A resting SpO_2 of < 95% has been reported to be a predictor of exercise-induced desaturation, especially in patients with a ≥ 36% reduction in DLCO[14] Zafar et al.[15] found no significant correlation between changes (decreases) in SpO_2 and resting SpO_2. Our study also found no significant correlation between baseline SpO_2 and decreases in SpO_2 ($r = 0.08$; $p = 0.46$).

The basis on which the theory of exercise intolerance in COPD is built is multifactorial: increased respiratory muscle work and oxygen uptake; lower limb skeletal muscle dysfunction; and dynamic lung hyperinflation; acting either alone or in combination.[8] Zafar et al.,[15] studying 30 patients with COPD, reported a good correlation between oxygen desaturation during the 6MWT and dynamic hyperinflation, but no correlation with the 6MWD. Our results also showed no correlation between desaturation and the 6MWD; however, we did not assess hyperinflation in the present study.

We found that the 6MWD showed no correlation with changes in SpO_2. There have been reports of skeletal muscle changes in patients with COPD, with the predominance of glycolytic fibers over oxidative fibers being highlighted. As a result, patients predominantly use the anaerobic metabolism at a low level of exercise,[16,17] characterizing a change in the metabolic pathway and reducing the aerobic load. The occurrence of some factors, such as inflammatory stress, physical deconditioning, prolonged use of corticosteroids, and hypoxemia, contributes to altering muscle contractile activity, triggering a series of adaptations that involve muscle fiber changes. According to one group of authors,[18] the work of breathing in the group of COPD patients who recruit abdominal muscles is twice that in the group of COPD patients who do not do so, being associated with increased dyspnea and decreased exercise tolerance. This is a possible explanation for our results, since patients may have a predominance of glycolytic fibers, may not recruit abdominal muscles, or both.

Previous studies[19,20] have shown that the time to desaturation during the 6MWT is an indicator of the possibility of desaturation during activities of daily living, culminating in severe hypoxemia and the need for oxygen therapy. Jenkins & Cecins[21] analyzed the adverse events that occurred during the 6MWT in a group of 572 patients with COPD who completed the 6MWT; 345 (47%) of the patients experienced significant desaturation (a ≥ 4% decrease). The study by Jenkins & Cecins[21] highlights the importance of continuous monitoring SpO_2 during the 6MWT. The telemetry system used at the HCPA enabled us to monitor the behavior of SpO_2 during the 6MWT in real time.

One group of authors[20] showed that, of 83 patients with COPD who performed the 6MWT, 48 experienced early desaturation (SpO_2 < 90% before the first minute) and that, over a 5-year follow-up period, 65% of those patients developed severe hypoxemia and required home oxygen therapy, compared with 11% of the patients who did not experience early desaturation ($p < 0.001$). Early desaturation is also associated with desaturation during a 24-h period and during most activities of daily living. In our sample of patients who experienced desaturation during the 6MWT, we noticed that most experienced desaturation of ≥ 4% within the first minute (71% and 63% of the sevCOPD and modCOPD group patients, respectively), which indicates the need for a more rigorous assessment of the routine activities of these individuals.

In one study,[22] 224 patients with COPD were divided into two groups: those with and those without oxygen desaturation during the 6MWT. The patients were followed for 3 years, and the desaturation group was found to have a more rapid decline in FEV_1 ($p = 0.006$), which suggests that exercise-induced desaturation can be a predictor of pulmonary function decline in patients with COPD. In our study, FEV_1 was found to be a good indicator of exercise-induced desaturation, showing a significant moderate negative correlation with the ΔSpO_2 ($r = -0.398$; $p < 0.001$) and time to the minimum SpO_2 ($r = -0.448$; $p < 0.001$).

Oxygen desaturation is a monitoring parameter that qualifies the performance of patients on the 6MWT and aids in determining the degree of disease-related impairment during physical exertion. Analysis of desaturation curves allows a comprehensive view of the time to a decrease in SpO_2, the intensity of that decrease, and the

 http://dx.doi.org/10.1590/S1806-37132014000300004

recovery time, which can assist in determining clinical severity. However, to our knowledge, no other studies have reported this type of data, which precludes a comparison with our results.

The present study underscores the importance of oxygen desaturation analysis with continuous monitoring during the 6MWT in patients with COPD. In the sevCOPD group, in comparison with the modCOPD group, SpO_2 was lower and most patients experienced early desaturation (within the first minute), suggesting a worse prognosis. The FEV_1 variable was found to be a good marker of exercise-induced desaturation, showing a moderate correlation with the minimum SpO_2, ΔSpO_2, and time to the minimum SpO_2.

References

1. Brunetto AF, Pitta FO, Probst VS, Paulin E, Yamaguti WP, Ferreira LF. Influência da saturação de O2 na velocidade do teste de distância percorrida de seis minutos em pacientes com DPOC grave. Rev Bras Fisioter. 2003;7(2):123-9.
2. Rondelli RR, Oliveira AN, Dal Corso S, Malaguti C. Uma atualização e proposta de padronização do teste de caminhada de seis minutos. Fisioter Movimento. 2009;22(2):249-59.
3. Pires SR, Oliveira AC, Perreira VF, Britto RR. Teste de caminhada de seis minutos em diferentes faixas etárias e índice de massa corporal. Rev Bras Fisioter. 2007;11(2):147-51. http://dx.doi.org/10.1590/S1413-35552007000200010
4. Ziegler B, Rovedder PM, Lukrafka JL, Oliveira CL, Menna-Barreto SS, Dalcin Pde T. Submaximal exercise capacity in adolescent and adult patients with cystic fibrosis. J Bras Pneumol. 2007;33(3):263-9. http://dx.doi.org/10.1590/S1806-37132007000300006
5. Dumke A. Estudo do comportamento da saturação periférica de oxigênio durante o teste de caminhada de 6 minutos em pacientes com doenças pulmonares crônicas [dissertation] Porto Alegre: Faculdade de Medicina da Universidade Federal do Rio Grande do Sul; 2006.
6. Rodrigues SL, Mendes HF, Viegas CA. Teste da caminhada de seis minutos: estudo do efeito do aprendizado em portadores de doença pulmonar obstrutiva crônica. J Pneumol. 2004;30(2):121-5.
7. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7. http://dx.doi.org/10.1164/ajrccm.166.1.at1102
8. Russo R, Iamonti VC, Jardim JR. Intolerância ao exercício no paciente com DPOC. Pneumol Paulista. 2012;26(1):38-41.
9. Global Initiative for Chronic Obstructive Lung Disease [homepage on the Internet]. Bethesda: Global Initiative for Chronic Obstructive Lung Disease. [cited 2013 Oct 21]. Global Strategy for the Diagnosis, Management and Prevention of Chronic Obstructive Pulmonary Disease. Updated 2013. [Adobe Acrobat document, 99p.]. Available from: http://www.goldcopd.org/uploads/users/files/GOLD_Report_2013_Feb20.pdf
10. Sociedade Brasileira de Pneumologia e Tisiologia. Diretrizes para testes de função pulmonar. J Pneumol. 2002;28(Suppl 3):S83-S238.
11. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. Am Rev Respir Dis. 1981;123(6):659 64.
12. Puente Maestú L, García de Pedro J. Lung function tests in clinical decision-making. Arch Bronconeumol. 2012;48(5):161-9 http://dx.doi.org/10.1016/j.arbr.2011.12.007
13. Hagarty EM, Skorodin MS, Langbein WE, Hultman CI, Jessen JA, Maki KC. Comparison of three oxygen delivery systems during exercise in hypoxemic patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1997;155(3):893-8. http://dx.doi.org/10.1164/ajrccm.155.3.9117023
14. Knower MT, Dunagan DP, Adair NE, Chin R Jr. Baseline oxygen saturation predicts exercise desaturation below prescription threshold in patients with chronic obstructive pulmonary disease. Arch Intern Med. 2001;161(5):732-6. http://dx.doi.org/10.1001/archinte.161.5.732
15. Zafar MA, Tsuang W, Lach L, Eschenbacher W, Panos RJ. Dynamic Hyperinflation correlates with exertional oxygen desaturation in patients with chronic obstructive pulmonary disease Lung. 2013;191(2):177-82 http://dx.doi.org/10.1007/s00408-012-9443-3
16. Gosker HR, van Mameren H, van Dijk PJ, Engelen MP, van der Vusse GJ, Wouters EF, et al. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. Eur Respir J. 2002;19(4):617-25. http://dx.doi.org/10.1183/09031936.02.00762001
17. Engelen MP, Schols AM, Does JD, Gosker HR, Deutz NE, Wouters EF. Exercise-induced lactate increase in relation to muscle substrates in patients with chronic obstructive pulmonary disease.. Am J Respir Crit Care Med. 2000;162(5):1697-704. http://dx.doi.org/10.1164/ajrccm.162.5.9910066
18. Aliverty A, Macklem PT. Last Word on Point:Counterpoint: The major limitation to exercise performance in COPD is 1) inadequate energy supply to the respiratory and locomotor muscles, 2) lower limb muscle dysfunction, 3) dynamic hyperinflation. J Appl Physiol (1985). 2008;105(2):763.
19. García-Talavera I, García CH, Macario CC, de Torres JP, Celli BR, Aguirre-Jaime A. Time to desaturation in the 6-min walking distance test predicts 24-hour oximetry in COPD patients with a PO2 between 60 and 70mmHg. Respir Med. 2008;102(7):1026-32. http://dx.doi.org/10.1016/j.rmed.2008.02.004
20. Garcia-Talavera I, Tauroni A, Trujillo JL, Pitti R, Eiroa L, Aguirre-Jaime A, et al. Time to desaturation less than one minute predicts the need for long-term home oxygen therapy. Respir Care. 2011;56(11):1812-7. http://dx.doi.org/10.4187/respcare.01164
21. Jenkins S, Cecins N. Six-minute walk test: observed adverse events and oxygen desaturation in a large cohort of patients with chronic lung disease. Intern Med J. 2011;41(5):416-22. http://dx.doi.org/10.1111/j.1445-5994.2010.02169.x
22. Kim C, Seo JB, Lee SM, Lee JS, Huh JW, Lee JH, et al. Exertional desaturation as a predictor of rapid lung function decline in COPD. Respiration. 2013;86(2):109-16 http://dx.doi.org/10.1159/000342891

About the authors

Maria Ângela Fontoura Moreira
Head. Pulmonary Physiology Clinic, Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Gabriel Arriola de Medeiros
Intern. Pulmonary Physiology Clinic, Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Francesco Pinto Boeno
Intern. Pulmonary Physiology Clinic, Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Paulo Roberto Stefani Sanches
Engineer. Department of Research and Development in Biomedical Engineering, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Danton Pereira da Silva Júnior
Engineer. Department of Research and Development in Biomedical Engineering, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

André Frotta Müller
Engineer. Department of Research and Development in Biomedical Engineering, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

 http://dx.doi.org/10.1590/S1806-37132014000300004

Hospitalized patients with COPD: analysis of prior treatment*

Pacientes portadores de DPOC hospitalizados: análise do tratamento prévio

Irai Luis Giacomelli, Leila John Marques Steidle, Frederico Fernandes Moreira, Igor Varela Meyer, Ricardo Goetten Souza, Mariângela Pimentel Pincelli

Abstract

Objective: Although COPD is a prevalent disease, it is undertreated, and there are no available data regarding previous treatment of COPD in Brazil. This study aimed to determine the appropriateness of maintenance treatment in COPD patients prior to their hospitalization and to identify variables associated with inappropriate treatment. **Methods:** This was an observational, cross-sectional, analytical study involving 50 inpatients with COPD at two hospitals in the city of Florianópolis, Brazil. The patients completed a questionnaire on parameters related to the maintenance treatment of COPD. Non-pharmacological management and pharmacological treatment were assessed based on the recommendations made by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 2011 and by the Brazilian National Ministry of Health in the chronic respiratory diseases section of its *Caderno de Atenção Básica* (CAB, Primary Care Guidebook). **Results:** In most of the patients, the COPD was classified as being severe or very severe. Regarding non-pharmacological management, 33% of the patients were smokers, only 32% had been advised to receive the flu vaccine, 28% had received pneumococcal vaccine, and only 6.5% of the patients in the B, C, and D categories received pulmonary rehabilitation. Regarding GOLD and CAB recommendations, pharmacological treatment was inappropriate in 50% and 74% of the patients, respectively. Based on GOLD recommendations, 38% were undertreated. A low level of education, low income, not receiving oxygen therapy, and not receiving the flu vaccine were associated with inappropriate treatment. **Conclusions:** The application of various non-pharmacological management recommendations was unsatisfactory. Regarding the GOLD recommendations, the high rate of inappropriate maintenance treatment was mainly due to undertreatment. In Brazil, even in severe COPD cases, optimizing treatment to achieve greater benefits continues to be a challenge.

Keywords: Pulmonary disease, chronic obstructive/therapy; Pulmonary disease, chronic obstructive/prevention and control; Clinical protocols.

Resumo

Objetivo: Embora a DPOC seja uma enfermidade prevalente, ela é subtratada, e dados sobre o tratamento prévio são desconhecidos em nosso meio. Buscou-se verificar a adequação às recentes diretrizes no que se refere ao tratamento de manutenção em pacientes com DPOC antes de sua hospitalização e identificar possíveis variáveis associadas à inadequação do tratamento. **Métodos:** Estudo transversal, observacional e analítico, que incluiu 50 portadores de DPOC, internados em dois hospitais na cidade de Florianópolis (SC). Aplicou-se um questionário sobre parâmetros relacionados ao tratamento de manutenção da DPOC. Avaliou-se o manejo não farmacológico e a adequação do tratamento farmacológico à terapia preconizada pelo *Global Initiative for Chronic Obstructive Lung Disease* (GOLD) 2011 e pelo Caderno de Atenção Básica (CAB) do Ministério da Saúde do Brasil sobre doenças respiratórias crônicas. **Resultados:** Na maioria dos pacientes, a DPOC foi classificada como grave ou muito grave. Em relação ao manejo não farmacológico, 33% eram tabagistas, apenas 32% foram orientados a receber vacinação anti-influenza, 28% receberam vacina anti-pneumocócica, e somente 6,5% dos pacientes nas categorias GOLD B, C e D realizaram reabilitação respiratória. O tratamento farmacológico foi inadequado em 50% e 74% da amostra, respectivamente, em relação às recomendações do GOLD e do CAB. Baseado nas recomendações do GOLD, 38% eram subtratados. Baixa escolaridade, baixa renda, não utilização de oxigenoterapia e ausência de vacinação anti-influenza associaram-se a inadequação do tratamento. **Conclusões:** Não foram seguidas satisfatoriamente várias recomendações do manejo não farmacológico. Segundo o GOLD, a elevada inadequação do tratamento de manutenção foi principalmente devida ao subtratamento. No Brasil, mesmo nos casos mais graves, a otimização do tratamento da DPOC para se obter benefícios mais evidentes continua a ser um desafio.

Descritores: Doença pulmonar obstrutiva crônica/terapia; Doença pulmonar obstrutiva crônica/prevenção & controle; Protocolos clínicos.

---

*Study carried out at the Polydoro Ernani de São Thiago University Hospital, Federal University of Santa Catarina, and at the Nereu Ramos Hospital, Florianópolis, Brazil.
Correspondence to: Irai Luis Giacomelli. Departamento de Clínica Médica, Hospital Universitário da UFSC, Campus Universitário, Trindade, CEP 88040-970, Florianópolis, SC, Brasil.
Tel/Fax: 55 48 3233-2152. E-mail: iraigiacomelli@gmail.com
Financial support: None.
Submitted: 27 January 2014. Accepted, after review: 26 March 2014.

Introduction

A respiratory disease characterized by chronic airflow obstruction that is not fully reversible, with systemic manifestations, COPD is preventable and treatable, being associated with an abnormal inflammatory response (primarily to the inhalation of cigarette smoke, noxious particles, and toxic gases).[1]

The prevalence of COPD is high, as demonstrated by studies conducted in Brazil. Menezes et al. evaluated individuals over 40 years of age living in the metropolitan area of São Paulo, Brazil, and found that 15.8% had COPD.[2] It has been estimated that there are over seven million adults with COPD in Brazil.[2] The disease accounts for a large number of deaths, being the fifth leading cause of death in Brazil, and estimates indicate that COPD will have become the fourth leading cause of death in the country by the next decade.[3]

Underdiagnosis of COPD is common. In the study by Menezes et al., 88% of the patients with COPD had never undergone spirometry and therefore had an unconfirmed diagnosis of COPD.[2] In a study evaluating patients treated at primary care clinics in the city of Aparecida de Goiânia, Brazil, 71% had never undergone spirometry and therefore had an unconfirmed diagnosis of COPD.[4]

Undertreatment of COPD is also common. Of the COPD patients in the metropolitan area of São Paulo in the last decade, only 2% reported having received a physician diagnosis of COPD, and only 18% reported that they were receiving treatment for the disease.[2] The consequences of delayed, inappropriate treatment are disastrous and include increased exacerbations, loss of lung function, increased morbidity, and increased mortality.[2,5] However, data on the maintenance treatment of COPD are scarce in Brazil.[5]

In the present study, we analyzed non-pharmacological management and the appropriateness of pharmacological maintenance treatment in COPD patients prior to their hospitalization at either of two referral hospitals for the treatment of respiratory diseases in the southern Brazilian state of Santa Catarina. We chose to evaluate inpatients because we assumed that they were more likely to present with COPD that is more severe and less likely to be underdiagnosed and undertreated (as a result of their having previously sought health care).[6,7]

In this context of unquestionable epidemiological relevance; that is, given that COPD is underdiagnosed and undertreated, the present study is warranted and was aimed at analyzing non-pharmacological treatment and the appropriateness of pharmacological maintenance treatment in COPD patients prior to their hospitalization. Non-pharmacological management and pharmacological treatment were assessed on the basis of the recommendations made by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) in 2011[1] and by the Brazilian National Ministry of Health in the chronic respiratory diseases section of its *Caderno de Atenção Básica* (CAB, Primary Care Guidebook).[8] In addition, we sought to identify variables associated with inappropriate treatment in order to raise awareness to this problem and improve the treatment of COPD patients.

Methods

This was an observational, cross-sectional, analytical study conducted between December of 2012 and June of 2013 at the Polydoro Ernani de São Thiago University Hospital and the Nereu Ramos Hospital, both of which are located in the city of Florianópolis, Brazil. The aforementioned hospitals were chosen because they are referral centers for the treatment of respiratory diseases and are therefore expected to provide accurate diagnosis and appropriate treatment of COPD.

The inclusion criteria were as follows: having been diagnosed with COPD on the basis of the 2011 GOLD criteria,[1] the diagnosis being confirmed by spirometry; having been admitted to the pulmonology ward of either hospital; and agreeing to participate in the study by giving written informed consent.

We used spirometric data obtained up to six months before admission, the most recent data being chosen. In the absence of earlier spirometric data, we used the spirometric data obtained after hospital discharge.

The exclusion criteria were as follows: having been diagnosed with bronchial asthma, allergic rhinitis, or chronic lung diseases other than COPD; having any disease potentially affecting lung function; having never undergone spirometry; and not meeting the criteria for subsequent spirometry.

During hospitalization, patients completed a structured questionnaire assessing the

 http://dx.doi.org/10.1590/S1806-37132014000300005

following: demographic data; socioeconomic data; non-pharmacological treatment-related factors, including smoking cessation, influenza vaccination, pneumococcal vaccination, home oxygen therapy, and pulmonary rehabilitation; and pharmacological treatment-related factors, including the use of short-acting β_2 agonists, long-acting β_2 agonists (LABAs), short-acting anticholinergics, long-acting anticholinergics, inhaled corticosteroids (ICs), and theophylline before admission.

A total of 190 inpatients were initially selected. Of those, 53 had been diagnosed with COPD on the basis of spirometric data. Of those 53 patients, 3 were excluded because they also had asthma. The final sample consisted of 50 patients.

In order to analyze the appropriateness of pharmacological treatment, we categorized patients, on the basis of the 2011 GOLD criteria, as follows: A (low risk and fewer symptoms); B (low risk and more symptoms); C (high risk and fewer symptoms); and D (high risk and more symptoms). Symptoms were assessed with the modified Medical Research Council scale.[1] Exacerbations were defined as events requiring maintenance treatment modification.[1] Patients were further classified as having mild COPD, moderate COPD, severe COPD, or very severe COPD on the basis of the CAB.[8]

We analyzed the appropriateness of treatment, as well as undertreatment and overtreatment, by comparing the treatment given with the treatment recommended in the 2011 GOLD guidelines. Undertreatment was defined as the complete absence of pharmacological treatment or the use of drug combinations other than those recommended for each category (A, B, C, or D). Overtreatment was defined as the unwarranted use of long-acting bronchodilators and ICs. On the basis of the 2011 GOLD parameters, the sum of the proportions of undertreatment and overtreatment resulted in the proportion of inappropriate treatment. A second analysis of the overall appropriateness of pharmacological treatment was performed on the basis of the CAB parameters. We opted to use the CAB guidelines and the 2011 GOLD guidelines because they represent the latest clinical protocols for COPD nationwide and worldwide, respectively.

Statistical analysis was performed with the Statistical Package for the Social Sciences, version 15 (SPSS Inc., Chicago, IL, USA). Categorical variables were expressed as absolute numbers and proportions. We compared the distribution of categorical variables between the patients receiving appropriate treatment and those receiving inappropriate treatment on the basis of the 2011 GOLD guidelines using the chi-square test and, when necessary, Fisher's exact test. In addition, we used univariate analysis in order to identify factors potentially associated with inappropriate treatment in comparison with the treatment recommended in the 2011 GOLD guidelines, determining the crude OR and its confidence interval for that outcome. The level of significance was set at $p < 0.05$.

The present study was approved by the Human Research Ethics Committee of the Polydoro Ernani de São Thiago University Hospital (Protocol no. 2425). All participants gave written informed consent.

Results

We evaluated 50 inpatients with COPD (21 patients admitted to one of the aforementioned hospitals and 29 admitted to the other). Most of the patients were male, were over 65 years of age, were normal weight or overweight, had had up to 8 years of schooling, were retired, had a per capita family income of up to one time the national minimum wage, were former smokers, and had a smoking history of ≥ 20 pack-years. One third of the patients were active smokers. As can be seen in Table 1, most of the patients were classified as having disease that is more severe, 76% being classified into category C or D on the basis of the 2011 GOLD criteria and 64% being classified as having severe or very severe disease on the basis of the CAB criteria. Regarding knowledge of disease, 64% reported having emphysema, 64% reported having chronic bronchitis, and 40% reported having COPD.

Regarding non-pharmacological maintenance treatment before admission, 14 patients were still smokers. Most (78%) reported that they had been advised by their physicians to quit smoking. Among those who had quit smoking, 95% had done so with no medications or cognitive behavioral therapy (Table 2).

Regarding immunization, 88% of the patients had received influenza vaccination, 32% had been advised by their physicians to receive influenza vaccination, 28% had received pneumococcal vaccination, and a similar proportion of patients

Table 1 - Demographic and socioeconomic data, as well as associated factors, together with the classification of the 50 COPD patients included in the study.

Variable		n	%
Gender	Male	33	66
Age, years	≤ 64	17	34
	≥ 65	33	66
Schooling, years	≤ 8	45	90
	≥ 9	5	10
Number of times the national minimum wage/ number of people	≤ 1	31	62
	> 1	19	38
Occupational status	Not retired	17	34
	Retired	33	66
Body mass index, kg/m^2	< 18.5	5	10
	18.5-24.9	22	44
	25.0-29.9	14	28
	> 30.0	9	18
Smoking status	Smoking	14	28
	Former smoker	33	66
	Passive smoker	3	06
Duration of dyspnea, years	< 10	19	38
	≥ 10	31	62
Physician-diagnosed emphysema, bronchitis, or COPD	Yes	47	94
2011 GOLD classification	A	2	04
	B	10	20
	C	5	10
	D	33	66
CAB classification (spirometry)	Mild	1	02
	Moderate	17	34
	Severe	26	52
	Very severe	6	12

GOLD: Global Initiative for Chronic Obstructive Lung Disease; CAB: *Caderno de Atenção Básica - doenças respiratórias crônicas* (Primary Care Guidebook, respiratory diseases section).

reported having been advised by their physicians to receive pneumococcal vaccination. Home oxygen therapy was used by 16% of the patients, and only half of those used it daily for ≥ 15 h. Only 6.5% of the patients in categories B, C, and D received pulmonary rehabilitation.

Of the sample as a whole, 50% received inappropriate pharmacological treatment in comparison with that recommended in the 2011 GOLD guidelines. The proportions of inappropriate treatment in categories C and D were 60% and 33%, respectively. Of the sample as a whole, 74% received inappropriate treatment in comparison with that recommended in the CAB. Of the patients who were classified as having severe or very severe disease on the basis of the CAB guidelines, 69% and 50%, respectively, received inappropriate treatment (Table 3).

Table 2 - Non-pharmacological management prior to hospitalization in the 50 patients included in the study.

Variable	n/N	%
Having quit smoking	33/47	70
Having been advised by a physician to quit smoking	36/47	78
Having received influenza vaccination	40/50	80
Having been advised by a physician to receive influenza vaccination	16/50	32
Having received pneumococcal vaccination	14/50	28
Having been advised by a physician to receive pneumococcal vaccination	13/50	26
Being on home oxygen therapy	8/50	16
Undergoing pulmonary rehabilitation (GOLD categories B, C, and D)	3/48	6.5

GOLD: Global Initiative for Chronic Obstructive Lung Disease.

Table 4 shows the use of different classes of maintenance medication among the different 2011 GOLD categories of patients. Of the sample as a whole, 14% received no pharmacological treatment at all. In addition, only 3 (6%) used theophylline, all of whom were category D patients.

Of the sample as a whole, 38% were undertreated, 8% (patients in categories A and B) receiving no pharmacological treatment at all. For the remainder (30%), undertreatment was identified by the lack of use of short- or long-acting bronchodilators, as well as by a slight predominance of lack of IC use in patients in categories C and D. Overtreatment was found in 12% of the patients (in categories A and B), all of whom used ICs and 1 of whom used an LABA + IC combination (LABA + IC).

In the univariate analysis, inappropriate pharmacological treatment (on the basis of the 2011 GOLD criteria) was significantly associated with up to 8 years of schooling, a per capita family income of up to one time the national minimum wage, and category A or B (i.e., less frequent exacerbations and FEV_1 ≥ 50% of predicted),[1] as can be seen in Table 5. Appropriate pharmacological treatment was significantly associated with the use of home oxygen therapy (OR = 9.33; 95% CI: 1.05-82.78) and influenza vaccination (OR = 5.41; 95% CI: 1.02-28.79), as well as with self-reported physician-diagnosed emphysema.

 http://dx.doi.org/10.1590/S1806-37132014000300005

Discussion

The objective of the present study was to examine the appropriateness of maintenance treatment prior to hospitalization in patients with COPD that is more severe, a distinct strategy being used in order to select inpatients. Several aspects of the recommended non-pharmacological treatment were not followed. One third of the patients were smokers, a small proportion (28%) had received pneumococcal vaccination, and very few (6.5%) of the patients in category B, C, or D were undergoing pulmonary rehabilitation. Of the sample as a whole, 50% received inappropriate treatment in comparison with that recommended in the 2011 GOLD guidelines, undertreated patients having predominated. The proportion of patients receiving inappropriate treatment was even higher (i.e., 74%) when the CAB was used as a reference for treatment. Inappropriate treatment was found to be associated with a low level of education and low income. Appropriate treatment was found to be associated with physician-diagnosed emphysema, use of home oxygen therapy, and influenza vaccination.

The present study is the first of its kind in Brazil. In the international literature, there are few studies addressing the appropriateness of COPD treatment.[2] In the present study, data

Table 3 - Inappropriate pharmacological treatment for each COPD severity category in the 50 patients included in the study.

Classification	Category	Patients receiving inappropriate treatment (n/N)	%
2011 GOLD	A	2/2	100
	B	9/10	90
	C	3/5	60
	D	11/33	33
	Total	25/50	50
CAB	Mild	1/1	100
	Moderate	15/17	88
	Severe	18/26	69
	Very severe	3/6	50
	Total	37/50	74

GOLD: Global Initiative for Chronic Obstructive Lung Disease; and CAB: *Caderno de Atenção Básica - doenças respiratórias crônicas* (Primary Care Guidebook, respiratory diseases section).

Table 4 - Pharmacological treatment regimens used for the different categories of COPD patients, classified on the basis of the 2011 Global Initiative for Chronic Obstructive Lung Disease guidelines.[a]

Categories	Patients (n)	SABA	SAA	SABA + SAA	LABA	LAA	LABA + LAA	IC	LABA + IC	LAA + IC	LABA + LAA + IC
A	2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
B	10	2 (20)	0 (0)	3 (30)	1 (10)	0 (0)	0 (0)	0 (0)	1 (10)	0 (0)	3 (30)
C	5	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	0 (0)	1 (20)	0 (0)	1 (20)
D	33	16 (48)	6 (2)	7 (21)	1 (3)	2 (6)	1 (3)	2 (6)	4 (12)	2 (6)	13 (39)
Total	50	19 (38)	4 (2)	10 (20)	2 (4)	2 (4)	2 (4)	2 (4)	7 (14)	2 (4)	17 (34)

SABA: short-acting β_2 agonist; SAA: short-acting anticholinergic; LABA: long-acting β_2 agonist; LAA: long-acting anticholinergic; and IC: inhaled corticosteroid. [a]Values expressed as n (%).

Table 5 - Variables associated with inappropriate treatment in comparison with the treatment recommended in the 2011 Global Initiative for Chronic Obstructive Lung Disease guidelines.

Variable		Treatment[a]		p	OR	95% CI
		inappropriate	appropriate			
Per capita income, number of times the national minimum wage	≤ 1	14 (28)	6 (12)	0.021	4.03	1.20-13.53
	> 1	11 (22)	19 (38)			
Schooling, years	≤ 8	25 (50)	20 (40)	0.018	1.25	1.03-1.52
	> 8	0 (0)	5 (10)			
Physician-diagnosed emphysema	Yes	11 (22)	21 (42)	0.003	0.15	0.04-0.57
	No	14 (28)	4 (8)			
GOLD category	A or B	11 (22)	1 (2)	0.001	18.86	2.20-161.99
	C or D	14 (28)	24 (48)			

GOLD: Global Initiative for Chronic Obstructive Lung Disease.

collection was standardized, the same researchers having administered the questionnaire at both hospitals.

In a study of the distribution of COPD patients by disease severity in Brazil,[2] a household survey conducted in the city of São Paulo showed that the prevalence rates of mild, moderate, severe, and very severe COPD were 10.1%, 4.6%, 0.9%, and 0.2%, respectively. As expected (because of the strategy adopted), the prevalence rates in the present study were different from those in the general population: severe and very severe COPD (52% and 12%, respectively); and categories C and D (10% and 66%, respectively). Therefore, patients with COPD that is more severe predominated, the benefits of maintenance treatment being greater in such patients.[5]

The sociodemographic characteristics of the COPD patients in the present study were similar to those of those in the study by Menezes et al., who also found a higher prevalence of COPD among males who were over 65 years of age and had a significant smoking history.[2]

Various studies have shown that clinical protocols are not widely implemented. A study conducted in Brazil showed that 34% of general practitioners do not use clinical protocols for the management of COPD.[9] Failure to follow the guidelines for the non-pharmacological maintenance treatment of COPD is consistent with the results of the present study; for example, 33% of the patients had not quit smoking, although smoking cessation has an impact on the rates of disease progression and mortality. Similar data are found in the international literature.[2] In the present study, the proportion of patients who had been advised by their physicians to receive influenza and pneumococcal vaccination was low. Similarly, Menezes et al. found that only 30.6% of the COPD patients had received influenza vaccination, the proportions of vaccinated patients with severe and very severe COPD being 39% and 46%, respectively.[2] A study evaluating COPD patients with respiratory failure admitted to the ICU also showed low vaccination rates, only 66.66% having received influenza vaccination and only 45.83% having received pneumococcal vaccination.[10] Only 6.5% of the patients in the present study received pulmonary rehabilitation, which is recommended in the 2011 GOLD guidelines for patients in categories B, C, and D. Other studies have shown low rates of pulmonary rehabilitation. In one study, only 14% of the patients hospitalized for COPD exacerbation had received prior pulmonary rehabilitation.[11]

In the present study, it was impossible to evaluate the need for home oxygen therapy, given that we had no access to previous blood gas analyses. In addition, it is impossible to confirm the need for home oxygen therapy during clinical instability.[1] Given the large proportion of patients with FEV_1 < 50% of predicted (i.e., 64%) and the large proportion of category D patients (i.e., 66%), we can assume that the proportion of patients requiring home oxygen therapy was larger than that actually found (i.e., 16%). However, it is of note that half of the patients who were on home oxygen therapy did not receive it for as many hours per day as recommended, i.e. > 15 h.[1]

On the basis of the guidelines analyzed in the present study, pharmacological treatment was found to be inappropriate in 50-74% of the patients. Our data regarding inappropriate COPD treatment are consistent with those found in the literature. One group of authors evaluated patients who had moderate, severe, or very severe COPD and who had health insurance; the authors found that 43% of the patients had received medication prescriptions that were not in accordance with clinical protocols and that 51% had not obtained the medication.[12]

One reason for the difference between the two guidelines used in the present study in terms of the proportion of patients receiving inappropriate treatment is that the CAB does not recommend IC use for patients with moderate obstruction. However, in the present study, the patients with moderate obstruction had had a high number of exacerbations in the previous year and were therefore included in GOLD category C or D, for which IC use is recommended in the GOLD guidelines, meaning that a proportion of patients classified as having moderate COPD and into categories C and D used ICs. Using the 2011 GOLD guidelines as a reference for pharmacological treatment, we found that 38% of the patients were undertreated, undertreatment constituting the most common instance of inappropriate pharmacological treatment. In one third of those patients, treatment inappropriateness was due to the complete absence of pharmacological treatment. In the remaining patients, treatment inappropriateness was due to the lack of use of

 http://dx.doi.org/10.1590/S1806-37132014000300005

at least one of the classes of drugs recommended in the GOLD guidelines.

It is known that COPD is underdiagnosed—88% of the COPD patients in the metropolitan area of São Paulo had an unconfirmed diagnosis of COPD—and undertreated.[2] The present study included only patients with a diagnosis of COPD confirmed by spirometry; a confirmed diagnosis might be, in and of itself, a determining factor for better treatment. It is possible that the problem of undertreatment is even worse in patients with milder disease treated at less advanced centers and without a confirmed diagnosis.

Overtreatment (i.e., inappropriate IC use) was found in 12% of the patients (GOLD categories A and B). This finding is relevant because overtreatment affords no clear benefits and increases the risk of hospitalization for pneumonia, as well as increasing the incidence of oral candidiasis, ecchymosis, and cataracts.[13-16] A recent systematic review showed that there is evidence for IC use only in patients with severe COPD and symptoms and in those with moderate or severe COPD, symptoms, and more than two COPD exacerbations per year.[5] Incorrect prescription of LABA + IC is common among patients with moderate disease treated at primary or secondary health care facilities.[17-20] Studies have shown that 45% of patients with moderate COPD receive ICs only, whereas 60% receive LABA + IC.[21,22] In the present study, half of the patients in categories A and B used LABA + IC. One study showed that 50% of COPD patients admitted to the ICU for respiratory failure used systemic corticosteroids, meaning that at least 50% of the patients had received inappropriate non-pharmacological treatment.[10] This reinforces the hypothesis that the use of clinical protocols is low, given that none of the major clinical protocols in Brazil include the use of systemic corticosteroids as a treatment option.[1,8]

Variables such as a low level of education and low income were significantly associated with a higher proportion of patients receiving inappropriate treatment. Given the context, this was expected. Inappropriate treatment was found to be significantly associated with being in category A or B. Given that the proportion of patients in those categories was low in the present study, it is impossible to determine whether treatment is most inappropriate in patients with disease that is less severe (in whom the number of hospitalizations is lower); if this is indeed the case, then health care is primarily focused on advanced disease. In contrast, appropriate treatment was significantly associated with home oxygen therapy and influenza vaccination. The external validity of the present study could have been higher if the number of participants had been larger. In addition, for the evaluation of maintenance treatment, different recruitment strategies can be used in order to increase the representation of all GOLD categories.

In the present study, a physician diagnosis of emphysema was associated with an increased rate of patients receiving appropriate treatment. This might be due to the fact that patients give greater weight to the term emphysema and therefore seek treatment more often.

The present study was conducted before the approval of the 2013 Brazilian National Ministry of Health clinical protocol and therapeutic guidelines for COPD and, therefore, before the proposal for providing COPD patients with specific medication.[23] The present study can therefore serve as a historical control for future epidemiological studies and contribute to the reorganization of COPD care.

On the basis of the evidence presented here, we conclude that the non- pharmacological management of COPD is currently unsatisfactory, as demonstrated by the significant proportion of active smokers, the low use of pneumococcal vaccination, and the lack of use of pulmonary rehabilitation. The same is true for the pharmacological treatment of COPD, the high rate of patients receiving inappropriate treatment being mainly due to undertreatment. Even in severe COPD cases, optimizing treatment to achieve greater benefits continues to be a challenge. Therefore, there is a need for further educating physicians and patients regarding COPD, as well as a need for more smoking cessation programs and pulmonary rehabilitation centers, together with a need for increasing the availability of medications for the treatment of COPD.

References

1. Global Initiative for Chronic Obstructive Lung Disease - GOLD [homepage on the Internet]. Bethesda: Global Initiative for Chronic Obstructive Lung Disease [cited 2011 Dec 26]. Global Strategy for the Diagnosis, Management, and Prevention of COPD - Revised 2011. [Adobe Acrobat document, 90p.]. Available from: http://www.goldcopd.org/uploads/users/files/GOLD_Report_2011_Feb21.pdf.

2. Menezes AM, Perez-Padilla R, Jardim JR, Mui-o A, Lopez MV, Valdivia G, et al. Chronic obstructive pulmonary disease in five Latin American cities (the PLATINO study): a prevalence study. Lancet. 2005;366(9500):1875-81. http://dx.doi.org/10.1016/S0140-6736(05)67632-5
3. Sociedade Brasileira de Pneumologia e Tisiologia. II Consenso Brasileiro de Doença Pulmonar Obstrutiva Crônica (DPOC) - 2004. J Bras Pneumol. 2004;30(5):1-42.
4. Queiroz MC, Moreira MA, Rabahi MF. Underdiagnosis of COPD at primary health care clinics in the city of Aparecida de Goiânia, Brazil. J Bras Pneumol. 2012;38(6):692-9. http://dx.doi.org/10.1590/S1806-37132012000600003
5. Menezes AM, Macedo SE, Noal RB, Fiterman JC, Cukier A, Chatikin JM, et al. Pharmacological treatment of COPD. J Bras Pneumol. 2011;37(4):527-43. http://dx.doi.org/10.1590/S1806-37132011000400016
6. Parker CM, Voduc N, Aaron SD, Webb KA, O'Donnell DE. Physiological changes during symptom recovery form moderate exacerbations of COPD. Eur Respir J. 2005;26(3):420-8. http://dx.doi.org/10.1183/09031936.05.00136304
7. Barberà JA, Roca J, Ferrer A, Félez MA, Díaz O, Roger N, et al. Mechanisms of worsening gas exchange during acute exacerbations of chronic obstructive pulmonary disease. Eur Respir J. 1997;10(6):1285-91. http://dx.doi.org/10.1183/09031936.97.10061285
8. Brasil. Ministério da Saúde. Secretaria de Políticas de Saúde. Departamento de Atenção Básica. Doenças respiratórias crônicas. Cadernos de atenção básica no 25. Brasília: Ministério da Saúde; 2010.
9. Aisanov Z, Bai C, Bauerle O, Colodenco FD, Feldman C, Hashimoto S, et al. Primary care physician perceptions on the diagnosis and management of chronic obstructive pulmonary disease in diverse regions of the world. Int J Chron Obstruct Pulmon Dis. 2012;7:271-82.
10. Pincelli MP, Grumann AC, Fernandes C, Cavalheiro AG, Haussen DA, Maia IS. Characteristics of COPD patients admitted to the ICU of a referral hospital for respiratory diseases in Brazil. J Bras Pneumol. 2011;37(2):217-22.
11. Donner CF, Virchow JC, Lusuardi M. Pharmacoeconomics in COPD and inappropriateness of diagnostics, management and treatment. Respir Med. 2011;105(6):828-37. http://dx.doi.org/10.1016/j.rmed.2010.12.017
12. Price L, Billups SJ, Rice MA, Hartsfield C. Investigation of barriers to clinical practice guideline-recommended pharmacotherapy in the treatment of COPD. Pharmacy Practice. 2007;5(2):74-7.
13. Chung KF, Caramori G, Adcock IM. Inhaled corticosteroids as combination therapy with beta-adrenergic agonists in airways disease: present and future. Eur J Clin Pharmacol. 2009;65(9):853-71. http://dx.doi.org/10.1007/s00228-009-0682-z
14. Clark DJ, Grove A, Cargill RI, Lipworth BJ. Comparative adrenal suppression with inhaled budesonide and fluticasone propionate in adult asthmatic patients. Thorax. 1996;51(3):262-6. http://dx.doi.org/10.1136/thx.51.3.262
15. Calverley P, Pauwels R, Vestbo J, Jones P, Pride N, Gulsvik A, et al. Combined salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease: a randomized controlled trial. Lancet. 2003;361(9356):449-56. http://dx.doi.org/10.1016/S0140-6736(03)12459-2
16. Ernst P, Gonzalez AV, Brassard P, Suissa S. Inhaled corticosteroid use in chronic obstructive pulmonary disease and the risk of hospitalization for pneumonia. Am J Respir Crit Care Med. 2007;176(2):162-6. http://dx.doi.org/10.1164/rccm.200611-1630OC
17. Glaab T, Banik N, Rutschmann OT, Wencker M. National survey of guideline-compliant COPD management among pneumologists and primary care physicians. COPD. 2006;3(3):141-8. http://dx.doi.org/10.1080/15412550600829299
18. Rutschmann OT, Janssens JP, Vermeulen B, Sarasin FP. Knowledge of guidelines for the management of COPD: a survey of primary care physicians. Respir Med. 2004;98(10):932-7. http://dx.doi.org/10.1016/j.rmed.2004.03.018
19. Jones RC, Dickson-Spillmann M, Mather MJ, Marks D, Shackell BS. Accuracy of diagnostic registers and management of chronic obstructive pulmonary disease: the Devon primary care audit. Respir Res. 2008;9:62. http://dx.doi.org/10.1186/1465-9921-9-62
20. Bourbeau J, Sebalt RJ, Day A, Bouchard J, Kaplan A, Hernandez P, et al. Practice patterns in the management of chronic obstructive pulmonary disease in primary practice: the CAGE study. Can Respir J. 2008;15(1): 13-9.
21. Decramer M, Celli B, Kesten S, Lystig T, Mehra S, Tashkin DP, et al. Effect of tiotropium on outcomes in patients with moderate chronic obstructive pulmonary disease (UPLIFT): a prespecified subgroup analysis of a randomized controlled trial. Lancet. 2009;374(9696):1171-8. http://dx.doi.org/10.1016/S0140-6736(09)61298-8
22. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. N Engl J Med. 2008;359(15):1543-54. http://dx.doi.org/10.1056/NEJMoa0805800
23. Portal da Saúde [homepage on the Internet]. Brasília: Ministério da Saúde [cited 2012 Nov 13]. Protocolo clínico e diretrizes terapêuticas doença pulmonar obstrutiva crônica. Available from: http://portal.saude.gov.br/portal/saude/profissional/visualizar_texto.cfm?idtxt=35115

About the authors

Irai Luis Giacomelli
Medical Student. Federal University of Santa Catarina, Florianópolis, Brazil.

Leila John Marques Steidle
Adjunct Professor. Federal University of Santa Catarina, Florianópolis, Brazil.

Frederico Fernandes Moreira
Medical Student. Federal University of Santa Catarina, Florianópolis, Brazil.

Igor Varela Meyer
Medical Student. Federal University of Santa Catarina, Florianópolis, Brazil.

Ricardo Goetten Souza
Medical Student. Federal University of Santa Catarina, Florianópolis, Brazil.

Mariângela Pimentel Pincelli
Professor. Department of Clinical Medicine, Federal University of Santa Catarina, Florianópolis, Brazil.

Effects of acute and chronic administration of methylprednisolone on oxidative stress in rat lungs*,**

Efeitos da administração aguda e crônica de metilprednisolona no estresse oxidativo em pulmões de ratos

Ronaldo Lopes Torres, Iraci Lucena da Silva Torres, Gabriela Laste, Maria Beatriz Cardoso Ferreira, Paulo Francisco Guerreiro Cardoso, Adriane Belló-Klein

Abstract

Objective: To determine the effects of acute and chronic administration of methylprednisolone on oxidative stress, as quantified by measuring lipid peroxidation (LPO) and total reactive antioxidant potential (TRAP), in rat lungs. **Methods:** Forty Wistar rats were divided into four groups: acute treatment, comprising rats receiving a single injection of methylprednisolone (50 mg/kg i.p.); acute control, comprising rats i.p. injected with saline; chronic treatment, comprising rats receiving methylprednisolone in drinking water (6 mg/kg per day for 30 days); and chronic control, comprising rats receiving normal drinking water. **Results:** The levels of TRAP were significantly higher in the acute treatment group rats than in the acute control rats, suggesting an improvement in the pulmonary defenses of the former. The levels of lung LPO were significantly higher in the chronic treatment group rats than in the chronic control rats, indicating oxidative damage in the lung tissue of the former. **Conclusions:** Our results suggest that the acute use of corticosteroids is beneficial to lung tissue, whereas their chronic use is not. The chronic use of methylprednisolone appears to increase lung LPO levels.

Keywords: Lung; Methylprednisolone; Glucocorticoids; Lipid peroxidation; Antioxidant response elements.

Resumo

Objetivo: Determinar os efeitos da administração aguda e crônica de metilprednisolona no estresse oxidativo, por meio da quantificação da peroxidação lipídica (POL) e do potencial antioxidante reativo total (PART), em pulmões de ratos. **Métodos:** Quarenta ratos Wistar foram divididos em quatro grupos: tratamento agudo, com ratos recebendo uma dose única de metilprednisolona (50 mg/kg i.p.); controle agudo, com ratos recebendo injeção unida de salina; tratamento crônico, com ratos recebendo metilprednisolona v.o. na água do bebedouro (6 mg/kg por dia durante 30 dias; e controle crônico, com ratos recebendo água de bebedouro normal). **Resultados:** Os níveis de PART foram significativamente maiores no grupo tratamento agudo que no grupo controle agudo, sugerindo uma melhora do sistema de defesa pulmonar. Os níveis de POL foram significativamente maiores no grupo tratamento crônico que no grupo controle crônico, indicando dano oxidativo no tecido pulmonar. **Conclusões:** Nossos resultados sugerem que o uso agudo de corticoides foi benéfico aos tecidos pulmonares, enquanto seu uso crônico não o foi. O uso crônico de metilprednisolona parece aumentar os níveis pulmonares da POL.

Descritores: Pulmão; Metilprednisolona; Glucocorticoides; Peroxidação de lipídeos; Elementos de resposta antioxidante.

Introduction

Corticosteroids are extensively used in a wide range of respiratory tract disorders, such as asthma, allergic rhinitis, and COPD.[1] It has been observed that acute treatment with corticosteroids can suppress inflammatory processes and reactive oxygen species (ROS) production. [2] In a recent study,[3] it was shown that the administration of dexamethasone decreases lung tissue malondialdehyde production after ischemia/reperfusion injury and protects cellular levels

*Study carried out in the Department of Pharmacology, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Correspondence to: Iraci Lucena da Silva Torres. Departamento de Farmacologia, ICBS, UFRGS, Rua Sarmento Leite, 500, Sala 202, CEP 90050-170, Porto Alegre, RS, Brasil.
Tel. 55 51 3308-3183. Fax: 55 51 3308-3121. E-mail: iracitorres@gmail.com

Financial support: This study received financial support from the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development) and from the *Fundação de Amparo à Pesquisa do Rio Grande do Sul* (FAPERGS, Foundation for the Support of Research in the state of Rio Grande do Sul).
Submitted: 21 October 2013. Accepted, after review: 12 May 2014.

**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

 http://dx.doi.org/10.1590/S1806-37132014000300006

of antioxidant enzymes. In addition, short-term administration of prednisolone or dexamethasone has been shown to inhibit ROS generation in platelets, and there is evidence that steroids inhibit oxidative phosphorylation.[4] It has been suggested that the long-term use of corticosteroids at low doses (1-2 mg/kg per day) can benefit the lungs and reduce the risk of systemic side effects in patients with acute respiratory distress syndrome,[5] whereas acute administration of high doses of corticosteroids has been found to produce no benefits in such patients.[6]

Chronic treatment with corticosteroids can induce a variety of symptoms and signs (side effects), including truncal obesity, facial swelling ("moon face"), cutaneous striae, hirsutism, cataract, osteoporosis, myopathy, diabetes mellitus, immunosuppression, and cardiovascular disorders.[7] Excess corticosteroid use can also induce overproduction of ROS by endothelial cells.[8]

It is well known that corticosteroids have anti-inflammatory effects, some of which can be mediated by ROS, which are products of normal metabolic processes in cells. The major sources of ROS are leakages from the electron transport chain in mitochondria and endoplasmic reticulum. Another important source of ROS is a membrane-associated NADH/NADPH oxidase. At low concentrations, ROS act as physiological mediators of cellular responses and regulators of gene expression.[4] The imbalance between the production of ROS and antioxidant defenses leads to oxidative stress.[9] Oxidative stress has been implicated as an important pathologic factor in pulmonary, neurodegenerative, and autoimmune diseases, as well as in metabolic disorders, cancer, and aging.[10-12] It is well known that ROS generate a biochemical cascade, producing lipid peroxidation (LPO), protein oxidation, DNA damage, and cell death, all of which can contribute to the occurrence of pathological conditions associated with a marked increase in ROS and other free radicals,[13] such as ischemia/reperfusion-induced lung injury.[14] Therefore, ROS play a crucial role in the cascade of events that lead to lung failure.

Taking all of the above into account, we conducted the present study with the objective of determining the effect of acute and chronic administration of methylprednisolone on oxidative stress. To that end, we quantified LPO and total reactive antioxidant potential (TRAP) in rat lungs.

Methods

Forty experimentally naive adult (60-day-old) male Wistar rats (200-250 g) were randomized by weight and housed in groups of five in polypropylene home cages (49 × 34 ×16 cm). All animals were maintained on a standard 12/12-h light/dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.) in a temperature-controlled environment (22 ± 2°C) and were given *ad libitum* access to water and chow. All experiments and procedures were approved by the institutional animal care and use committee and were in compliance with the Brazilian guidelines involving the use of animals in research (Law no. 11,794) and with international guidelines. Vigorous attempts were made to minimize animal suffering and to decrease external sources of pain and discomfort, as well as to use only the number of animals required in order to produce reliable scientific data.

We used methylprednisolone sodium succinate (Solu-Medrol®, Pharmacia, New York, NY, USA). The lyophilized powder (500 mg) was dissolved in 8 mL of 0.9% saline solution. The drug solution was prepared immediately prior to its administration.

In the acute treatment experiment, the animals were divided into two groups (n = 10 each). The rats in one group (the acute treatment group) received a single injection of methylprednisolone (50 mg/kg, i.p.) in a volume of 1 mL/kg of the solution, whereas those in the other group (the acute control group) were injected with an equal volume of saline (i.p.).

In the chronic treatment experiment, the animals were divided into two groups (n = 10 each). The rats in one group (the chronic treatment group) received methylprednisolone (6 mg/kg per day, p.o.) in drinking water for 30 days, whereas those in the other group (the chronic control group) received drinking water only. Each 500 mL of the drinking water contained 31 mg of methylprednisolone sodium succinate (0.0625 mg/mL). Considering a mean consumption of 25 mL/day per rat, each chronic treatment group rat consumed 1.56 mg of methylprednisolone per day.

At 24 h after acute administration or at the end of the chronic treatment period, the animals were killed by decapitation. The lungs were extracted and frozen by immersion in liquid nitrogen. Samples were stored at −80°C until analysis. The lungs were weighed and homogenized at 1:5 w/v in ice-cold (1.15% KCl and 20 mmol/L phenylmethylsulfonyl fluoride) fluid using an

Ultra-Turrax homogenizer (IKA, Toronto, Ontario, Canada). To remove the particulate fraction, the homogenates were centrifuged at 1,000 g for 20 min at 0-4°C, and the supernatant was used for LPO, TRAP, and protein content assays.[15]

The level of TRAP was determined by measuring luminol chemiluminescence intensity induced by the thermolysis of 2,2'-azobis(2-amidinopropane) dihydrochloride.[16] The results are expressed as µM of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid per mg of protein. We quantified LPO using chemiluminescence. The method is highly sensitive and capable of detecting small amounts of peroxidation products. Chemiluminescence was measured in a liquid scintillation counter using the out-of-coincidence mode (LKB Rack Beta Liquid Scintillation Spectrometer 1215; LKB Produkter AB, Bromma, Sweden). The reactions were started by the addition of 3 mmol/L tert-butyl hydroperoxide, and the data are expressed as counts per second (cps) per mg of protein in the homogenate.[17] Protein levels were measured with the method devised by Lowry et al.,[18] and bovine serum albumin was used as the standard.

The data are expressed as mean ± SE and statistically evaluated using the Student's t-test. Values of $p < 0.05$ were considered significant.

Results

We first evaluated the effect of acute treatment with methylprednisolone on the levels of TRAP and LPO in rat lungs. A significant (20%) increase was observed in total TRAP levels in the treated group ($p < 0.05$; Figure 1). No significant difference was found between the groups regarding LPO levels ($p > 0.05$; Figure 2).

We found no difference between the chronic treatment group and the chronic control group in terms of the total TRAP levels ($p > 0.05$; Figure 3). The degree of pulmonary oxidative damage, as assessed by chemiluminescence, was significantly (38%) greater in the chronic treatment group than in the chronic control group ($p < 0.05$; Figure 4).

Discussion

Antioxidant concentrations in the lungs can be quantified by determining the level of TRAP.[10,14] The relative concentration of antioxidants determines the total tissue antioxidant capacity. The TRAP level primarily represents non-enzymatic water-soluble antioxidants in the tissue. In addition, the level of LPO, which plays an important role in the induction of free radical formation and apoptosis,[19] is widely used as a marker of oxidative stress.

The results of the present study show that the duration of corticosteroid treatment alters the oxidative system responses in the lungs of rats. Acute treatment with methylprednisolone induced a significant increase in TRAP levels in rat lungs without any changes in LPO levels. However, when the treatment was maintained for 30 days, we observed an increase in LPO levels without any changes in TRAP levels, which increases the risk of oxidative lung injury. Nevertheless, when animals were submitted to methylprednisolone treatment for 15 days at a lower dose, none of those effects were observed (data not shown).

The increased antioxidant potential induced by short-term administration of methylprednisolone might represent a mechanism of protection against ROS generation after exposure to corticosteroids. ROS can be generated as a consequence of the intracellular metabolism of foreign compounds, toxins, or drugs by the cytochrome P450 monooxygenase system, as well as because of exposure to environmental factors, such as excessive iron salts or UV irradiation.[20] Intracellular antioxidants, cell membranes, and extracellular fluids can be upregulated and mobilized in order to neutralize excessive and inappropriate ROS formation. To provide extracellular antioxidant defense mechanisms, respiratory tract epithelial cells synthesize and secrete various antioxidant enzymes, such as extracellular forms of superoxide dismutase[21] and glutathione peroxidase,[22] as well as several metal-binding proteins (e.g., transferrin and ceruloplasmin) that minimize the involvement of transition metal ions (e.g., iron and copper) in oxidative reactions.[21] In addition, the extracellular epithelial lining fluid also contains various non-enzymatic antioxidant systems, including vitamin C (ascorbate) and vitamin E (alpha-tocopherol).[23] The TRAP assay employed in the current study is widely used,[10,14,24] and it mostly measures non-enzymatic water-soluble antioxidants, such as glutathione, ascorbic acid, and uric acid. The measurement of all of these antioxidants is essential for assessing antioxidant status. However, the number of different antioxidants in biological samples makes it difficult to measure each separately.

 http://dx.doi.org/10.1590/S1806-37132014000300006

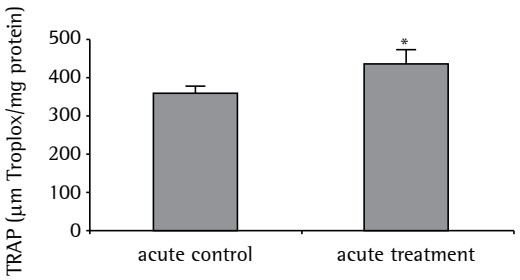


Figure 1 - Mean levels of total reactive antioxidant potential (TRAP) in the lungs of rats subjected to acute administration of methylprednisolone (acute treatment group) or injected with an equal volume of saline (acute control group). Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid. *p < 0.05, Student's t-test.

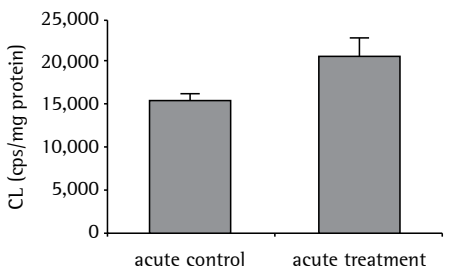


Figure 2 - Mean levels of lipid peroxidation in the lungs of rats subjected to acute administration of methylprednisolone (acute treatment group) or injected with an equal volume of saline (acute control group). CL: chemiluminescence; and cps: counts per second.

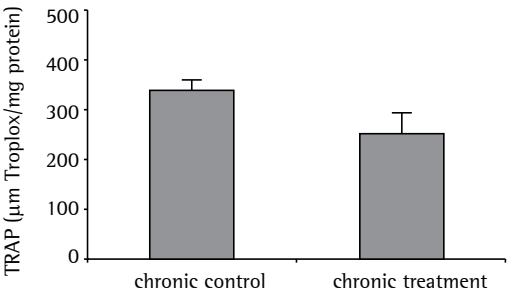


Figure 3 - Mean levels of total reactive antioxidant potential (TRAP) in the lungs of rats subjected to chronic administration of oral methylprednisolone (chronic treatment group) or not (chronic control group). Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

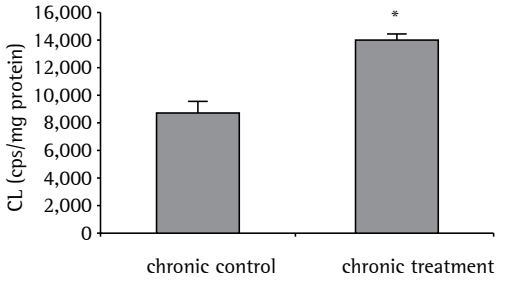


Figure 4 - Mean levels of lipid peroxidation in the lungs of rats subjected to chronic administration of oral methylprednisolone (chronic treatment group) or not (chronic control group). CL: chemiluminescence; and cps: counts per second. *p < 0.05, Student's t-test.

In addition, the possible interaction among different antioxidants can make measurements of individual antioxidants less representative than is the overall antioxidant status.[24]

Our results corroborate those of previous studies, suggesting that short-term administration of corticosteroids is protective against oxidative injury in different tissues in experimental models.[24] The short-term administration of prednisolone and dexamethasone has been shown to inhibit ROS generation in platelets, and there is evidence that corticosteroids also inhibit oxidative phosphorylation.[4] In contrast, we found that 30 days of methylprednisolone treatment increased LPO levels. The chemiluminescence assay is the easiest method and can be applied to crude biological extracts. Although its specificity has been questioned,[25] this particular assay is widely used for ex vivo and in vitro measurements,[10] and it is accepted as an empirical window for the examination of the complex process of LPO.[25] However, the imbalance between production of ROS and antioxidant defenses in the body is called oxidative stress, which has major health implications.[19] If there are too many ROS or too few antioxidants for protection, oxidative stress develops, which can cause permanent damage.[26] Although the differences were less than significant, we found that long-term administration of a corticosteroid induced a decrease in TRAP levels and an increase in LPO levels, suggesting that oxidative stress occurred.

One of the earliest and most important components of tissue injury after reperfusion of ischemic organs is ROS production. The major ROS include the superoxide radical, the hydroxyl radical, and hydrogen peroxide. ROS-induced injury targets proteins, enzymes, nucleic acids, cytoskeleton, cell membranes, and lipid peroxides, resulting in decreased mitochondrial function and LPO.[27] The damage caused by ROS leads to the loss of microvascular integrity and decreased

blood flow. The pathogenesis of the various forms of lung injury has been shown to involve peroxidative breakdown of polyunsaturated fatty acids (due to the effects on membrane function); inactivation of membrane-bound receptors and enzymes; and increased tissue permeability.[28] There is increasing evidence that aldehydes, which are generated endogenously during the LPO process, are involved in many of the pathophysiological events associated with oxidative stress in cells and tissues.[29] In addition to their cytotoxic properties, lipid peroxides have been increasingly recognized as being important in signal transduction for a number of important events in the lung inflammatory response.[30] The oxidative pathway was reported to play a significant role in the etiology of remote lung injury in a rabbit model of hepatoenteric ischemia-reperfusion, as well as in other animal models.[31]

It is important to emphasize that, by choosing two different administration regimens of methylprednisolone (acute and chronic), we sought to simulate the parenteral administration of high doses, which might be warranted in emergencies, such as in severe acute asthma, and a moderate oral dose, which is used under less urgent circumstances in humans. It should be borne in mind that drug metabolism is more rapid in small animals than in humans, and larger doses are therefore necessary.[32] Nevertheless, the fact that we used different drug dose regimens in the two treatments represents a limitation of the present study, because it constitutes a confounding variable.

In conclusion, our results suggest that the acute use of corticosteroids is beneficial to lung tissue, whereas their chronic use is not. In addition, we found that acute administration of methylprednisolone increased antioxidant levels in the lung tissue in rats, which is an important finding, considering the use of this medication in acute events and in lung transplantation. Conversely, the negative effect that chronic treatment with methylprednisolone has on LPO might play a role in the mechanisms of the adverse effects involved in pathological conditions associated with the chronic use of glucocorticoids. Future studies using rat models of ischemia/reperfusion injury in lungs might elucidate the differences between acute and chronic use of corticosteroids, in terms of the mechanisms by which they act on a pathological condition.

References

1. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids--new mechanisms for old drugs. N Engl J Med. 2005;353(16):1711-23. http://dx.doi.org/10.1056/NEJMra050541
2. Simons SS Jr. What goes on behind closed doors: physiological versus pharmacological steroid hormone actions. Bioessays. 2008; 30(8):744-56. http://dx.doi.org/10.1002/bies.20792
3. Taghizadieh M, Hajipour B, Asl NA, Khodadadi Ali, Somi MH. Co-administration of melatonin and dexamethasone attenuates lung tissue injury after liver ischemia/reperfusion. Life Sci J. 2013;10(6s):314-20.
4. Sanner BM, Meder U, Zidek W, Tepel M. Effects of glucocorticoids on generation of reactive oxygen species in platelets. Steroids. 2002;67(8):715-9. http://dx.doi.org/10.1016/S0039-128X(02)00024-7
5. Wajanaponsan N, Reade MC, Milbrandt EB. Steroids in late ARDS? Crit Care. 2007;11(4):310. http://dx.doi.org/10.1186/cc5954
6. Bernard GR, Luce JM, Sprung CL, Rinaldo JE, Tate RM, Sibbald WJ, et al. High-dose corticosteroids in patients with the adult respiratory distress syndrome. N Engl J Med. 1987; 317(25):1565-70. http://dx.doi.org/10.1056/NEJM198712173172504
7. Schäcke H, Döcke WD, Asadullah K. Mechanisms involved in the side effects of glucocorticoids. Pharmacol Ther. 2002;96(1):23-43. http://dx.doi.org/10.1016/S0163-7258(02)00297-8
8. Iuchi T, Akaike M, Mitsui T, Ohshima Y, Shintani Y, Azuma H, et al. Glucocorticoid excess induces superoxide production in vascular endothelial cell and elicits vascular endothelial dysfunction. Circ Res. 2003;92(1):81-7. http://dx.doi.org/10.1161/01.RES.0000050588.35034.3C
9. Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry (Mosc.). 2005;70:200-14. http://dx.doi.org/10.1007/s10541-005-0102-7
10. Latini A, Scussiato K, Rosa RB, Liesuy S, Bello-Klein A, Dutra-Filho CS, et al. D-2-hydroxyglutaric acid induces oxidative stress in cerebral cortex of young rats. Eur J Neurosci. 2003;17(10):2017-22. http://dx.doi.org/10.1046/j.1460-9568.2003.02639.x
11. Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature. 2008;451(7182):1069-75. http://dx.doi.org/10.1038/nature06639
12. Dourado VZ, Tanni SE, Vale AS, Faganello MM, Sanchez FF, Godoy I. Systemic manifestations in chronic obstructive pulmonary disease. J Bras Pneumol. 2006;32(2):161-71.
13. Liu J, Mori A. Stress, aging, and brain oxidative damage. Neurochem Res. 1999;24(11):1479-97. http://dx.doi.org/10.1023/A:1022597010078
14. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84 http://dx.doi.org/10.1016/j.biocel.2006.07.001
15. Llesuy SF, Milei I, Molina H, Boveris A, Milei S. Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4'-epiadriamycin in mice. Tumori. 1985;71(3):241-9.
16. Evelson P, Travacio M, Repetto M, Escobar J, Llesuy S, Lissi EA. Evaluation of total reactive antioxidant potential

 http://dx.doi.org/10.1590/S1806-37132014000300006

(TRAP) of tissue homogenates and their cytosols. Arch Biochem Biophys. 2001;388(2):261-6. http://dx.doi.org/10.1006/abbi.2001.2292
17. Gonzalez Flecha B, Llesuy S, Boveris A. Hydroperoxide-initiated chemiluminescence: an assay for oxidative stress in biopsies of heart, liver, and muscle. Free Radic Biol Med. 1991;10(2):93-100. http://dx.doi.org/10.1016/0891-5849(91)90002-K
18. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.
19. Friederich M, Hansell P, Palm F. Diabetes, oxidative stress, nitric oxide and mitochondria function. Curr Diabetes Rev. 2009;5(2):120-44. http://dx.doi.org/10.2174/157339909788166800
20. Ichihashi M, Ueda M, Budiyanto A, Bito T, Oka M, Fukunaga M, et al. UV-induced skin damage. Toxicology. 2003;189(1-2):21-39. http://dx.doi.org/10.1016/S0300-483X(03)00150-1
21. Folz RJ, Guan J, Seldin MF, Oury TD, Enghild JJ, Crapo JD. Mouse extracellular superoxide dismutase: primary structure, tissue-specific gene expression, chromosomal localization, and lung in situ hybridization. American Am J Respir Cell Mol Biol. 1997;17(4):393-403. http://dx.doi.org/10.1165/ajrcmb.17.4.2826
22. Avissar N, Finkelstein JN, Horowitz S, Willey JC, Coy E, Frampton MW. Extracellular glutathione peroxidase in human lung epithelial lining fluid and in lung cells. Am J Physiol. 1996;270(2 Pt 1):L173-82.
23. Ely DR, Dapper V, Marasca J, Corrêa JB, Gamaro GD, Xavier MH, et al. Effects of restraint stress on feeding behavior of rats. Physiol Behav. 1997;61(3):395-8. http://dx.doi.org/10.1016/S0031-9384(96)00450-7
24. Lissi E, Salim-Hanna M, Pascual C, del Castillo MD. Evaluation of total antioxidant potential (TRAP) and total antioxidant reactivity from luminol-enhanced chemiluminescence measurements. Free Radic Biol Med. 1995;18(2):153-8. http://dx.doi.org/10.1016/0891-5849(94)00117-3
25. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid-peroxidation and peroxidative tissue-injury. Free Radic Biol Med. 1990;9(6):515-40. http://dx.doi.org/10.1016/0891-5849(90)90131-2
26. Halliwell B. Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? Lancet. 1994;344(8924):721-4. http://dx.doi.org/10.1016/S0140-6736(94)92211-X
27. McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985;312(3):159-63. http://dx.doi.org/10.1056/NEJM198501173120305
28. Rahman I. Oxidative stress in pathogenesis of chronic obstructive pulmonary disease: cellular and molecular mechanisms. Cell Biochem Biophys. 2005;43(1):167-88. http://dx.doi.org/10.1385/CBB:43:1:167
29. Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995; 41:1819–1828.
30. Uchida K, Shiraishi M, Naito Y, Torii N, Nakamura Y, Osawa T. Activation of stress signaling pathways by the end product of lipid peroxidation. Clin Chem. 1995;41(12 Pt 2):1819-28.
31. Okutan H, Savas C, Delibas N. The antioxidant effect of melatonin in lung injury after aortic occlusion-reperfusion. Interact Cardiovasc Thorac Surg. 2004;3(3):519-22. http://dx.doi.org/10.1016/j.icvts.2004.05.005
32. Martignoni M, Groothuis GM, de Kanter R. Species differences between mouse, rat, dog, monkey and human CYP-mediated drug metabolism, inhibition and induction. Expert Opin Drug Metab Toxicol. 2006;2(6):875-94. http://dx.doi.org/10.1517/17425255.2.6.875

About the authors

Ronaldo Lopes Torres
Physician. *Hospital Divina Providência*, Porto Alegre, Brazil.

Iraci Lucena da Silva Torres
Adjunct Professor. Department of Pharmacology, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Gabriela Laste
Doctoral Student. Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Maria Beatriz Cardoso Ferreira
Associate Professor. Department of Pharmacology, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

Paulo Francisco Guerreiro Cardoso
Professor. Department of Cardiorespiratory Diseases, Thoracic Surgery Section, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

Adriane Belló-Klein
Associate Professor. Department of Physiology. Institute of Basic Health Sciences, Federal University of Rio Grande do Sul, Porto Alegre, Brazil

Pulmonary function in advanced uncomplicated singleton and twin pregnancy*,**

Função pulmonar em mulheres com gestação única ou gemelar avançada e sem complicações

Anwar Hasan Siddiqui, Nazia Tauheed, Aquil Ahmad, Zehra Mohsin

Abstract

Objective: Pregnancy brings about significant changes in respiratory function, as evidenced by alterations in lung volumes and capacities, which are attributable to the mechanical impediment caused by the growing foetus. This study was undertaken in order to identify changes in respiratory function during normal pregnancy and to determine whether such changes are more pronounced in twin pregnancy than in singleton pregnancy. **Methods:** Respiratory function was assessed in 50 women with twin pregnancies and in 50 women with singleton pregnancies (during the third trimester in both groups), as well as in 50 non-pregnant women. We measured the following pulmonary function test parameters: FVC; FEV_1; PEF rate; FEV_1/FVC ratio; $FEF_{25-75\%}$; and maximal voluntary ventilation. **Results:** All respiratory parameters except the FEV_1/FVC ratio were found to be lower in the pregnant women than in the non-pregnant women. We found no significant differences between women with twin pregnancies and those with singleton pregnancies, in terms of respiratory function. **Conclusions:** Despite its higher physiological demands, twin pregnancy does not appear to impair respiratory function to any greater degree than does singleton pregnancy.

Keywords: Respiratory function tests; Respiratory mechanics; Pregnancy, twin; Pregnancy.

Resumo

Objetivo: A gravidez traz mudanças significativas na função respiratória, evidenciada por alterações nos volumes e capacidades pulmonares, que são atribuíveis ao impedimento mecânico causado pelo feto em crescimento. Este estudo foi realizado a fim de identificar alterações na função respiratória durante a gravidez normal e determinar se tais alterações são mais pronunciadas em gestação gemelar que em gestação única. **Métodos:** Foi avaliada a função respiratória de 50 mulheres com gestações gemelares e de 50 mulheres com gestações únicas (durante o terceiro trimestre em ambos os grupos), bem como de 50 mulheres não grávidas. Medimos os seguintes parâmetros de função pulmonar: CVF, FEV_1, taxa do PFE, relação VEF_1/CVF, $FEF_{25-75\%}$ e ventilação voluntária máxima. **Resultados:** Todos os parâmetros, exceto a relação VEF_1/CVF, foram menores nas mulheres grávidas do que nas mulheres não grávidas.Não foram encontradas diferenças significativas entre as mulheres com gestações gemelares e aquelas com gestações únicas em relação à função respiratória. **Conclusões:** Apesar das demandas fisiológicas maiores da gestação gemelar, essa não parece causar um comprometimento maior da função respiratória do que a gestação única.

Descritores: Testes de função respiratória; Mecânica respiratória; Gravidez de gêmeos; Gravidez.

Introduction

Pregnancy causes many changes in the human body, not all of which are visible. Like other organ systems, the respiratory system, which is highly efficient and sensitive, undergoes profound changes as a result of maternal adaptation to pregnancy. The respiratory system represents the best example of selective adaption of a system during pregnancy.[1] The anatomical and physiological adaptation of the respiratory system in pregnancy must be studied in the interest of properly diagnosing and managing associated respiratory pathologies during pregnancy.[2]

*Study carried out at the Antenatal Clinics of the Department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College, Aligarh, India.

Correspondence to: Anwar Hasan Siddiqui. Department of Physiology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, 202002 India.
Tel. 91 991 717-3366. E-mail: only.anwar@gmail.com

Financial support: None.
Submitted: 2 December 2013. Accepted, after review: 27 March 2014.

**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

 http://dx.doi.org/10.1590/S1806-37132014000300007

In pregnant women, alterations in pulmonary function are attributable to hormonal changes and to the mechanical impediment caused by the growing foetus. In the mucosa of the upper airway, elevated levels of oestrogen cause hyperaemia, hypersecretion, and oedema, leading to nasal obstruction, especially in the third trimester.[3] In addition, progesterone can cause a type of chemoreceptor resetting that results in a slight increase in PaO_2 and a consequent decrease in $PaCO_2$, leading to a state of compensated respiratory alkalosis.[4] The increasing size of the foetus with advancing gestation constitutes a mechanical impediment to the normal process of maternal ventilation. As the uterus expands, there is a $\leq$ 4 cm cephalad displacement of the diaphragm, with a compensatory increase in the transverse and anteroposterior diameters of the chest, caused by hormonal effects that relax the ligaments.[5]

It has been found that tidal volume increases progressively throughout pregnancy, because of increased diaphragm excursion, although inspiratory capacity and vital capacity remain almost unchanged.[6] The increased demand for oxygen without any compensatory increase in the respiratory rate increases the risk of developing maternal hypoxia.

Knowledge of the expected changes in pulmonary parameters is fundamental to the understanding of how any disease state affects pregnancy and vice versa. Pulmonary function tests (PFTs) permit an accurate and reproducible assessment of the functional state of the respiratory system and allow quantification of the severity of lung diseases. This information is also essential for the assessment of whether a patient is a candidate for anaesthesia, as well as of the dangers associated with obstetrical analgesia, given that all of the narcotics and hypnotics used for such analgesia are respiratory depressants.[7]

Due primarily to advances in assisted reproductive techniques, the incidence of twin pregnancy has shown a rising trend over the last decade.[8] Because the increased maternal and foetal demands for oxygen are higher in twin pregnancies, we hypothesized that respiratory changes would be more pronounced in twin pregnancy than in singleton pregnancy. In addition, because the uterus is larger in twin pregnancy, the cephalad displacement of the diaphragm might be expected to be greater, as might the laxity of the ligaments of the ribs, both of which could affect lung volumes. Therefore, it seems likely that pregnancy-related changes in respiratory function would be greater in women with twin pregnancy than in those with singleton pregnancy, although that has not been tested. Despite numerous reports of changes in PFT results during pregnancy, not much work has been done on twin pregnancies. The aim of this study was to provide pertinent data by comparing women with twin pregnancies, women with singleton pregnancies, and non-pregnant women, in terms of respiratory function.

Methods

This was a cross-sectional study involving 40 women with twin pregnancies and 60 women with singleton pregnancies. In all of the pregnant women, respiratory function was assessed at 36 weeks of gestation. In a control group of 50 non-pregnant women, age-matched to the pregnant women, respiratory function was assessed in the first half of the menstrual cycle. The pregnant women were recruited from among those seen at the antenatal clinics of the Department of Obstetrics and Gynaecology at Jawaharlal Nehru Medical College, in the city of Aligarh, India. The controls were volunteers recruited from among the relatives of pregnant women seen at the same antenatal clinics, as well as from among the hospital staff and students. All of the women recruited were between 20 and 32 years of age and had a moderate income, most being homemakers. Of the 40 women with twin pregnancies, 35 were primiparous, as were 48 of the 60 women with singleton pregnancies and 43 of the 50 non-pregnant women. All of the women evaluated were healthy, non-smokers without lung disease, cardiovascular disease, or current respiratory infection. None were taking medication that is believed to alter respiratory function, although some were taking supplemental iron, calcium, or both. Women with acute complications of pregnancy, such as preeclampsia and polyhydramnios, were excluded. The study was approved by the local institutional ethics committee, and all of the participants gave written informed consent. For each subject, a detailed history was taken, a physical examination was performed, and baseline investigations were conducted, in order to rule out cardiorespiratory disease and anaemia.

All PFTs were performed with a computerized spirometer (Medspiror; RMS, Chandigarh, India). Before the PFTs were performed, the procedures were thoroughly explained to the subjects, and the need to maintain an effective seal with the lips around the mouthpiece was emphasized, as was the need to use the nose clip during the procedure. Each subject was instructed to relax for at least 5 min prior to the PFTs.

For each subject, we measured the following parameters: FVC; FEV_1; $FEF_{25\%-75\%}$; PEF rate; FEV_1/FVC ratio; and maximal voluntary ventilation (MVV). All tests were performed in triplicate, and the highest of the three measurements was considered for analysis.

The Kolmogorov-Smirnov test was used in order to assess whether the data were normally distributed. To assess the statistical significance of differences, we employed one-way ANOVA with the Tukey-Kramer post hoc test for multiple comparisons, using the Statistical Package for the Social Sciences, version 17 (SPSS, Inc., Chicago, IL, USA). All normally distributed data are expressed as mean ± standard deviation unless otherwise stated. Medians (with 95% confidence intervals) are used in order to describe skewed data. All analyses were two-tailed, and values of $p < 0.05$ were considered statistically significant. We calculated that, in order to achieve a power of 80% for the detection of a one standard deviation difference between groups for each measurement, at the 5% level of significance, it would be necessary to recruit at least 16 patients into each group.

Results

The three groups—twin pregnancy, singleton pregnancy, and control—were comparable on the basis of age, height, weight, blood pressure, and haemoglobin levels (Tables 1 and 2). We observed a significant difference between the study subjects (both groups) and the control subjects in terms of body weight and body mass index. The American Thoracic Society PFT guidelines, established in March of 1991, are based on the height, age, gender, and race of the individual under testing, suggesting that pregnancy-related weight gain has no significant effect on lung function. We found that haemoglobin levels were significantly lower in the twin pregnancy group than in the control group.

In the present study, the values for all PFT parameters were lower among the pregnant women (both groups) than among the non-pregnant women (Table 3). Comparisons between various group pairings (Table 4) showed that all of the PFT parameters, with the exception of the FEV_1/FVC ratio and MVV, were significantly lower for the pregnant women (twin or singleton pregnancy) than for the non-pregnant women. The MVV values were also lower among the pregnant women, although the difference was not significant. As can be seen in Figure 1, there were no significant differences in lung function between the twin and singleton pregnancy groups.

Discussion

In the present study, we have demonstrated that lung function in the last trimester of pregnancy does not differ significantly between women with twin pregnancies and those with singleton pregnancies. Nevertheless, the values for most respiratory parameters were seen to be significantly lower among pregnant women (twin or singleton pregnancy) than among non-pregnant women.

Table 1 - Descriptive statistics of baseline variables in the groups evaluated.[a]

Variable	Group		
	NP	SP	TP
	(n = 50)	(n = 60)	(n = 40)
Subject age, years	26.72 ± 4.16	26.84 ± 2.95	27.62 ± 3.16
Subject height, cm	154.71 ± 3.11	153.13 ± 2.45	154.45 ± 3.41
Subject weight, kg	54.54 ± 4.92	62.78 ± 5.83	64.78 ± 6.10
Subject BMI, kg/m^2	22.37 ± 2.80	27.00 ± 3.42	27.13 ± 2.56
Subject SBP, mmHg	118.24 ± 9.14	119.52 ± 9.38	123.63 ± 8.92
Subject DBP, mmHg	77.42 ± 6.52	76.56 ± 5.82	75.24 ± 5.32
Subject haemoglobin, g/dL	11.82 ± 0.54	11.43 ± 0.43	11.29 ± 0.40
Gestational age of foetus, days	-	252 ± 2.52	255 ± 2.17

NP: non-pregnant (control) group; SP: singleton pregnancy group; TP: twin pregnancy group; BMI: body mass index; SBP: systolic blood pressure; and DBP: diastolic blood pressure. [a]Values expressed as mean ± SD.

 http://dx.doi.org/10.1590/S1806-37132014000300007

The decrease in FVC among the pregnant women evaluated in our study can be attributed to the mechanical pressure of the enlarged gravid uterus, which results in the upward displacement of the diaphragm and a consequent restriction of lung mobility. In addition, the elevation of the diaphragm brings about a relative decrease in the negative intrapleural pressure, which hampers forceful expiration.[9] Apart from the mechanical factor, hormonal changes during pregnancy have a significant influence on tracheobronchial smooth muscle tone, the reduction of which can decrease FVC.

Our finding that FEV_1, $FEF_{25-75\%}$, the PEF rate and MVV were lower among the pregnant women might be due to the decline in alveolar $PaCO_2$ during pregnancy, which effectively acts as a bronchoconstrictor. Pregnancy is associated with hyperventilation, as the increase in oxygen demand by the growing foetus far exceeds the supply obtained by normal breathing. Hyperventilation in pregnancy is attributed to the effects that progesterone has on the respiratory drive; progesterone not only increases the sensitivity but also reduces the threshold of the respiratory centre.[10] Hyperventilation causes the alveolar $PaCO_2$ to drop, resulting in bronchoconstriction. The lower PEF rates and MVV values obtained for the pregnant women evaluated in the present study can also be attributed to the decline in the strength of contraction of the main respiratory muscle (viz., the anterior abdominal muscle) and the internal intercostal muscles during the pregnant state. Studies suggest that this decrease in the muscular force of contraction is due to maternal weight gain, as well as pregnancy-related oedema, altered eating habits, and inadequate nutrition, all of which limit maternal respiratory effort during pregnancy.[11-13] Another factor that might have contributed to lowering the PEF rate and the MVV is the relatively low haemoglobin level observed in the pregnant women. Although none of the subjects had a haemoglobin level < 10 g/dL, even a borderline change in haemoglobin can make a difference.

We found that the FEV_1/FVC ratio was lower among pregnant women than among non-pregnant women, although the difference was less than significant. That might be because, despite the fact that both FEV_1 and FVC were lower in the study subjects than in the control subjects, the

Table 2 - Results of one-way ANOVA comparing baseline variables between different group pairs.

Variable	NP vs. SP	NP vs. TP	SP vs. TP
	p	p	p
Age	0.346	0.925	0.236
Height	0.244	0.751	0.198
Weight	0.015	0.005	0.061
Body mass index	0.023	0.014	0.138
Systolic blood pressure	0.543	0.064	0.142
Diastolic blood pressure	0.150	0.098	0.248
Haemoglobin	0.175	0.028	0.079

NP: non-pregnant (control) group; SP: singleton pregnancy group; and TP: twin pregnancy group.

Table 3 - Descriptive statistics of pulmonary function test results for the groups of women evaluated.[a]

Variable	Group		
	NP	SP	TP
	(n = 50)	(n = 60)	(n = 40)
FVC			
% of predicted value	92.48 ± 8.43	86.48 ± 4.37	85.56 ± 7.85
Actual value, L	2.64 ± 0.42	2.47 ± 0.29	2.44 ± 0.34
FEV_1			
% of predicted value	94.53 ± 6.24	88.64 ± 5.62	86.34 ± 4.39
Actual value, L	2.37 ± 0.18	2.17 ± 0.25	2.14 ± 0.18
FEV_1/FVC ratio	85.19 ± 2.61	85.52 ± 2.32	85.73 ± 2.21
$FEF_{25-75\%}$			
% of predicted value	92.12 ± 6.61	86.79 ± 5.76	85.64 ± 6.23
Actual value, L	3.48 ± 0.42	3.21 ± 0.27	3.19 ± 0.34
PEF rate, L/min	417 ± 8.61	313.52 ± 8.05	311.52 ± 6.79
MVV, L/min	104.32 ± 14.45	98.53 ± 13.62	97.68 ± 14.21

NP: non-pregnant (control) group; SP: singleton pregnancy group; TP: twin pregnancy group; and MVV: maximal voluntary ventilation. [a]Values expressed as mean ± SD.

Table 4 - Results of one-way ANOVA comparing pulmonary function test results between different group pairs.

Variable	NP vs. SP	NP vs. TP	SP vs. TP
	p	p	p
FVC	0.013	0.004	0.381
FEV_1	0.034	0.029	0.257
FEV_1/FVC ratio	0.306	0.321	0.432
PEF rate	0.001	0.001	0.062
$FEF_{25-75\%}$	0.004	0.006	0.247
MVV	0.543	0.477	0.982

NP: non-pregnant (control) group; SP: singleton pregnancy group; TP: twin pregnancy group; and MVV: maximal voluntary ventilation.

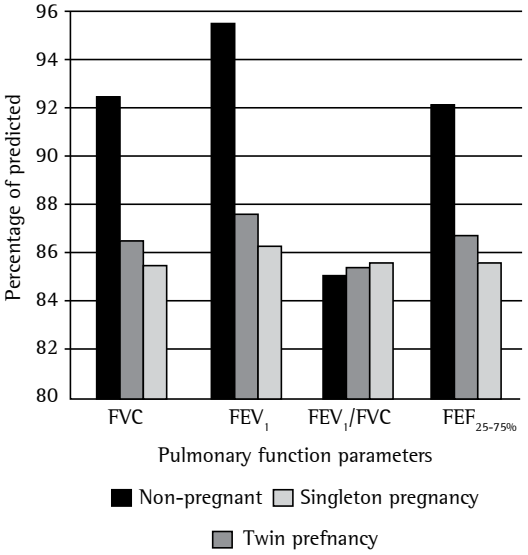


Figure 1 - Comparison of pulmonary function parameters among the three groups evaluated.

pregnancy-related decline was not as great for FEV_1 as it was for FVC.

Our study has at least one limitation. Because the study sample was relatively small, our findings and conclusions might not be generalisable to the general population. A study with a larger sample size might provide more conclusive evidence.

In the present study, the measures of pulmonary function evaluated did not differ significantly between the twin and singleton pregnancy groups. It is known that the decline in alveolar $PaCO_2$ during pregnancy increases airway resistance,[14] which is reduced by pregnancy-related increases in the circulating levels of relaxin, progesterone, and cortisol.[15] In twin pregnancy, there might be a balance between these two opposing forces, which would explain why we found no significant differences in comparison with singleton pregnancy. Studies have shown that, in pregnant women, the plasma level of relaxin correlates positively with the number of foetuses.[15] It is evident that the respiratory changes in pregnancy are mediated and determined mainly by the hormonal changes occurring in the body, especially changes in the levels of progesterone and oestrogen. Although twin pregnancy is associated with greater oxygen demand and more uterine distension, the lung volume changes observed in the women with twin pregnancies evaluated in the present study were similar to those seen in the women with singleton pregnancies. This effect can be thought to be mediated by higher levels of progesterone in twin pregnancy. Therefore, we conclude that no significant differences in lung function exist between women with twin pregnancies and those with singleton pregnancies. In healthy women, the respiratory system copes well with the extra demands placed upon it by a twin pregnancy, and no special consideration is required with respect to the dose adjustment of inhalational anaesthetics.

References

1. Dutta DC. Physiological changes during pregnancy. In: Obstetrics and Gynecology. 4th edition. Calcutta: New Central Book Agency; 1998. p. 46-57.
2. Elkus R, Popovich J Jr. Respiratory physiology in pregnancy. Clin Chest Med. 1992;13(4):555-65.
3. Hegewald MJ, Crapo RO. Respiratory physiology in pregnancy. Clin Chest Med. 2001;32(1)1-13. http://dx.doi.org/10.1016/j.ccm.2010.11.001
4. Christopher FC, Gertic FM, Johnston D. Physiological changes associated with pregnancy. Update in Anaesthesia. 1998;9:72-6. [Adobe Acrobat document, 6p.]. Available from: http://e-safe-anaesthesia.org/e_library/02/Pregnancy-physiological_changes_Update_2008.pdf
5. Kolarzyk E, Szot WM, Lyszczarz J. Lung function and breathing regulation parameters during pregnancy. Arch Gynecol Obstet. 2005;272(1):53-8. http://dx.doi.org/10.1007/s00404-004-0691-1
6. Contreras G, Gutiérrez M, Beroíza T, Fantín A, Oddó H, Villarroel L, et al. Ventilatory drive and respiratory muscle function in pregnancy. Am Rev Respir Dis. 1991;144(4):837-41. http://dx.doi.org/10.1164/ajrccm/144.4.837
7. Phatak MS, Kurhade GA. A longitudinal study of antenatal changes in lung function tests and importance of postpartum exercises in their recovery. Indian J Physiol Pharmacol. 2003;47(3):352-6.
8. Alikani M, Cekleniak NA, Walters E, Cohen J. Monozygotic twinning following assisted conception: an analysis of 81 consecutive cases. Hum Reprod. 2003;18(9):1937-43. http://dx.doi.org/10.1093/humrep/deg369
9. Wise RA, Polito AJ, Krishnan V. Respiratory physiologic changes in pregnancy. Immunol Allergy Clin North Am. 2006;26(1):1-12. http://dx.doi.org/10.1016/j.iac.2005.10.004

 http://dx.doi.org/10.1590/S1806-37132014000300007

10. DeCherney AH, Pernoll ML. Maternal physiology during pregnancy. In: Benson RC, DeCherney AH, Nathan L, editors. Current obstetrics & gynecologic: diagnosis and treatment. 9th edition. New York: Lange Medical Books/McGraw Hill; 2003. p. 159-60.
11. Singhal U, Saxena K. Effect of anaemia on respiratory and metabolic parameters during third trimester of pregnancy. Indian J Physiol Pharmacol. 1987;31(2):130-5.
12. Puranik BM, Kurhade GA, Kaore SB, Patwardhan SA, Kher JR. PEFR in pregnancy: a longitudinal study. Ind J Physiol Pharmacol. 1995;39(2):135-9.
13. Pereira A, Krieger BP. Pulmonary complications of pregnancy. Clin Chest Med. 2004;25(2):299-310. http://dx.doi.org/10.1016/j.ccm.2004.01.010
14. Jensen D, Wolfe LA, Slatkovska L, Webb KA, Davies GA, O'Donnell DE. Effects of human pregnancy on the ventilatory chemoreflex response to carbon dioxide. Am J Physiol Regul Integr Comp Physiol. 2005;288(5):R1369-75. http://dx.doi.org/10.1152/ajpregu.00862.2004
15. Johnson MR, Abbas AA, Allman AC, Nicolaides KH, Lightman SL. The regulation of plasma relaxin levels during human pregnancy. J Endocrinol. 1994;142(2):261-5. http://dx.doi.org/10.1677/joe.0.1420261

About the authors

Anwar Hasan Siddiqui
Assistant Professor. Department of Physiology, Jawaharlal Nehru Medical College, Aligarh, India.

Nazia Tauheed
Assistant Professor. Department of Anaesthesiology and Critical Care, Jawaharlal Nehru Medical College, Aligarh, India.

Aquil Ahmad
Assistant Professor. Department of Physiology, Jawaharlal Nehru Medical College, Aligarh, India.

Zehra Mohsin
Associate Professor. Department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College, Aligarh, India.

Reliability of a rapid hematology stain for sputum cytology*

Confiabilidade da coloração hematológica rápida para citologia de escarro

Jéssica Gonçalves, Emilio Pizzichini, Marcia Margaret Menezes Pizzichini, Leila John Marques Steidle, Cristiane Cinara Rocha, Samira Cardoso Ferreira, Célia Tânia Zimmermann

Abstract

Objective: To determine the reliability of a rapid hematology stain for the cytological analysis of induced sputum samples. **Methods:** This was a cross-sectional study comparing the standard technique (May-Grünwald-Giemsa stain) with a rapid hematology stain (Diff-Quik). Of the 50 subjects included in the study, 21 had asthma, 19 had COPD, and 10 were healthy (controls). From the induced sputum samples collected, we prepared four slides: two were stained with May-Grünwald-Giemsa, and two were stained with Diff-Quik. The slides were read independently by two trained researchers blinded to the identification of the slides. The reliability for cell counting using the two techniques was evaluated by determining the intraclass correlation coefficients (ICCs) for intraobserver and interobserver agreement. Agreement in the identification of neutrophilic and eosinophilic sputum between the observers and between the stains was evaluated with kappa statistics. **Results:** In our comparison of the two staining techniques, the ICCs indicated almost perfect interobserver agreement for neutrophil, eosinophil, and macrophage counts (ICC: 0.98-1.00), as well as substantial agreement for lymphocyte counts (ICC: 0.76-0.83). Intraobserver agreement was almost perfect for neutrophil, eosinophil, and macrophage counts (ICC: 0.96-0.99), whereas it was moderate to substantial for lymphocyte counts (ICC = 0.65 and 0.75 for the two observers, respectively). Interobserver agreement for the identification of eosinophilic and neutrophilic sputum using the two techniques ranged from substantial to almost perfect (kappa range: 0.91-1.00). **Conclusions:** The use of Diff-Quik can be considered a reliable alternative for the processing of sputum samples.

Keywords: Sputum\analysis; Sputum\cytology; Azure stains.

Resumo

Objetivo: Determinar a confiabilidade da coloração hematológica rápida para a análise do escarro induzido. **Métodos:** Estudo transversal comparando a técnica padrão (coloração May-Grünwald-Giemsa) com a coloração hematológica rápida (panótico rápido). Participaram do estudo 50 indivíduos (21 asmáticos, 19 portadores de DPOC e 10 controles). Após a coleta do escarro induzido, foram preparadas 4 lâminas, sendo 2 coradas por May-Grünwald-Giemsa e 2 por panótico rápido. As lâminas foram lidas de forma independente por dois pesquisadores capacitados para o exame de escarro induzido e cegados para a identificação das lâminas. A confiabilidade para as contagens celulares dos dois métodos foi avaliada pela determinação dos coeficientes de correlação intraclasse (CCI) para as concordâncias intraobservador e interobservador. As concordâncias na identificação de escarro neutrofílico e eosinofílico entre observadores e entre as duas colorações foram calculadas por estatística kappa. **Resultados:** Nas duas colorações, os CCI apontaram concordância interobservador quase perfeita para as contagens de neutrófilos, eosinófilos e macrófagos (variação do CCI: 0,98-1,00) e substancial para as contagens de linfócitos (variação do CCI: 0,76-0,83). Na análise intraobservador, a concordância foi quase perfeita para as contagens de neutrófilos, eosinófilos e macrófagos (variação do CCI: 0,96-0,99) e de moderada a substancial para as contagens de linfócitos (CCI = 0,65 e 0,75 para observadores 1 e 2, respectivamente). A concordância interobservador na identificação de escarro eosinofílico e neutrofílico para os dois métodos de coloração variou entre substancial e quase perfeita (variação kappa: 0,91-1,00). **Conclusões:** O panótico rápido pode ser considerado uma alternativa confiável para o processamento de amostras de escarro.

Descritores: Escarro\análise; Escarro\citologia; Corantes azur.

*Study carried out at the Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Correspondence to: Jéssica Gonçalves. Universidade Federal de Santa Catarina, Hospital Universitário, Núcleo de Pesquisa em Asma e Inflamação das Vias Aéreas, Campus Universitário, Trindade, CEP 88040-970, Florianópolis, SC, Brasil.
Tel. 55 48 3721-8134. E-mail: jessicagoncalvesufsc@gmail.com

Financial support: None.
Submitted: 1 October 2013. Accepted, after review: 30 April 2014.

 http://dx.doi.org/10.1590/S1806-37132014000300008

Introduction

The understanding of the mechanisms of diseases and their correct diagnosis has been made possible by analysis of body fluids in several areas of medicine. In the past, sputum analysis was considered not to be reliable or reproducible enough to assist in the understanding of the mechanisms of respiratory diseases.[1] More recently, significant advances related to the processing of sputum samples have allowed the method of sputum examination to become feasible, reproducible, valid, and responsive to interventions. Several researchers have used this method to study the various aspects of airway inflammation in asthma. The use of sputum examination has been further extended to COPD, cystic fibrosis, chronic cough, idiopathic pulmonary fibrosis, and other respiratory diseases.

Inflammation is central in the pathogenesis of airway diseases and is considered responsible for their symptoms, airflow obstruction, exacerbations, and secondary structural changes.[2] Therefore, airway inflammation plays an extremely significant role in two major obstructive respiratory tract diseases: asthma and COPD.[3] Both in asthma and in COPD, there is great heterogeneity in clinical and inflammatory characteristics, which result in different clinical phenotypes.[4] Consequently, there is a need to characterize the phenotype of patients in order to optimize their clinical management, particularly in more severe cases.[4-6]

Examination of induced sputum is currently considered reliable, reproducible, discriminative of different types of inflammation, and responsive to interventions. Therefore, it has been an important tool in the management of inflammatory diseases of the airways.[7] In addition, sputum induction is a safe minimally invasive technique,[8] making it possible to identify the type of inflammation and its intensity.[9,10] However, its use in clinical practice is still very restricted because of the method for sputum induction and for the processing of sputum samples, which is laborious and time-consuming and requires highly trained personnel.

Induced sputum slides for differential cell counts, both in research and in clinical practice, have been stained with May-Grünwald-Giemsa or with Wright-Giemsa. These stains are part of a group called "Romanowsky stains".[11] Diff-Quik (a rapid hematology stain), which is also based on the Romanowsky technique,[12] uses similar reagents, but the staining time is considerably shorter; whereas the standard stain requires 34 min, the rapid stain is performed within 2 min.

Shortening the total processing time would be extremely important for improving the viability of the technique. In addition, the cost of Diff-Quik is considerably lower than that of the standard stain. Therefore, the validation of this technique is important, especially for developing countries, such as Brazil.

The objective of the present study was to assess the reliability of Diff-Quik for the cytological analysis of induced sputum samples.

Methods

The study included 50 patients, of whom 21 were adult patients with uncontrolled asthma, characterized by an Asthma Control Questionnaire[13] score greater than 1.7 in the previous week and objectively confirmed (in the 3 previous years) by reversible airflow limitation (a > 12% increase in FEV_1 and a > 200 mL increase in FEV_1 after inhalation of a short-acting bronchodilator) in participants with airflow limitation (an FEV_1/FVC ratio < 0.7); 19 COPD patients aged > 40 years who had a history of respiratory symptoms associated with moderate or severe airflow obstruction (an FEV_1 < 50% of predicted and an FEV_1/FVC ratio < 0.7), were receiving any type of medication for COPD, and were (current or former) heavy smokers with a smoking history of > 20 pack-years; and 10 healthy nonsmokers who had no respiratory symptoms and whose diagnostic status was objectively confirmed by normal spirometry results. The study excluded patients who had respiratory infection in the four previous weeks, those who had severe diseases of other systems, those who had other known pulmonary diseases, and pregnant women.

The study was conducted at the Center for Research on Asthma and Airway Inflammation, located at the Federal University of Santa Catarina University Hospital in the city of Florianópolis, Brazil, and was approved by the local Human Research Ethics Committee (Process no. 2093; FR 437236, issued on November 28, 2011). All participants gave written informed consent after being given a detailed explanation of the study.

Participants underwent pre- and post-bronchodilator spirometry with a computerized spirometer (Koko; PDS Instrumentation, Inc., Louisville, CO, USA), in accordance with the

American Thoracic Society guidelines[14] The reference values used were those of Crapo et al.[15]

Subsequently, sputum induction was performed in accordance with the method described by Pizzichini et al.[16] The procedure involved inhalation of an isotonic saline aerosol (0.9%) followed by serial inhalation of increasing concentrations of hypertonic saline aerosol (3%, 4%, and 5%) via a Fisoneb ultrasonic nebulizer (Fisons, Pickering, Ontario, Canada). Aerosol inhalation was continued for 1-2 min, according to the level of bronchoconstriction severity before the procedure, and was followed by measurement of FEV_1. Participants were instructed to rinse their mouths with water, swallow the water, and blow their noses in order to reduce contamination by saliva or postnasal discharge. They were then asked to cough and expectorate the sputum into a clean container. These procedures were repeated consecutively, with the solution concentration being increased every 7 min for 21 min or until there was a $\geq$ 20% decrease in FEV_1.

The processing of sputum samples was started within 2 h of collection, which is the longest time reported in the literature.[2] The thick portions of the expectorated material were selected with the naked eye or under visualization with an inverted microscope, and the sputum was separated from the saliva. The selected fractions were treated with 0.1% DTT at a ratio of four times the fraction volume. This mixture was homogenized with a Pasteur pipette and agitated on a desktop shaker for 15 min. Dulbecco's PBS was added thereto in an amount that was four times the initial volume of sputum selected, and the resulting suspension was filtered to remove cell debris and undissolved mucus. Subsequently, total leukocyte counts were performed with a modified Neubauer hemocytometer, excluding squamous cells. Cell viability was determined by the trypan blue exclusion test. The sample was adjusted to 1.0×10^6 cells/mL, and we prepared four slides, which were coded. In the present study, all of the slides were prepared using the cytocentrifugation method (cytospin). After air-drying, two slides were stained with May-Grünwald-Giemsa, and the other two were stained with Diff-Quik (the technique under study).

May-Grünwald-Giemsa staining was performed with an automated system (Sysmex sp1000i™; Sysmex Co., Kobe, Japan). For this technique, the slides were fixed by immersion in analytical grade methanol, and then they were immersed in a May-Grünwald stain solution and a dilute May-Grünwald solution (1:1). Immediately afterward, the slides were immersed in a freshly prepared Giemsa stain solution (dilution 1:10) and subsequently dried. The whole procedure lasted exactly 34 min, as recommended by the equipment manufacturer.

Diff-Quik was performed manually. The process included initial immersion of the slides in solution no. 1 (0.1% triarylmethane), moving up and down continuously for 5-10 seconds (5-10 one-second immersions). Subsequently, extensions were immersed in solution no. 2 (0.1% xanthene), repeating the same procedure. After draining, the slides were immersed in solution no. 3 (0.1% thiazine), repeating the same procedure. The slides were rinsed with distilled water and allowed to air-dry.[12] This staining procedure took a maximum of 2 min to complete. Two researchers, trained in reading induced sputum slides, independently counted 400 non-squamous cells on the slides stained either with May-Grünwald-Giemsa or Diff-Quik. Because the slides were coded, the slide readers were prevented from identifying their respective pairs or previous reading results.

The reliability for cell counting using the two techniques (standard stain vs. tested stain) was evaluated by determining the intraclass correlation coefficients (ICCs) for intraobserver and interobserver agreement, and Bland & Altman plots were used.[17] The interpretation of ICCs was based on the classification proposed by Landis & Koch.[18] Agreement in the identification of eosinophilic sputum (eosinophils $\geq$ 3%)[6] and neutrophilic sputum (neutrophils > 64%)[19] between the observers and between the stains was evaluated with kappa statistics.[18] Differences between the characteristics of the groups studied were examined by ANOVA and the Bonferroni test in the post hoc analysis. The statistical tests were two-tailed, and the level of significance was set at 5%. The Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses.

Results

Sputum induction was performed in 62 individuals, 50 (80.6%) of whom were able to produce an adequate sample, with cell viability greater than 50%. Twelve induced sputum samples were considered inadequate because

 http://dx.doi.org/10.1590/S1806-37132014000300008

of excessive salivary contamination (> 20% of squamous cells), low cell viability (< 50%), or insufficient material to prepare the slides. The demographic, clinical, and functional characteristics of the participants are shown in Table 1. The groups were distinct and well characterized, as demonstrated by their demographic, clinical, and functional characteristics.

The cellular characteristics of induced sputum were as expected for the different groups studied. The sputum of asthma patients was characterized by a significantly higher proportion of eosinophils than that found in the sputum of COPD patients and healthy controls. In contrast, the sputum of COPD patients showed a significant increase in total cell counts and in the proportion of neutrophils when compared with that of controls. The control group showed a significantly higher proportion of macrophages than did the other two groups. Figure 1 shows the proportions of neutrophils, eosinophils, and macrophages in the different groups studied. In Figure 2, the median proportions of neutrophils, eosinophils, and macrophages, in the study sample as a whole, are separated by type of stain used. No significant differences were found for the cell counts on the slides stained by either of the two techniques used.

The results for interobserver agreement for induced sputum differential cell counts on the cytospin slides stained by the May-Grünwald-Giemsa technique show that the medians and percentiles were similar between the two observers, and the ICCs indicated almost perfect agreement for eosinophil, neutrophil, and macrophage counts (ICC = 1.00, 0.99, and 0.98, respectively). Interobserver agreement for lymphocyte counts was substantial (ICC = 0.76). For the slides stained by the Diff-Quik technique, the medians and percentiles were also very close between the two observers, and the ICC indicated almost perfect interobserver agreement for eosinophil, neutrophil, macrophage, and lymphocyte counts (ICC = 1.00, 0.99, 0.99, and 0.83, respectively).

Regarding intraobserver agreement, the ICC values for the two observers for the differential cell counts on the pairs of cytospin slides stained by either of the two studied techniques indicated that it was almost perfect for neutrophils (ICC = 0.97 for both), eosinophils (ICC = 0.99 and 0.98), and macrophages (ICC = 0.96 for both). For lymphocyte counts, intraobserver agreement was substantial for observer 1 (ICC = 0.75) and moderate for observer 2 (ICC = 0.65). These results are shown graphically in Figure 3.[17]

Interobserver agreement for the identification of eosinophilic and neutrophilic sputum using the two techniques ranged from substantial to almost perfect, as shown in Table 2. However, although intraobserver agreement was substantial, it was lower than was interobserver agreement.

Discussion

The results of the present study show that cytospin slides stained either by the May-Grünwald-Giemsa technique or with Diff-Quik yield similar cell counts, with high intraobserver and intraobserver agreement. These results demonstrate the reliability of the Diff-Quik technique for use in the processing of induced sputum samples. This fact is relevant because the Diff-Quik technique is simpler, allows a reduction in sample processing

Table 1 - Demographic, clinical, and functional characteristics of the participants.[a]

Characteristic	Groups			p
	Asthma	COPD	Control	
	(n = 21)	(n = 19)	(n = 10)	
Age, years[b]	47.3 (22-68)	62.8 (52-77)	38.4 (21-58)	< 0.001*,*** and 0.2**
Female gender[c]	12 (57.0)	5 (26.3)	7 (70.0)	0.03
Pre-BD FEV_1,% of predicted	55.3 ± 11.9	50.2 ± 18.2	102.2 ± 7.8	0.1* and < 0.001**,***
Post-BD FEV_1,% of predicted	64.9 ± 11.7	52.7 ± 18.2	104.5 ± 8.7	0.02* and < 0.001**,***
Pre-BD FEV_1/FVC, %	58.3 ± 9.5	52.5± 13.8	80.4 ± 5.0	0.4* and < 0.001**,***
Post-BD FEV_1/FVC,%	61.3 ± 9.2	53.2 ± 14.4	82.4 ± 4.6	0.06* and < 0.001**,***
Pre-BD ΔFEV_1, L	0.29 ± 0.29	0.07 ± 0.09	0.08 ± 0.08	0.003*, 0.02**, and 1.0***
Post-BD ΔFEV_1, %	19.1 ± 17.6	6.1 ± 7.4	2.5 ± 2.4	0.005*, 0.002**, and 1.0***

Pre-BD: pre-bronchodilator; and post-BD: post-bronchodilator. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Value expressed as mean (range). [c]Value expressed as n (%). *Asthma group vs. COPD group. **Asthma group vs. control. ***COPD group vs. control.

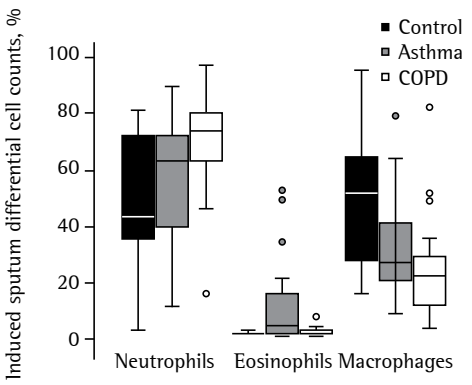


Figure 1 - Induced sputum differential cell counts in the three groups studied.

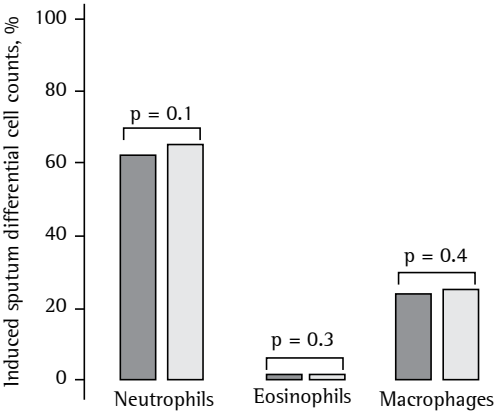


Figure 2 - Median differential cell counts on the cytospin slides stained either with May-Grünwald-Giemsa (dark gray bar) or Diff-Quik (light gray bar).

time of up to 32 min without impairing sample quality, and is considerably cheaper.

To our knowledge, this was the first study to evaluate the reliability of the Diff-Quik technique for use in induced sputum cytology, by comparing it with a standard staining technique, i.e., the May-Grünwald-Giemsa technique. It is important to evaluate the reliability and reproducibility of the results in order to confirm the accuracy of the results obtained by using Diff-Quik. In the present study, the reliability of Diff-Quik was tested by two distinct strategies. The first strategy was to calculate the ICCs for the cell counts performed by two independent observers, blinded to the identification of the slides. Although the stains used in the present study could be identified by the appearance of the slides, differing codes were used to prevent the identification of the respective pairs of slides. Previous studies[20-22] have shown that intraobserver and interobserver agreement for cell counts on cytospin slides stained with Wright-Giemsa and May-Grünwald-Giemsa could be considered perfect, but that it depended on the degree of salivary contamination on the slides.[21] The results obtained with Diff-Quik in the present study are in line with those of the aforementioned studies. The second strategy was to examine the reliability of Diff-Quik for identifying eosinophilic sputum and neutrophilic sputum. This is relevant because, in clinical practice, sputum examination is used to identify phenotypes of severe asthma, predict response to treatment, and decrease the number of asthma exacerbations by control of eosinophilic inflammation.

Intraobserver agreement for the identification of eosinophilic and neutrophilic sputum by the two staining techniques was found to be substantial. In addition, interobserver agreement for the identification of eosinophilic and neutrophilic sputum was almost perfect. These results again demonstrate the reliability of Diff-Quik, because they confirm its accuracy for identifying the different inflammatory phenotypes. However, the results also showed that interobserver agreement was higher than intraobserver agreement for the identification of the phenotypes. This difference could be due to variability in cell content on the slides stained by either of the two techniques. Although intraobserver agreement was substantial, this particular result suggests caution and a need for further studies to identify the reason for this variability.

Interobserver reproducibility for differential leukocyte counts in induced sputum samples has been previously reported.[20-22] In 1997, one group of authors reported high interobserver reproducibility for all cell types studied. Those authors also found lower agreement for lymphocytes than for the other cell types. The lower agreement for the proportions of lymphocytes was considered to be due to the very small amount of this cell type in the induced sputum samples. In the present study, we also found lower agreement for lymphocyte counts. However, this was not emphasized because it is a known fact that these variations are not clinically relevant.

In one study,[21] there was good interobserver agreement for neutrophil, eosinophil, and macrophage counts, and, again, this agreement was lower for lymphocyte counts; the justification for the lower repeatability rate was related not only to the scarcity of lymphocytes in the samples

 http://dx.doi.org/10.1590/S1806-37132014000300008

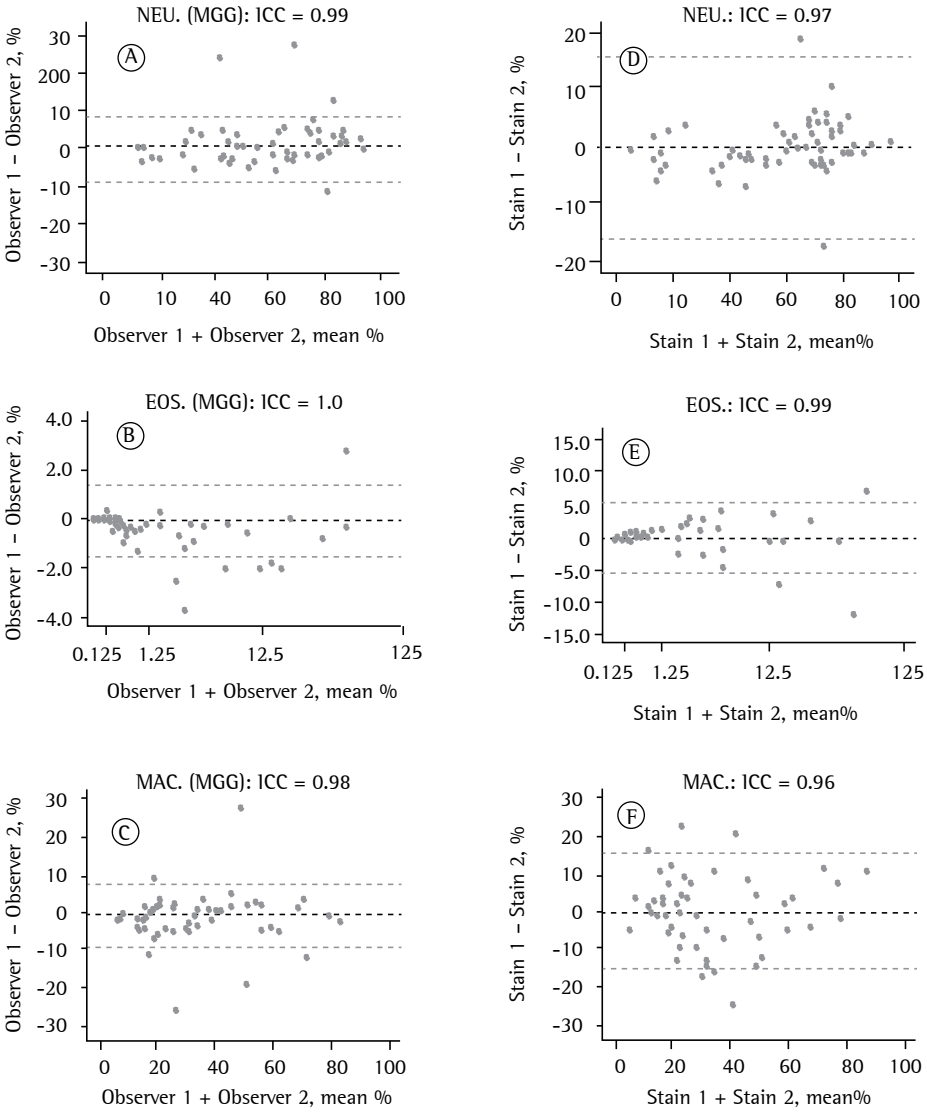


Figure 3 - Bland & Altman plots. Interobserver reproducibility for the proportion of neutrophils (in A), eosinophils (in B), and macrophages (in C) on the cytospin slides prepared from induced sputum samples and stained with May-Grünwald-Giemsa (MGG). Intraobserver reproducibility for the proportion of neutrophils (in D), eosinophils (in E), and macrophages (in F) on the cytospin slides prepared from induced sputum samples and stained by either of the two staining techniques. The plots refer to the differences between the readings by observers 1 and 2 (y axis) in relation to the mean readings by observers 1 and 2 (x axis). The central broken line indicates absence of differences, and the peripheral broken lines indicate two standard deviations of the mean of the differences. ICC: intraclass correlation coefficient; NEU.: neutrophils; EOS.: eosinophils; and MAC.: macrophages.

but also to the difficulty in identifying these cells. One group of authors[21] confirmed the results of a previous study,[20] demonstrating that the reproducibility of induced sputum differential cell counts is affected by salivary contamination and low cell viability. In the present study, samples with > 20% salivary contamination or < 50% viability were considered inadequate for analysis.

Methods for refinement of sputum examination have greatly contributed to its accuracy and reproducibility.[23] However, previous studies have primarily focused on the steps preceding the preparation of cytospin slides and the liquid phase of sputum.[24-27]

In 2003, a study comparing the results and costs of three techniques for analysis of induced

Table 2 - Interobserver and intraobserver agreement for the identification of eosinophilic and neutrophilic sputum in the evaluation of slides stained by either of the two studied techniques.

Interobserver agreement	Sputum type	kappa	p
Diff-Quik stain	Eosinophilic	1.000	< 0.001
	Neutrophilic	1.000	< 0.001
May-Grünwald-Giemsa stain	Eosinophilic	0.905	< 0.001
	Neutrophilic	0.960	< 0.001
Intraobserver agreement	Sputum type	kappa	p
Observer 1	Eosinophilic	0.746	< 0.001
	Neutrophilic	0.801	< 0.001
Observer 2	Eosinophilic	0.758	< 0.001
	Neutrophilic	0.760	< 0.001

sputum samples was published.[28] The techniques consisted of one of the following: preparation of smears from sputum not treated with DTT; preparation of smears from sputum treated with DTT; or preparation of cytospin slides from sputum treated with DTT. Although the first two techniques reduced the time and cost of sputum sample analysis, Spearman's correlation coefficient, which ranged from 0.57 to 0.64 for eosinophil counts and from 0.51 to 0.57 for neutrophil counts ($p < 0.01$ for both), can be considered inadequate for these types of techniques. The authors concluded that the technique that uses cytospin slides prepared from samples treated with DTT is more suitable for research purposes and for use in specialized centers.[28]

Some of the aspects that hinder the widespread use of induced sputum are its complexity and the sample processing time. Two previous studies have attempted to reduce the complexity of sample processing,[29,30] both using a sputum filtration device (Accufilter; Cellometrics, Hamilton, Ontario, Canada), which would be an alternative for standardizing the processing of induced sputum samples. The device consists of a kit with a tube used for weighing and treating sputum selected from saliva, connected to a filter and to a reception tube containing DTT, saline solution, and trypan blue. One of those studies[30] sought to determine the validity of using this device in the analysis of sputum samples, by comparing it with the standard method. The study reported ICCs that indicate good reproducibility for eosinophil and neutrophil counts between the methods; however, the ICCs indicated a reduction in cell viability and total cell counts, as well as an increase in the proportion of squamous epithelial cells, with the use of that device.

In summary, the high degree of agreement for the cell counts on the cytospin slides stained either by the May-Grünwald-Giemsa technique or with Diff-Quik attests to the reliability of the latter stain, which justifies the recommendation that it be used when the objective is to reduce sample processing time and induced sputum costs.

References

1. Djukanovic R, Sterk PJ, editors. An atlas of induced sputum--an aid for research and diagnosis. New York: Parthenon Publishing Group; 2004.
2. Pizzichini E, Efthimiadis A, Pizzichini MM, Hargreave FE. Sputum Examination for indices of airway inflammation: laboratory procedures. Lund, Sweden: Astra Draco AB; 1997.
3. Silva LC, Hetzel JL. Pneumonias obstrutivas. In: Condutas em Pneumologia. Silva LC, editor. Rio de Janeiro: Revinter; 2001. p. 261-352.
4. Pavord ID, Sterk PJ, Hargreave FE, Kips JC, Inman MD, Louis R, et al. Clinical applications of assessment of airway inflammation using induced sputum. Eur Respir J Suppl. 2002;37:40s-43s. PMid:12361362
5. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet. 1999;353(9171):2213-4. http://dx.doi.org/10.1016/S0140-6736(99)01813-9
6. Jayaram L, Pizzichini MM, Cook RJ, Boulet LP, Lemière C, Pizzichini E, et al. Determining asthma treatment by monitoring sputum cell counts: effect on exacerbations. Eur Respir J. 2006;27(3):483-94. http://dx.doi.org/10.1183/09031936.06.00137704
7. Polombini BC, Moreira JS, Miorim MC, Villanova CA. Exame físico-valorização do exame de escarro. In: Silva LC, editor. Condutas em Pneumologia. Rio de Janeiro: Revinter; 2001. p. 67-78.
8. Pizzichini E, Pizzichini MM, Leigh R, Djukanovic R, Sterk PJ. Safety of sputum induction. Eur Respir J Suppl. 2002;37:9s-18s. PMid:12361366
9. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. Thorax. 1997;52(6):498-501. http://dx.doi.org/10.1136/thx.52.6.498
10. Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA, Hargreave FE, et al. Use of induced sputum cell counts to investigate airway inflammation in asthma.

 http://dx.doi.org/10.1590/S1806-37132014000300008

Thorax. 1992;47(1):25-9. http://dx.doi.org/10.1136/thx.47.1.25
11. Horobin RW. How Romanowsky stains work and why they remain valuable - including a proposed universal Romanowsky staining mechanism and a rational troubleshooting scheme. Biotech Histochem. 2011;86(1):36-51. http://dx.doi.org/10.3109/10520295.2010.515491
12. Laborclin Produtos para Laboratório Ltda [homepage on the Internet]. Pinhais: Laborclin [cited 2013 Jan 1]. Instruções de uso - panótico rápido. [Adobe Acrobat document, 2p.]. Available from: http://www.interlabdist.com.br/dados/produtos/bula/doc/13764277764820b3e23b11e.pdf
13. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. Eur Respir J. 1999;14(4):902-7. http://dx.doi.org/10.1034/j.1399-3003.1999.14d29.x
14. Standardization of Spirometry, 1994 update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36. http://dx.doi.org/10.1164/ajrccm.152.3.7663792
15. Crapo RO, Morris AH, Gardner RM. Reference spirometric values using techniques and equipment that meet ATS recommendations. Am Rev Respir Dis. 1981;123(6):659-64. PMid:7271065
16. Pizzichini E, Pizzichini MM, Efthimiadis A, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effects of selection of sputum to minimize salivary contamination. Eur Respir J. 1996;9(6):1174-80. http://dx.doi.org/10.1183/09031936.96.09061174
17. Bland JM, Altman DG. Comparing methods of measurement: why plotting difference against standard method is misleading. Lancet. 1995;346(8982):1085-7. http://dx.doi.org/10.1016/S0140-6736(95)91748-9
18. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-74. http://dx.doi.org/10.2307/2529310
19. Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. Am J Respir Crit Care Med. 2000;161(2 Pt 1):475-8. http://dx.doi.org/10.1164/ajrccm.161.2.9903097
20. Efthimiadis A, Pizzichini MM, Pizzichini E. The influence of cell viability and squamous epithelial cell contamination on the repeatability of sputum differential cell counts (abstract). Am J Respir Crit Care Med. 1995;151:A384.
21. Ward R, Woltmann G, Wardlaw AJ, Pavord ID. Between-observer repeatability of sputum differential cell counts. Influence of cell viability and squamous cell contamination. Clin Exp Allergy. 1999;29(2):248-52. http://dx.doi.org/10.1046/j.1365-2222.1999.00483.x
22. Spanevello A, Migliori GB, Sharara A, Ballardini L, Bridge P, Pisati P, et al. Induced sputum to assess airway inflammation: a study of reproducibility. Clin Exp Allergy. 1997;27(10):1138-44. http://dx.doi.org/10.1111/j.1365-2222.1997.tb01150.x
23. Efthimiadis A, Spanevello A, Hamid Q, Kelly MM, Linden M, Louis R, et al. Methods of sputum processing for cell counts, immunocytochemistry and in situ hybridisation. Eur Respir J Suppl. 2002;37:19s-23s. PMid:12361358
24. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med. 1996;154(2 Pt 1):308-17. http://dx.doi.org/10.1164/ajrccm.154.2.8756799
25. Kelly MM, Leigh R, Horsewood P, Gleich GJ, Cox G, Hargreave FE. Induced sputum: validity of fluid-phase IL-5 measurement. J Allergy Clin Immunol. 2000;105(6 Pt 1):1162-8. http://dx.doi.org/10.1067/mai.2000.106375
26. Popov TA, Pizzichini MM, Pizzichini E, Kolendowicz R, Punthakee Z, Dolovich J, et al. Some technical factors influencing the induction of sputum for cell analysis. Eur Respir J. 1995;8(4):559-65. PMid:7664854
27. Efthimiadis A, Pizzichini MM, Pizzichini E, Dolovich J, Hargreave FE. Induced sputum cell and fluid-phase indices of inflammation: comparison of treatment with dithiothreitol vs phosphate-buffered saline. Eur Respir J. 1997;10(6):1336-40. http://dx.doi.org/10.1183/09031936.97.10061336
28. Saraiva-Romanholo BM, Barnabé V, Carvalho AL, Martins MA, Saldiva PH, Nunes Mdo P. Comparison of three methods for differential cell count in induced sputum. Chest. 2003;124(3):1060-6. http://dx.doi.org/10.1378/chest.124.3.1060
29. Pizzichini E, Pizzichini MM, Hargreave FE. Induced sputum (IS) cell counts: a new device to standardize processing in clinical trials and practice. In: International Conference of American Thoracic Society, 2004, Orlando. Am J Respir Crit Care Med. 2004;169:A817.
30. Chaboillez S, Dasgupta A, Prince P, Boulet LP, Lemiere C. A kit to facilitate and standardize the processing of sputum for measurement of airway inflammation. Can Respir J. 2013;20(4):248-52. PMid:23717819

About the authors

Jéssica Gonçalves
Pharmacist. Clinical Analysis Department, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Emilio Pizzichini
Adjunct Professor. Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Marcia Margaret Menezes Pizzichini
Professor. Graduate Program in Medical Sciences, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Leila John Marques Steidle
Adjunct Professor. Department of Clinical Medicine, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Cristiane Cinara Rocha
Coordinating Nurse. Center for Research on Asthma and Airway Inflammation, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Samira Cardoso Ferreira
Pharmacist. Clinical Analysis Department, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

Célia Tânia Zimmermann
Laboratory Technician. Clinical Analysis Department, Federal University of Santa Catarina University Hospital, Florianópolis, Brazil.

 http://dx.doi.org/10.1590/S1806-37132014000300008

Indoor air quality and health in schools*

Qualidade do ar interno e saúde em escolas

Ana Maria da Conceição Ferreira, Massano Cardoso

Abstract

Objective: To determine whether indoor air quality in schools is associated with the prevalence of allergic and respiratory diseases in children. **Methods:** We evaluated 1,019 students at 51 elementary schools in the city of Coimbra, Portugal. We applied a questionnaire that included questions regarding the demographic, social, and behavioral characteristics of students, as well as the presence of smoking in the family. We also evaluated the indoor air quality in the schools. **Results:** In the indoor air of the schools evaluated, we identified mean concentrations of carbon dioxide (CO_2) above the maximum reference value, especially during the fall and winter. The CO_2 concentration was sometimes as high as 1,942 ppm, implying a considerable health risk for the children. The most prevalent symptoms and respiratory diseases identified in the children were sneezing, rales, wheezing, rhinitis, and asthma. Other signs and symptoms, such as poor concentration, cough, headache, and irritation of mucous membranes, were identified. Lack of concentration was associated with CO_2 concentrations above the maximum recommended level in indoor air ($p = 0.002$). There were no other significant associations. **Conclusions:** Most of the schools evaluated presented with reasonable air quality and thermal comfort. However, the concentrations of various pollutants, especially CO_2, suggest the need for corrective interventions, such as reducing air pollutant sources and improving ventilation. There was a statistically significant association between lack of concentration in the children and exposure to high levels of CO_2. The overall low level of pollution in the city of Coimbra might explain the lack of other significant associations.

Keywords: Air pollution, indoor; Child welfare; Signs and symptoms, respiratory.

Resumo

Objetivo: Determinar se há uma associação entre a qualidade do ar interno em escolas e a prevalência de patologias alérgicas e respiratórias nas crianças que as frequentam. **Métodos:** Foram avaliados 1.019 alunos de 51 escolas de ensino básico na cidade de Coimbra, Portugal. A avaliação foi realizada através de um questionário com questões referentes a características demográficas, sociais e comportamentais dos alunos, assim como presença de hábitos tabágicos na família. Foi ainda avaliada a qualidade do ar interno nas escolas. **Resultados:** Foram identificadas concentrações médias de dióxido de carbono (CO_2) no interior das salas de aula acima da concentração máxima de referência, principalmente no período de outono/inverno, chegando a valores de 1.942 ppm, o que implica elevado risco potencial para a saúde das crianças. Os sintomas/patologias respiratórias mais prevalentes nas crianças foram crises de espirros, rinite alérgica, estertores/sibilos e asma. Outros sinais e sintomas verificados foram falta de concentração, tosse, dores de cabeça e irritação das mucosas. A falta de concentração das crianças foi associada ao ar interno das salas de aula com valores acima do máximo recomendado para CO_2 ($p = 0,002$). Não houve outras associações significativas. **Conclusões:** A maioria das escolas estudadas apresentava razoável qualidade do ar e conforto térmico, embora a concentração de vários poluentes, sobretudo CO_2, sugere a necessidade de intervenções corretivas, como redução de fontes emissoras de poluentes e melhorias da ventilação. Houve uma associação estatisticamente significativa entre a falta de concentração nas crianças e exposição a valores elevados de CO_2. O baixo nível de poluição na cidade de Coimbra pode explicar a falta de outras associações significativas.

Descritores: Poluição do ar em ambientes fechados; Bem-estar da criança; Sinais e sintomas respiratórios.

Introduction

People spend, on average, over 80% of their time in buildings, being therefore exposed to higher concentrations of pollutants indoors than outdoors. Children are vulnerable to such exposure, being at an increased risk of developing respiratory diseases, such as asthma.[1,2] Asthma is

*Study carried out at the Coimbra School of Health Technology, Coimbra, Portugal.

Correspondence to: Ana Maria da Conceição Ferreira. Rua 5 de Outubro, São Martinho do Bispo, 3000, Coimbra, Portugal.
Tel. 351 23 980-2430. E-mail: anaferreira@estescoimbra.pt

Financial support: None.
Submitted: 7 August 2013. Accepted, after review: 15 April 2014.

the leading cause of hospitalization and school absenteeism, negatively affecting learning and academic performance in students in Western countries.[3,4]

Numerous strategies can be implemented in order to reduce the risk of exposure to pollutants; good indoor air quality is indispensable and is achieved through appropriate room ventilation, as well as through ventilation and exhaust of combustion fumes and gases. Temperature control and humidity control are also indispensable. Other, practical, recommendations include daily breathing exercises and outdoor leisure activities.[5]

Given that children spend a long time in school buildings, we can predict that the conditions in such buildings affect the incidence of respiratory symptoms.[6,7] Several studies involving children have shown a positive association between exposure to air pollutants and increased morbidity and mortality from respiratory problems.[8-10]

The objective of the present study was to determine whether indoor air quality in elementary schools in the city of Coimbra, Portugal, is associated with the prevalence of allergic and respiratory diseases in children.

Methods

The study focused on public and private elementary (1st-4th grade) schools in the Municipality of Coimbra. The schools were selected on the basis of a comparative analysis of the 81 schools and 230 classrooms (the network of public and private elementary schools) in the Municipality of Coimbra, the 2008/2015 Education Charter of the Municipality of Coimbra being taken into consideration. Various generic, demographic, and social indicators were used in the analysis. When there was only one elementary school in a given parish, that school was necessarily chosen so that all of the parishes of the Municipality of Coimbra were represented. Other aspects were taken into consideration, including school size (larger schools being selected), school surroundings, human activity, nearby traffic, and industrial activity in the area. A non-probabilistic convenience sampling procedure was used in order to select the sample. The inclusion criteria were as follows: selection of at least one school per parish; use of the aforementioned comparison criteria; and authorization from the *Direção Regional de Educação do Centro* (DREC, Central Regional Education Board), school clusters, and school principals. The sample consisted of 51 schools, which corresponded to 81 classrooms (35 1st-grade classrooms, 34 4th-grade classrooms, and 12 mixed classrooms). Of the total of schools, 32 were located in predominantly urban parishes, 17 were located in moderately urban parishes, and 2 were located in predominantly rural parishes.[11]

Indoor air quality was evaluated in the fall/winter and spring/summer. In order to characterize indoor air quality, we measured temperature, relative humidity (RH), and the concentrations of the following: carbon monoxide (CO); carbon dioxide (CO_2); ozone (O_3); nitrogen dioxide; sulfur dioxide (SO_2); volatile organic compounds (VOCs); formaldehyde; particulate matter of 2.5 μm in diameter ($PM_{2.5}$); and PM of 10 μm in diameter (PM_{10}). The aforementioned measurements were performed in the fall/winter (between November of 2010 and February of 2011) and in the spring/summer (between March of 2011 and June of 2011).

According to Portuguese National technical standards NT-SCE-02,[12] pollutants should be measured in the representative period of activity, either 2-3 h after the initiation of activities or after equilibrium conditions have been reached. All measurements of indoor air quality were performed during regular classes, i.e., within approximately 2 h after the beginning of classes (in the morning or in the afternoon), by placing the equipment in the most central position in each classroom and at the level of the airways of the students in the sitting position. All measurements were performed in accordance with Portuguese National technical standards NT-SCE-02,[12] at 1 m from the floor and at least 3 m from the walls, between 10:30 a.m. and 5:30 p.m., for a period of 30 min, samples for the measurement of PM, VOCs, and the remaining parameters of indoor air quality being collected every 30 s, every 15 s, and every 60 s, respectively. All measurements were performed over the course of one week. On average, two measurements per day were performed in each classroom, depending on the size of the classroom.

Outdoor air quality was measured during recess, the measurements being performed at 1 m from the ground and at least 1 m from the external walls of the schools studied.[13]

For real-time measurement of air quality parameters, the following portable devices were used: VelociCalc 9555-P (TSI Inc., Shoreview,

 http://dx.doi.org/10.1590/S1806-37132014000300009

MN, USA), in order to measure temperature, RH, and the concentrations of CO and CO_2; series 500 handheld monitor (Aeroqual Ltd., Auckland, New Zealand), in order to measure the concentration of O_3; QRAE (ERA Systems Europe ApS, Kastrup, Denmark), in order to measure the concentrations of nitrogen dioxide and SO_2; Formaldemeter htV (PPM Technology, Caernarfon, UK), in order to measure formaldehyde; Voyager (Photovac Inc., Waltham, MA, USA), in order to measure the concentrations of VOCs; and DUSTTRACK (TSI Inc.), in order to measure the concentration of PM. The devices were calibrated before sampling, the "blank" (or zero) standard being used whenever necessary in order to compare the results obtained in cases of measurements performed after changing sensors. We took into consideration the conversion of the readings on the basis of the variations in temperature and pressure.

Information on the students was collected by a questionnaire that resulted from different pre-tests. Those pre-tests focused on the time it took parents or legal guardians to complete the questionnaire, as well as on their understanding of the questions depending on the topics covered. The final version covered the following topics: family characteristics (nuclear family, single-parent family, or extended family); housing characteristics (place of residence, mean length of stay, type of housing, and thermal conditions, among others); and information regarding symptoms/diseases and physical activity levels in the children studied. Envelopes containing the questionnaires were delivered to the teachers by the principal investigator, and the teachers instructed the students to deliver the questionnaires to their parents/legal guardians.

A non-probabilistic convenience sampling procedure was used in order to select elementary school children in the 1st-4th grade. Of a total of 4,319 children, 1,019 were selected.

We measured the weight and height of the children and subsequently calculated the body mass index, by dividing the weight (in kg) by height (in m^2). Children with a body mass index above the 95th percentile were classified as overweight on the basis of the 2000 US Centers for Disease Control and Prevention percentile distribution for gender and age.[14]

The results are presented by school, and the proportions were compared by the chi-square test and Fisher's exact test. For comparison of continuous variables, ANOVA or its non-parametric equivalent (i.e., the Kruskal-Wallis test) was used. The relative risk was estimated by calculating ORs and their 95% CIs. All statistical analyses were performed with the IBM SPSS Statistics software package, version 19.0 (IBM Corporation, Armonk, NY, USA).

In the present study, we strategically divided the schools into two categories, on the basis of CO_2 levels: no health risk (i.e., mean CO_2 concentrations below the maximum reference value established by Portuguese government Decree-Law no. 79/2006, i.e., $\leq$ 984 ppm); and health risk (i.e., mean CO_2 concentrations > 984 ppm).

Results

The mean age of 1st-grade students was 6.20 $\pm$ 0.42 years, and the mean age of 4th-grade students was 9.25 $\pm$ 0.48 years. Most (51.63%) of the children were male. There was a relatively uniform distribution of the 493 female children between the two grades studied. A similar trend was observed in male students.

By measuring weight and height, we found that 5% of the children included in the study were obese.

We also found that 84.6% of the children practiced sports. In 7 of the schools studied, 100% of the children practiced sports.

Regarding the level of education of the parents/legal guardians (by area of residence, i.e., parish), of the 1,014 respondents, 436 (43%) had finished college, and 48 (4.7%) had had only 4 years of schooling. Most of the parents/legal guardians resided in predominantly urban and moderately urban parishes (61% and 26%, respectively).

Regarding the age of the households where the children lived, we found that there were statistically significant differences: 71.3% of the households were less than 21 years of age, and 8.2% were over 40 years of age. In addition, 25.4% of the households had mold, and there were significant differences among the households in terms of the presence of moisture and a heating system (53.7% of the children having been found to live in households without heating systems).

The proportion of students living in households with heating and fewer signs of moisture was higher in the schools whose students had

parents/legal guardians who had a higher level of education.

Table 1 shows the mean concentrations of indoor air quality parameters in the elementary schools in the two sampling periods, i.e., fall/winter and spring/summer.

Mean concentrations of CO and CO_2 were significantly higher in the fall/winter than in the spring/summer ($p < 0.001$). There was a reduction of 0.28 ppm in the concentration of CO from one sampling period to another. Regarding CO_2 levels, there was a reduction of 425.36 ppm in the spring/summer. We found mean CO_2 concentrations that were well above the maximum reference value (i.e., 984 ppm) and therefore posed health risks to the children studying in those schools.

There were significant differences between the mean formaldehyde concentration measured in the fall/winter and that measured in the spring/summer. Mean formaldehyde concentrations were found to be significantly higher in the spring/summer than in the fall/winter, formaldehyde levels having increased by 0.0103 ppm. Although there were no significant differences between the two sampling periods in terms of the remaining parameters, the concentrations of PM_{10}, O_3, VOCs, and SO_2 were found to be lower in the spring/summer than in the fall/winter. Conversely, the concentration of $PM_{2.5}$ was found to be higher, although not significantly so.

In all but 2 classrooms, mean air temperatures in the fall/winter were found to be well below the reference value. In general, mean air temperatures in the spring/summer were found to be above the reference value, which was due to the external temperature and the fact that the classrooms had no cooling system.

In both sampling periods, RH values were found to be between the lower and upper limits (30-70%). However, in the fall/winter period, 7 schools had RH values above 70%.

Regarding the concentration of air pollutants in the outdoor air of the schools in the two sampling periods, mean concentrations of CO, CO_2, $PM_{2.5}$, PM_{10}, and formaldehyde varied significantly. CO and CO_2 levels were found to be significantly lower in the fall/winter than in the spring/summer. Conversely, $PM_{2.5}$, PM_{10}, and formaldehyde levels were found to be significantly higher in the spring/summer than in the fall/winter.

Table 2 shows the most prevalent symptoms and diseases in the children studied.

The most prevalent symptoms/diseases in the 1st-grade children were as follows: sneezing attacks, in 24%; lack of concentration, in 20%; rales and wheezing, in 17%; cough, in 16%; and allergic rhinitis, in 16%. In the 4th-grade children, the most prevalent symptoms/diseases were as follows: sneezing attacks, in 27%; lack of concentration, in 24%; allergic rhinitis, in 20%; and cough, in 16%. When we compared the children who were in the 1st grade with those who were in the 4th grade in terms of the prevalence of each symptom, we found that rales and wheezing were more common in the 1st-grade children (having been found in 55%), as was cough (in 51%). The remaining symptoms/diseases were found to be more common in the children who were in the 4th grade.

Of all environmental parameters analyzed, CO_2 levels showed the worst results, posing serious health risks. In the indoor air of the schools evaluated, mean CO_2 concentrations were in general well above the maximum reference value (984 ppm), being sometimes as high as 1,942 ppm. Given that CO_2 concentrations in indoor air were found to be much higher in the fall/winter than in the spring/summer, we sought to estimate the risk of symptoms/diseases in the elementary school children. The classrooms were classified as posing health risks or as posing no health risks on the basis of the reference value. The symptoms/diseases were reported by the parents/legal guardians through the questionnaire (Table 3).

We found no significant association between the presence/absence of asthma and exposure to classrooms with/without health risks ($p = 0.831$). However, the prevalence of asthma was 11.8% in the total population of children studied.

Chronic bronchitis occurred in 22 children (2.2%); however, we found no significant association between the disease and exposure to high CO_2 levels in the classrooms during the fall/winter ($p > 0.05$). Rales/wheezing were reported in 155 children (prevalence, 15.2%), with no significant associations ($p > 0.05$). Although we found no association between sneezing attacks and exposure to high CO_2 levels ($p > 0.05$), we found that, of the 856 children studying in classrooms with high CO_2 levels (health risk), 223 (26.1%) had sneezing attacks.

 http://dx.doi.org/10.1590/S1806-37132014000300009

Table 1 - Distribution of the concentrations of pollutants in the indoor air of the 81 classrooms studied, by season.[a]

Pollutants	Sampling period		Δfall/winter – spring/summer (mean)	Maximum reference value according to Portuguese law
	Fall/winter	Spring/summer		
CO, ppm	0.42 ± 0.53*	0.14 ± 0.13	0.28	10.7
CO_2, ppm	1578.16* ± 712.49	1152.80 ± 595.41	425.36	984
$PM_{2.5}$, mg/m^3	0.08 ± 0.04	0.10 ± 0.03	–0.02	Not mentioned
PM_{10}, mg/m^3	0.12 ± 0.05	0.11 ± 0.03	0.006	0.15
O_3, ppm	0.002 ± 0.060	0.0009 ± 0.0040	0.001	0.10
VOCs, ppb	97.82 ± 73.72	90.51 ± 65.66	7.31	260
SO_2, ppm	0.005 ± 0.020	0.004 ± 0.030	0.001	Not mentioned
Formaldehyde, ppm	0.01 ± 0.01*	0.02 ± 0.02	–0.01	0.08

CO: carbon monoxide; CO_2: carbon dioxide; $PM_{2.5}$: particulate matter of 2.5 μm in diameter; PM_{10}: particulate matter of 10 μm in diameter; O_3: ozone; VOCs: volatile organic compounds; and SO_2: sulfur dioxide. [a]Values expressed as mean ± SD. *p < 0.0001 (Student's t-test for paired samples or Wilcoxon t-test).

We found no association of allergic rhinitis, cough, or breathing difficulties with exposure to classrooms with or without health risks because of CO_2 levels (p > 0.05); however, of the total of children controlled for each symptom, 184 (18.9%) had rhinitis, 164 (16.1%) had cough, and 103 (10.1%) had breathing difficulties.

We sought to understand the distribution of non-respiratory symptoms by classroom with or without health risks during the fall/winter. The classification into presence or absence of health risks followed the methods described in the previous analysis (Table 4).

On the basis of the reports by the parents/legal guardians of the 1,019 children included in the present study, we calculated the prevalence of the following signs and symptoms: stress, 1.8%; dizziness, 2.0%; irritability, 4.2%; headache, 8%; mucosal irritation, 4.9%; and insomnia, 4.0%. None of the parameters evaluated were found to be significantly associated with the presence or absence of health risks in the classrooms (p > 0.05). Lack of concentration was found to be associated with exposure to indoor air in which CO_2 levels were > 984 ppm (p = 0.002). The probability of having poor concentration was 2.143 times higher in the children who were exposed to CO_2 levels > 984 ppm than in those who were not. Of the total of children investigated in the present study, 227 (22.3%) were found to have poor concentration.

We sought to determine whether asthma was associated with household exposure to tobacco smoke (Table 5). We found that 361 (35.43%) of the parents/legal guardians were smokers, and, of those, 252 (69.8%) had the habit of smoking in the household. Although we found no association between tobacco exposure and asthma (p > 0.05), 30 (11.9%) of the 252 children exposed to tobacco smoke had asthma.

Discussion

Children constitute a risk group and are vulnerable to poor indoor environmental quality. The development of respiratory diseases is associated with poor air quality in school buildings.[6,15]

In the present study, the concentrations of the pollutants analyzed were in general below the maximum reference value, the exception being the concentration of CO_2. However, we found significant concentrations of certain parameters, namely PM_{10} and VOCs.

The results of the present study showed inadequate classroom air renewal. Because of the total volume of the classrooms, the total number of classroom occupants, and the climatic conditions, classroom ventilation during breaks is insufficient to reduce CO_2 levels to acceptable levels. Several recent studies, some of which were conducted in Portugal,[2,16,17] showed high CO_2 levels in schools as a result of high occupancy and inadequate ventilation.[18-21] These results raise several questions to be answered by governments and those responsible for this area, especially after the latest restructuring carried out at the level of schools and school clusters. Large clusters increase the number of students per classroom and, consequently, reduce the number of classes, leading us to ask the following question: Won't this reduce indoor air quality and therefore have a negative impact on the health of children?

Table 2 - Signs, symptoms, and diseases in 1st- and 4th-grade children in the city of Coimbra, Portugal.

Signs, symptoms, and diseases		School grade						Total	
		1st grade			4th grade				
		n	% column	% line	n	% column	% line	n	% column
Asthma	No	451	89.3	50.2	448	87.2	49.8	899	88.2
	Yes	54	10.7	45.0	66	12.8	55.0	120	11.8
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Chronic bronchitis	No	495	98.0	49.6	502	97.7	50.4	997	97.8
	Yes	10	2.0	45.5	12	2.3	54.5	22	2.2
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Rales/wheezing	No	420	83.2	48.6	444	86.4	51.4	864	84.8
	Yes	85	16.8	54.8	70	13.6	45.2	155	15.2
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Sneezing attacks	No	382	75.6	50.3	377	73.3	49.7	759	74.5
	Yes	123	24.4	47.3	137	26.7	52.7	260	25.5
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Allergic rhinitis	No	426	84.4	51.0	409	79.6	49.0	835	81.9
	Yes	79	15.6	42.9	105	20.4	57.1	184	18.1
	Total	505	100.0	49.6	514	100.0	50.4	1019	100.0
Breathing difficulties	No	459	90.9	50.1	457	88.9	49.9	916	89.9
	Yes	46	9.1	44.7	57	11.1	55.3	103	10.1
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Stress	No	499	98.8	49.9	502	97.7	50.1	1001	98.2
	Yes	6	1.2	33.3	12	2.3	66.7	18	1.8
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Dizziness	No	497	98.4	49.7	502	97.7	50.3	999	98.0
	Yes	8	1.6	40.0	12	2.3	60.0	20	2.0
	Total	505	100.0	49.6	514	100.0	50.4	1019	100.0
Irritability	No	487	96.4	49.9	489	95.1	50.1	976	95.8
	Yes	18	3.6	41.9	25	4.9	58.1	43	4.2
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Headache	No	472	93.5	50.4	465	90.5	49.6	937	92.0
	Yes	33	6.5	40.2	49	9.5	59.8	82	8.0
	Total	505	100.0	49.6	514	100.0	50.4	1019	100.0
Conjunctival irritation	No	483	95.6	49.8	486	94.6	50.2	969	95.1
	Yes	22	4.4	44.0	28	5.4	56.0	50	4.9
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Insomnia	No	486	96.2	49.7	492	95.7	50.3	978	96.0
	Yes	19	3.8	46.3	22	4.3	53.7	41	4.0
	Total	505	100.0	49.6	514	100.0	50.4	1019	100.0
Cough	No	421	83.4	49.2	434	84.4	50.8	855	83.9
	Yes	84	16.6	51.2	80	15.6	48.8	164	16.1
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0
Lack of concentration	No	402	79.6	50.8	390	75.9	49.2	792	77.7
	Yes	103	20.4	45.4	124	24.1	54.6	227	22.3
	Total	505	100.0	49.6	514	100.0	50.4	1.019	100.0

In the present study, the most prevalent symptoms/diseases were sneezing attacks, lack of concentration, allergic rhinitis, cough, rales/wheezing, and asthma. Other studies have reported similar results.[6,22,23] In addition, lack of concentration was associated with CO_2 levels > 984 ppm in indoor air ($p = 0.002$). The probability of having poor concentration was 2.143 times

 http://dx.doi.org/10.1590/S1806-37132014000300009

Table 3 - Estimation of the risk of respiratory symptoms and diseases in the children who, during the fall/winter, were exposed to a mean carbon dioxide concentration that was above or below the maximum reference value.

Respiratory symptoms and diseases	Maximum reference value for carbon dioxide concentration							c^2	df	p	OR	95% CI
		Above		Below		Total						
		n	%	n	%	n	%					
Asthma	Yes	100	11.7	20	12.3	120	11.8	0.046	1	0.831	0.946	0.567-1.579
	No	756	88.3	143	87.7	899	88.2					
	Total	856	100.0	163	100.0	1019	100.0					
Chronic bronchitis	Yes	18	2.1	4	2.5	22	2.2	0.080	1	0.777	0.854	0.285-2.556
	No	838	97.9	159	97.5	997	97.8					
	Total	856	100.0	163	100.0	1019	100.0					
Rales/wheezing	Yes	133	15.5	22	13.5	155	15.2	0.442	1	0.506	1.179	0.725-1.917
	No	723	84.5	141	86.5	864	84.8					
	Total	856	100.0	163	100.0	1019	100.0					
Sneezing attacks	Yes	223	26.1	37	22.7	260	25.5	0.810	1	0.368	1.200	0.807-1.784
	No	633	73.9	126	77.3	759	74.5					
	Total	856	100.0	163	100.0	1019	100.0					
Allergic rhinitis	Yes	160	18.7	24	14.7	184	18.1	1.457	1	0.227	1.331	0.835-2.122
	No	696	81.3	139	85.3	835	81.9					
	Total	856	100.0	163	100.0	1019	100.0					
Cough	Yes	141	16.5	23	14.1	164	16.1	0.565	1	0.452	1.200	0.745-1.933
	No	715	83.5	140	85.9	855	83.9					
	Total	856	100.0	163	100.0	1019	100.0					
Breathing difficulties	Yes	85	9.9	18	11.0	103	10.1	0.187	1	0.666	0.888	0.518-1.522
	No	771	90.1	145	89.0	916	89.9					
	Total	856	100.0	163	100.0	1019	100.0					

df: degrees of freedom.

higher in the children who were exposed to CO_2 levels above the reference range than in those who were not. In one study, high CO_2 levels in schools were associated with rales and cough in children.[24]

Exposure to tobacco smoke in indoor environments results in an increased risk for bronchitis and asthma, among others.[25,26] Of the 361 parents/legal guardians who were smokers, 30.2% did not smoke at home and 69.8% did. We therefore sought to determine whether there was a relationship between parents who smoked at home and symptoms/diseases in the children. We found that most of the parents/legal guardians who smoked at home had children with asthma (76.9%), chronic bronchitis, rales/wheezing (69.0%), sneezing attacks (56.0%), allergic rhinitis (65.0%), stress (66.7%), dizziness (85.7%), irritability (71.4%), headache (75.0%), irritation of the mucous membranes of the eyes (66.7%), dry cough (53%), insomnia (72.7%), breathing difficulties (70.5%), and lack of concentration (62.2%). The health effects of passive exposure to tobacco smoke have been the subject of numerous investigations. It is known that children are particularly susceptible, being at an increased risk of developing allergic airway disease, particularly bronchial asthma, and the diseases is more severe in children.[27,28] Therefore—and on the basis of our results, which are worrisome from an environmental standpoint in schools—it is desirable that children be exposed to lower levels of all contaminants at home, including tobacco smoke contaminants.

Nowadays, people spend most of their time in enclosed spaces, such as school buildings. Poor indoor air quality in such buildings is associated with the development of respiratory diseases. In the present study, the most prevalent symptoms were sneezing attacks and lack of concentration.

Most of the schools studied had reasonable air quality and thermal comfort. However, the concentrations of various pollutants, especially CO_2, suggest the need for corrective interventions, such as reducing air pollutant sources and improving ventilation. Several studies have shown high CO_2

Table 4 - Estimation of the risk of non-respiratory signs and symptoms in the children who, during the fall/winter, were exposed to a mean carbon dioxide concentration that was above or below the maximum reference value.

Signs and symptoms	Maximum reference value for carbon dioxide concentration							c^2	df	p	OR	95% CI
		Above		Below		Total						
		n	%	n	%	n	%					
Stress	Yes	14	1.6	4	2.5	18	1.8	0.529	1	0.467	0.661	0.215-2.034
	No	842	98.4	159	97.5	1001	98.2					
	Total	856	100.0	163	100.0	1019	100.0					
Dizziness	Yes	17	2.0	3	1.8	20	2.0	0.015	1	0.902	1.081	0.313-3.730
	No	839	98.0	160	98.2	999	98.0					
	Total	856	100.0	163	100.0	1019	100.0					
Irritability	Yes	40	4.7	3	1.8	43	4.2	2.718	1	0.099	2.614	0.799-8.554
	No	816	95.3	160	98.2	976	95.8					
	Total	856	100.0	163	100.0	1019	100.0					
Headache	Yes	70	8.2	12	7.4	82	8.0	0.123	1	0.726	1.121	0.593-2.118
	No	786	91.8	151	92.6	937	92.0					
	Total	856	100.0	163	100.0	1019	100.0					
Mucosal irritation	Yes	42	4.9	8	4.9	50	4.9	0.0001	1	0.999	1.000	0.460-2.171
	No	814	95.1	155	95.1	969	95.1					
	Total	856	100.0	163	100.0	1019	100.0					
Insomnia	Yes	33	3.9	8	4.9	41	4.0	0.393	1	0.531	0.777	0.352-1.714
	No	823	96.1	155	95.1	978	96.0					
	Total	856	100.0	163	100.0	1019	100.0					
Lack of concentration	Yes	206	24.1	21	12.9	227	22.3	9.888	1	0.002	2.143	1.320-3.478
	No	650	75.9	142	87.1	792	77.7					
	Total	856	100.0	163	100.0	1019	100.0					

df: degrees of freedom.

Table 5 - Association between asthma in children and smoking parents/legal guardians (N = 361) smoking at home.

Parents/legal guardians smoking at home	Asthma in children			p	OR	95% CI
	Yes	No	TOTAL			
Yes	30 (11.9)	222 (88.1)	252 (100.0)	0.305	1.502	0.687-3.280
No	9 (8.3)	100 (91.7)	109 (100.0)			
TOTAL	39 (10.8)	322 (89.2)	361 (100.0)			

levels in schools as a result of high occupancy or inadequate ventilation.[2,18-21] We found a statistically significant association between poor concentration and exposure to high CO_2 levels. One possible explanation for the lack of other significant associations is the overall low level of pollution in the city of Coimbra.

Potential limitations of the present study include the fact that the information regarding symptoms/diseases in the children was reported by their parents/legal guardians. The perception that parents/legal guardians have of their children might not correspond to reality.

The present study allowed us to assess the risks to which the population is exposed and provide guidelines for the development of measures to minimize these risks. We hope that our findings will contribute to environmental health planning in school buildings and the improvement of political strategies to promote quality of life.

References

1. Cruz H, Viegas JC. Medição experimental do escoamento do ar através de janelas abertas. ENVIRH [serial on the Internet]. Lisboa: ENVIRH. IV Conferência Nacional em Mecânica dos Fluidos, Termodinâmica e Energia, 2012 May 28-29; Lisboa. [Adobe Acrobat document,

 http://dx.doi.org/10.1590/S1806-37132014000300009

10p.]. Available from: http://envirh.fcm.unl.pt/images/stories/docs/cn03.pdf
2. Viegas JC, Papoila AL, Martins P, Aelenei D, Cano M, Proença C, et al. Ventilação, qualidade do ar e saúde em creches e infantários resultados preliminares do Projeto ENVIRH. ENVIRH [serial on the Internet] IV Congresso Nacional Construção, 2012 Dec 18-20; Coimbra [Adobe Acrobat document, 12p.]. Available from: http://envirh.fcm.unl.pt/images/stories/docs/cn10.pdf
3. Moonie SA, Sterling DA, Figgs L, Castro M. Asthma status and severity affects missed school days. J Sch Health. 2006;76(1):18-24. http://dx.doi.org/10.1111/j.1746-1561.2006.00062.x
4. Daisey JM, Angell WJ, Apte MG. Indoor air quality, ventilation and health symptoms in schools: an analysis of existing information. Indoor Air. 2003;13(1):53-64. http://dx.doi.org/10.1034/j.1600-0668.2003.00153.x
5. Borrego C, Neuparth N, Carvalho AC, Carvalho A, Miranda AI, Costa AM, et al. A saúde e o ar que respiramos: um caso de estudo em Portugal. Lisboa: Fundação Calouste Gulbenkian; 2008.
6. Sundell J. On the history of indoor air quality and health. Indoor Air. 2004;14 Suppl 7:51-8. http://dx.doi.org/10.1111/j.1600-0668.2004.00273.x
7. Kim JL, Elfman L, Mi Y, Johansson M, Smedje G, Norbäck D. Current asthma and respiratory symptoms among pupils in relation to dietary factors and allergens in the school environment. Indoor Air. 2005;15(3):170-82. http://dx.doi.org/10.1111/j.1600-0668.2005.00334.x
8. Braga AL, Conceição GM, Pereira LA, Kishi HS, Pereira JC, Andrade MF, et al. Air pollution and pediatric respiratory hospital admissions in São Paulo, Brazil. J Environ Med. 1999;1:95-102. http://dx.doi.org/10.1002/(SICI)1099-1301(199904/06)1:2<95::AID-JEM16>3.0.CO;2-S
9. Braga AL, Saldiva PH, Pereira LA, Menezes JJ, Conceição GM, Lin CA, et al. Health effects of air pollution exposure on children and adolescents in São Paulo, Brazil. Pediatr Pulmonol. 2001;31(2):106-13. http://dx.doi.org/10.1002/1099-0496(200102)31:2<106::AID-PPUL1017>3.0.CO;2-M
10. Lin AC, Martins MA, Farhat SC, Pope CA 3rd, Conceição GM, Anastácio MV, et al. Air pollution and respiratory illness of children in São Paulo, Brazil. Paediatr Perinat Epidemiol. 1999;13(4):475-88. http://dx.doi.org/10.1046/j.1365-3016.1999.00210.x
11. Portugal. Conselho Superior de Estatística. Deliberação nº. 2717/2009, 28 de setembro de 2009. 8a Deliberação da Secção Permanente de Coordenação Estatística. Revisão da Tipologia de áreas urbanas. Diário da República, 2a série, Lisboa, no. 188, 2009.
12. Portugal. Decreto-Lei nº 80/2006 de 4 de Abril. Estabelece a Norma Técnica NTSCE02-Metodologia para auditorias periódicas de qualidade do ar interior em edifícios existentes no âmbito do RSECE. Diario da Republica . 4 abr 2006.
13. Carvalho R, Coelho D, Ferreira C, Nunes T. A Monitorização da qualidade do ar interior (QAI) em Portugal - Estudo comparativo de metodologias de amostragem e medição de QAI. Aveiro: Universidade de Aveiro; 2009.
14. Obesidade online (webpage on the Internet). Lisboa: obesidade online. [cited 2011 Jul 5]. Available from www.obesidade.online.pt
15. Shusterman D, Murphy MA. Nasal hyperreactivity in allergic and non-allergic rhinitis: a potential risk factor for non-specific building-related illness. Indoor Air. 2007;17(4):328-33. http://dx.doi.org/10.1111/j.1600-0668.2007.00482.x
16. Almeida SM, Canha N, Silva A, Freitas MC, Pegas P, Alves C, et al. Children exposure to atmospheric particles in indoor of Lisbon primary schools. Atmos Environ. 2011;45(40);7594-9.
17. Pegas PN. Indoor air quality in elementary schools of Lisbon and Aveiro [thesis]. Aveiro: Universidade de Aveiro; 2012.
18. van Dijken F, van Bronswijk JE, Sundell J. Indoor environment and pupils' health in primary schools. Build Res Inform. 2006;34(5):437-46.
19. Borodinecs A, Budjko Z. Indoor air quality in nursery schools in Latvia. Proceedings of the 9th International & Exhibition Healthy Buildings; 2009 Sep 13-17; Syracuse, USA.
20. Mumovic D, Palmer J, Davies M, Orme M, Ridley I, Oreszczyn T, et al. Winter indoor air quality, thermal comfort and acoustic performance of newly built secondary schools in England. Build Environ. 2009;44(7):1466-77.
21. Al-Rashidi K, Loveday D, Al-Mutawa N. Impact of ventilation modes on carbon dioxide concentration levels in Kuwait classrooms. Energy Build. 2012;47:540-9.
22. Saijo Y, Kishi R, Sata F, Katakura Y, Urashima Y, Hatkeyama A, et al. Symptoms in relation to chemicals and dampness in newly built dwellings. Int Arch Occup Environ Health. 2004;77(7):461-70.
23. Armstrong BC, White E, Saracci R. Principles of exposure measurement in Epidemiology. Oxford: Oxford University Press; 1994.
24. Fernandes EO, Silva GV, Martins A, Samúdio MJ, Guedes J, Barros H, et al. Saúde ambiental em ambiente escolar. In: Borrego C, Carvalho A, Miranda AI, Costa AM, Monteiro A., Amorim JH, et al, editors. Investigação em ambiente e saúde, desafios e estratégias. Aveiro: Universidade de Aveiro/CESAM; 2009. p. 83-7.
25. Horak E, Morass B, Ulmer H. Association between environmental tobacco smoke exposure and wheezing disorders in Austrian preschool children. Swiss Med Wkly. 2007;137(43-44):608-13.
26. Kabir Z, Manning PJ, Holohan J, Keogan S, Goodman PG, Clancy L. Second hand smoke exposure in cars and respiratory health effects in children. Eur Respir J. 2009; 34(3):629-33.
27. Bernstein JA, Alexis N, Bacchus H, Bernstein IL, Fritz P, Horner E, et al. The health effects of non-industrial indoor air pollution. J Allergy Clin Immunol. 2008;121(3):585-91. PMid:17990155
28. Moraes AP. Qualidade do ar interno com ênfase na concentração de aerodispersóides nos edifícios [dissertation]. São Paulo: Universidade de São Paulo; 2006.

Sobre os autores

Ana Maria da Conceição Ferreira
President. Scientific Committee, Environmental Health Course, Coimbra School of Health Technology, Coimbra, Portugal.

Massano Cardoso
Full Professor. University of Coimbra School of Medicine, Coimbra, Portugal.

 http://dx.doi.org/10.1590/S1806-37132014000300009

Association between serum selenium level and conversion of bacteriological tests during antituberculosis treatment*,**

Associações entre níveis de selênio sérico e conversão de testes bacteriológicos durante o tratamento antituberculose

Milena Lima de Moraes, Daniela Maria de Paula Ramalho, Karina Neves Delogo, Pryscila Fernandes Campino Miranda, Eliene Denites Duarte Mesquita, Hedi Marinho de Melo Guedes de Oliveira, Antônio Ruffino-Netto, Paulo César de Almeida, Rachel Ann Hauser-Davis, Reinaldo Calixto Campos, Afrânio Lineu Kritski, Martha Maria de Oliveira

Abstract

Objective: To determine whether serum selenium levels are associated with the conversion of bacteriological tests in patients diagnosed with active pulmonary tuberculosis after eight weeks of standard treatment. **Methods:** We evaluated 35 healthy male controls and 35 male patients with pulmonary tuberculosis, the latter being evaluated at baseline, as well as at 30 and 60 days of antituberculosis treatment. For all participants, we measured anthropometric indices, as well as determining serum levels of albumin, C-reactive protein (CRP) and selenium. Because there are no reference values for the Brazilian population, we used the median of the serum selenium level of the controls as the cut-off point. At 30 and 60 days of antituberculosis treatment, we repeated the biochemical tests, as well as collecting sputum for smear microscopy and culture from the patients. **Results:** The mean age of the patients was 38.4 ± 11.4 years. Of the 35 patients, 25 (71%) described themselves as alcoholic; 20 (57.0%) were smokers; and 21 (60.0%) and 32 (91.4%) presented with muscle mass depletion as determined by measuring the triceps skinfold thickness and arm muscle area, respectively. Of 24 patients, 12 (39.2%) were classified as moderately or severely emaciated, and 15 (62.5%) had lost > 10% of their body weight by six months before diagnosis. At baseline, the tuberculosis group had lower serum selenium levels than did the control group. The conversion of bacteriological tests was associated with the CRP/albumin ratio and serum selenium levels 60 days after treatment initiation. **Conclusions:** Higher serum selenium levels after 60 days of treatment were associated with the conversion of bacteriological tests in pulmonary tuberculosis patients.
Keywords: Selenium; Nutritional status; Tuberculosis; Immunity.

Resumo

Objetivo: Determinar se os níveis séricos de selênio estão associados à conversão dos testes bacteriológicos em pacientes diagnosticados com tuberculose pulmonar ativa após oito semanas de tratamento-padrão. **Métodos:** No início do estudo, avaliamos 35 controles saudáveis, do sexo masculino, e 35 pacientes do sexo masculino com tuberculose pulmonar. Estes foram também avaliados após 30 e 60 dias de tratamento antituberculose. Todos os participantes submeteram-se a medições antropométricas e quantificação dos níveis séricos de albumina, proteína C reativa (PCR) e selênio. Como não há valores de referência para a população brasileira, usamos a mediana dos resultados de selênio sérico dos controles como ponto de corte. Aos 30 e 60 dias do tratamento antituberculose, todos os testes bioquímicos foram repetidos, e foram coletadas amostras de escarro para baciloscopia e cultura. **Resultados:** A média de idade dos pacientes foi de 38,4 ± 11.4 anos. Dos 35 pacientes, 25 (71,0%) referiram alcoolismo, 20 (57,0%) eram fumantes, e 21 (60,0%) e 32 (91,4%) apresentavam depleção muscular pela medição da dobra cutânea tricipital e da área muscular do braço, respectivamente. De 24 pacientes, 12 (39,2%) foram classificados em moderadamente ou gravemente magros, e 15 (62,5%) apresentaram perda de peso > 10% em até seis meses antes do diagnóstico. No início do estudo, o grupo com tuberculose apresentou menores níveis de selênio sérico que os controles. A conversão dos testes bacteriológicos associou-se à relação PCR/albumina e aos níveis de selênio sérico 60 dias após o início do tratamento. **Conclusões:** Níveis maiores de selênio sérico após 60 dias de tratamento associaram-se à conversão bacteriológica em pacientes com tuberculose pulmonar.
Descritores: Selênio; Estado nutricional; Tuberculose; Imunidade.

*Study carried out in the Department of Clinical Medicine, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.
Correspondence to: Milena Lima de Moraes. Centro de Pesquisa em Tuberculose, Rua Professor Rodolpho Paulo Rocco, 255, 4º andar, Ilha do Fundão, CEP 21941-913, Rio de Janeiro, RJ, Brasil.
Tel. 55 21 2562-2426. Fax: 55 21 2550-0693. E-mail: milena_mila2003@yahoo.com.br
Financial support: This study received financial support from the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development; grant MCT/CNPq/INCT 573548/2008-0 and MCT/CNPq/INCT 478033/2009-5), and from the *Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro* (FAPERJ, Foundation for the Support of Research in the State of Rio de Janeiro; grant E 26/110974/2011).
Submitted: 5 August 2013. Accepted, after review: 30 April 2014.
**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

Introduction

The World Health Organization considers tuberculosis a serious public health problem. In 2010, 9.4 million new tuberculosis cases occurred, with 1.7 million associated deaths, among which 500,000 were HIV-positive patients. In Brazil, tuberculosis is the leading cause of mortality among patients with HIV/AIDS, a result arising from late diagnosis.[1] Since 2006, the Global Plan to Stop TB has been prioritizing the critical points in the field of tuberculosis, especially the development of new diagnostic tests, vaccines, drugs, and biomarkers of therapeutic response, of healing, and of disease recurrence.[2]

Among the risk factors associated with the occurrence of tuberculosis are precarious working conditions and changes in host defense against the infection by *Mycobacterium tuberculosis*, such as malnutrition, smoking, diabetes mellitus, and alcohol abuse.[3]

The degree of malnutrition is associated with the severity of pulmonary tuberculosis in adults. Tuberculosis patients usually present malnutrition and a decrease in micronutrient levels, regardless of their HIV status.[4]

Recently, one group of authors[5] reported that a two-month intervention with vitamin E and selenium supplements reduced oxidative stress and increased total antioxidant capacity in patients with pulmonary tuberculosis undergoing standard treatment. A similar improvement in the immune status of patients with tuberculosis who received selenium supplementation was also reported in another study.[6]

The objective of the present study was to determine whether serum selenium levels are associated with the conversion of bacteriological tests in patients diagnosed with active pulmonary tuberculosis after eight weeks of standard treatment. The conversion (to negative) of cultures of sputum collected eight weeks after treatment initiation has been used as a useful marker of the sterilizing activity of tuberculosis treatment,[7] and a substantial improvement in serum selenium levels in these patients would indicate that selenium can be a biomarker of therapeutic response.

Methods

Study subjects

Between March of 2007 and March of 2008, we included male patients with pulmonary tuberculosis admitted to either of the two referral hospitals for tuberculosis in the city of Rio de Janeiro, Brazil, namely the *Hospital Estadual Santa Maria* and the *Instituto Estadual de Doenças do Tórax Ary Parreiras*. We decided to include only male patients in the study because the great majority of the patients treated in these hospitals are males, and the inclusion of very few female patients could become a confounding factor in the data analysis. The patients enrolled in the present study had been hospitalized for clinical reasons; however, in most cases, the duration of hospital stay was prolonged for at least 60 days due to social reasons. The inclusion criteria were as follows: being 19-60 years of age; having a positive culture for *M. tuberculosis* or positive smear microscopy in spontaneous sputum in association with chest X-rays and symptoms indicative of tuberculosis; receiving treatment with first-line antituberculosis drugs; not having diabetes mellitus or renal disease (undergoing peritoneal dialysis or hemodialysis); having tested negative for HIV; and reporting no comorbidities.

Because there are no established reference values for selenium levels in serum for the Brazilian population, we determined the serum selenium levels of 35 HIV-negative healthy subjects residing in the city of Rio de Janeiro (using similar inclusion criteria) in order to define a cut-off point. All subjects gave written informed consent. The study was approved by the Research Ethics Committee of the Federal University of Rio de Janeiro (Protocol no. 004/05, of April 28, 2005). The patients enrolled in the pilot study were not included in the present study.

Data collection

A pilot study was conducted in order to determine the adequacy of the questionnaire applied to the study subjects. The interviewers were trained regarding data collection. Anthropometric measurements taken by different interviewers showed a high level of inter-rater agreement (> 95%).

The pulmonary tuberculosis patients completed a questionnaire regarding demographic data, socioeconomic data, and tobacco use, as well as the criteria used in the Cut down, Annoyed, Guilty, and Eye-opener (CAGE) questionnaire.[8] Anthropometric measurements were collected at baseline, as well as at 30 and 60 days after antituberculosis treatment initiation. Blood and

 http://dx.doi.org/10.1590/S1806-37132014000300010

sputum samples were also collected at the same time points. At 30- and 60-day sample collection time points, some of the patients no longer presented sputum production, and therefore no sputum smear microscopy/culture were performed for those patients. The healthy subjects also completed the questionnaire, underwent anthropometric assessment, and had their blood samples collected.

The anthropometric evaluation consisted of two body weight measurements using a calibrated platform scale with a stadiometer (Filizola, São Paulo, Brazil) with a sensitivity of 100 g and maximum weight of 150 kg. The subjects were weighed barefoot and wearing light clothing. Height was measured twice (stadiometer with a sensitivity of 0.5 cm and maximum height of 191 cm).

The body mass index (BMI) was calculated by the formula weight/height^2 and classified according to the World Health Organization recommendations: underweight, < 18.5 kg/m^2; normal weight, 18.5-24.9 kg/m^2; and overweight, ≥ 25.0 kg/m^2.[9] All measurements were collected in accordance with the techniques recommended by Gibson[10] in order to avoid possible bias. The patients with pulmonary tuberculosis also reported their usual weight (in the last 6 months) so that their weight loss until the beginning of the study (baseline) could be estimated.

The triceps skinfold thickness (TST) was measured three times with an adipometer (Lange Beta Technology Inc., Cambridge, MD, USA) with a sensitivity of 0.5 mm. Measurements were taken at the midpoint of the back of the non-dominant arm, between the acromion and olecranon, with the subjects standing with their arms relaxed and extended alongside the body.

The measurement of arm circumference (AC) was performed twice, with a flexible and inelastic millimeter tape at the same height as the midpoint used for the TST measurement. After that, the arm muscle area (AMA) was calculated using the following equation[11]:

$$AMA\ (cm^2) = [(AC(cm) - \varpi \times TST(mm) \div 10)^2 - 10]/4\varpi.$$

Mean TST and AMA results were calculated, and the cut-off values used were those by Frisancho.[12]

Peripheral blood samples were collected in the morning with subjects fasting for 12 h. The samples were collected in metal- and EDTA-free tubes. The samples were centrifuged at 3,000 g for 15 min for further quantification of albumin, C-reactive protein (CRP), and selenium. All quantifications were performed immediately after sample collection, except for the determination of selenium levels. In this case, a portion of the serum obtained was stored at −70°C for later quantification.

Albumin quantification was determined colorimetrically (Advia®; Siemens Healthcare Diagnostics, Eschborn, Germany). According to the manufacturer, normal albumin values should range from 3.4 to 4.8 g/dL. CRP was measured by nephelometry using a CardioPhase hsCRP assay (Dade Behring Holding GmbH, Liederbach, Germany) and a BNII nephelometer (Siemens Healthcare, Indianapolis, IN, USA). According to the manufacturer, normal values lay below 0.3 mg/dL.

In the present study, we evaluated the CRP/albumin ratio as a substitute for the prognostic inflammatory nutritional index because it maintains the same diagnostic sensitivity regarding the levels of complication risks.[13] According to one study, the levels of complication risks are as follows: no risk, if the ratio is < 0.4; low risk, from 0.4 to 1.1; medium risk, from 1.2 to 2.0; and high risk, > 2.0.[13]

The determination of selenium levels was performed by graphite furnace atomic absorption spectrometry, using a ZEEnit 60 spectrometer (Analytik Jena, Jena, Germany) equipped with a selenium hollow cathode lamp operating at a wavelength of 196.0 nm. After the thawing and homogenizing of the serum samples, 200 mL aliquots were transferred to polyethylene tubes, free of trace elements, and 1 mL of a 0.1% v/v Triton ×100 solution was added. This solution (10 mL) was used for the instrumental analysis, together with a mixture (10 mL) containing palladium (0.15% m/v) and magnesium (0.10% m/v) as matrix modifier. External calibration was performed with calibration solutions prepared in the stock solution, and the temperature protocol is shown in Table 1. All measurements were conducted at least in triplicate.

Sputum samples of the subjects included in the study were collected in disposable vials. Smear microscopy and cultures for mycobacteria were performed in accordance with the recommendations by the Brazilian National Ministry of Health.[14]

Table 1 - Temperature program used in order to determine selenium levels in serum.

Step	Temperature, °C	Ramp, °C/s	Duration, s
Drying	90	10	10
Drying	120	15	20
Pyrolysis	500	10	20
Pyrolysis	1,100	30	30
Auto zero	1,100	0	6
Atomization[a]	2,200	2,000	3
Cleaning	2,300	1,000	3

[a]Measurement.

Cultures contaminated by other microorganisms were designated as contaminated and considered negative in the data analysis. The strains were identified as *M. tuberculosis* on the basis of the characteristics of the colonies (rough, opaque, and creamy) and biochemical testing (ability to produce niacin, nitrate reduction, and thermal inactivation of catalase).[14] In the present study, the individuals were diagnosed with pulmonary tuberculosis at baseline when cultures were positive for *M. tuberculosis* or when there were positive results in sputum smear microscopy associated with X-ray findings and symptoms indicative of tuberculosis. Patients who presented with X-ray findings and symptoms indicative of tuberculosis but negative cultures or smear results at baseline were not included in the study.

Susceptibility testing was performed on the clinical specimens from 28 patients who had positive cultures using the method of proportions, which is considered the gold standard. In addition, we used the indirect proportion method (one strain per patient) in order to determine the susceptibility of the *M. tuberculosis* strains to isoniazid, rifampin, streptomycin, and ethambutol. All of the tested strains were susceptible to the drugs tested.

New sputum samples were collected 30 and 60 days after treatment initiation, and new smear microscopy testing and cultures for mycobacteria were performed. Depending on the results of the tests, the patients could be reallocated to either of the two groups: tuberculosis-positive (TB+) group, when smears or cultures were positive for *M. tuberculosis*; and tuberculosis-negative (TB–) group, when smears and cultures were negative for *M. tuberculosis*. The individuals who were unable to produce sputum spontaneously at the moments of collection were not included in either group.

Statistical analysis

The Kolmogorov-Smirnov test was used in order to verify the normality of the variables, and the Levene test was used in order to determine the equality of variances. A logarithmic transformation was used for the variables that showed non-normal distribution. We used Tukey's test and Games-Howell test to compare pairs of groups with equal and different variances, respectively. When appropriate, ANOVA and Student's t-test were used in order to estimate differences between quantitative variables. To evaluate the association between categorical variables, we used the chi-square test with continuity correction when indicated. A p-value < 0.05 was considered significant. The Statistical Package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA), was used for data analysis.

Results

We included 35 pulmonary tuberculosis patients in the study group at baseline. Among these, 6 were recurrent tuberculosis patients. After 30 days of treatment, only 29 patients presented spontaneous sputum production, and, after 60 days of treatment, 34 patients showed spontaneous sputum production (Figure 1).

The general characteristics of the pulmonary tuberculosis patients are presented in Table 2. The mean age of the patients was 38.4 ± 11.4 years. Among the 35 male study subjects included in the study, 25 (71%) reported alcoholism according to the CAGE questionnaire, and 20 (57%) were smokers. We determined the BMI of 24 of the patients, and 12 (39%) were classified as being severely or moderately emaciated. Of the 35 patients, 21 (60%) and 32 (91%) were found to have with muscle mass depletion on the basis of their TST and AMA, respectively. Of the 24 patients who provided information regarding their weight by 6 months prior to their inclusion in the study, 15 (63%) had lost > 10% of their body weight. Statistically significant differences were found between the pulmonary tuberculosis patients and the healthy controls at baseline.

When we compared the three study time points (baseline, 30 days, and 60 days), we found that the conversion to a negative-culture status was associated with the CRP levels and the CRP/albumin ratio results at 30 and 60 days, as well

 http://dx.doi.org/10.1590/S1806-37132014000300010

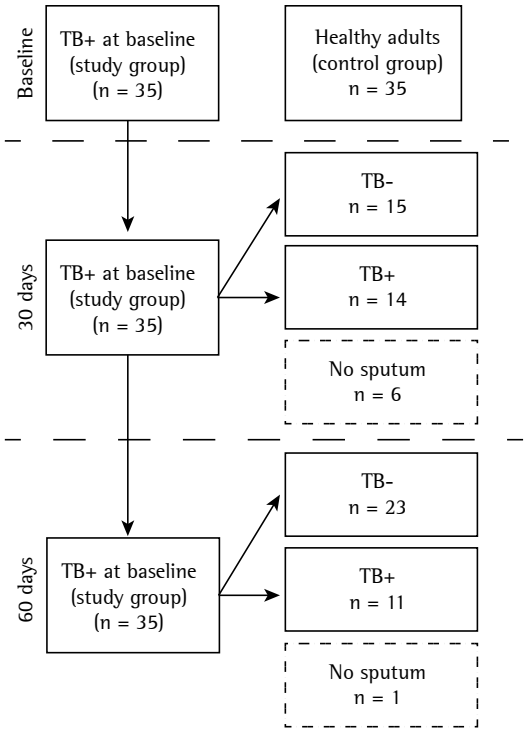


Figure 1 - Study and control groups at baseline, at 30 days after antituberculosis treatment initiation, and at 60 days after antituberculosis treatment initiation. TB+: positive sputum culture or positive sputum smear microscopy results at that study time point; and TB–: negative sputum culture and negative sputum smear microscopy results at that study time point.

as with albumin and selenium levels at 60 days (Table 3). No differences were observed between the TB+ and TB– groups for any of these variables at 30 days.

Table 4 presents the distribution of patients in the TB+ and TB– groups in relation to the results of the biochemical tests and serum selenium levels at the three study time points in order to determine the existence of any associations. In order to evaluate the association between the results of bacteriological tests (culture and smear microscopy) and serum selenium levels, we used the cut-off point based on the median of the results obtained in the healthy control group.

Discussion

In the present study, the clinical characteristics of the patients are similar to those described in other studies carried out in referral hospitals for the treatment of tuberculosis in developing nations, with high rates of alcoholism and tobacco use.[15]

Table 2 - General characteristics of the patients with pulmonary tuberculosis (N = 35).[a]

Characteristic	Result
Age, years[b]	38.43 ± 11.42
Alcoholism	25 (71)
Smoking status	
Smokers	20 (57)
Former smokers	7 (20)
Never smokers	8 (23)
Weight loss, kg[b,c]	11.03 ± 9.69
Weight loss, %[c]	
> 10	15 (63)
5-10	4 (17)
< 5%	2 (8)
No loss	3 (12)
Classification according to BMI[d]	
Severe thinness	6 (19.4)
Moderate thinness	6(19.4)
Mild thinness	6 (19.4)
Normal weight	12 (38.7)
Overweight or obese	1 (3.2)
Classification according to TST	
Depletion	21 (60.0)
Normal	14 (40.0)
Classification according to AMA	
Depletion	32 (91.4)
Normal	3 (8.6)

BMI: body mass index; TST: triceps skinfold thickness; and AMA: arm muscle area. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD. [c]n = 24. [d]n = 31

The relationship between tuberculosis and malnutrition has been revisited, since malnutrition may predispose to the development of active tuberculosis, and tuberculosis can contribute to malnutrition.[16] The mean weight loss in the study group prior to antituberculosis treatment initiation was 11.03 ± 9.69 kg. This can be considered even more significant when categorized by the percentage of body weight loss, because 63% of the patients presented with a weight loss ≥ 10%, which is considered a predisposing factor for tuberculosis.[17]

In the present study, the assessment of the nutritional status based on anthropometric parameters (BMI, TST, and AMA) confirmed the depleted nutritional status in the study group, as described in the literature.[18] For any infection, there is a complex interplay between the host response and the virulence of the microorganism, which modulates the metabolic response, as well as the degree and pattern of

Table 3 - Anthropometric variables, biochemical test results, and serum selenium levels in the groups studied at the three study time points.[a]

Variable	Baseline		30 days after treatment initiation			60 days after treatment initiation		
	Control	TB+ group	TB− group	TB+ group	TB+ group at baseline	TB− group	TB+ group	TB+ group at baseline
	(n = 35)	(n = 35)	(n = 15)	(n = 14)	(n = 35)	(n = 23)	(n = 11)	(n = 35)
BMI, kg/m^2	25.27 ± 3.59	18.21 ± 2.53*	19.60 ± 2.18*	19.40 ± 2.46*	19.49 ± 2.86*	20.64 ± 3.25*	20.41 ± 3.10*	20.53 ± 3.11*
TST, mm	12.71 ± 4.99	5.11 ± 2.51*	6.28 ± 2.48*	5.87 ± 1.82*	6.13 ± 2.57*	7.42 ± 4.32*	7.15 ± 2.83*	7.35 ± 3.80*
AMA, cm^2	55.15 ± 12.11	26.10 ± 7.92*	28.54 ± 9.86*	29.07 ± 9.24*	28.74 ± 9.69*	32.29 ± 12.37*	30.52 ± 12.09*	31.77 ± 11.94*
Alb, g/dL	4.86 ± 0.19	3.64 ± 0.62*	3.99 ± 0.38*	4.02 ± 0.60*	4.02 ± 0.47*	4.27 ± 0.50*†	3.95 ± 0.37*	4.16 ± 0.48*†
CRP, mg/dL	0.16 ± 0.16	6.35 ± 4.12*	2.31 ± 1.88*†	4.33 ± 3.36*	3.66 ± 3.55*	1.95 ± 1.70*†	4.43 ± 3.69*‡	2.68 ± 2.71*†
CRP/alb ratio	0.03 ± 0.03	1.93 ± 1.58*	0.60 ± 0.52*†	1.22 ± 1.20*	0.99 ± 1.11*†	0.48 ± 0.44*†	1.20 ± 1.06*‡	0.70 ± 0.76
Se, µg/L	100.12 ± 12.11	80.13 ± 46.92*	93.55 ± 56.40*	77.31 ± 40.64*	88.26 ± 54.56*	104.53 ± 55.35	70.89 ± 38.66*‡	97.60 ± 54.59†‡

TB+: positive sputum culture or positive sputum smear microscopy results at that study time point; TB−: negative sputum culture and negative smear sputum microscopy results at that study time point; BMI: body mass index; TST: triceps skinfold thickness; AMA: arm muscle area; Alb: albumin; CRP: C-reactive protein; Se: selenium. [a]Values expressed as mean ± SD. *p < 0.05 vs. control. †p < 0.05 vs. TB+ group at baseline. ‡p < 0.05 TB+ group vs. TB− group. ††p < 0.05 TB+ group vs. TB+ group at baseline. Tukey test (equal variances), Games-Howell test (different variances).

Table 4 - Distribution of the patients in the TB+ and TB− groups in relation to the results of the biochemical tests and serum selenium levels at the three study time points.[a]

Variable	Baseline	30 days after treatment initiation			60 days after treatment initiation		
	TB+ group	TB− group	TB+ group	p*	TB− group	TB+ group	p*
	(n = 35)	(n = 15)	(n = 14)		(n = 23)	(n = 11)	
Albumin, g/dL				0.792			0.338
< 3.4	11 (100)	1 (33.3)	2 (66.7)		2 (100)	0 (0.0)	
3.4-4.8[b]	24 (100)	13 (54.2)	11 (45.8)		19 (63.3)	11 (36.7)	
>4.8	0 (0.0)	1 (50.0)	1 (50.0)		2 (100)	0 (0.0)	
CRP, mg/dL				0.617			0.683
< 0.3[b]	1 (100)	2 (100)	0 (0.0)		2 (100)	0 (0.0)	
≥ 0.3	34 (100)	13 (50.0)	13 (50.0)		21 (65.6)	11 (34.4)	
CRP/albumin ratio[c]				0.206			0.041
< 0.4	2 (100)	3 (30.0)	7 (70.0)		12 (75.0)	4 (25.0)	
0.4-1.1	11 (100)	5 (50.0)	5 (50.0)		9 (81.8)	2 (18.2)	
1.2-2.0	8 (100)	2 (50.0)	2 (50.0)		2 (50.0)	2 (50.0)	
> 2.0	13 (100)	3 (100)	0 (0.0)		0 (0.0)	3 (100)	
Selenium							
< cut-off point[d]	24 (100)	9 (47.4)	10 (52.6)	0.518	12 (54.5)	10 (45.5)	0.027
≥ cut-off point[d]	11 (100)	6 (60.0)	4 (40.0)		11 (91.7)	1 (8.3)	

TB−: negative sputum culture and negative sputum smear microscopy results at that study time point; TB+: positive sputum culture or positive sputum smear microscopy results at that study time point; and CRP: C-reactive protein. [a]Values expressed as n (%). [b]Normal values. [c]Used in order to determine the level of complication risks.[13] [d]Based on the median of the results obtained in the healthy control group. *Chi-square test.

tissue loss. In tuberculosis patients, reduced appetite, malabsorption of macronutrients and micronutrients, and altered metabolism lead to cachexia.[16] However, no association between the nutritional parameters studied and culture conversion at 60 days of antituberculosis treatment was observed. Nevertheless, we found that low BMI, TST, and AMA persisted in the tuberculosis

 http://dx.doi.org/10.1590/S1806-37132014000300010

patients (even in those whose results converted to negative) after 60 days of treatment.

The use of BMI as an indicator of nutrition in the relationship between nutritional status and tuberculosis has been reported.[19] The evaluation of TST and AMA in patients with tuberculosis, however, is less often described in the literature. Nevertheless, one group of authors[20] described differences in lean body mass and fat mass gain in tuberculosis patients after 6 months of treatment. This fact points to the importance of not only evaluating the overall weight gain, but also differentiating it between lean and fat body mass.

Regarding the biochemical tests studied, we found that albumin levels improved during antituberculosis treatment. Patients with newly diagnosed tuberculosis have been described to present with lower albumin levels when compared with healthy control groups,[18] which corroborates the results in the present study. In a study in Tanzania, the albumin levels of patients with tuberculosis also increased significantly after 60 days of antituberculosis treatment, equaling to the levels found in the control group, which is at odds with our findings.[21] In another study conducted in Brazil, tuberculosis patients were followed for 6 months, and no improvement in albumin levels throughout the study was observed.[22]

Higher levels of albumin have been considered as a predictor of a better outcome in patients with pulmonary tuberculosis. Albumin has also been identified as an indicator of protein status when tuberculosis is diagnosed.[23] However, cytokines present during the acute phase response (APR) to the infection can suppress the synthesis of albumin, thereby reducing its circulating levels. Therefore, it is difficult to interpret low albumin levels in patients with active tuberculosis without other parameters to assess APR and malnutrition, since low albumin levels may reflect both APR to infection and protein deficiency. Thus, the discrepancy across studies might be due to variations in nutritional status, the intensity of APR in the studied populations, or the small number of patients included.

Because CRP synthesis is increased in the host systemic response to infection, statistically significant differences were observed between the TB+ and TB– groups at baseline, at 30 days of treatment, and at 60 days of treatment, confirming the association between bacteriological conversion and decreased in CRP levels.

One group of authors evaluated CRP levels in patients with pulmonary tuberculosis during 6 months of treatment; at 3 and 6 months after treatment initiation, there was a significant reduction in CRP levels.[22] CRP has been identified as an important indicator in the diagnosis of individuals with suspected tuberculosis and positive smear microscopy.[24] In our study, a statistically significant association between lower CRP/albumin ratio values and negative cultures for mycobacteria was also found. The CRP/albumin ratio has been described to be increased in patients with other APR-related diseases.[14]

The tuberculosis infection is a condition known to induce oxidative stress in the infected organism, such as the production of reactive oxygen species (ROS) derived from free radicals. These ROS are associated with dysfunction in pulmonary tuberculosis. A way of suppressing these ROS is by means of antioxidant enzymes, which scavenge free radicals and protect cells from oxidative damage. Various of these enzymes, such as glutathione peroxidase, have selenium as an essential element.[25] Thus, a reduction in micronutrient intake (such as vitamins, zinc, and selenium) leads to impaired immune responses.

Studies show that patients with active tuberculosis have lower concentrations of various micronutrients, including selenium, in blood.[26] In the present study, the healthy subjects showed higher selenium levels when compared with the study group at baseline. Among the pulmonary tuberculosis patients, we found an association between positive culture results and low selenium levels even after 60 days of treatment. Micronutrient deficiency is a frequent cause of secondary immunodeficiency and morbidity due to related infections, including tuberculosis. This trace element has an important role in the maintenance of immune processes and, therefore, may have a fundamental role in the defense against the mycobacteria. Low selenium levels have been considered a significant risk factor for the development of mycobacterial disease in HIV-positive patients.[27] In one study with 22 pulmonary tuberculosis patients who were newly diagnosed with positive sputum,[28] the authors found a significant difference between selenium levels between the control and study groups at baseline, as we found in the present

study. However, in that study, no bacteriological tests were performed 60 days later. In the present study, it is noteworthy that the selenium levels remained low in the TB+ group individuals. One group of authors in India evaluated the circulating concentrations of antioxidant enzymes that have selenium as an essential component and are markers of oxidative stress in patients with pulmonary tuberculosis.[29] The results showed lower antioxidant potential as determined by low levels of superoxide dismutase, catalase, and glutathione, as well as increased lipid peroxidation (malonaldehyde), in the patients with tuberculosis. However, the antioxidant potential and selenoenzymes levels increased with the treatment, as observed in the present study.

In another study, conducted in Malawi[30] and involving 500 newly diagnosed pulmonary tuberculosis patients (including 370 coinfected with HIV), it was observed that micronutrient deficiencies were common in all patients, and 88% of the sample was deficient in selenium. These decreased selenium concentrations were also associated with the severity of anemia, which is common in active tuberculosis patients. It is thus suggested that selenium deficiency might contribute to anemia via increased oxidative stress in tuberculosis patients. According to one group of authors,[5] a two-month intervention with vitamin E and selenium supplementation reduced oxidative stress and increased the total antioxidant capacity in patients with treated pulmonary tuberculosis. However, in that study,[5] the association between selenium supplementation and negative smear microscopy results or cultures at the end of 2 months of treatment was not reported.

In summary, in our study, we found poor nutritional status (based on BMI, TST, and AMA) in patients with pulmonary tuberculosis, but these parameters were not associated with sputum culture conversion at 60 days of antituberculosis treatment. The relationship between CRP and albumin levels might be a useful tool for assessing the bacteriological conversion in patients with tuberculosis. In addition, low serum selenium levels after 60 days of treatment were associated with positive sputum culture and positive sputum smear microscopy. Our results corroborate the findings in other studies that showed improvement of the immune status of tuberculosis patients who received selenium supplementation.[27,30] Thus, despite the limitations of the present study (small sample of tuberculosis patients and inclusion of male patients only), our results suggest that selenium levels and CRP/albumin ratio can be used as biomarkers of therapeutic response in pulmonary tuberculosis. Further studies are necessary in order to confirm or refute our results. In addition, studies on the interaction between *M. tuberculosis* and serum selenium levels are needed in order to help us understand whether (and how) tuberculosis modulates selenium levels.

References

1. World Health Organization [homepage on the Internet]. Geneva: World Health Organization [cited 2013 May 8]. Global tuberculosis control: WHO report 2011. [Adobe Acrobat document, 258p.]. Available from: http://www.who.int/tb/publications/global_report/2011/gtbr11_full.pdf
2. Wallis RS, Kim P, Cole S, Hanna D, Andrade BB, Maeurer M, et al. Tuberculosis biomarkers discovery: developments, needs, and challenges. Lancet Infect Dis. 2013;13(4):362-72. http://dx.doi.org/10.1016/S1473-3099(13)70034-3
3. Lönnroth K, Jaramillo E, Williams BG, Dye C, Raviglione M. Drivers of tuberculosis epidemics: the role of risk factors and social determinants. Soc Sci Med. 2009;68(12):2240-6. http://dx.doi.org/10.1016/j.socscimed.2009.03.041
4. van Lettow M, Harries AD, Kumwenda JJ, Zijlstra EE, Clark TD, Taha TE, et al. Micronutrient malnutrition and wasting in adults with pulmonary tuberculosis with and without HIV co-infection in Malawi. BMC Infect Dis. 2004;4(1):61. http://dx.doi.org/10.1186/1471-2334-4-61
5. Seyedrezazadeh E, Ostadrahimi A, Mahboob S, Assadi Y, Ghaemmagami J, Pourmogaddam M. Effect of vitamin E and selenium supplementation on oxidative stress status in pulmonary tuberculosis patients. Respirology. 2008;13(2):294-8. http://dx.doi.org/10.1111/j.1440-1843.2007.01200.x
6. Villamor E, Mugusi F, Urassa W, Bosch RJ, Saathoff E, Matsumoto K, et al. A trial of the effect of micronutrient supplementation on treatment outcome, T cell counts, morbidity, and mortality in adults with pulmonary tuberculosis. J Infect Dis. 2008;197(11):1499-505. http://dx.doi.org/10.1086/587846
7. Wilson D, Nachega J, Morroni C, Chaisson R, Maartens G. Diagnosing smear-negative tuberculosis using case definitions and treatment response in HIV-infected adults. Int J Tuberc Lung Dis. 2006;10(1):31-8.
8. Ewing JA. Detecting alcoholism: the CAGE questionnaire. JAMA. 1984;252(14):1905-7. http://dx.doi.org/10.1001/jama.1984.03350140051025
9. World Health Organization. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. WHO Technical Report Series 854. Geneva: World Health Organization; 1995.
10. Gibson RS. Principles of nutritional assessment. 2nd ed. New York: Oxford University Press; 2005. p. 245-93.
11. Heymsfield SB, McManus C, Smith J, Stevens V, Nixon DW. Anthropometric measurement of muscle mass: revised equations for calculating bone-free arm muscle area. Am J Clin Nutr. 1982;36(4):680-90. PMID: 7124671

12. Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. Am J Clin Nutr. 1981,34(11):2540-5. PMID: 6975564
13. Corrêa CR, Angeleli AY, Camargo NR, Barbosa L, Burini RC. Comparação entre a relação PCR/albumina e o índice prognóstico inflamatório nutricional (IPIN). J Bras Patol Med Lab. 2002;38(3):183-90. http://dx.doi.org/10.1590/S1676-24442002000300004
14. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Guia de vigilância epidemiológica. Brasília: Ministério da Saúde; 2005.
15. Rehm J, Samokhvalov AV, Neuman MG, Room R, Parry C, Lönnroth K, et al. The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. BMC Public Health. 2009;9:450 http://dx.doi.org/10.1186/1471-2458-9-450
16. Macallan DC, Mcnurlan MA, Kurpad AV, de Souza G, Shetty PS, Calder AG, et al. Whole body protein metabolism in human pulmonary tuberculosis and undernutrition: evidence for anabolic block in tuberculosis. Clin Sci (Lond). 1998;94(3):321-31. PMID: 9616267
17. American Thoracic Society; Centers for Disease Control and Prevention; Infectious Diseases Society of America. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: controlling tuberculosis in the United States. Am J Respir Crit Care Med. 2005;172(9):1169-227. http://dx.doi.org/10.1164/rccm.2508001
18. Wiid I, Seaman T, Hoal EG, Benade AJ, Van Helden PD. Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy. IUBMB Life. 2004;56(2):101-6. http://dx.doi.org/10.1080/15216540410001671259
19. Lönnroth K, Williams BG, Cegielski P, Dye C. A consistent log-linear relationship between tuberculosis incidence and body mass index. Int J Epidemiol. 2010;39(1):149-55. http://dx.doi.org/10.1093/ije/dyp308
20. Schwenk A, Hodgson L, Wright A, Ward LC, Rayner CF, Grubnic S, et al. Nutrient partitioning during treatment of tuberculosis: gain in body fat mass but not in protein mass. Am J Clin Nutr. 2004;79(6):1006-12.
21. Mugusi FM, Rusizoka O, Habib N, Fawzi W. Vitamin A status of patients presenting with pulmonary tuberculosis and asymptomatic HIV-infected individuals, Dar es Salaam, Tanzania. Int J Tuberc Lung Dis. 2003;7(8):804-7.
22. Peresi E, Silva SM, Calvi SA, Marcondes-Machado J. Cytokines and acute phase serum proteins as markers of inflammatory regression during the treatment of pulmonary tuberculosis. J Bras Pneumol. 2008;34(11):942-9. http://dx.doi.org/10.1590/S1806-37132008001100009
23. Mehta JB, Fields CL, Byrd RP Jr, Roy TM. Nutritional status and mortality in respiratory failure caused by tuberculosis. Tenn Med. 1996;89(10):369-71.
24. Wilson D, Badri M, Maartens G. Performance of serum C-reactive protein as a screening test for smear-negative tuberculosis in an ambulatory high HIV prevalence population. PLoS One. 2011;6(1):e15248. http://dx.doi.org/10.1371/journal.pone.0015248
25. Kassu A, Yabutani T, Mahmud ZH, Mohammad A, Nguyen N, Huong BT, et al. Alterations in serum levels of trace elements in tuberculosis and HIV infections. Eur J Clin Nutr. 2006;60(5):580-6. http://dx.doi.org/10.1038/sj.ejcn.1602352
26. Papathakis PC, Piwoz E. Nutrition and tuberculosis: a review of the literature and consideration for TB control programs. Washington (DC): U.S. Agency for International Development; 2008. p. 46.
27. Shor-Posner G, Miguez MJ, Pineda LM, Rodriguez A, Ruiz P, Castillo G, et al. Impact of selenium status on the pathogenesis of mycobacterial disease in HIV-1-infected drug users during the era of highly active antiretroviral therapy. J Acquir Immune Defic Syndr. 2002;29(2):169-73. http://dx.doi.org/10.1097/00042560-200202010-00010
28. Ciftci TU, Ciftci B, Yis O, Guney Y, Bilgihan A, Ogretensoy M. Changes in serum selenium, copper, zinc levels and cu/zn ratio in patients with pulmonary tuberculosis during therapy. Biol Trace Elem Res. 2003;95(1):65-71. http://dx.doi.org/10.1385/BTER:95:1:65
29. Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. Indian J Tuberc. 2004;51:213-8.
30. van Lettow M, West CE, van der Meer JW, Wieringa FT, Semba RD. Low plasma selenium concentrations, high plasma human immunodeficiency virus load and high interleukin-6 concentrations are risk factors associated with anemia in adults presenting with pulmonary tuberculosis in Zomba district, Malawi. Eur J Clin Nutr. 2005;59(4):526-32. http://dx.doi.org/10.1038/sj.ejcn.1602116

About the authors

Milena Lima de Moraes
Postdoctoral Student. McGill University, Montreal, QC, Canada.

Daniela Maria de Paula Ramalho
Doctoral Student. Graduate Program in Clinical Medicine, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Karina Neves Delogo
Master's Student in Science. Graduate Program in Clinical Medicine, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Pryscila Fernandes Campino Miranda
Doctoral Student in Science. Graduate Program in Clinical Medicine, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Eliene Denites Duarte Mesquita
Physician. Tuberculosis Research Center, Academic Program in Tuberculosis, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Hedi Marinho de Melo Guedes de Oliveira
Physician. Tuberculosis Research Center, Academic Program in Tuberculosis, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Antônio Ruffino-Netto
Full Professor. University of São Paulo at Ribeirão Preto School of Medicine, Ribeirão Preto, Brazil.

Paulo César de Almeida
Adjunct Professor. Graduate Course in Nutrition, Ceará State University, Fortaleza, Brazil.

Rachel Ann Hauser-Davis
Biologist. Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro, Brazil.

Reinaldo Calixto Campos
Associate Professor. Department of Chemistry, Pontifical Catholic University of Rio de Janeiro, Rio de Janeiro, Brazil.

Afrânio Lineu Kritski
Full Professor. Academic Program in Tuberculosis, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

Martha Maria de Oliveira
Researcher. Tuberculosis Research Center, Academic Program in Tuberculosis, Federal University of Rio de Janeiro School of Medicine, Rio de Janeiro, Brazil.

 http://dx.doi.org/10.1590/S1806-37132014000300010

Tuberculosis in hospitalized patients: clinical characteristics of patients receiving treatment within the first 24 h after admission*

Tuberculose em pacientes hospitalizados: características clínicas dos pacientes que iniciaram tratamento nas primeiras 24 h de permanência hospitalar

Denise Rossato Silva, Larissa Pozzebon da Silva, Paulo de Tarso Roth Dalcin

Abstract

Objective: To evaluate clinical characteristics and outcomes in patients hospitalized for tuberculosis, comparing those in whom tuberculosis treatment was started within the first 24 h after admission with those who did not. **Methods:** This was a retrospective cohort study involving new tuberculosis cases in patients aged ≥ 18 years who were hospitalized after seeking treatment in the emergency room. **Results:** We included 305 hospitalized patients, of whom 67 (22.0%) received tuberculosis treatment within the first 24 h after admission (≤24h group) and 238 (88.0%) did not (>24h group). Initiation of tuberculosis treatment within the first 24 h after admission was associated with being female (OR = 1.99; 95% CI: 1.06-3.74; p = 0.032) and with an AFB-positive spontaneous sputum smear (OR = 4.19; 95% CI: 1.94-9.00; p < 0.001). In the ≤24h and >24h groups, respectively, the ICU admission rate was 22.4% and 15.5% (p = 0.258); mechanical ventilation was used in 22.4% and 13.9% (p = 0.133); in-hospital mortality was 22.4% and 14.7% (p = 0.189); and a cure was achieved in 44.8% and 52.5% (p = 0.326). **Conclusions:** Although tuberculosis treatment was initiated promptly in a considerable proportion of the inpatients evaluated, the rates of in-hospital mortality, ICU admission, and mechanical ventilation use remained high. Strategies for the control of tuberculosis in primary care should consider that patients who seek medical attention at hospitals arrive too late and with advanced disease. It is therefore necessary to implement active surveillance measures in the community for earlier diagnosis and treatment.

Keywords: Tuberculosis; Hospitalization; Time-to-treatment; Emergency medicine; Delayed diagnosis.

Resumo

Objetivo: Comparar as características clínicas e os desfechos de pacientes hospitalizados por tuberculose que iniciaram tratamento nas primeiras 24 h de permanência hospitalar com as daqueles que iniciaram tratamento após 24 h. **Métodos:** Estudo de coorte retrospectivo de casos novos de tuberculose com idade ≥ 18 anos que necessitaram internação hospitalar após atendimento no setor de emergência. **Resultados:** Foram incluídos 305 pacientes hospitalizados, dos quais 67 (22,0%) iniciaram o tratamento nas primeiras 24 h (grupo ≤24h), e 238 (88,0%) o iniciaram após (grupo >24h). Ser do sexo feminino (OR = 1,99; IC95%: 1,06-3,74; p = 0,032) e ter pesquisa de BAAR positiva no escarro espontâneo (OR = 4,19; IC95%: 1,94-9,00; p < 0,001) se associaram com o tratamento nas primeiras 24 h. Na comparação dos grupos ≤24h e >24h, a taxa de internação em UTI foi de, respectivamente, 22,4% e 15,5% (p = 0,258), enquanto a ventilação mecânica foi utilizada em 22,4% e 13,9% (p = 0,133), a taxa de óbito hospitalar foi de 22,4% e 14,7% (p = 0,189), e a taxa de cura foi de 44,8% e 52,5% (p = 0,326). **Conclusões:** Embora o tratamento antituberculose tenha sido iniciado rapidamente em uma proporção considerável dos pacientes hospitalizados, as taxas de mortalidade hospitalar, internação em UTI e uso de ventilação mecânica permaneceram elevadas. Estratégias para o controle de tuberculose na atenção primária devem considerar que pacientes atendidos em hospitais chegam muito tardiamente e com doença avançada, sendo necessário implementar medidas de busca ativa na comunidade para o diagnóstico e o tratamento mais precoce.

Descritores: Tuberculose; Hospitalização; Tempo para o tratamento; Medicina de emergência; Diagnóstico tardio.

---

*Study carried out under the auspices of the Graduate Program in Pulmonology, Federal University of Rio Grande do Sul School of Medicine, and in the Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Correspondence to: Paulo de Tarso Roth Dalcin. Rua Honório Silveira Dias, 1529/901, Higienópolis, CEP 90540-070, Porto Alegre, RS, Brasil.
Tel. 55 51 3330-0521. E-mail: pdalcin@terra.com.br

Financial support: This study received financial support from the *Fundo de Incentivo à Pesquisa do Hospital de Clínicas de Porto Alegre* (FIPE-HCPA, Research Incentive Fund of the Porto Alegre *Hospital de Clínicas*). Larissa Pozzebon da Silva is the recipient of a scholarship from the *Programa Institucional de Bolsas de Iniciação Científica* (PIBIC, Institutional Program for Scientific Initiation Scholarships) of the Brazilian *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq, National Council for Scientific and Technological Development).
Submitted: 8 January 2014. Accepted, after review: 26 March 2014.

Introduction

Tuberculosis remains a major public health problem worldwide. It is estimated that one third of the world population is infected with *Mycobacterium tuberculosis*. In 2011, 9 million new tuberculosis cases were estimated to have occurred worldwide, with 1.4 million deaths. Brazil ranks 22nd among the 22 countries with the highest reported incidence of tuberculosis, with 42 cases/100,000 population in 2011.[1]

Disease control in the community depends on early diagnosis and treatment. Although tuberculosis control programs recommend that the diagnosis of tuberculosis be made at primary health care clinics, most patients are diagnosed in hospitals.[2,3] In Porto Alegre, Brazil, 39% of all tuberculosis patients have been diagnosed in hospitals.[4]

A previous study conducted in a university hospital in Porto Alegre[5] showed that the median time elapsed between hospital admission and diagnosis of tuberculosis was 6 days, the major factors associated with delayed diagnosis being extrapulmonary disease and negative sputum smears. The emergency room has always been the gateway for such patients.

In this context, it is important to analyze the characteristics of patients diagnosed with tuberculosis and receiving tuberculosis treatment within the first 24 h after hospital admission. We hypothesize that such patients do not need hospitalization, and that strategies for the screening and management of tuberculosis in the primary care setting can be developed on the basis of the present study, contributing to reducing the rates of emergency room treatment and the burden of hospitalization for tuberculosis.

The objective of the present study was to analyze clinical characteristics and major outcomes in patients who were hospitalized for tuberculosis and who started tuberculosis treatment within the first 24 h after admission.

Methods

This was a retrospective cohort study of patients who were diagnosed with and hospitalized for tuberculosis after seeking treatment in the emergency room of the *Hospital de Clínicas de Porto Alegre* (HCPA, Porto Alegre *Hospital de Clínicas*), located in the city of Porto Alegre, Brazil.

The study was approved by the local research ethics committee. The authors signed a data use agreement, protecting the confidentiality of patient information.

The study population consisted of new tuberculosis patients who were diagnosed after seeking treatment in the HCPA emergency room. We included patients who were 18 years of age or older and who were identified as new cases of tuberculosis on the basis of consensus criteria.[6] The diagnosis of pulmonary tuberculosis was based on the criteria established by the Third Brazilian Thoracic Association Guidelines on Tuberculosis[6]: a) positive Ziehl-Neelsen staining for AFB (two positive sputum smears); b) positive Ziehl-Neelsen staining for AFB (one positive sputum smear and one positive sputum culture for *M. tuberculosis*); c) positive Ziehl-Neelsen staining for AFB and radiological findings consistent with pulmonary tuberculosis; d) a single positive sputum culture for *M. tuberculosis*; or e) epidemiological, clinical, and radiological findings consistent with pulmonary tuberculosis, together with a favorable response to treatment with antituberculosis drugs. The diagnosis of extrapulmonary tuberculosis was based on clinical examination findings and ancillary test results (depending on the site of disease). The exclusion criteria were as follows: reported tuberculosis cases in which the diagnosis was subsequently changed; and cases of patients who had started treatment before hospitalization.

The patients were retrospectively identified on the basis of data obtained from individual tuberculosis report forms in the *Sistema de Informação de Agravos de Notificação* (SINAN, Brazilian Case Registry Database), and patient charts were reviewed. At our hospital, computerized physician order entry of antituberculosis drugs automatically generates the SINAN report form; therefore, we were able to identify all of the patients who started treatment.

Patient charts were reviewed by the investigators, who completed a standardized questionnaire including the following items: demographic data (age, gender, race, and level of education); comorbidities; smoking status; alcohol use; injection drug use; use of immunosuppressive drugs; history of tuberculosis; clinical form of tuberculosis; symptoms at admission; diagnostic methods; treatment regimen used; HIV infection; time from admission to initiation of treatment;

 http://dx.doi.org/10.1590/S1806-37132014000300011

length of hospital stay; ICU admission; need for and duration of mechanical ventilation; outcome of hospitalization (discharge or death); and outcome after discharge (cure, treatment nonadherence, or death). Post-discharge data were obtained by reviewing patient charts, by searching the SINAN database, or by telephoning the outpatient clinics where patients were being followed.

Data were entered into a Microsoft Excel® 2010 spreadsheet, after which they were processed and analyzed with the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA).

The study variables were analyzed descriptively. Quantitative data were presented as mean ± SD or as median (interquartile range). Qualitative data were expressed as number of cases and proportion.

For statistical analysis, patients were divided into two groups: the ≤24h group, comprising those who received tuberculosis treatment within the first 24 h after hospital admission and the >24h group, comprising those who did not.

For continuous variables, we used the independent sample t-test or the Mann-Whitney U test. For qualitative variables, we used the chi-square test (Yates' correction or Fisher's exact test being used when necessary).

The non-collinear variables that reached significance ($p < 0.01$) in the univariate analysis were included in a stepwise forward conditional binary logistic regression model (adjusted for gender and age) for each outcome.

All statistical tests were two-tailed, and the level of significance was set at 5%.

Results

Between January of 2008 and January of 2011, 305 patients diagnosed with tuberculosis were included in the study.

Table 1 shows the characteristics of the patients studied and a comparison between the ≤24h and >24h groups. The mean age was 42.0 ± 17.2 years, and most (64.6%) of the patients were male and White (75.4%). Of the 305 tuberculosis patients, 110 (36.1%) had pulmonary tuberculosis, 143 (46.9%) had extrapulmonary tuberculosis, and 52 (17.0%) had concomitant pulmonary and extrapulmonary disease. A total of 191 patients (62.6%) were HIV-positive. The mean length of hospital stay was 27.7 ± 21.8 days. None of the patients were discharged within the first 24 h after hospital admission, 6 (2%) were discharged 24-48 h after admission, and 9 (3%) were discharged 24-72 h after admission. The mean time elapsed between admission and initiation of treatment was 8.8 ± 10.8 days. Tuberculosis treatment was initiated on the same day as diagnosis. Therefore, the mean length of hospital stay after diagnosis and initiation of treatment was 18.9 ± 19.1 days. Although there was no difference between HIV-positive and HIV-negative patients regarding the length of hospital stay ($p = 0.921$), the hospital stay was longer in patients with one or more comorbidities than in those without comorbidities (29.3 ± 23.1 days vs. 23.2 ± 16.9 days; $p = 0.030$).

A total of 67 patients (22.0%) received tuberculosis treatment within the first 24 h after hospital admission. The proportion of males was higher in the >24h group than in the ≤24h group (68.5% vs. 50.7%; $p = 0.011$). The proportion of patients with pulmonary tuberculosis alone was higher in the ≤24h group than in the >24h group (61.2% vs. 29.0%; $p < 0.001$), whereas the proportion of patients with extrapulmonary tuberculosis alone was higher in the >24h group than in the ≤24h group (54.2% vs. 20.9%; $p < 0.001$). Cough was more common in the ≤24h group than in the >24h group (64.2% vs. 37.4%; $p < 0.001$), as were night sweats (35.8% vs. 21.0%; $p = 0.019$). The proportion of patients with AFB-positive spontaneous sputum smears was significantly higher in the ≤24h group than in the >24h group (53.7% vs. 14.7%; $p < 0.001$). The proportion of patients with routine chest X-rays showing cavitary disease was significantly higher in the ≤24h group than in the >24h group (22.4% vs. 7.6%; $p = 0.001$), whereas the proportion of patients with normal chest X-rays was significantly higher in the >24h group than in the ≤24h group (16.4% vs. 4.5%; $p = 0.015$). The total length of hospital stay was shorter in the ≤24h group than in the >24h group (19.6 ± 22.6 days vs. 29.9 ± 21.1; $p = 0.001$). There were no differences between the two groups of patients regarding the following outcomes: ICU admission; mechanical ventilation use; in-hospital mortality; one-year mortality; and cure rate ($p > 0.05$ for all).

Table 2 shows the binary logistic regression analysis of the characteristics associated with initiation of tuberculosis treatment within the first 24 h after hospital admission. Initiation of

Table 1 - Patient characteristics and comparison between the group of patients who received tuberculosis treatment within the first 24 h after hospital admission and that of those who did not.[a]

Characteristics	Total	Group ≤24h	Group >24h	p
	N = 305	n = 67	n = 238	
Age, years[b]	42.0 ± 17.2	38.5 ± 16.5	42.9 ± 17.5	0.067
Age > 60 years	42 (13.8)	7 (10.4)	35 (14.7)	0.428
Gender				
Male	197 (64.6)	34 (50.7)	163 (68.5)	0.011
Female	108 (35.4)	33 (49.3)	75 (31.5)	
Race				
White	230 (75.4)	48 (71.6)	182 (76.5)	0.516
Non-White	75 (24.6)	19 (28.4)	56 (23.5)	
Smoking status				
Nonsmoker	162 (53.1)	33 (49.3)	129 (54.2)	0.285
Former smoker	52 (17.0)	9 (13.4)	43 (18.1)	
Smoker	91 (29.8)	25 (37.3)	66 (27.7)	
Alcoholism	89 (29.2)	20 (29.9)	69 (29.0)	1.000
Illicit drug use	81 (26.6)	21 (31.3)	60 (25.2)	0.397
Form of tuberculosis				
Pulmonary tuberculosis	110 (36.1)	41 (61.2)	69 (29.0)	< 0.001
Extrapulmonary tuberculosis	143 (46.9)	14 (20.9)	129 (54.2)	< 0.001
Combined pulmonary and extrapulmonary tuberculosis	52 (17.0)	12 (17.9)	40 (16.8)	0.977
Symptoms				
Cough	132 (43.3)	43 (64.2)	89 (37.4)	< 0.001
Weight loss	151 (49.5)	40 (59.7)	111 (46.6)	0.080
Night sweats	74 (24.3)	24 (35.8)	50 (21.0)	0.019
Fever	190 (62.3)	39 (58.2)	151 (63.4)	0.523
Dyspnea	46 (15.1)	11 (16.4)	35 (14.7)	0.879
Chest pain	34 (11.1)	9 (13.4)	25 (10.5)	0.512
AFB-positive spontaneous sputum smear				
Yes	71 (23.3)	36 (53.7)	35 (14.7)	< 0.001
No	93 (30.5)	14 (20.9)	79 (33.2)	
No sputum	141 (46.2)	17 (25.4)	124 (52.1)	
Chest X-ray				
Cavitary disease	33 (10.8)	15 (22.4)	18 (7.6)	0.001
Consolidation	52 (17.0)	16 (23.9)	36 (15.1)	0.134
Pleural effusion	58 (19.0)	7 (10.4)	51 (21.4)	0.065
Normal	42 (13.8)	3 (4.5)	39 (16.4)	0.015
Miliary pattern	37 (12.1)	10 (14.9)	27 (11.3)	0.405
Comorbidities				
Diabetes mellitus	19 (6.2)	2 (3.0)	17 (7.1)	0.266
Chronic kidney disease	8 (2.6)	0 (0.0)	8 (3.4)	0.207
Transplantation	7 (2.3)	0 (0.0)	7 (2.9)	0.354
Chronic liver disease	6 (2.0)	1 (1.5)	5 (2.1)	0.744
Neoplasia	13 (4.3)	2 (3.0)	11 (4.6)	0.740
HIV	191 (62.6)	39 (58.2)	152 (63.9)	0.482
Any comorbidity	224 (73.4)	47 (70.1)	177 (74.4)	0.593
Length of hospital stay, days[b]	27.7 ± 21.8	19.6 ± 22.6	29.9 ± 21.1	0.001
ICU admission	52 (17.0)	15 (22.4)	37 (15.5)	0.258
Mechanical ventilation	48 (15.7)	15 (22.4)	33 (13.9)	0.133
Outcomes				
In-hospital mortality	50 (16.4)	15 (22.4)	35 (14.7)	0.189
One-year mortality	97 (31.8)	23 (34.3)	74 (31.1)	0.723
Cure	155 (50.8)	30 (44.8)	125 (52.5)	0.326

[a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD.

 http://dx.doi.org/10.1590/S1806-37132014000300011

tuberculosis treatment within the first 24 h after admission was independently associated with being female (OR = 1.99; 95% CI: 1.06-3.74; p = 0.032) and with an AFB-positive spontaneous sputum smear (OR = 4.19; 95% CI: 1.94-9.00; p < 0.001).

Discussion

This retrospective cohort study evaluated new cases of tuberculosis treated in the emergency room of a university hospital and requiring hospitalization. Of the sample as a whole, 22.0% were diagnosed with tuberculosis and started treatment within the first 24 h after hospital admission. Diagnosis of tuberculosis and initiation of tuberculosis treatment within the first 24 h after admission were associated with being female and with an AFB-positive spontaneous sputum smear. However, there were no differences between the ≤24h group and the >24h group regarding the following outcomes: ICU admission; mechanical ventilation use; in-hospital mortality; one-year mortality; and cure rate. In addition, for the sample as a whole, the mean hospital stay was long (27.7 days), and 97% of the patients required hospitalization for more than 3 days. The fact that the mean length of hospital stay after diagnosis and initiation of treatment was 18.9 days underscores the severity of the clinical situation resulting from tuberculosis or comorbidities.

In the present study, an AFB-positive sputum smear was significantly associated with diagnosis within the first 24 h after hospital admission. Various studies have shown that a negative sputum smear is associated with delayed diagnosis.[7-12] A cross-sectional study of adults with newly diagnosed tuberculosis showed that hospitalization and delayed treatment were more likely to occur in smear-negative patients.[12] In a referral hospital in Rwanda, a negative sputum smear was considered a risk factor for further health care system delay.[9] Previous studies have shown that, in addition to delayed diagnosis, indicators of atypical manifestations (such as negative sputum smears) were associated with increased mortality.[13,14]

Being female was associated with a diagnosis of tuberculosis within the first 24 h after hospital admission. In a cross-sectional study conducted in Ethiopia,[15] it was demonstrated that female patients took longer to seek medical attention than did male patients, although there was less delay in diagnosis in female patients after their entry into the health care system, a finding that is consistent with ours. Other studies have shown that being female is a risk factor for a delay in seeking medical attention (patient delay).[16-21] Therefore, in the present study, female patient delay in seeking medical attention was possibly associated with a more severe and more advanced disease presentation, which raised diagnostic suspicion in the emergency room.

Although it is recommended that the diagnosis of tuberculosis be made at primary health care clinics, a significant proportion of the population is diagnosed in public hospitals.[10,22] In 2007 in Porto Alegre, 38.98% of all tuberculosis cases were reported by hospitals.[23] The fact that tuberculosis is often diagnosed in hospitals is generally believed to be due to a lack of resources in primary care or the need for tests that are more specific in order to establish a diagnosis. In

Table 2 - Binary logistic regression for characteristics associated with tuberculosis treatment initiation within the first 24 h after hospital admission.

Variable	b	Wald	Significance	OR	95% CI
Age	0.01	2.26	0.133	1.02	1.00-1.03
Female gender	0.69	4.60	0.032	1.99	1.06-3.74
Pulmonary tuberculosis	0.73	2.88	0.090	2.08	0.89-4.83
Extrapulmonary tuberculosis	0.04	0.005	0.942	1.04	0.36-3.00
Cough	0.34	0.94	0.333	1.40	0.71-2.76
Night sweats	0.41	1.30	0.254	1.50	0.75-3.00
AFB-positive spontaneous sputum smear	1.43	13.37	< 0.001	4.19	1.94-9.00
Cavitary disease	−0.10	0.05	0.828	0.90	0.36-2.26
Pleural effusion	−0.50	1.00	0.317	0.61	0.23-1.62
Normal chest X-ray	−0.57	0.63	0.428	0.57	0.14-2.31
Constant	−0.57	0.26	0.611	0.57	-

fact, studies have shown that delayed diagnosis is closely related to poor access to health care (which is due to the fact that health care facilities are far from where patients live), difficulties in performing tests, and the prescription of drugs other than antituberculosis drugs.[2,11] However, in our study, 22% of all patients were diagnosed with tuberculosis and started tuberculosis treatment within the first 24 h after hospital admission, and positive sputum smears were associated with treatment initiation within the first 24 h after admission. This means that the diagnosis of tuberculosis was quickly established, often on the basis of sputum smear microscopy, which is a simple test that is available at primary health care clinics. Another possible explanation for this finding is the inability of primary health care professionals to recognize tuberculosis symptoms, which delays the diagnostic process. A previous study showed that cough, expectoration, and hemoptysis were less common in females than in males,[24] leading physicians to suspect less of tuberculosis in the former. In addition, as discussed above, being female is usually associated with a delay in seeking medical attention, which might be due to the fact that females have to balance work and home duties or to the stigma attached to the disease; even after their entry into the health care system, difficulties in collecting sputum samples constitute an obstacle to early diagnosis in the primary care setting.[25-29]

The major limitation of the present study is its retrospective design; the accuracy of data collection is lower in retrospective studies than in prospective studies. Another limitation is that this was a single-center study. Nevertheless, it is important to investigate the factors associated with delayed diagnosis, because it has an impact on tuberculosis transmission. Therefore, it is crucial that the sources of delay in diagnosis be evaluated under tuberculosis control programs at hospitals.

In conclusion, although a diagnosis of tuberculosis was established and tuberculosis treatment was initiated within the first 24 h after hospital admission in 22.0% of the new cases of tuberculosis treated in our emergency room and requiring hospitalization, the rates of in-hospital mortality, ICU admission, and mechanical ventilation use remained high. Diagnosis of tuberculosis and initiation of tuberculosis treatment within the first 24 h after admission were associated with being female and with an AFB-positive spontaneous sputum smear. Strategies for the control of tuberculosis in primary care should consider that patients who seek medical attention at hospitals arrive too late and with advanced disease. It is therefore necessary to implement active surveillance measures in the community for earlier diagnosis and treatment.

References

1. World Health Organization. Global Tuberculosis Control 2010. Geneva: World Health Organization; 2012.
2. dos Santos MA, Albuquerque MF, Ximenes RA, Lucena-Silva NL, Braga C, Campelo AR, et al. Risk factors for treatment delay in pulmonary tuberculosis in Recife, Brazil. BMC Public Health. 2005;5:25. http://dx.doi.org/10.1186/1471-2458-5-25
3. Sreeramareddy CT, Panduru KV, Menten J, Van den Ende J. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. BMC Infect Dis. 2009;9:91. http://dx.doi.org/10.1186/1471-2334-9-91
4. Prefeitura Municipal de Porto Alegre [homepage on the Internet]. Porto Alegre: a Prefeitura [cited 2014 Jan 8]. Boletim epidemiológico no. 45 de maio de 2011. [Adobe Acrobat document, 8p.]. Available from: http://lproweb.procempa.com.br/pmpa/prefpoa/cgvs/usu_doc/10018-boletim_epidemiologico_setembro_2011_45[1].pdf
5. Rossato Silva D, Müller AM, Dalcin Pde T. Factors associated with delayed diagnosis of tuberculosis in hospitalized patients in a high TB and HIV burden setting: a cross-sectional study. BMC Infect Dis. 2012;12:57. http://dx.doi.org/10.1186/1471-2334-12-57
6. Conde MB, Melo FA, Marques AM, Cardoso NC, Pinheiro VG, Dalcin Pde T, et al. III Brazilian Thoracic Association Guidelines on tuberculosis. J Bras Pneumol. 2009;35(10):1018-48.
7. Farah MG, Rygh JH, Steen TW, Selmer R, Heldal E, Bjune G. Patient and health care system delays in the start of tuberculosis treatment in Norway. BMC Infect Dis. 2006;6:33. http://dx.doi.org/10.1186/1471-2334-6-33
8. Lin CY, Lin WR, Chen TC, Lu PL, Huang PM, Tsai ZR, et al. Why is in-hospital diagnosis of pulmonary tuberculosis delayed in southern Taiwan? J Formos Med Assoc. 2010;(4):269-77. http://dx.doi.org/10.1016/S0929-6646(10)60052-6
9. Lorent N, Mugwaneza P, Mugabekazi J, Gasana M, Van Bastelaere S, Clerinx J, et al. Risk factors for delay in the diagnosis and treatment of tuberculosis at a referral hospital in Rwanda. Int J Tuberc Lung Dis. 2008;12(4):392-6.
10. Sherman LF, Fujiwara PI, Cook SV, Bazerman LB, Frieden TR. Patient and health care system delays in the diagnosis and treatment of tuberculosis. Int J Tuberc Lung Dis. 1999;3(12):1088-95.
11. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health. 2008;8:15. http://dx.doi.org/10.1186/1471-2458-8-15
12. Whitehorn J, Ayles H, Godfrey-Faussett P. Extra-pulmonary and smear-negative forms of tuberculosis are associated with treatment delay and hospitalisation. Int J Tuberc Lung Dis. 2010;14(6):741-4.

 http://dx.doi.org/10.1590/S1806-37132014000300011

13. Naalsund A, Heldal E, Johansen B, Kongerud J, Boe J. Deaths from pulmonary tuberculosis in a low-incidence country. J Intern Med. 1994;236(2):137-42. http://dx.doi.org/10.1111/j.1365-2796.1994.tb01275.x
14. Penner C, Roberts D, Kunimoto D, Manfreda J, Long R. Tuberculosis as a primary cause of respiratory failure requiring mechanical ventilation. Am J Respir Crit Care Med. 1995;151(3 Pt 1):867-72. http://dx.doi.org/10.1164/ajrccm/151.3_Pt_1.867
15. Wondimu T, Michael KW, Kassahun W, Getachew S. Delay in initiating tuberculosis treatment and factors associated among pulmonary tuberculosis patients in East Wollega, Western Ethiopia. Ethiop J Health Develop 2007;21(2):148-56. http://dx.doi.org/10.4314/ejhd.v21i2.10043
16. Lawn SD, Afful B, Acheampong JW. Pulmonary tuberculosis: diagnostic delay in Ghanaian adults. Int J Tuberc Lung Dis. 1998;2(8):635-40.
17. Lienhardt C, Rowley J, Manneh K, Lahai G, Needham D, Milligan P, et al. Factors affecting time delay to treatment in a tuberculosis control programme in a sub-Saharan African country: the experience of The Gambia. Int J Tuberc Lung Dis. 2001;5(3):233-9.
18. Ngamvithayapong J, Yanai H, Winkvist A, Diwan V. Health seeking behaviour and diagnosis for pulmonary tuberculosis in an HIV-epidemic mountainous area of Thailand. Int J Tuberc Lung Dis. 2001;5(11):1013-20.
19. Pronyk RM, Makhubele MB, Hargreaves JR, Tollman SM, Hausler HP. Assessing health seeking behaviour among tuberculosis patients in rural South Africa. Int J Tuberc Lung Dis. 2001;5(7):619-27.
20. Rajeswari R, Chandrasekaran V, Suhadev M, Sivasubramaniam S, Sudha G, Renu G. Factors associated with patient and health system delays in the diagnosis of tuberculosis in South India. Int J Tuberc Lung Dis. 2002;6(9):789-95.
21. Xu X, Liu JH, Cao SY, Zhao Y, Dong XX, Liang Y, et al. Delays in care seeking, diagnosis and treatment among pulmonary tuberculosis patients in Shenzhen, China. Int J Tuberc Lung Dis. 2013;17(5):615-20. http://dx.doi.org/10.5588/ijtld.12.0231
22. Zerbini E, Chirico MC, Salvadores B, Amigot B, Estrada S, Algorry G. Delay in tuberculosis diagnosis and treatment in four provinces of Argentina. Int J Tuberc Lung Dis. 2008;12(1):63-8.
23. Prefeitura Municipal de Porto Alegre [homepage on the Internet]. Porto Alegre: a Prefeitura [cited 2014 Jan 8]. Boletim epidemiológico no. 36 de fevereiro de 2008. [Adobe Acrobat document, 8p.]. Available from: http://lproweb.procempa.com.br/pmpa/prefpoa/sms/usu_doc/boletim_36_fevereiro_2008.pdf
24. Long NH, Diwan VK, Winkvist A. Difference in symptoms suggesting pulmonary tuberculosis among men and women. J Clin Epidemiol. 2002;55(2):115-20. http://dx.doi.org/10.1016/S0895-4356(01)00455-3
25. Allebeck P. Delay in tuberculosis care: one link in a long chain of social inequities. Eur J Public Health. 2007;17(5):409. http://dx.doi.org/10.1093/eurpub/ckm046
26. Boeree MJ, Harries AD, Godschalk P, Demast Q, Upindi B, Mwale A, et al. Gender differences in relation to sputum submission and smear-positive pulmonary tuberculosis in Malawi. Int J Tuberc Lung Dis. 2000;4(9):882-4.
27. Gosoniu GD, Ganapathy S, Kemp J, Auer C, Somma D, Karim F, et al. Gender and socio-cultural determinants of delay to diagnosis of TB in Bangladesh, India and Malawi. Int J Tuberc Lung Dis. 2008;12(7):848-55.
28. Hudelson P. Gender differentials in tuberculosis: the role of socio-economic and cultural factors. Tuber Lung Dis. 1996;77(5):391-400. http://dx.doi.org/10.1016/S0962-8479(96)90110-0
29. Karim F, Islam MA, Chowdhury AM, Johansson E, Diwan VK. Gender differences in delays in diagnosis and treatment of tuberculosis. Health Policy Plan. 2007;22(5):329-34. http://dx.doi.org/10.1093/heapol/czm026

About the authors

Denise Rossato Silva
Adjunct Professor. Federal University of Rio Grande do Sul School of Medicine; and Pulmonologist. Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Larissa Pozzebon da Silva
Undergraduate Student. Federal University of Rio Grande do Sul School of Nursing, Porto Alegre, Brazil.

Paulo de Tarso Roth Dalcin
Associate Professor. Federal University of Rio Grande do Sul School of Medicine; and Pulmonologist. Department of Pulmonology, Porto Alegre *Hospital de Clínicas*, Porto Alegre, Brazil.

Nicotine dependence and smoking habits in patients with head and neck cancer*

Dependência nicotínica e perfil tabágico em pacientes com câncer de cabeça e pescoço

Adriana Ávila de Almeida, Celso Muller Bandeira, Antonio José Gonçalves, Alberto José Araújo

Abstract

Objective: To assess smoking habits and nicotine dependence (ND) in patients with head and neck cancer **Methods:** This study involved 71 smokers or former smokers with squamous cell carcinoma in the oral cavity, pharynx, or larynx who were treated at a university hospital in the city of São Paulo between January and May of 2010. We used the Fagerström Test for Nicotine Dependence to evaluate smoking habits and ND in the sample. Data regarding cancer treatment were collected from medical records. Depending on the variables studied, we used the chi-square test, Fisher's exact test, Student's t-test, or Spearman's correlation test. **Results:** Of the 71 patients, 47 (66.2%) presented with high or very high ND, 40 (56.3%) smoked more than 20 cigarettes/day, and 32 (45.1%) smoked their first cigarette within 5 min of awakening. Advanced disease stage correlated significantly with the number of cigarettes smoked per day ($p = 0.011$) and with smoking history ($p = 0.047$). We found that ND did not correlate significantly with gender, disease stage, smoking cessation, or number of smoking cessation attempts, nor did the number of cigarettes smoked per day correlate with smoking cessation or gender. Treatment for smoking cessation was not routinely offered. **Conclusions:** In most of the patients studied, the level of ND was high or very high. The prevalence of heavy smoking for long periods was high in our sample. A diagnosis of cancer is a motivating factor for smoking cessation. However, intensive smoking cessation treatment is not routinely offered to smoking patients diagnosed with cancer.

Keywords: Head and neck neoplasms; Tobacco use disorder; Smoking cessation.

Resumo

Objetivo: Avaliar o perfil tabágico (PT) e a dependência nicotínica (DN) em pacientes com câncer de cabeça e pescoço**. Métodos:** Estudo realizado com 71 pacientes portadores de carcinoma epidermoide de cavidade oral, faringe e laringe, tabagistas ou ex-tabagistas, atendidos em um hospital universitário da cidade de São Paulo entre janeiro e maio de 2010. Utilizou-se o Teste de Fagerström para Dependência de Nicotina para avaliar PT e DN na amostra. Informações sobre o tratamento oncológico foram coletadas dos prontuários. Foram utilizados os testes do qui-quadrado, exato de Fisher ou t de Student, assim como o teste de correlação de Spearman conforme as variáveis estudadas. **Resultados:** Dos 71 pacientes, 47 (66,2%) apresentavam DN elevada ou muito elevada, 40 (56,3%) fumavam mais de 20 cigarros/dia, e 32 (45,1%) fumavam seu primeiro cigarro até 5 min após acordar. Houve associações significativas do estádio avançado da doença com a quantidade de cigarros fumados por dia ($p = 0,011$) e a carga tabágica ($p = 0,047$). Não houve diferenças significativas da DN em relação a sexo, estádio da doença, cessação tabágica ou tentativas anteriores de cessação, nem como do número de cigarros fumados ao dia em relação a cessação ou sexo. O tratamento do tabagismo não foi rotineiramente oferecido. **Conclusões:** A DN foi elevada ou muito elevada na maioria dos pacientes estudados. A prevalência de grandes fumantes por longos períodos foi alta em nossa amostra. O diagnóstico de câncer é um fator motivador para a cessação tabágica; entretanto, o tratamento intensivo do tabagismo ainda não é rotineiramente oferecido a fumantes diagnosticados com câncer.

Descritores: Neoplasias de cabeça e pescoço; Transtorno por uso de tabaco; Abandono do hábito de fumar.

*Study carried out at the *Santa Casa de Misericórdia de São Paulo* School of Medical Sciences, São Paulo, Brazil.
Correspondence to: Adriana Ávila de Almeida. Avenida dos Astronautas, 1758, CEP 12227-010, São José dos Campos, São Paulo, SP, Brasil.
Tel. 55 12 3208-6140. E-mail: celdrica2003@yahoo.com.br
Financial support: None.
Submitted: 29 August 2013. Accepted, after review: 8 May 2014.

 http://dx.doi.org/10.1590/S1806-37132014000300012

Introduction

The World Health Organization considers smoking to be the leading preventable cause of death worldwide and a chronic recurrent disease caused by nicotine dependence (ND).[1] Most smokers are unaware of the damage caused by chronic tobacco use, and nearly half will die of a tobacco-related disease.[1] Tobacco is the most important risk factor for cancer and more than 50 diseases. More than 5,300 components, of which at least 70 are carcinogens, have been identified in tobacco smoke.[2]

Squamous cell carcinoma of the oral cavity, pharynx, and larynx are among the 10 most common types of cancer in men.[3,4] Cancer of the oral cavity is considered a public health problem worldwide. In Brazil, it ranks fifth among men, and it is estimated that, in 2014, there will be 11,280 new cases among men and 4,010 new cases among women. Worldwide, laryngeal cancer ranks second among respiratory tract tumors, with 160,000 new cases per year. In Brazil, it ranks seventh among men, and its incidence is higher from the fourth decade of life onward. According to data from the Brazilian National Cancer Institute, in 2014, it is estimated that there will be 6,870 new cases of laryngeal cancer among men and 770 new cases of laryngeal cancer among women.[5]

Chief among the major risk factors are smoking and alcoholism. Studies have shown that the risk of developing cancer of the oral cavity and larynx is much higher in smokers and drinkers, depending on the duration of smoking, the number of cigarettes smoked per day, and the frequency of alcohol intake.[3-5]

Continuing to smoke after a diagnosis of cancer contributes to a higher risk of complications during treatment and to decreased responses to radiotherapy and chemotherapy, as well as leading to worsening of other tobacco-related diseases. Maintaining tobacco use increases the risk of recurrence and of developing a second primary tumor, with decreased quality of life and overall survival.[6-9]

A complex behavior, ND is influenced by genetic, social, and environmental factors, and is therefore considered a chronic disease that requires repeated interventions.[10-12] Some studies have shown that, in cancer patients, ND is higher.[13-16]

The Fagerström Test for Nicotine Dependence (FTND) has emerged as a quick, easily administered instrument to assess ND, is widely used worldwide, and has been validated in several languages and populations.[13,17,18]

The assessment of ND in patients with tobacco-related cancer makes it possible to identify which patients may have difficulty in quitting smoking.[8,18,19]

Considering that the vast majority of patients with head and neck cancer are heavy smokers and that quitting smoking has tangible benefits in cancer treatment, we decided to conduct a study on smoking habits and ND in this population in order to provide data for interventions and approaches that are more effective for smoking cessation in cancer patients.

Methods

This was an observational study involving patients with squamous cell carcinoma of the oral cavity, pharynx, or larynx who were treated at the Head and Neck Surgery Outpatient Clinic of the *Santa Casa de São Paulo* School of Medical Sciences, in the city of São Paulo, Brazil. The study design was submitted to and approved by the local ethics committee.

We consecutively recruited 71 subjects who met the eligibility criteria and were treated between January and May of 2010. All participants were interviewed by the same investigator and were informed of the objectives of the study. After reading the written informed consent form, they agreed to complete the questionnaire regarding ND and smoking habits.

Data regarding cancer staging and treatment were collected from medical records. Laryngectomized patients who were unable to speak completed the questionnaire by reading the questions and indicating or pointing to responses. Patients were operated on the *Santa Casa de São Paulo* over a time period ranging from one month to 15 years. We excluded patients with tumors of the nasopharynx or lip because these tumors do not have smoking as their major risk factor.

Active smokers were defined as subjects who had smoked at least 100 cigarettes in their lifetime and who, at the time of the interview, continued to smoke daily or occasionally. Smoking history was quantified by pack-years of cigarettes smoked.

For the purposes of statistical analysis, patients were separated into two groups according to their FTND scores: 0 to 5 points (very low to

moderate dependence); and 6 to 10 points (high or very high dependence).

The analysis of smoking habits included questions regarding current cigarette use, age at smoking initiation, type of tobacco used, amount of tobacco used, and duration of use. Patients were asked questions regarding the number of smoking cessation attempts and the use of pharmacological treatment for smoking cessation. We assessed emotional factors (anxiety, sadness, and happiness) associated with the act of smoking, as well as behavioral factors (coffee consumption, alcohol consumption, having meals, talking on the phone, and the work environment). In addition, we obtained data regarding withdrawal symptoms after smoking cessation, contact with smokers, and number of relapses.

The presence of withdrawal symptoms was defined as the presence of one or more of the symptoms included in the definitions of tobacco withdrawal syndrome established in the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (irritability, depression, restlessness, insomnia, anxiety, hunger, and lack of concentration).[20]

Patients were asked questions regarding regular alcohol use, and the Cut down, Annoyed, Guilty, and Eye-opener (CAGE) questionnaire was used to assess risky alcohol use.[21] Alcohol abuse and dependence was defined as responding affirmatively to two or more questions.

For statistical purposes, patients were classified into two groups: early stages (stages I and II); and advanced stages (stages III and IV). For the same reason, patients were gathered in two groups according to the number of smoking cessation attempts: those who had not made any attempts (no attempts); and those who had made at least one attempt (one or more attempts). The responses to the first question on the FTND, i.e., the one that assesses the time to smoke the first cigarette of the day, were divided into "within 30 min of awakening" and "after 30 min of awakening".

All data were analyzed by the Statistical Package for the Social Sciences, version 13 (SPSS Inc., Chicago, IL, USA). The results were assessed by the chi-square test, Fisher's exact test, Student's t-test, or Spearman's correlation test, as appropriate. For all tests, the level of significance was set at 5%.

Results

The present study included 71 patients with squamous cell carcinoma of the oral cavity, pharynx, or larynx. Table 1 shows the general characteristics of the patients studied. In our sample, 59 patients (83.1%) were male and 52 (73.2%) were White. The mean age of the sample was 61.4 ± 8.1 years. The mean age at smoking initiation was 15.6 ± 3.7 years (range, 6-30). The mean duration of tobacco use was 40.4 ± 10.7 years. Of the 71 patients, 50 (70.4%) had advanced stages of disease. Commercial cigarettes were the most commonly used type of tobacco (in (98.6%), 70 patients (98.6%) reported contact with smokers at home or at work, and 31 (43.7%) had never tried to quit smoking.

The mean FTND score was 5.8 ± 2.3 points for the sample as a whole. Of the respondents, 47 (66.2%) had a high or very high FTND score (Tables 1 and 2). We found that ND did not correlate significantly with gender (p = 0.970), disease stage (p = 0.620), smoking cessation (p = 0.251), or number of smoking cessation attempts (p = 0.792; Table 3).

Of the 63 patients (88.7%) who ceased smoking, 43 (60.5%) did so after diagnosis or during treatment. Of those who ceased smoking, 40 (63.5%) had a high or very high FTND score.

We found that smoking cessation did not show statistically significant correlations with level of ND (p = 0.251), with number of cigarettes smoked per day (p = 0.507), or with time to smoke the first cigarette of the day (p = 0.673; Tables 3 and 4).

In our sample, 40 patients (56.3%) smoked more than 20 cigarettes/day, and the mean smoking history was 60.5 ± 29.8 pack-years. Although the number of cigarettes smoked per day did not correlate with the success of cessation attempts (p = 0.507) or with gender (p = 0.261), it correlated significantly with disease stage (p = 0.011; Table 4).

We found that smoking history correlated significantly with disease stage (p = 0.047) but not with time to smoke the first cigarette of the day (p = 0.270) or with smoking cessation (p = 0.960; Table 5). When we examined the association between smoking history and disease stage by using Spearman's correlation test, we found no significant correlation (r = 0.184; p = 0.125).

 http://dx.doi.org/10.1590/S1806-37132014000300012

Table 1 - General characteristics of the 71 study patients.[a]

Characteristic	Result
Age, years[b]	61.4 ± 8.1
Gender	
Male	59 (83.1)
Female	12 (16.9)
Race	
White	52 (73.2)
Non-White	19 (26.8)
Disease site	
Larynx	38 (53.5)
Pharynx	22 (31.0)
Mouth	11 (15.5)
Disease stage	
I	15 (21.1)
II	6 (8.5)
III	17 (23.9)
IV	33 (46.5)
Cancer treatment	
Surgery + radiotherapy	10 (14.0)
Surgery + radiotherapy + chemotherapy	18 (25.4)
Surgery	27 (38.0)
Chemotherapy	1 (1.4)
Radiotherapy + chemotherapy	9 (12.7)
Radiotherapy	6 (8.5)
Age at smoking initiation, years[b]	
Overall	15.6 ± 3.7
Males	15.5 ± 3.6
Females	16.1 ± 4.5
Duration of smoking, years[b]	40.4 ± 10.7
FTND, score	5.8 ± 2.3
Low to moderate ND (0-5)	24 (33.8)
High or very high ND (6-10)	47 (66.2)
Pharmacological treatment	
Nicotine patch	2 (2.8)
Bupropion	1 (1.4)
None	68 (95.8)
Number of cigarettes smoked	
Up to 20 cigarettes/day	31 (43.7)
More than 20 cigarettes/day	40 (56.3)
Factors associated with tobacco use	
Work	66 (93.0)
Coffee	60 (84.5)
Meals	64 (90.1)
Talking on the phone	19 (26.8)
Anxiety	65 (91.5)
Happiness	58 (81.7)
Sadness	54 (76.1)
Alcohol	61 (85.9)
Number of smoking cessation attempts	
None	31 (43.7)
One	25 (35.3)
Two	5 (7.0)
Three or more	10 (14.0)
Type of cigarette smoked	
Commercial	70 (98.6)
Hand-rolled	1 (1.4)
Contact with smokers	70 (98.6)
Smoking history, pack-years[b]	60.5 ± 29.8
Daily alcohol intake	66 (93.0)

FTND: Fagerström Test for Nicotine Dependence; and ND: nicotine dependence. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD.

Of the 71 study participants, only 3 used smoking cessation medication. Two were successfully treated with nicotine replacement therapy, and one used bupropion but relapsed. Of the 61 subjects who did not use medication, 43 (70.5%) reported withdrawal symptoms during smoking cessation, and, of those, 29 (46%) presented with high or very high dependence. Anxiety was the most commonly reported withdrawal symptom among those who ceased smoking.

Regarding alcohol use, 66 subjects (93%) responded affirmatively to at least two of the four questions on the CAGE questionnaire.

The workplace, meal times, and coffee or alcohol consumption were considered triggers for smoking for the vast majority of respondents, whereas anxiety was the emotional factor most commonly associated with the act of smoking (Table 1).

Discussion

Squamous cell carcinoma of the head and neck is one of the most common types of cancer worldwide, and the prevalence of heavy smoking is high in this group.[3,13,15] Considering that continuing to smoke after a diagnosis of cancer contributes to a higher risk of complications during treatment and of development of a second primary tumor,[6-8,22] the present study was designed to assess ND and smoking habits in this population.

The assessment of ND in cancer patients can contribute to the development of an approach that is more effective and has longer-term effects in terms of smoking cessation.[8,15,22,23] Although the FTND does not assess motivational or emotional components, it is able to predict withdrawal symptoms and guide the pharmacological treatment during smoking cessation.[22,24]

In our sample, we found 47 subjects (66.2%) with high or very high ND. These results confirm the findings of other studies that also reported high ND in head and neck cancer patients.[13,19] We found that ND did not correlate with gender, disease stage, smoking cessation, or number of smoking cessation attempts. We also found that the number of cigarettes smoked per day did not correlate significantly with smoking cessation or gender.

Questions 1 and 4 on the FTND are the ones of greatest importance.[17,24] In our sample, 55

Table 2 - Distribution of the 71 patients with head and neck cancer, by responses given to the Fagerström Test for Nicotine Dependence.[17]

Question	Score (points)	Response[a]
1. How soon after you wake up do you smoke your first cigarette?		
Within 5 minutes	3	32 (45.1)
6 to 30 minutes	2	23 (32.4)
31 to 60 minutes	1	5 (7.0)
After 60 minutes	0	11 (15.5)
2. Do you find it difficult to refrain from smoking in places where it is forbidden (i.e., in church, at the library, etc.)?		
Yes	1	33 (46.5)
No	0	38 (53.5)
3. Which cigarette would you hate most to give up?		
The first one in the morning	1	36 (50.7)
Any other	0	35 (49.3)
4. How many cigarettes per day do you smoke?		
31 or more	3	32 (45.1)
21 to 30	2	8 (11.2)
11 to 20	1	22 (31.0)
10 or less	0	9 (12.7)
5. Do you smoke more frequently during the first hours after waking?		
Yes	1	13 (18.3)
No	0	58 (81.7)
6. Do you smoke if you are so ill that you are in bed most of the day?		
Yes	1	42 (59.2)
No	0	29 (40.8)

[a]Values expressed as n (%).

Table 3 - Distribution of the patients with head and neck cancer by nicotine dependence and by the variables gender, disease stage, smoking cessation, and number of smoking cessation attempts.

Variable	Category	Nicotine dependence		Total	p
		Very low/low	High/very high		
Gender	Female	4 (33.3)	8 (66.7)	12 (100.0)	0.970*
	Male	20 (33.9)	39 (66.1)	59 (100.0)	
Disease stage	Early	8 (38.1)	13 (61.9)	21 (100.0)	0.620**
	Advanced	16 (32.0)	34 (68.0)	50 (100.0)	
Smoking cessation	Yes	23 (36.5)	40 (63.5)	63 (100.0)	0.251*
	No	1 (12.5)	7 (87.5)	8 (100.0)	
Number of attempts	None	11 (35.5)	20 (64.5)	31 (100.0)	0.792**
	≥ 1	13 (32.5)	27(67.5)	40 (100.0)	

*Fisher's exact test. **Chi-square test.

Table 4 - Distribution of the patients with head and neck cancer by number of cigarettes smoked per day and by the variables gender, disease stage, and smoking cessation.

Variable	Category	Number of cigarettes/day		Total	p
		Up to 20 cigarettes	≥ 20 cigarettes		
Gender	Female	7 (58.3)	5 (41.7)	12 (100.0)	0.261**
	Male	24 (40.7)	35 (59.3)	59 (100.0)	
Disease stage	Early	14 (66.3)	7 (33.3)	21 (100.0)	0.011**
	Advanced	17 (34.0)	33 (66.0)	50 (100.0)	
Smoking cessation	Yes	28 (44.5)	35 (55.5)	63 (100.0)	0.507*
	No	3 (37.5)	5 (62.5)	8 (100.0)	

*Fisher's exact test. **Chi-square test.

 http://dx.doi.org/10.1590/S1806-37132014000300012

Table 5 - Distribution of the patients with head and neck cancer by smoking history and by the variables time to smoke the first cigarette of the day, disease stage, and smoking cessation.

Variable	Category	Smoking history, pack-years		Total	p
		≤ 40	> 40		
Time to smoke the first cigarette of the day	Within 30 min of awakening	13 (23.6%)	42 (76.4%)	55 (100%)	0.270**
	After 30 min of awakening	6 (37.5%)	10 (62.5%)	16 (100%)	
Disease stage	Early	9 (42.9%)	12 (57.1%)	21 (100%)	0.047**
	Advanced	10 (20.0%)	40 (80.0%)	50 (100%)	
Smoking cessation	Yes	17 (27.0%)	46 (73.0%)	63 (100%)	0.960*
	No	2 (25.0%)	6 (75.0%)	8 (100%)	

*Fisher's exact test.

subjects (77.5%) reported smoking their first cigarette within 30 min of awakening, and 32 (45.1%) smoked more than 30 cigarettes per day. These data are consistent with those of a study[24] of 301 regular smokers, which showed that a shorter time to smoke the first cigarette of the day and a greater number of cigarettes smoked per day translated into a greater level of ND.

Many patients continue to smoke even though they are aware that smoking is a risk factor for morbidity and a factor increasing morbidity during treatment.[8,9,13-16,22] However, in our sample, 63 patients (88.7%) ceased tobacco use. Of those, only 2 received pharmacological treatment (nicotine patch). This result confirms literature reports that smoking after a cancer diagnosis is yet to be routinely addressed as a disease requiring treatment.[8,9,16,23]

Our results for age at smoking initiation were similar to those reported in the literature—90% of smokers start smoking before 19 years of age. Earlier age at smoking initiation is known to translate into a greater chance of becoming dependent.[25] In our sample, the mean age at smoking initiation was 15.6 ± 3.7 years (range, 6-30 years).

Smoking is a recurrent disease, and multiple cessation attempts are common until permanent cessation success is achieved.[10,26] In our sample, 40 subjects had made at least one attempt.

The mean smoking history was 60.5 ± 29.8 pack-years. There was a significant association between smoking history and disease stage (p = 0.047; chi-square test). However, when we examined the association between smoking history and disease stage by using Spearman's correlation test, we found no significant correlation (r = 0.184; p = 0.125). This difference can be explained by the fact that Spearman's correlation test requires linear correlation of distribution, which cannot be seen when considering subgroups of early disease patients and advanced disease patients.

Analysis of smoking history revealed that the number of cigarettes smoked ever did not affect the success of cessation attempts (p = 0.960).

In our study, 66 subjects (93%) were considered alcohol dependent by the CAGE questionnaire.[21] In addition, alcohol was reported as a trigger for tobacco use by 61 subjects (85.9%), which is a cause for concern, especially in cases of alcohol abuse and smoking. Because smoking and alcoholism are important risk factors, they should be treated concurrently in patients with head and neck cancer.[3,4,7,13,22]

The time of diagnosis and the course of treatment are a great opportunity for promoting behavioral changes, and we should encourage cessation of tobacco and alcohol use; however, this approach should be tailored to each patient.[13-15,22] The basic model of intervention should progressively increase in intensity according to the needs of the patient, also including adjuvant pharmacological treatment.[8,12,13,19,22,23]

Cancer treatment is frequently accompanied by the discomfort of surgery, radiotherapy, and/or chemotherapy. These factors alone could contribute to patients experiencing anxiety or mood swings, or an exacerbation of these problems, which could add to or be mistaken for the withdrawal symptoms due to concurrent cessation of tobacco and (in many cases) alcohol use.[14]

Smoking cessation interventions in oncology should focus not only on the risks of continued tobacco use, but also, and mainly, on supporting long-term abstinence and reducing relapse

risk factors, which are very common in this population.[8,9,23,27]

It is essential that oncologists understand that smoking and alcoholism are diseases that should be approached and treated properly and are components of the cancer treatment process as a whole.[9,16,23,27]

The present study has some limitations to be considered. First, although there are scales for assessing withdrawal symptoms, we concluded that it would be difficult to apply them because of the long period of continued abstinence of the former smokers in the present study. Second, although the literature reports similar results for the FTND in smokers and former smokers, it is possible that the long interval between smoking cessation and the administration of the questionnaire influenced patients' responses, thereby reducing the sensitivity of the test.[28-30]

In conclusion, the level of ND was high or very high in most patients in our study, and the prevalence of heavy smoking (more than 20 cigarettes/day) for long periods was high. Although most respondents were successful in smoking cessation, withdrawal symptoms were common and pharmacological treatment was not routinely offered. There was a significant association between smoking history and advanced disease stage. The approach to head and neck cancer patients who smoke is a window of opportunity for offering smoking cessation treatment. A diagnosis of cancer is a motivator for smoking cessation. However, intensive smoking cessation treatment is not routinely performed.

References

1. World Health Organization [homepage on the Internet]. Geneva: World Health Organization [cited 2014 Feb 26]. WHO report on the global tobacco epidemic, 2011: warning about the dangers of tobacco. [Adobe Acrobat document, 164p.]. Available from: http://whqlibdoc.who.int/publications/2011/9789240687813_eng.pdf
2. World Health Organization. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Vol 100E--Tobacco Smoking [monograph on the Internet]. Lyon: IARC; 2012 [cited 2014 Feb 26]. [Adobe Acrobat document, 170p.]. Available from: http://monographs.iarc.fr/ENG/Monographs/vol100E/mono100E-6.pdf
3. Curado MP, Hashibe M. Recent changes in the epidemiology of head and neck cancer. Curr Opin Oncol. 2009;21(3):194-200. http://dx.doi.org/10.1097/CCO.0b013e32832a68ca
4. Wünsch-Filho V. The epidemiology of oral and pharynx cancer in Brazil. Oral Oncol. 2002;38(8):737-46. http://dx.doi.org/10.1016/S1368-8375(02)00066-0
5. Ministério da Saúde. Instituto Nacional do Câncer José Alencar Gomes da Silva [homepage on the Internet]. Rio de Janeiro: INCA [cited 2014 Feb 26].Estimativa 2014 - Incidência de Câncer no Brasil 2014. [Adobe Acrobat document, 126p.]. Available from: http://www.inca.gov.br/estimativa/2014/estimativa-24012014.pdf
6. Chen AM, Chen LM, Vaughan A, Sreeraman R, Farwell DG, Luu Q, et al. Tobacco smoking during radiation therapy for head-and-neck cancer is associated with unfavorable outcome. Int J Radiat Oncol Biol Phys. 2011;79(2):414-9. http://dx.doi.org/10.1016/j.ijrobp.2009.10.050
7. León X, del Prado Venegas M, Orús C, López M, García J, Quer M. Influence of the persistence of tobacco and alcohol use in the appearance of second neoplasm in patients with a head and neck cancer. A case-control study. Cancer Causes Control. 2009;20(5):645-52. http://dx.doi.org/10.1007/s10552-008-9277-8
8. Toll BA, Brandon TH, Gritz ER, Warren GW, Herbst RS; AACR Subcommittee on Tobacco and Cancer. Assessing tobacco use by cancer patients and facilitating cessation: an American Association for Cancer Research policy statement. Clin Cancer Res. 2013;19(8):1941-8. http://dx.doi.org/10.1158/1078-0432.CCR-13-0666
9. Pinto FR, Matos LL, Gumz Segundo W, Vanni CM, Rosa DS, Kanda JL. Tobacco and alcohol use after head and neck cancer treatment: influence of the type of oncological treatment employed. Rev Assoc Med Bras. 2011;57(2):171-6. http://dx.doi.org/10.1590/S0104-42302011000200014
10. Reichert J, Araújo AJ, Gonçalves CM, Godoy I, Chatkin JM, Sales MP, et al. Smoking cessation guidelines--2008. J Bras Pneumol. 2008;34(10):845-80. http://dx.doi.org/10.1590/S1806-37132008001000014
11. Chatkin JM. The influence of genetics on nicotine dependence and the role of pharmacogenetics in treating the smoking habit. J Bras Pneumol. 2006;32(6):573-9. http://dx.doi.org/10.1590/S1806-37132006000600016
12. Henningfield JE, Shiffman S, Ferguson SG, Gritz ER. Tobacco dependence and withdrawal: science base, challenges and opportunities for pharmacotherapy. Pharmacol Ther. 2009;123(1):1-16. http://dx.doi.org/10.1016/j.pharmthera.2009.03.011
13. Gritz ER, Carr CR, Rapkin DA, Chang C, Beumer J, Ward PH. A smoking cessation intervention for head and neck cancer patients: trial design, patient accrual, and characteristics. Cancer Epidemiol Biomarkers Prev. 1991;1(1):67-73.
14. Gritz ER, Schacherer C, Koehly L, Nielsen IR, Abemayor E. Smoking withdrawal and relapse in head and neck cancer patients. Head Neck. 1999;21(5):420-7. http://dx.doi.org/10.1002/(SICI)1097-0347(199908)21:5<420::AID-HED7>3.0.CO;2-U
15. Cox LS, Africano NL, Tercyak KP, Taylor KL. Nicotine dependence treatment for patients with cancer. Cancer. 2003;98(3):632-44. http://dx.doi.org/10.1002/cncr.11538
16. Duffy SA, Louzon SA, Gritz ER. Why do cancer patients smoke and what can providers do about it? Community Oncol. 2012;9(11):344-52. http://dx.doi.org/10.1016/j.cmonc.2012.10.003
17. Heatherton TF, Kozlowski LT, Frecker RC, Fagerström KO. The Fagerström Test for Nicotine Dependence: a revision of the Fagerström Tolerance Questionnaire. Br J Addict. 1991;86(9):1119-27. http://dx.doi.org/10.1111/j.1360-0443.1991.tb01879.x
18. Mikami I, Akechi T, Kugaya A, Okuyama T, Nakano T, Okamura H, et al. Screening for nicotine dependence

among smoking-related cancer patients. Jpn J Cancer Res. 1999;90(10):1071-5. http://dx.doi.org/10.1111/j.1349-7006.1999.tb00679.x
19. Sharp L, Johansson H, Fagerström K, Rutqvist LE. Smoking cessation among patients with head and neck cancer: cancer as a 'teachable moment'. Eur J Cancer Care (Engl). 2008;17(2):114-9. http://dx.doi.org/10.1111/j.1365-2354.2007.00815.x
20. Associação Americana de Psiquiatria. Manual Diagnóstico e Estatístico de Transtornos Mentais--DSM-4, 4a edição. Porto Alegre; Artmed; 1994.
21. Masur J, Monteiro MG. Validation of the "CAGE" alcoholism screening test in a Brazilian psychiatric inpatient hospital setting. Braz J Med Biol Res. 1983;16(3):215-8.
22. Gritz ER, Carr CR, Rapkin D, Abemayor E, Chang LJ, Wong WK, et al. Predictors of long-term smoking cessation in head and neck cancer patients. Cancer Epidemiol Biomarkers Prev. 1993;2(3):261-70.
23. Balogh EP, Dresler C, Fleury ME, Gritz ER, Kean TJ, Myers ML, et al. Reducing tobacco-related cancer incidence and mortality: summary of an institute of medicine workshop. Oncologist. 2014;19(1):21-31. http://dx.doi.org/10.1634/theoncologist.2013-0230
24. Halty LS, Hüttner MD, Oliveira Netto IC, Santos VA, Martins G. Análise da utilização do Questionário de Tolerância de Fagerström (QTF) como instrumento de medida da dependência nicotínica. J Pneumologia. 2002;28(4):180-6. http://dx.doi.org/10.1590/S0102-35862002000400002
25. Lando HA, Hipple BJ, Muramoto M, Klein JD, Prokhorov AV, Ossip DJ, et al. Tobacco is a global paediatric concern. Bull World Health Organ [serial on the Internet]. 2010 Jan [cited 2012 Jan 20];88(1):[about 3p.]. Available from: http://www.who.int/bulletin/volumes/88/1/09-069583/en/.
26. 2008 PHS Guideline Update Panel, Liaisons, and Staff. Treating tobacco use and dependence: 2008 update U.S. Public Health Service Clinical Practice Guideline executive summary. Respir Care. 2008;53(9):1217-22.
27. Gritz ER, Toll BA, Warren GW. Tobacco use in the oncology setting: advancing clinical practice and research. Cancer Epidemiol Biomarkers Prev. 2014;23(1):3-9. http://dx.doi.org/10.1158/1055-9965.EPI-13-0896
28. Meneses-Gaya IC, Zuardi AW, Loureiro SR, Crippa JA. Psychometric properties of the Fagerström Test for Nicotine Dependence. J Bras Pneumol. J Bras Pneumol. 2009;35(1):73-82. http://dx.doi.org/10.1590/S1806-37132009000100011
29. Vink JM, Willemsen G, Beem AL, Boomsma DI. The Fagerström Test for Nicotine Dependence in a Dutch sample of daily smokers and ex-smokers. Addict Behav. 2005;30(3):575-9. http://dx.doi.org/10.1016/j.addbeh.2004.05.023
30. Hudmon KS, Pomerleau CS, Brigham J, Javitz H, Swan GE. Validity of retrospective assessments of nicotine dependence: a preliminary report. Addict Behav. 2005;30(3):613-7. http://dx.doi.org/10.1016/j.addbeh.2004.08.006

About the authors

Adriana Ávila de Almeida
Pulmonologist. *Santa Casa de Misericórdia de São Paulo* School of Medical Sciences, São Paulo, Brazil.

Celso Muller Bandeira
Head and Neck Surgeon. *Santa Casa de Misericórdia de São Paulo* School of Medical Sciences, São Paulo, Brazil.

Antonio José Gonçalves
Full Professor. Department of Surgery, *Santa Casa de Misericórdia de São Paulo* School of Medical Sciences, São Paulo, Brazil.

Alberto José Araújo
Pulmonologist and Coordinator. Center for the Study and Treatment of Smoking, Thoracic Diseases Institute, Clementino Fraga Filho University Hospital, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

Performance of ICU ventilators during noninvasive ventilation with large leaks in a total face mask: a bench study*,**

Desempenho de ventiladores de UTI durante ventilação não invasiva com grandes vazamentos em máscara facial total: estudo em simulador mecânico

Maria Aparecida Miyuki Nakamura, Eduardo Leite Vieira Costa, Carlos Roberto Ribeiro Carvalho, Mauro Roberto Tucci

Abstract

Objective: Discomfort and noncompliance with noninvasive ventilation (NIV) interfaces are obstacles to NIV success. Total face masks (TFMs) are considered to be a very comfortable NIV interface. However, due to their large internal volume and consequent increased CO_2 rebreathing, their orifices allow proximal leaks to enhance CO_2 elimination. The ventilators used in the ICU might not adequately compensate for such leakage. In this study, we attempted to determine whether ICU ventilators in NIV mode are suitable for use with a leaky TFM. **Methods:** This was a bench study carried out in a university research laboratory. Eight ICU ventilators equipped with NIV mode and one NIV ventilator were connected to a TFM with major leaks. All were tested at two positive end-expiratory pressure (PEEP) levels and three pressure support levels. The variables analyzed were ventilation trigger, cycling off, total leak, and pressurization. **Results:** Of the eight ICU ventilators tested, four did not work (autotriggering or inappropriate turning off due to misdetection of disconnection); three worked with some problems (low PEEP or high cycling delay); and one worked properly. **Conclusions:** The majority of the ICU ventilators tested were not suitable for NIV with a leaky TFM.

Keywords: Ventilators, mechanical; Positive-pressure Respiration; Noninvasive ventilation; Equipment safety; Equipment failure; Masks.

Resumo

Objetivo: O desconforto e a falta de adaptação às interfaces de ventilação não invasiva (VNI) são obstáculos ao sucesso da VNI. A máscara facial total (MFT) é uma interface de VNI considerada muito confortável. No entanto, devido a seu grande volume interno e, consequentemente, ao aumento da reinalação de CO_2, a MFT tem orifícios que permitem vazamentos proximais para melhorar a eliminação de CO_2. É possível que os ventiladores usados na UTI não compensem esse vazamento adequadamente. Neste estudo, buscamos determinar se ventiladores de UTI com módulo de VNI podem ser usados com MFT com grandes vazamentos. **Métodos:** Estudo em simulador mecânico conduzido em um laboratório universitário de pesquisa. Oito ventiladores de UTI equipados para realizar VNI e um ventilador específico para VNI foram conectados a uma MFT com grandes vazamentos. Todos foram testados com dois níveis de *positive end-expiratory pressure* (PEEP, pressão expiratória final positiva) e três níveis de pressão de suporte. As variáveis analisadas foram disparo do ventilador, ciclagem, vazamento total e pressurização. **Resultados:** Dos oito ventiladores de UTI, quatro não funcionaram (autodisparo ou desligamento inapropriado por detecção incorreta de desconexão), três funcionaram com alguns problemas (valores baixos de PEEP ou grande atraso na ciclagem do ventilador) e apenas um funcionou adequadamente. **Conclusões:** A maioria dos ventiladores de UTI testados mostrou-se inadequada para VNI com MFT com grandes vazamentos.

Descritores: Ventiladores mecânicos; Respiração com pressão positiva; Ventilação não invasiva; Segurança de equipamentos; Falha de equipamento; Máscaras.

*Study carried out in *Laboratório de Investigação Médica* 09 (LIM 09, Laboratory for Medical Research 09), Department of Pulmonology, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.
Correspondence to: Mauro R. Tucci. Laboratório de Investigação Médica 09 (LIM-09), University of São Paulo School of Medicine, Avenida Dr. Arnaldo, 455, 2º andar, sala 2144, CEP 01246-903, São Paulo, SP, Brasil.
Tel. 55 11 3061-7361. E-mail: mrotucci@gmail.com
Financial support: This study received financial support from the *Fundação de Amparo à Pesquisa do Estado de São Paulo* (FAPESP, São Paulo Research Foundation) and the *Financiadora de Estudos e Projetos* (FINEP, Brazilian Financing Agency for Studies and Projects).
Submitted: 17 September 2013. Accepted, after review: 5 April 2014.
**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

 http://dx.doi.org/10.1590/S1806-37132014000300013

Introduction

Noninvasive ventilation (NIV) has been used successfully in order to manage respiratory failure of different etiologies.[1] Adherence to treatment is a major concern and has a profound impact on NIV success. In acute respiratory failure, for example, 40-60% of NIV trials fail due to mask discomfort and patient noncompliance.[2-4]

The total face mask (TFM) is an alternative interface designed to increase patient tolerance. It covers the entire face, delivering effective ventilation via nasal and oral routes. By means of its increased contact surface with the skin, it also minimizes gas leakage whilst avoiding pressure sores to the face.[5-7] However, TFM has the disadvantage of having a large internal volume (875 mL).[8] Therefore, in order to minimize CO_2 rebreathing, the mask has two built-in exhalation ports that allow air leakage.[5,9,10] These intentional air leaks are often adequately compensated by NIV ventilators but might not be handled as well by ICU ventilators.[11]

The main hypothesis of our study was that ICU ventilators do not perform adequately with TFM with large air leaks. We evaluated the performance of ICU ventilators in delivering NIV via TFM with large air leaks on a bench model and compared the results obtained with a mechanical ventilator dedicated for NIV.

Methods

This was an experimental study conducted in 2008 in the Laboratory for Medical Research 09, specializing in Pulmonology, at the University of São Paulo School of Medicine, in the city of São Paulo, Brazil.

The NIV model (Figure E1, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf) was adapted from previously described models[12,13] and consisted of a two-chamber test lung (TTL 2600; Michigan Instruments, Grand Rapids, MI, USA) partially connected by a lift bar. The first chamber (drive chamber) was connected to the drive ventilator and, during the inspiratory phase, was insufflated, moving the second chamber (chest chamber) together and producing a negative pressure in its interior, which was transmitted to a second one-chamber test lung (Takaoka, São Paulo, Brazil). This simulator consists of a bellows device in a rigid box, the bellows representing the "lung", and the space between the bellows and the box representing the pleural space with direct communication with the chest chamber. The "lung" was connected to a mannequin head made of PVC. A TFM (Philips Respironics, Murryville, PA, USA) was attached to the mannequin head and connected to the test ventilator. The mechanical lung model compliance was 50 mL/cmH_2O at an inspiratory volume of 500 mL.

Two pressure transducers (Valydine, Northridge, CA, USA) were connected to the model (Figure E1): one for proximal pressure (between the mask and the proximal pneumotachograph) and one for pleural pressure (between the two-chamber test lung and the one-chamber test lung). The flow was measured with two pneumotachographs (Hans-Rudolph, Kansas City, MO, USA): one for proximal flow (between the proximal pressure transducer and the Y connector from the ventilator circuit) and one for distal flow (between the upper airway of the mannequin and the one-chamber test lung). The resistance of the proximal pneumotachograph varied with the flow values. For flows of 0.5 L/s, 1.0 L/s, 2.0 L/s, and 3.0 L/s, resistance was 1.29 cmH_2O . L^{-1} . s^{-1}, 1.44 cmH_2O . L^{-1} . s^{-1}, 1.91 cmH_2O . L^{-1} . s^{-1}, and 2.40 cmH_2O . L^{-1} . s^{-1}, respectively. The analogical signals from the transducers were recorded at 200 Hz and analyzed off-line with a customized Labview software program (National Instruments, Austin, TX, USA).

A Newport e500 ventilator (Newport Medical Instruments, Costa Mesa, CA, USA) was used in order to provide the inspiratory effort for the NIV model. Respiratory rate was 12 breaths/min in pressure control mode. Inspiratory time was 1.0 s, driving pressure was 17 cmH_2O, positive end-expiratory pressure (PEEP) was 0 cmH_2O, and inspiratory slope was +2, developing a tidal volume in the mechanical lung model of 300 mL and an airway occlusion pressure after 0.1 s ($P_{0.1}$) of 3.4 cmH_2O.

Ventilators tested

One NIV ventilator (BiPAP Vision; Philips Respironics) and eight ICU ventilators, all equipped with noninvasive mode, were tested (Table E1, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf): Puritan Bennett 840 (Covidien, Boulder, CO, USA); Servo-i (Maquet,

Solna, Sweden); Vela (Viasys Healthcare, Palm Springs, CA, USA); Savina (Drägerwerk AG & Co., Lübeck, Germany); Esprit (Philips Respironics); GALILEO Gold (Hamilton Medical, Rhäzuns, Switzerland); Horus (Taëma, Anthony, France); and e500 (Newport Medical Instruments).

Whenever available, the pressure trigger was used and set to the most sensitive level that did not result in autotriggering. When the pressure trigger was unavailable or autotriggering was unavoidable, we used the flow trigger. When adjustable, inspiratory rise time was set, initially, at 50% of the maximum value, expiratory threshold was set at 25% of peak inspiratory flow (PIF), and maximum inspiratory time was set at 1.5 s. The evaluations were performed using pressure support model, with PEEPs of 5 cmH_2O (PEEP5) and 10 cmH_2O (PEEP10), each with three different pressure support levels: 5 cmH_2O (PS5); 10 cmH_2O (PS10); and 15 cmH_2O (PS15). Initially, each ventilator was tested with a sealed facial mask in order to verify the NIV functionality of the ventilator. The TFM was subsequently tested with the two exhalation ports open to allow air to escape, as recommended by the manufacturer.[5]

Measured variables

First, we evaluated whether each ventilator worked properly with the TFM. The "no operation" of the ventilator was defined as the presence of constant autotriggering or of the inspiratory flow turning off (misinterpretation of disconnection due to massive leakage) even after trying different settings of triggering and inspiratory rise time. If the ventilator worked, we recorded the additional adjustments necessary to make it work properly.

We measured the following variables (Figure 1): proximal inspiratory pressure (PIP) at the end of the inspiratory phase, in cmH_2O, measured at the proximal sensor; PEEP, in cmH_2O; inspiratory leakage, in L/s, determined by the difference between proximal flow and distal flow at PIF; expiratory leakage, in L/s, by measuring proximal flow at the end of the expiratory phase; PIF, in L/s; tidal volume, in L, calculated by the integration of the flow signal from the distal flow transducer; trigger delay, in ms, determined by the time elapsed between the onset of inspiratory effort (in pleural pressure) and the onset of inspiratory flow; cycling-off delay, in ms, measured by the time from the end of the driving inspiratory effort to the end of the ventilator inspiratory flow; and inspiratory pressure-time product at 500 ms and at 1 s (PTP500 and PTPt, respectively), determined by computing the area under the pressure-time curve between the onset of inspiratory effort and these two times.

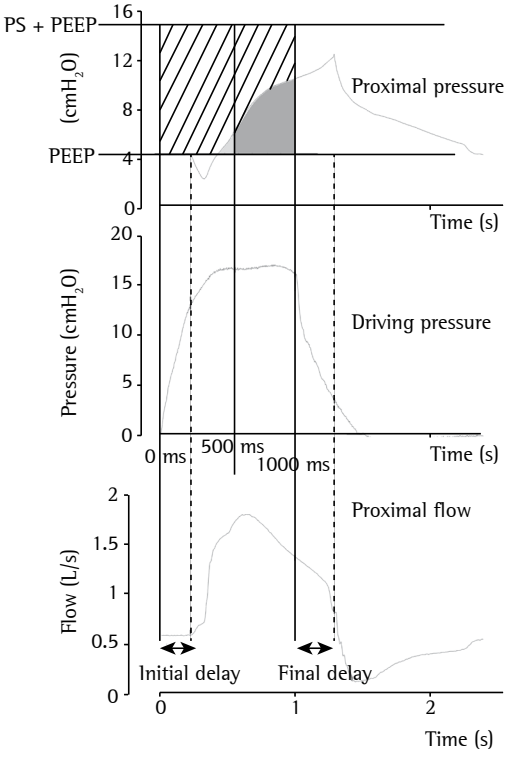


Figure 1 - Representation of the variables measured. Pressure (upper) and flow (lower) tracings of a hypothetical measurement with positive end-expiratory pressure (PEEP) of 5 cmH_2O and pressure support (PS) of 10cmH_2O. In the middle, tracing of the pressure for the drive ventilator. The upper tracing shows the inspiratory pressure-time product at 500 ms (PTP500; light gray area) and at 1 s (PTPt; dark gray area plus light gray area), both expressed in percentage of ideal area (line-shaded areas).

Statistical analysis

For each experimental condition, the software calculated a representative mean cycle through a point-by-point averaging of five cycles. Data for each variable are shown as one value (from the mean cycle) for each condition. Data for the various conditions are shown as mean ± SD.

The cycles were very stable, and the variances for each variable were negligible; therefore, in

 http://dx.doi.org/10.1590/S1806-37132014000300013

our analysis, we did not use formal statistical hypothesis tests but a nominal comparison of the values of each mean cycle, such as in other similar studies.[13,14] Because of the low variability of the NIV mechanical model, any clinically relevant difference in the measurements would certainly result in a difference of more than 2.8 times the measurement error (standard deviation of various cycles of one condition).[15] The calculated values (2.8 times the measurement error) were as follows: tidal volume, 0.020 L; PIP, 0.17 cmH_2O; PEEP, 0.15 cmH_2O; PTP500, 4.3%; PTPt, 2.5%; PIF, 0.05 L/s; expiratory leak, 0.03 L/s; trigger delay, 9.7 ms; and cycling-off delay, 7.5 ms.

Results

The NIV ventilator and only four of the ICU ventilators equipped with NIV mode worked with the TFM: Horus, Vela, e500, and Servo-i (Table 1). Of these, all but the Servo-i had problems to trigger or to cycle off. The main problems in the other ventilators, which were considered to be non-operational, were the misinterpretation of disconnection and autotriggering. With the sealed oronasal mask (i.e., in the absence of significant system leakage), all ventilators worked well.

In all ICU ventilators, except for the Horus, we needed to turn the NIV mode on. For the Horus, e500, and Vela to work in some settings, we needed to adjust the expiratory trigger sensitivity, the inspiratory pressure slope, or the inspiratory sensitivity.

The Horus ventilator did not work in NIV mode. Using the default "invasive mode", we had to set it to pressure support mode, with its expiratory cycling threshold and inspiratory pressure-slope adjusted to maximum (30 L/min and 150 cmH_2O/s, respectively). Inspiratory trigger

Table 1 - Performance of the ventilators tested[a] with the total face mask and the major problems observed.

Ventilator	Proper operation	Cause of no operation	Problems during operation
BiPAP Vision	Yes		
Puritan Bennett 840[b,c]	No	AT	
Savina[b,d]	No	AT	
GALILEO Gold[b,e]	No	FTO	
Servo-i[b,f]	Yes		Premature cycling at PEEP5 and PS5 and at PEEP10 and PS5
e500[b,e]	No	FTO (nonoperational at PEEP10)	LCF at PEEP5 and the 3 PS settings
Esprit[b,g]	No	AT, FTO	
Horus (NIV deactivated)	No	FTO (nonoperational at PEEP5)	CIT and LCF at PEEP10 and the 3 PS settings
Horus[b,e]	No	AT	
Vela[b,h]	No		CIT

NIV: noninvasive ventilation; AT: autotriggering (the ventilator tested maintained a respiratory rate larger than that of the drive ventilator (12 breaths/min) and the inspiratory time was variable); FTO: inspiratory flow turning off (inappropriate turning-off after some cycles due to misinterpretation of disconnection); PS5: pressure support of 5 cmH_2O; PEEP5: positive end-expiratory pressure of 5 cmH_2O; PEEP10: positive end-expiratory pressure of 10 cmH_2O; LCF: leakage compensation failure (ventilator cannot maintain PEEP level); and CIT: cycling by maximum inspiratory time adjusted in 1.5 s. [a]BiPAP Vision (Philips Respironics, Murryville, PA, USA); Puritan Bennett 840 (Covidien, Boulder, CO, USA); Savina (Drägerwerk AG & Co., Lübeck, Germany); Galileo Gold (Hamilton Medical, Rhäzuns, Switzerland); Servo-i (Maquet, Solna, Sweden); e500 (Newport Medical Instruments, Costa Mesa, CA, USA); Esprit (Philips Respironics); Horus (Taëma, Anthony, France); and Vela (Viasys Healthcare, Palm Springs, CA, USA). [b]Noninvasive ventilation activated. Updates on ventilator capabilities between 2008 and 2013: [c]Puritan Bennett™ has a new optional software with leak compensation up to 65 L/min for adults. [d]No change in leak compensation. The new model (Savina 300) has improved leak compensation. [e]No updates for leak compensation. [f]The new version of the NIV mode compensates for leaks up to 65 L/min for adults. [g]The new software version and new model (V200) have leak compensation up to 60 L/min and autoadaptative triggering and cycling-off (autotracking). [h]Leak compensation up to 40 L/min in Vela Plus and Vela Comprehensive models.

sensitivity could not be properly adjusted with the pressure-trigger option and worked only within a narrow range with the flow-trigger option (approximately 1.7 L/min).

The e500 inspiratory pressure slope was set to automatic mode, as was the expiratory cycling threshold at 50% of peak flow. To avoid autotriggering, it was necessary to set the pressure trigger at values close to –3.2 cmH_2O.

The Vela NIV mode has an automatic selection of inspiratory triggering sensitivity. Expiratory cycling threshold had to be set at maximum (30% of peak flow). However, when PEEP was set at 10 cmH_2O and pressure support was set at 15 cmH_2O, there was a high inspiratory cycling-off delay, creating large tidal volumes that exceeded the lung model capacity.

The Servo-i worked properly. Its NIV mode has automatic inspiratory triggering sensitivity. The inspiratory pressure slope was adjusted to 50% of maximum (0.2 s), and the expiratory cycling threshold was set at 25% of peak flow.

The inspiratory flow slope of the NIV ventilator (BiPAP Vision) was adjusted to 50% of maximum (0.2 s). The inspiratory-triggering and expiratory-triggering sensitivity were automatic.

Regarding PEEP and expiratory leakage, the Servo-i, Vela, and BiPAP Vision compensated for the TFM leaks during exhalation, maintaining PEEP values close to the set values (Figure 2). The mean flows delivered by these ventilators in order to compensate for leaks during exhalation were 0.65 ± 0.12 L/s and 0.89 ± 0.16 L/s for PEEP5 and PEEP10, respectively. The e500 and Horus compensated for the leakage poorly and therefore were not able to maintain the target PEEP (Figure 2). The maximum leakage-compensation flows for the e500 and Horus were, respectively, 0.52 ± 0.01 L/s (PEEP5 with leakage compensation option switched on) and 0.41 ± 0.01 L/s (PEEP10).

The Horus ventilator had the lowest PIP at the mask (Figure E2, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf). All of the other ventilators reached similar PIPs. The BiPAP Vision presented with the highest PTP500 and PTPt values (Figure 3). The e500 and Horus had the lowest values for PTP500.

The PIF values increased in parallel with increases in inspiratory pressures (Figure 4), and the volume of leaks increased in parallel with increases in PEEP and pressure support. The mean values of PIF for all ventilators were 1.69 ± 0.31 L/s, 2.07 ± 0.26 L/s, and 2.36 ± 0.31 L/s, respectively, at PS5, PS10, and PS15. The highest PIFs were reached by the BiPAP Vision and e500 (2.39 ± 0.32 L/s and 2.14 ± 0.32 L/s, respectively). The Servo-i and the Vela ventilators had intermediate values (2.00 ± 0.32 L/s and 1.82 ± 0.33 L/s, respectively), and the smallest PIFs were attained by the Horus (1.60 ± 0.29 L/s).

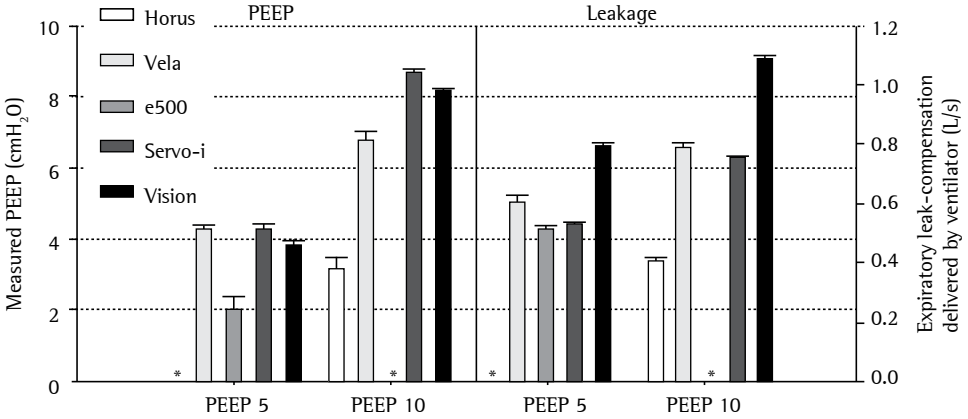


Figure 2 - Measured positive end-expiratory pressure (PEEP; left panel) and expiratory flow delivered by the ventilator (right panel) to compensate for air leakage at PEEP of 5 cmH_2O (PEEP5) and 10 cmH_2O (PEEP10), expressed as mean ± SD. As shown, the Horus and the e500 ventilators did not compensate adequately for leaks, delivering less than 0.6 L/s of compensatory flow, and were not capable of keeping the set PEEP level. *Not measured due to autotriggering.

 http://dx.doi.org/10.1590/S1806-37132014000300013

Regarding tidal volumes, they became higher with the increase in pressure support levels (Figure E3, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf). The mean tidal volumes for all ventilators using PEEP5 at PS5, PS10, and PS15 were 472 ± 25 mL, 609 ± 32 mL, and 726 ± 124 mL, respectively, whereas the mean tidal volumes for the ventilators using PEEP10 at the same three pressure support modes were 489 ± 25 mL, 641 ± 28 mL, and 768 ± 90 mL, respectively.

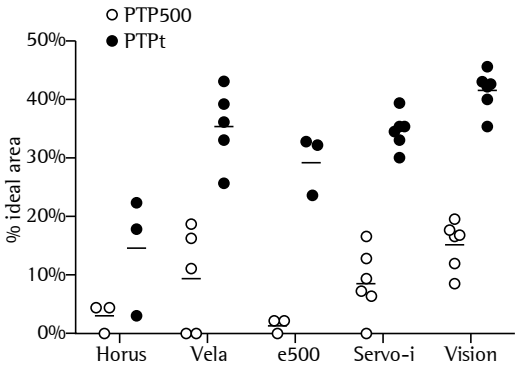


Figure 3 - Pressurization characteristics of five of the ventilators tested, demonstrated by inspiratory pressure-time product at 500 ms (PTP500; open circles) and at 1 s (PTPt; filled circles), expressed in percentage of ideal area. The horizontal dashes indicate the means of all measures (positive end-expiratory pressures of 5 and 10 cmH_2O vs. pressure support of 5, 10 and 15 cmH_2O) available for the ventilators.

The mechanical lung model (without the test ventilators) had an intrinsic baseline delay of 30.5 ms and 24.9 ms, respectively, for trigger delay and cycling-off delay. Only the e500 and Horus presented with trigger delay values higher than 100 ms (276 ± 105 ms and 152 ± 36 ms, respectively). The Servo-i, Vela, and BiPAP Vision, respectively, had trigger delay values of 50 ± 12 ms, 53 ± 6 ms, and 69 ± 27 ms (Figure E4, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf). Neither PEEP nor pressure support altered the triggering delay. Although the Servo-i ventilator had the lowest cycling-off delay (8.5 ± 67 ms), it presented with premature cycling in some settings (−94 ms for PEEP5 at PS5, as well as −43 ms for PEEP10 at PS5). The BiPAP Vision ventilator had a mean cycling-off delay of 136 ± 92 ms. The Horus and Vela had mean values of 273 ± 231 ms and 228 ± 214 ms, respectively. In both, cycling sometimes occurred by reaching the adjusted maximum inspiratory time of 1.5 s. The e500 ventilator showed the highest cycling-off delay (590 ± 622 ms; Figure E5, available online at http://www.jornaldepneumologia.com.br/imagebank/images/jbp_v40n3_suplemment.pdf).

Discussion

The most important finding in the present study was that only one of the tested ICU ventilators was suitable for NIV using a TFM.

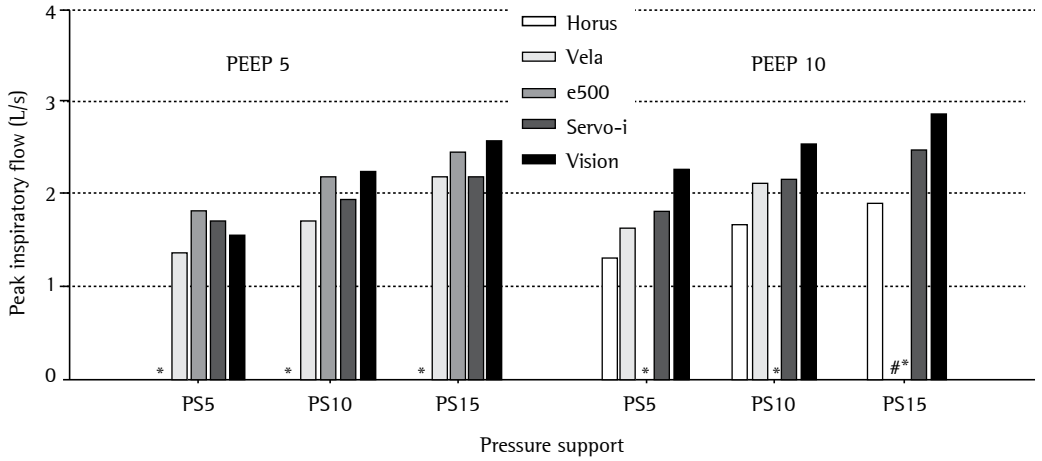


Figure 4 - Peak inspiratory flow of five of the ventilators tested. Measurements with positive end-expiratory pressure of 5 cmH_2O (PEEP5) and 10 cmH_2O (PEEP10) are on the left and right sides, respectively. The measurements were taken at pressure support levels of 5, 10, and 15 cmH_2O (PS5, PS10, and PS15, respectively). *The Horus ventilator at PEEP5 and the e500 ventilator at PEEP10 were not measured due to autotriggering. #Because of the limitation of the lung model, the Vela ventilator was not tested for PEEP10 with PS15.

Of the eight ICU ventilators, four were considered totally non-operational due to inappropriate turning-off (misinterpretation of disconnection) or autotriggering, whereas three of the remaining four had problems to compensate for the large leaks through the exhalation ports, resulting in inability to keep PEEP and inspiratory pressure, delayed inspiratory triggering, or delayed inspiration-to-expiration cycling. Only the Servo-i and the control NIV ventilator (BiPAP Vision) worked properly under all experimental conditions. It is important to emphasize that, in the absence of significant system leakage (sealed oronasal mask), all ventilators worked well (data not shown).

It is expected that TFMs cause large air leaks through their built-in orifices, which can sometimes be of a magnitude comparable to that of a patient disconnection. To increase safety, various manufacturers have limited the leak compensation for ICU ventilators to values equal to or lower than 30 L/min (or 0.5 L/s),[16] values above which the disconnection alarm of the ventilator goes off. In our study, the mean expiratory leak for the three ventilators that were able to maintain PEEP levels (Servo-i, Vela, and BiPAP Vision) was 45.6 ± 10.8 L/min with the smallest PEEP value (PEEP5), which is greater than when an oronasal mask is used.[17] These large air leaks most likely explain why four of the eight ventilators were considered non-operational.

Similar findings have been described previously. Miyoshi et al.[11] tested two ICU ventilators and reported that both worked properly with leaks up to 11.3 L/min; however, autotriggering and shutdown of the inspiratory flow occurred with leaks larger than 18 L/min. Another bench study, using an oronasal face mask and three customized leaks, compared nine ICU ventilators equipped with NIV mode and one NIV ventilator (BiPAP Vision). [16] When the air leak was increased to 37 L/min, only one ICU ventilator (Servo-i) and the NIV ventilator worked properly without adjustments, whereas four ICU ventilators either went to backup ventilation or were unable to synchronize.[16]

In our study, the e500 and the Horus ventilators were not able to maintain PEEP values with air leaks close to 30 L/min (or 0.5 L/s). Other authors have recognized the importance of the leak magnitude for PEEP maintenance.[18,19]

Usually, the inspiratory pressurization is evaluated by the PTP within the first 300 ms or 500 ms (PTP300 and PTP500, respectively), because PIF is reached within the first 250-300 ms and the level of pressure support is reached, in most ventilators, within the first 500 ms.[20] Due to the magnitude of the air leak found in our study, pressurization was delayed and PTP300 values were very low, rendering this measurement inappropriate for the evaluation of the quality of pressurization. We therefore chose PTP500 and PTPt as the indices of pressurization. Using these variables, we found that the pressurization capacity in the presence of air leaks varied widely among the ventilators, even after reaching the optimal setting of inspiratory pressure slope. This finding is in agreement with those in previous studies.[18,19]

Given the importance of the inspiratory pressure slope on the pressurization capacity, it has been suggested that slope adjustment should be automated for better performance.[21] Unexpectedly, the two ventilators with automatic adjustment of slope (e500 and Vela) were outperformed by Servo-i and BiPAP Vision in their ability to maintain pressurization. Nevertheless, it is true that the e500 showed the highest PIF and PIP, and it was the pressure loss during the expiratory phase that hindered its capacity to achieve optimal pressurization.

The trigger delay was smallest in the Servo-i, Vela, and BiPAP Vision. The ventilators that had the greatest difficulty in compensating for air leakage during the expiratory phase (e500 and Horus) were the ones with the highest trigger delays. Leaks in a suboptimally compensated ventilatory system can interfere with the synchrony between the patient and the ventilator because the ventilator relies on monitored pressure and flow in order to trigger each breath, and air leaks can change these signals. Delays of a magnitude similar to our findings were reported in an evaluation of portable ventilators with two pressure levels in a model with small inspiratory leaks (maximum of 0.16 L/s).[22]

In pressure support mode, the ventilator cycles from inspiration to expiration when the inspiratory flow decreases to a given value or, in most cases, to a proportion of PIF. When the ventilator cannot end the inspiration by this criterion, cycling-off occurs by secondary criteria, usually an upper limit threshold for the inspiratory time.[23] In our study, this backup criterion served as the primary mechanism under conditions of high pressures and large air leaks in the Horus,

 http://dx.doi.org/10.1590/S1806-37132014000300013

Vela, and e500. Limitations of the cycling-off criteria in ICU ventilators in the presence of air leakage were previously reported,[24] but this problem has been amended in new software versions of some ventilators and new ventilator models[25] (see footnotes in Table 1).

Of all tested ICU ventilators, only Servo-i had an acceptable performance with TFM, although it is noteworthy to mention that, in some settings, premature cycling occurred, a finding previously reported regarding this ventilator.[26] The use of TFM with the other ICU ventilators led to considerable asynchrony that would likely cause excessive patient discomfort and noncompliance with NIV. In addition, some ventilators did not offer sufficient assistance to satisfy the demands of the model, and this fact might potentially increase the respiratory muscle load and worsen respiratory failure in a clinical setting.

These findings suggest that, in order to adequately handle the air leaks that occur with the TFM, manufacturers will have to improve the algorithms in ICU ventilators,[27] allowing the reset of the upper limit of flow compensation, which is already true for some ventilators (see footnotes in Table 1). Because this type of software change might have safety implications (disconnection alarm), one possible solution would be to implement an adjustable upper limit of flow based on whether the patient is connected to a TFM or not. Another important consequence of air leaks, which can get higher than 1.7 L/s (100 L/min) in some instances, is the extra cost of wasted medical gases, especially with the use of high fractions of inspired oxygen. To overcome this problem, the true need of such large proximal leaks when using TFMs should be investigated as a mathematical model, and bench studies have shown that the dead space of a TFM is smaller than is its internal volume.[28,29]

Recently, new NIV interfaces (PerforMax and Fitlife; Philips Respironics)[30] have been marketed with similar characteristics but smaller internal volumes and without the two bores in the mask that act as exhalation valves. These interfaces use two types of elbow: a standard elbow for use with ICU ventilators without air leakage and an entrainment elbow for NIV ventilators. However, it remains unknown whether the use of these masks without leaks (standard elbows) would result in adequate alveolar ventilation even if they were attached to invasive mechanical ventilators (which have separate inspiratory and expiratory limbs). For example, in one report, patients required a high minute ventilation to avoid CO_2 retention when using a TFM without significant leaks.[31] In conditions in which high tidal volumes are considered inappropriate, one possible solution would be to combine the use of the entrainment elbow (with leaks) with invasive mechanical ventilators, thus optimizing CO_2 washout.

Some limitations of this study should be outlined. First, this is a bench study, and the results should be extrapolated for clinical practice with caution. Second, due to the large air leakage in the system, the ventilators had to generate high inspiratory flows, causing a significant pressure drop, as measured with the pneumotachograph (placed between the ventilator and the proximal pressure transducer), which led to a slight underestimation of the proximal pressures. However, although it was not possible to precisely estimate the absolute values of PEEP, PIP, and PTP produced in the ventilator circuit, it was possible to compare the ventilators. In addition, because the values of PEEP were inferior to the value generated by the ventilator, the expiratory leakage was likely to be a little higher than that measured. Finally, some ventilators or their NIV modes have been updated (see footnotes in Table 1) after the present study was conducted, and these changes might have affected their performances.

In conclusion, due to the large air leaks associated with the use of TFMs, the majority of the ICU ventilators tested were not suitable for NIV with TFMs.

Acknowledgements

The authors would like to thank the manufacturers of ventilators tested for providing them gratuitously.

References

1. Garpestad E, Brennan J, Hill NS. Noninvasive ventilation for critical care. Chest. 2007;132(2):711-20. http://dx.doi.org/10.1378/chest.06-2643
2. Schettino G, Altobelli N, Kacmarek RM. Noninvasive positive-pressure ventilation in acute respiratory failure outside clinical trials: experience at the Massachusetts General Hospital. Crit Care Med. 2008;36(2):441-7. http://dx.doi.org/10.1097/01.CCM.0000300084.67277.90
3. Carlucci A, Richard JC, Wysocki M, Lepage E, Brochard L; SRLF Collaborative Group on Mechanical Ventilation. Noninvasive versus conventional mechanical ventilation.

An epidemiologic survey. Am J Respir Crit Care Med. 2001;163(4):874-80. http://dx.doi.org/10.1164/ajrccm.163.4.2006027
4. Mehta S, Hill NS. Noninvasive ventilation. Am J Respir Crit Care Med. 2001;163(2):540-77. http://dx.doi.org/10.1164/ajrccm.163.2.9906116
5. Criner GJ, Travaline JM, Brennan KJ, Kreimer DT. Efficacy of a new full face mask for noninvasive positive pressure ventilation. Chest. 1994;106(4):1109-15. http://dx.doi.org/10.1378/chest.106.4.1109
6. Holanda MA, Reis RC, Winkeler GF, Fortaleza SC, Lima JW, Pereira ED. Influence of total face, facial and nasal masks on short-term adverse effects during noninvasive ventilation. J Bras Pneumol. 2009;35(2):164-73. http://dx.doi.org/10.1590/S1806-37132009000200010
7. Ozsancak A, Sidhom SS, Liesching TN, Howard W, Hill NS. Evaluation of the total face mask for noninvasive ventilation to treat acute respiratory failure. Chest. 2011;139(5):1034-41. http://dx.doi.org/10.1378/chest.10-1905
8. Schettino GP, Chatmongkolchart S, Hess DR, Kacmarek RM. Position of exhalation port and mask design affect CO2 rebreathing during noninvasive positive pressure ventilation. Crit Care Med. 2003;31(8):2178-82. http://dx.doi.org/10.1097/01.CCM.0000081309.71887.E9
9. Roy B, Cordova FC, Travaline JM, D'Alonzo GE Jr, Criner GJ. Full face mask for noninvasive positive-pressure ventilation in patients with acute respiratory failure. J Am Osteopath Assoc. 2007;107(4):148-56.
10. Cuvelier A, Pujol W, Pramil S, Molano LC, Viacroze C, Muir JF. Cephalic versus oronasal mask for noninvasive ventilation in acute hypercapnic respiratory failure. Intensive Care Med. 2009;35(3):519-26. http://dx.doi.org/10.1007/s00134-008-1327-x
11. Miyoshi E, Fujino Y, Uchiyama A, Mashimo T, Nishimura M. Effects of gas leak on triggering function, humidification, and inspiratory oxygen fraction during noninvasive positive airway pressure ventilation. Chest. 2005;128(5):3691-8. http://dx.doi.org/10.1378/chest.128.5.3691
12. Schettino GP, Tucci MR, Sousa R, Valente Barbas CS, Passos Amato MB, Carvalho CR. Mask mechanics and leak dynamics during noninvasive pressure support ventilation: a bench study. Intensive Care Med. 2001;27(12):1887-91. http://dx.doi.org/10.1007/s00134-001-1146-9
13. Lucato JJ, Tucci MR, Schettino GP, Adams AB, Fu C, Forti G Jr, et al. Evaluation of resistance in 8 different heat-and-moisture exchangers: effects of saturation and flow rate/profile. Respir Care. 2005;50(5):636-43.
14. Fu C, Caruso P, Lucatto JJ, de Paula Schettino GP, de Souza R, Carvalho CR. Comparison of two flow generators with a noninvasive ventilator to deliver continuous positive airway pressure: a test lung study. Intensive Care Med. 2005;31(11):1587-91. http://dx.doi.org/10.1007/s00134-005-2795-x
15. Bland JM, Altman DG. Measurement error. BMJ. 1996;313(7059):744. http://dx.doi.org/10.1136/bmj.313.7059.744
16. Ferreira JC, Chipman DW, Hill NS, Kacmarek RM. Bilevel vs ICU ventilators providing noninvasive ventilation: effect of system leaks: a COPD lung model comparison. Chest. 2009;136(2):448-56. http://dx.doi.org/10.1378/chest.08-3018
17. Louis B, Leroux K, Isabey D, Fauroux B, Lofaso F. Effect of manufacturer-inserted mask leaks on ventilator performance. Eur Respir J. 2010;35(3):627-36. http://dx.doi.org/10.1183/09031936.00188708
18. Vignaux L, Tassaux D, Jolliet P. Performance of noninvasive ventilation modes on ICU ventilators during pressure support: a bench model study. Intensive Care Med. 2007;33(8):1444-51. http://dx.doi.org/10.1007/s00134-007-0713-0
19. Mehta S, McCool FD, Hill NS. Leak compensation in positive pressure ventilators: a lung model study. Eur Respir J. 2001;17(2):259-67. http://dx.doi.org/10.1183/09031936.01.17202590
20. Tassaux D, Strasser S, Fonseca S, Dalmas E, Jolliet P. Comparative bench study of triggering, pressurization, and cycling between the home ventilator VPAP II and three ICU ventilators. Intensive Care Med. 2002;28(9):1254-61. http://dx.doi.org/10.1007/s00134-002-1421-4
21. Chatmongkolchart S, Williams P, Hess DR, Kacmarek RM. Evaluation of inspiratory rise time and inspiration termination criteria in new-generation mechanical ventilators: a lung model study. Respir Care. 2001;46(7):666-77.
22. Battisti A, Tassaux D, Janssens JP, Michotte JB, Jaber S, Jolliet P. Performance characteristics of 10 home mechanical ventilators in pressure-support mode: a comparative bench study. Chest. 2005;127(5):1784-92. http://dx.doi.org/10.1378/chest.127.5.1784
23. Yamada Y, Du HL. Effects of different pressure support termination on patient-ventilator synchrony. Respir Care. 1998;43(12):1048-057.
24. Calderini E, Confalonieri M, Puccio PG, Francavilla N, Stella L, Gregoretti C. Patient-ventilator asynchrony during noninvasive ventilation: the role of expiratory trigger. Intensive Care Med. 1999;25(7):662-7. http://dx.doi.org/10.1007/s001340050927
25. Carteaux G, Lyazidi A, Cordoba-Izquierdo A, Vignaux L, Jolliet P, Thille AW, et al. Patient-ventilator asynchrony during noninvasive ventilation: a bench and clinical study. Chest. 2012;142(2):367-76. http://dx.doi.org/10.1378/chest.11-2279
26. Vignaux L, Tassaux D, Carteaux G, Roeseler J, Piquilloud L, Brochard L, et al. Performance of noninvasive ventilation algorithms on ICU ventilators during pressure support: a clinical study. Intensive Care Med. 2010;36(12):2053-9. http://dx.doi.org/10.1007/s00134-010-1994-2
27. Hess DR. Patient-ventilator interaction during noninvasive ventilation. Respir Care. 2011;56(2):153-65; discussion 65-7. http://dx.doi.org/10.4187/respcare.01049
28. Fodil R, Lellouche F, Mancebo J, Sbirlea-Apiou G, Isabey D, Brochard L, et al. Comparison of patient-ventilator interfaces based on their computerized effective dead space. Intensive Care Med. 2011;37(2):257-62. http://dx.doi.org/10.1007/s00134-010-2066-3
29. Saatci E, Miller DM, Stell IM, Lee KC, Moxham J. Dynamic dead space in face masks used with noninvasive ventilators: a lung model study. Eur Respir J. 2004;23(1):129-35. http://dx.doi.org/10.1183/09031936.03.00039503
30. Belchior I, Gonçalves MR, Winck JC. Continuous noninvasive ventilation delivered by a novel total face mask: a case series report. Respir Care. 2012;57(3):449-53. http://dx.doi.org/10.4187/respcare.01275
31. Fraticelli AT, Lellouche F, L'her E, Taillé S, Mancebo J, Brochard L. Physiological effects of different interfaces during noninvasive ventilation for acute respiratory failure. Crit Care Med. 2009;37(3):939-45. http://dx.doi.org/10.1097/CCM.0b013e31819b575f

About the authors

Maria Aparecida Miyuki Nakamura
Research Physical Therapist. *Laboratório de Investigação Médica* 09 (LIM 09, Laboratory for Medical Research 09), Department of Pulmonology, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

Eduardo Leite Vieira Costa
Physician. Respiratory ICU, Department of Pulmonology, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*; and Researcher, *Instituto de Ensino e Pesquisa*, *Hospital Sírio-Libanês*, São Paulo, Brazil.

Carlos Roberto Ribeiro Carvalho
Full Professor of Pulmonology. Department of Cardiorespiratory Diseases, University of São Paulo School of Medicine, São Paulo, Brazil.

Mauro Roberto Tucci
Research Physician. *Laboratório de Investigação Médica* 09 (LIM 09, Laboratory for Medical Research 09), Department of Pulmonology, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

Combined pulmonary fibrosis and emphysema: an increasingly recognized condition*,**

Combinação de fibrose pulmonar e enfisema: uma doença cada vez mais reconhecida

Olívia Meira Dias, Bruno Guedes Baldi, André Nathan Costa, Carlos Roberto Ribeiro Carvalho

Abstract

Combined pulmonary fibrosis and emphysema (CPFE) has been increasingly recognized in the literature. Patients with CPFE are usually heavy smokers or former smokers with concomitant lower lobe fibrosis and upper lobe emphysema on chest HRCT scans. They commonly present with severe breathlessness and low DLCO, despite spirometry showing relatively preserved lung volumes. Moderate to severe pulmonary arterial hypertension is common in such patients, who are also at an increased risk of developing lung cancer. Unfortunately, there is currently no effective treatment for CPFE. In this review, we discuss the current knowledge of the pathogenesis, clinical characteristics, and prognostic factors of CPFE. Given that most of the published data on CPFE are based on retrospective analysis, more studies are needed in order to address the role of emphysema and its subtypes; the progression of fibrosis/emphysema and its correlation with inflammation; treatment options; and prognosis.

Keywords: Pulmonary fibrosis; Emphysema; Hypertension, pulmonary; Lung diseases, interstitial.

Resumo

A combinação de fibrose pulmonar e enfisema (CFPE) é cada vez mais reconhecida na literatura. Os pacientes são geralmente fumantes pesados ou ex-fumantes nos quais a TCAR de tórax revela enfisema nos lobos superiores e, concomitantemente, fibrose nos lobos inferiores. Esses pacientes comumente apresentam dispneia grave e baixa DLCO, não obstante os volumes pulmonares relativamente preservados em exames espirométricos. Hipertensão arterial pulmonar de moderada a grave e aumento da incidência de câncer de pulmão também são comuns nesses pacientes. Infelizmente, ainda não existe um tratamento eficaz para a CFPE. O objetivo desta revisão é discutir o que se sabe atualmente a respeito da patogênese, das características clínicas e dos fatores prognósticos da CFPE. Como a maioria dos dados publicados baseia-se em análise retrospectiva, são necessários mais estudos sobre o papel do enfisema e seus subtipos, a progressão da fibrose/enfisema e sua correlação com a inflamação, as opções de tratamento e o prognóstico em pacientes com CFPE.

Descritores: Fibrose pulmonar; Enfisema; Hipertensão pulmonar; Doenças pulmonares intersticiais.

Introduction

Idiopathic pulmonary fibrosis (IPF) and pulmonary emphysema are distinct clinicopathological entities that pulmonologists have long been familiar with. Since the advent of HRCT, the combination of these two conditions has been increasingly described and has been proven to be a prevalent and distinct entity rather than a rare coincidence.

The association of IPF and emphysema was initially described in 1990 by Wiggins et al.,[1] who described eight heavy smokers with fibrosis and upper lobe emphysema on HRCT scans, together with severe breathlessness, strikingly low DLCO, and preserved lung volumes. In 2005, Grubstein et al.[2] reported an association of fibrosis with emphysema in eight patients, their clinical and functional findings being similar to those of the aforementioned study. The authors also found moderate to severe pulmonary arterial hypertension (PAH) and postulated that smoking is a factor

*Study carried out in the Department of Pulmonology, *Instituto do Coração* – InCor, Heart Institute – University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.
Correspondence to: Olívia Meira Dias. Rua Doutor Enéas de Carvalho Aguiar, 44, 5º andar, CEP 05403-900, São Paulo, SP, Brasil. Tel. 55 11 3069-5000. E-mail: meiradias@yahoo.com.br
Financial support: None.
Submitted: 6 May 2014. Accepted, after review: 19 May 2014.
**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

 http://dx.doi.org/10.1590/S1806-37132014000300014

linking emphysema, pulmonary fibrosis, and pulmonary vascular disease.[2] The term combined pulmonary fibrosis and emphysema (CPFE) was first used in 2005 by Cottin et al.,[3] who characterized a homogeneous group of 61 patients with CT findings of emphysema in the upper zones and interstitial lung disease (ILD) with pulmonary fibrosis in the lower lobes.

When CPFE was first described, patients with other ILDs were excluded from the study.[3] Later on, CPFE was described in patients with other ILDs, such as connective tissue disease (CTD)-associated ILD,[4-7] as well as in patients with microscopic polyangiitis.[8]

Studies have shown that patients with CPFE associated with CTDs (especially rheumatoid arthritis and systemic sclerosis) are significantly younger than their idiopathic CPFE counterparts, are predominantly female, and have less DLCO impairment.[4] One group of authors found elevated serum antinuclear antibodies with or without positive perinuclear antineutrophil cytoplasmic antibodies in CPFE patients when compared with IPF patients without emphysema, those with positive autoimmune markers exhibiting greater infiltration of CD20+ B cells forming lymphoid follicles in fibrotic lung tissue and improved survival when compared with those with negative autoimmune markers.[9]

Given that tobacco exposure seems to modulate an underlying inflammatory response in patients with ILD, CPFE should be categorized as a pattern associated with other pulmonary diseases rather than as a primary idiopathic syndrome, a classification similar to the usual interstitial pneumonia (UIP) pattern in other fibrotic ILDs. In other words, the recognition of a CPFE pattern should also prompt the investigation of secondary autoimmune diseases and CTDs.

Patients with CPFE are predominantly male, with a history of heavy tobacco exposure, and usually present with severe breathlessness and cough. Physical examination reveals "Velcro" crackles at the lung bases and digital clubbing.[3,10] Pulmonary hypertension is a hallmark of the syndrome and determines poor prognosis.[10] Between January of 2006 and December of 2013, 17 patients were diagnosed with CPFE at our interstitial lung disease outpatient clinic, and the data are summarized in Table 1. In accordance with the literature, our patients were predominantly male (88%), the mean age at diagnosis being 68 years. All of the patients presented with tobacco exposure and dyspnea at diagnosis. Almost half of the patients had pulmonary hypertension diagnosed by echocardiography. Few (6%) had a diagnosis of lung cancer, and 12% died during the follow-up period.

Pathogenesis

The pathogenesis of CPFE has yet to be elucidated. Tobacco exposure per se can be an important fibrogenic stimulus, smoking having been shown to play a key role in the pathogenesis of several ILDs, including respiratory bronchiolitis-associated ILD (RB-ILD), desquamative interstitial pneumonia, pulmonary Langerhans cell histiocytosis, and, possibly, IPF.

Washko et al. conducted a lung cancer screening study involving a large cohort of COPD patients and found interstitial lung abnormalities on HRCT scans in up to 8% of smokers.[11] Likewise, Katzenstein et al. reported frequent and severe interstitial fibrosis in over half of lobectomy specimens excised for lung cancer from smokers with no clinical evidence of ILD, even in those patients in whom emphysema was the only CT finding.[12]

Those histological findings characterized a distinct, non-classifiable ILD, which Katzenstein et al. designated "smoking related-interstitial fibrosis", characterized by thickening of alveolar septa by fibrosis composed mostly of hyalinized eosinophilic collagen bundles and surrounding enlarged airspaces of emphysema, as well as by signs of respiratory bronchiolitis.[12] Although follow-up was short, the clinical progression

Table 1 - Characteristics of 17 patients with combined pulmonary fibrosis and emphysema treated at the Interstitial Lung Disease Outpatient Clinic of the University of São Paulo School of Medicine *Hospital das Clínicas* between 2006 and 2013, together with the clinical manifestations of the disease.[a]

Characteristic	Result
Male/Female	15 (88)/2 (12)
Age at diagnosis, years[b]	68 ± 7
Tobacco exposure	17 (100)
Dyspnea at diagnosis	17 (100)
Pulmonary hypertension at diagnosis	8 (47)
Hypoxemia at diagnosis	11 (65)
Lung cancer during follow-up	1 (6)
Deaths during follow-up	2 (12)

[a] Values expressed as n (%), except where otherwise indicated.
[b] Values expressed as mean ± SD.

seemed to be particularly different from that of IPF, with indolent fibrosis and better survival rates, reinforcing the idea of a different disease.[12]

It is reasonable to assume that the lung parenchyma shows different patterns of injury and repair in response to tobacco exposure. The different phenotypes of lesions secondary to tobacco exposure depend on the balance of apoptosis, proteolysis, and fibrosis. Patients in whom genes related to connective tissue synthesis, structural constituents of the cytoskeleton, and cell adhesion are overexpressed typically display a fibrogenic phenotype, such as that found in patients with UIP; however, a different inflammatory response to smoking-associated cellular damage (destruction and repair of cells, vessels, and pneumocytes) leads to destruction of lung parenchyma, culminating in pulmonary emphysema.[13] A combination of these two patterns of response can be found in patients with CPFE and has recently been demonstrated by gene expression analysis of fibrotic and emphysematous lesions in such patients.[13]

The role of environmental exposure as a potential trigger of lung injury is also plausible, given that some CPFE patients have had significant exposure to agrochemical compounds that cause airway damage and ILD in genetically susceptible smokers.[14] Some authors have described CPFE as an occupational disease, e.g., in patients exposed to talc[15] and in welders.[16]

The signaling pathways to these responses are unknown. Laboratory animal studies have demonstrated that oxidative stress inducing inflammatory cell activation, elevated matrix metalloproteinase levels causing proteolytic activity,[17,18] and overexpression of other mediators, such as PDGF,[19] TNF-α, and TGF-β,[20,21] are potential pathways explaining the lesions that lead to emphysema and fibrosis. A study analyzing inflammatory mediators in BAL fluid from patients with IPF showed significantly higher concentrations of chemokine (C-X-C motif) ligand 5 and chemokine (C-X-C motif) ligand 8 in those with concomitant HRCT findings of emphysema.[22] These chemokines are associated with neutrophil accumulation in airspaces and suggest a different pathway of inflammation leading to the development of emphysematous changes superimposed on pulmonary fibrosis.[22]

Genetic mutations have been described in CPFE patients with and without a significant smoking history.[6,23] This indicates that risk factors other than tobacco smoking are associated with the development of CPFE or that tobacco smoking can be a triggering factor in susceptible patients. Plausible genetic pathways have been confirmed in case reports in which mutations in the surfactant protein C gene were identified in a 32-year-old female who had never smoked[23] and an ABCA3 mutation was identified in a 41-year-old male nonsmoker,[24] with CT findings identical to those in CPFE patients. These mutations are known to cause dysfunction of surfactant homeostasis and, consequently, injury or death of alveolar epithelial type II cells and myofibroblast proliferation.[23] Finally, reports have described CPFE features in a family with inherited telomerase mutations.[25]

These findings reinforce the idea of a combination of genetic predisposition and a triggering exposure (smoking) in susceptible individuals leading to continued damage to alveolar epithelial cells that cannot be properly repaired, initiating a vicious cycle of attempts at alveolar regeneration and uncontrolled activation of fibrosis proliferation and parenchymal destruction.[26]

Imaging studies in CPFE

Imaging studies are essential for the diagnosis of CPFE. Although routine chest X-rays are not as sensitive as HRCT scans, they can reveal an interstitial pattern predominantly in the subpleural and basal lung regions, with hyperlucency in the lung apices corresponding to emphysematous areas.

The mainstay of the diagnosis of CPFE, HRCT scans typically show centrilobular or paraseptal emphysema in the upper lobes, as well as reticular opacities, traction bronchiectasis, septal thickening, ground-glass opacities, and honeycombing in the lower lobes[3] (Figures 1 and 2). Although UIP is the most common CT pattern, some patients have ground-glass opacities that are more extensive than expected for a UIP pattern and are therefore suggestive of nonspecific interstitial pneumonia, RB-ILD, and even desquamative interstitial pneumonia.[3]

Brillet et al.[27] described patterns of distribution of fibrosis and emphysema in patients with CPFE other than those initially described: a progressive transition from apical emphysema to a zone of transition between bullae and honeycombing; paraseptal emphysema with areas of fibrosis;

 http://dx.doi.org/10.1590/S1806-37132014000300014

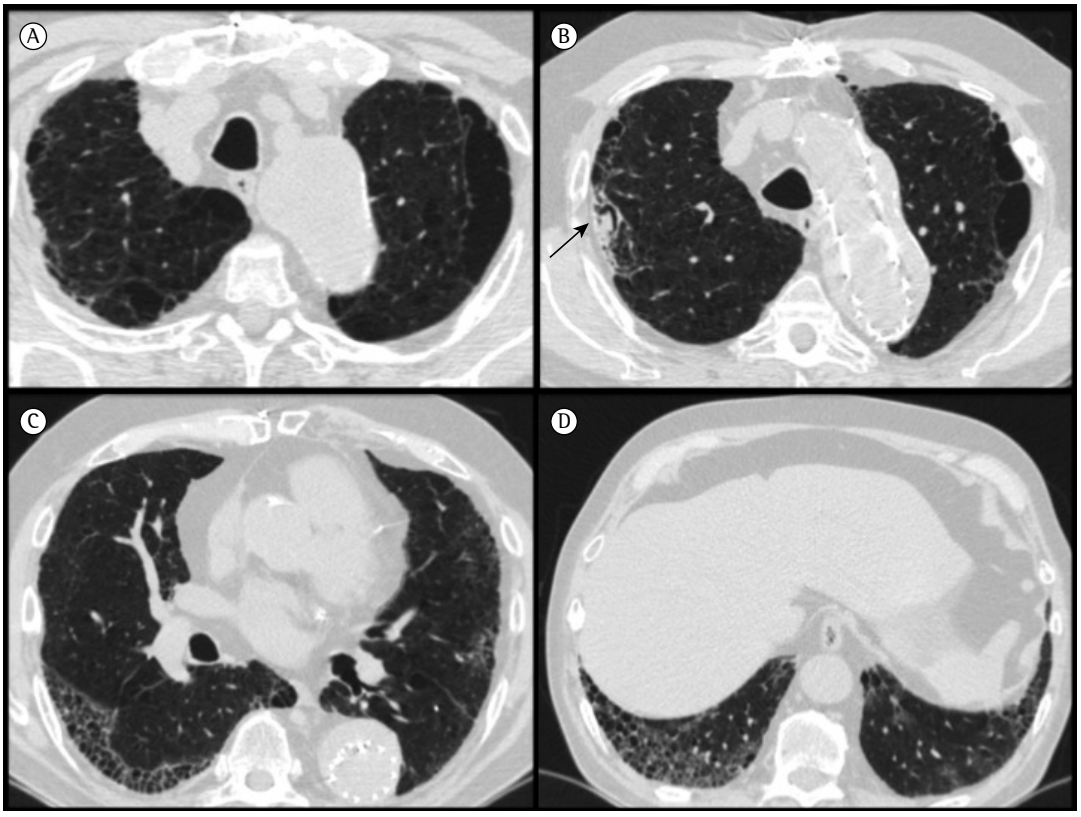


Figure 1 - CT scan of the chest of a 67-year-old female patient with combined pulmonary fibrosis and emphysema, showing centrilobular and paraseptal emphysema in the upper lobes (A and B), as well as ground-glass opacities, traction bronchiectasis, and honeycombing in the lower lobes (C and D). Note an aspergilloma in one of the paraseptal bullae in the right upper lobe (black arrow, in B).

and separate processes with independent areas of fibrosis and emphysema.[27]

Lung function in CPFE

Regarding the lung function of patients with CPFE, spirometry can be normal or show mild abnormalities, FVC, FEV_1, and TLC values usually being within normal ranges or slightly altered.[3,28] The FEV_1/FVC ratio can be within or slightly below the normal range. Severely impaired DLCO and hypoxemia during exercise are common.[3,28]

Silva et al. reported similar findings in a retrospective cohort of 11 Brazilian patients, spirometry having revealed normal lung volumes in 7.[29] At our facility, 17 CPFE patients were evaluated, and spirometry showed normal lung function and lung volumes in 12%, an obstructive pattern in 18%, and a restrictive pattern in 47%. All patients had reduced DLCO (Table 2).

In a five-year follow-up study of 16 CPFE patients, the annual decline in FVC, DLCO, and DLCO/alveolar volume was significantly higher than was that found in a group of COPD patients.[30] In another study, the rate of decline in lung volume was found to be considerably lower in patients with CPFE than in those with IPF.[31] Although DLCO values were lower in the patients with CPFE than in those with IPF, the annual rate of decline in DLCO was also significantly lower in the former. There were no differences between the two groups in terms of survival.[31]

One possible explanation for normal or subnormal spirometry results despite severe impairment in DLCO is that hyperinflation and greater lung compliance as a result of loss of elasticity in the areas of emphysema can compensate for the losses in volume and lung compliance caused by fibrosis. Another plausible explanation is that fibrosis prevents the early small airway closure observed in patients with emphysema.

Although a single spirometry test can underestimate the severity of the disease, Schmidt et al. demonstrated that a progressive approach,

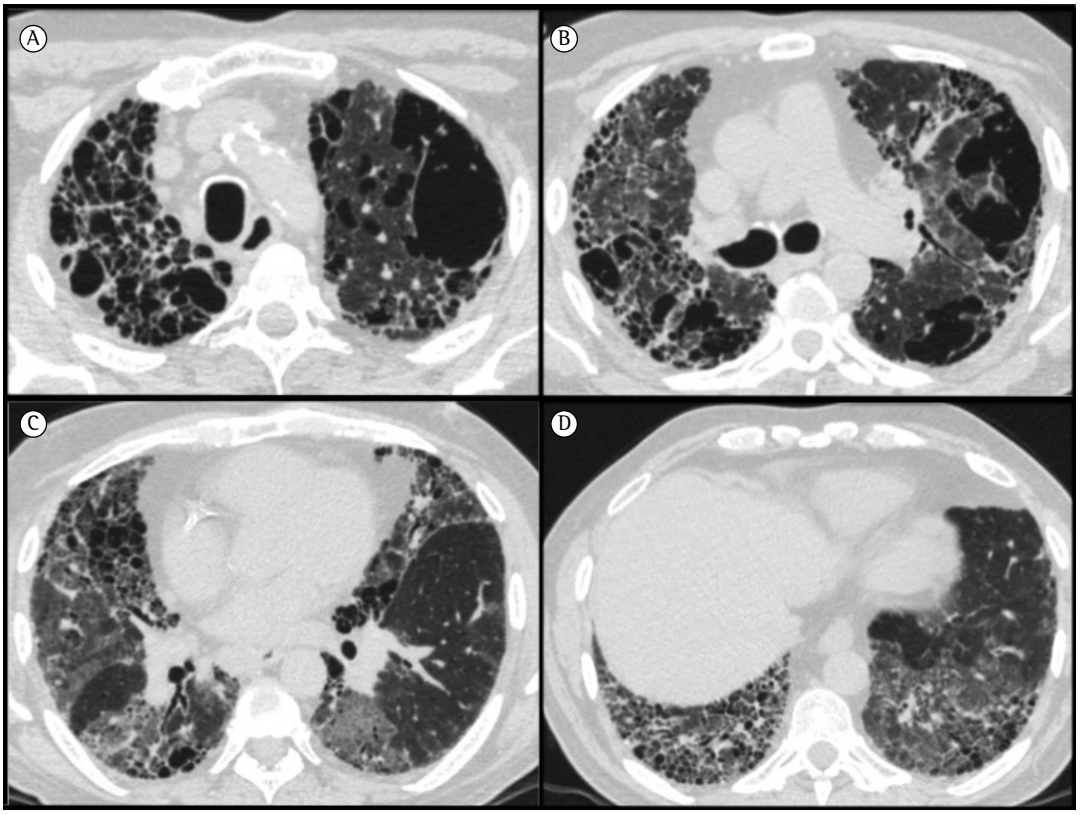


Figure 2 - CT scan of the chest of a 70-year-old male patient with combined pulmonary fibrosis and emphysema and acute exacerbation of interstitial disease. Note predominantly paraseptal emphysema in the lung apices, with architectural destruction of the lung parenchyma (A and B). Extensive areas of ground-glass opacity and honeycombing can be seen in the lower lobes (C and D).

Table 2 - Pulmonary function test results at diagnosis in 17 patients with combined pulmonary fibrosis and emphysema treated at the Interstitial Lung Disease Outpatient Clinic of the University of São Paulo *Hospital das Clínicas* between 2006 and 2013.[a]

Variable	Result
Obstructive pattern	3 (18)
Restrictive pattern	8 (47)
Air trapping	4 (24)
Normal spirometry and pulmonary volumes	2 (12)
Reduced DLCO	17 (100)
FVC, % predicted[b]	79 ± 16
FEV_1, % predicted[b]	79 ± 14
FEV_1/FVC[b]	0.77 ± 0.08
TLC, % predicted[b]	73 ± 17
RV/TLC[b]	0.36 ± 0.06
DLCO, % predicted[b]	30 ± 14

[a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD.

with a longitudinal decline in FEV_1, can accurately define disease progression and predict mortality in CPFE patients.[32] In addition, the authors found a correlation between the extent of emphysema on HRCT scans and the decline in FEV_1.[32]

PAH in CPFE

The prevalence of PAH is exceedingly high in patients with CPFE, and PAH correlates with worse survival.[3,33,34] The prevalence of PAH in CPFE patients varies from 47% to 90%, being considerably higher than that in patients with COPD or IPF alone.[18] Indeed, the five-year survival rate in a study involving CPFE patients was 25% in those with PAH (as measured by transthoracic echocardiography), being 75% in those without PAH.[28] In another study, the finding of severe PAH on echocardiography was associated with an increased risk of death.[35]

Mejia et al. conducted a study in Mexico, in which PAH was assessed by transthoracic echocardiography in a cohort of patients with IPF (with and without emphysema). Not only was PAH more prevalent in the patients with CPFE, but it was also responsible for a worse

 http://dx.doi.org/10.1590/S1806-37132014000300014

prognosis. Another important finding was that the amount of emphysema on CT scans was directly correlated with a higher estimated systolic pulmonary artery pressure.[34]

Although transthoracic echocardiography is an operator-dependent imaging modality and lacks accuracy in the diagnosis of PAH in patients with advanced lung disease, including COPD and IPF, it seems to have a good correlation with right heart catheterization studies in CPFE patients, being therefore an effective screening tool for PAH in such patients.[18,34]

Cottin et al. retrospectively characterized PAH by means of right heart catheterization in 40 patients with CPFE. Higher pulmonary vascular resistance, higher HR, lower cardiac index, and lower DLCO were associated with a worse prognosis, the one-year survival rate being 60%. Although an evaluation of the effect of treatment was not the primary objective of the study, none of the available treatments improved survival.[33]

Novel noninvasive methods for the diagnosis and quantification of PAH in CPFE patients have been proposed, including time-resolved magnetic resonance angiography,[35] which allows anatomic imaging of the pulmonary vasculature and evaluation of hemodynamic parameters.[35] Using this technique, Sergiacomi et al. prospectively studied 18 CPFE patients using pulmonary arterial mean transit time and time to peak enhancement as surrogate parameters for hemodynamic data (mean pulmonary artery pressure and pulmonary vascular resistance), which were obtained through right heart catheterization performed three days before time-resolved magnetic resonance angiography was performed.[35] Pulmonary arterial mean transit time and time to peak enhancement showed good correlation with the invasive parameters.[35]

Treatment and prognosis

As is the case with IPF, there is currently no effective treatment for CPFE, with the exception of smoking cessation and lung transplantation (for patients with advanced disease). Bronchodilators, however, can be prescribed to patients with a positive response to bronchodilators in pulmonary function tests. Cottin et al.[4] recommended the use of N-acetylcysteine (1.8 g/day) on the basis of the results of studies investigating IPF. Oral corticosteroids and immunosuppressants have been considered an option in the setting of CTD-associated CPFE; however, no randomized trials have been conducted.[4] Lung transplantation is the only option that can improve survival.

The major causes of death in patients with CPFE are chronic respiratory failure, PAH,[25,26] acute exacerbation,[3,4] and lung cancer.[36,37] Usui et al. found an 8.9% prevalence of CPFE in 1,143 consecutive patients with primary lung cancer in Japan.[36] The authors showed that, in comparison with patients with fibrosis or emphysema alone, CPFE patients had a worse survival and an increased incidence of acute exacerbation after surgery.[36]

Variations in DLCO, hypoxemia, digital clubbing,[10] mean pulmonary artery pressure,[26,27] and decline in FEV_1[32] are considered better surrogates for disease progression and higher risk of mortality, allowing earlier evaluation for transplantation. Recently, Chiba et al. demonstrated that two biomarkers of fibrosis, namely KL-6 and surfactant protein D, are good indicators of the extent of fibrosis in patients with CPFE.[38] High KL-6 and surfactant protein D levels were found to correlate negatively with all lung volumes and with DLCO.[38] Kishaba et al. demonstrated that high levels of KL-6 are predictors of acute exacerbations in patients with CPFE.[10]

The prognosis and overall mortality of CPFE patients in comparison with those of IPF and COPD patients is still a matter of debate. Different enrollment criteria, duration of follow-up, heterogeneity of patients (including type of emphysema), retrospective data analysis, and lead-time bias might explain the heterogeneity of results.

Todd et al.[39] and Todd & Atamas[40] examined the extent and type of emphysema in a subset of patients with pulmonary fibrosis and found that patients with a combination of fibrosis with centrilobular or mixed (centrilobular and paraseptal) emphysema had better survival rates than did those with pulmonary fibrosis without emphysema, those with trivial emphysema, and those with advanced paraseptal emphysema.[39,40] The pattern of emphysema and its extent seem to correlate with disease severity. The reasons for these findings remain unknown. One possible explanation is that patients with CPFE tend to have somewhat preserved lung volumes compared with patients with fibrosis alone; however, no retrospective studies found any correlation between preserved lung volumes

(as determined by pulmonary function tests) and better survival in patients with CPFE. In addition, there were no differences in DLCO among those groups of patients.[39,40]

Another hypothesis for centrilobular emphysema acting as a "protective factor" is based on the fact that centrilobular emphysema is essentially caused by tobacco exposure. Some in vitro studies have shown that the proinflammatory cytokines seen in cases of cigarette smoking and emphysema have antifibrotic properties; therefore, such patients might have smaller areas of fibrosis and a better prognosis.[19] Paraseptal emphysema might represent another lung response to smoking, leading to severe pulmonary fibrosis, or simply reflect a greater extent of fibrosis in lower lung zones, exerting traction on lung tissue located in the apices. However, the fibrotic areas seen on chest X-rays do not precede the onset of emphysema, indicating that both processes probably occur simultaneously.

Corroborating this theory, Kurashima et al. found that a greater extent of emphysema on CT scans correlated with better pulmonary function parameters and a better prognosis in comparison with those in a group of patients with IPF.[41] The authors did not classify emphysema into subtypes and suggested that emphysema is a protective factor in patients with CPFE.[41] Similar findings were reported by Ando et al.[42] in a study examining the relationship between pulmonary function and CT quantification of emphysema and fibrosis in CPFE, the authors having concluded that pulmonary fibrotic changes contribute more to the progression of CPFE than does emphysema.[42]

The mortality rates for CPFE and IPF are similar.[43,44] Recently, Ryerson et al. compared CPFE-IPF patients with non-CPFE IPF patients.[45] Although the patients with CPFE had a more extensive smoking history, greater oxygen requirements, higher pulmonary artery pressure, less restrictive physiology, and lower diffusing capacity, there was no significant difference in mortality between the two groups.[45]

Final considerations

Pulmonologists have been accustomed to recognizing fibrosis and emphysema as two well-defined diseases. However, a large body of evidence has shown that an overlap can exist, CPFE being therefore a new entity, with unique features. In view of the fact that most studies investigating CPFE have had a retrospective design, more studies are needed to address the role of emphysema and its subtypes, the progression of fibrosis/emphysema and its correlation with inflammation, treatment options (including the treatment of PAH), and prognosis in CPFE patients. A deeper understanding of the pathophysiology and progression of CPFE is urgently required in order to improve its management, given that there is currently no effective treatment for the disease.

References

1. Wiggins J, Strickland B, Turner-Warwick M. Combined cryptogenic fibrosing alveolitis and emphysema: the value of high resolution computed tomography in assessment. Respir Med. 1990;84(5):365-9. http://dx.doi.org/10.1016/S0954-6111(08)80070-4
2. Grubstein A, Bendayan D, Schactman I, Cohen M, Shitrit D, Kramer MR. Concomitant upper-lobe bullous emphysema, lower-lobe interstitial fibrosis and pulmonary hypertension in heavy smokers: report of eight cases and review of the literature. Respir Med. 2005;99(8):948-54. http://dx.doi.org/10.1016/j.rmed.2004.12.010
3. Cottin V, Nunes H, Brillet PY, Delaval P, Devouassoux G, Tillie-Leblond I et al. Combined pulmonary fibrosis and emphysema: a distinct underrecognised entity. Eur Respir J. 2005;26(4):586-93. http://dx.doi.org/10.1183/09031936.05.00021005
4. Cottin V, Nunes H, Mouthon L, Gamondes D, Lazor R, Hachulla E, et al. Combined pulmonary fibrosis and emphysema syndrome in connective tissue disease. Arthritis Rheum. 2011;63(1):295-304. http://dx.doi.org/10.1002/art.30077
5. Lubatti C, Zeni S, Ingegnoli F. Combined pulmonary fibrosis and emphysema syndrome in systemic sclerosis. Int J Rheum Dis. 2012;15(5):e122-3. http://dx.doi.org/10.1111/j.1756-185X.2012.01747.x
6. Cottin V, Freymond N, Cabane J, Cordier JF. Combined pulmonary fibrosis and emphysema syndrome in a patient age 28 years with severe systemic sclerosis. J Rheumatol. 2011;38(9):2082-3. http://dx.doi.org/10.3899/jrheum.101365
7. Cottin V, Cordier JF. Combined pulmonary fibrosis and emphysema in connective tissue disease. Curr Opin Pulm Med. 2012;18(5):418-27. http://dx.doi.org/10.1097/MCP.0b013e328356803b
8. Tzouvelekis A, Zacharis G, Oikonomou A, Koulelidis A, Steiropoulos P, Froudarakis M, et al. Combined pulmonary fibrosis and emphysema associated with microscopic polyangiitis. Eur Respir J. 2012;40(2):505-7. http://dx.doi.org/10.1183/09031936.00216311
9. Tzouvelekis A, Zacharis G, Oikonomou A, Mikroulis D, Margaritopoulos G, Koutsopoulos A, et al. Increased incidence of autoimmune markers in patients with combined pulmonary fibrosis and emphysema. BMC Pulm Med. 2013;13:31. http://dx.doi.org/10.1186/1471-2466-13-31
10. Kishaba T, Shimaoka Y, Fukuyama H, Yoshida K, Tanaka M, Yamashiro S, et al. A cohort study of mortality predictors

 http://dx.doi.org/10.1590/S1806-37132014000300014

and characteristics of patients with combined pulmonary fibrosis and emphysema. BMJ Open. 2012;15:2(3).
11. Washko GR, Hunninghake GM, Fernandez IE, Nishino M, Okajima Y, Yamashiro T, et al. Lung volumes and emphysema in smokers with interstitial lung abnormalities. N Engl J Med. 2011;364(10):897-906. http://dx.doi.org/10.1056/NEJMoa1007285
12. Katzenstein AL, Mukhopadhyay S, Zanardi C, Dexter E. Clinically occult interstitial fibrosis in smokers: classification and significance of a surprisingly common finding in lobectomy specimens. Hum Pathol. 2010;41(3):316-25. http://dx.doi.org/10.1016/j.humpath.2009.09.003
13. Hanaoka M, Ito M, Droma Y, Ushiki A, Kitaguchi Y, Yasuo M, et al. Comparison of gene expression profiling between lung fibrotic and emphysematous tissues sampled from patients with combined pulmonary fibrosis and emphysema. Fibrogenesis Tissue Repair. 2012;5(1):17. http://dx.doi.org/10.1186/1755-1536-5-17
14. Daniil Z, Koutsokera A, Gourgoulianis K. Combined pulmonary fibrosis and emphysema in patients exposed to agrochemical compounds. Eur Respir J. 2006;27(2):434. http://dx.doi.org/10.1183/09031936.06.00124505
15. Karkhanis VS, Joshi JM. Combined pulmonary fibrosis and emphysema in a tyre industry worker. Lung India. 2012;29(3):273-6. http://dx.doi.org/10.4103/0970-2113.99116
16. Roshan R, Guptal M, Kulshrestha R, Menon B, Chhabra SK. Combined pulmonary fibrosis and emphysema in a welder. Monaldi Arch Chest Dis. 2012;77(1):26-8.
17. Rogliani P, Mura M, Mattia P, Ferlosio A, Farinelli G, Mariotta S, et al. HRCT and histopathological evaluation of fibrosis and tissue destruction in IPF associated with pulmonary emphysema. Respir Med. 2008;102(12):1753-61. http://dx.doi.org/10.1016/j.rmed.2008.07.010
18. Portillo Carroz K, Roldán Sánchez J, Morera Prat J. Combined pulmonary fibrosis and emphysema [Article in Spanish]. Arch Bronconeumol. 2010;46(12):646-51. http://dx.doi.org/10.1016/j.arbres.2010.06.011
19. Hoyle GW, Li J, Finkelstein JB, Eisenberg T, Liu JY, Lasky JA, et al. Emphysematous lesions, inflammation, and fibrosis in the lungs of transgenic mice overexpressing platelet-derived growth factor. Am J Pathol. 1999;154(6):1763-75. http://dx.doi.org/10.1016/S0002-9440(10)65432-6
20. Lundblad LK, Thompson-Figueroa J, Leclair T, Sullivan MJ, Poynter ME, Irvin CG, et al. Tumor necrosis factor-alpha overexpression in lung disease: a single cause behind a complex phenotype. Am J Respir Crit Care Med. 2005;171(12):1363-70. http://dx.doi.org/10.1164/rccm.200410-1349OC
21. Lee CG, Cho S, Homer RJ, Elias JA. Genetic control of transforming growth factor-beta1-induced emphysema and fibrosis in the murine lung. Proc Am Thorac Soc. 2006;3(6):476-7. http://dx.doi.org/10.1513/pats.200603-040MS
22. Tasaka S, Mizoguchi K, Funatsu Y, Namkoong H, Yamasawa W, Ishii M, et al. Cytokine profile of bronchoalveolar lavage fluid in patients with combined pulmonary fibrosis and emphysema. Respirology. 2012;17(5):814-20. http://dx.doi.org/10.1111/j.1440-1843.2012.02182.x
23. Cottin V, Reix P, Khouatra C, Thivolet-Béjui F, Feldmann D, Cordier JF. Combined pulmonary fibrosis and emphysema syndrome associated with familial SFTPC mutation. Thorax. 2011;66(10):918-9. http://dx.doi.org/10.1136/thx.2010.151407
24. Epaud R, Delestrain C, Louha M, Simon S, Fanen P, Tazi A. Combined pulmonary fibrosis and emphysema syndrome associated with ABCA3 mutations. Eur Respir J. 2014;43(2):638-41. http://dx.doi.org/10.1183/09031936.00145213
25. Nunes H, Monnet I, Kannengiesser C, Uzunhan Y, Valeyre D, Kambouchner M, et al. Is telomeropathy the explanation for combined pulmonary fibrosis and emphysema syndrome?: report of a family with TERT mutation. Am J Respir Crit Care Med. 2014;189(6):753-4. http://dx.doi.org/10.1164/rccm.201309-1724LE
26. Chilosi M, Carloni A, Rossi A, Poletti V. Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. Transl Res. 2013;162(3):156-73. http://dx.doi.org/10.1016/j.trsl.2013.06.004
27. Brillet PY, Cottin V, Letoumelin P, Landino F, Brauner MW, Valeyre D, et al. Combined apical emphysema and basal fibrosis syndrome (emphysema/fibrosis syndrome): CT imaging features and pulmonary function tests [Article in French]. J Radiol. 2009;90(1 Pt 1):43-51. http://dx.doi.org/10.1016/S0221-0363(09)70077-0
28. Cottin V, Cordier JF. The syndrome of combined pulmonary fibrosis and emphysema. Chest. 2009;136(1):1-2 http://dx.doi.org/10.1378/chest.09-0538
29. Silva DR, Gazzana MB, Barreto SS, Knorst MM. Idiopathic pulmonary fibrosis and emphysema in smokers. J Bras Pneumol. 2008;34(10):779-86. http://dx.doi.org/10.1590/S1806-37132008001000005
30. Kitaguchi Y, Fujimoto K, Hayashi R, Hanaoka M, Honda T, Kubo K. Annual changes in pulmonary function in combined pulmonary fibrosis and emphysema: over a 5-year follow-up. Respir Med. 2013;107(12):1986-92. http://dx.doi.org/10.1016/j.rmed.2013.06.015
31. Akagi T, Matsumoto T, Harada T, Tanaka M, Kuraki T, Fujita M, et al. Coexistent emphysema delays the decrease of vital capacity in idiopathic pulmonary fibrosis. Respir Med. 2009;103(8):1209-15. http://dx.doi.org/10.1016/j.rmed.2009.02.001
32. Schmidt SL, Nambiar AM, Tayob N, Sundaram B, Han MK, Gross BH et al. Pulmonary function measures predict mortality differently in IPF versus combined pulmonary fibrosis and emphysema. Eur Respir J. 2011;38(1):176-83. http://dx.doi.org/10.1183/09031936.00114010
33. Cottin V, Le Pavec J, Prévot G, Mal H, Humbert M, Simonneau G, et al. Pulmonary hypertension in patients with combined pulmonary fibrosis and emphysema syndrome. Eur Respir J. 2010;35(1):105-11. http://dx.doi.org/10.1183/09031936.00038709
34. Mejía M, Carrillo G, Rojas-Serrano J, Estrada A, Suárez T, Alonso D, et al. Idiopathic pulmonary fibrosis and emphysema: decreased survival associated with severe pulmonary arterial hypertension. Chest. 2009;136(1):10-5. http://dx.doi.org/10.1378/chest.08-2306
35. Sergiacomi G, Bolacchi F, Cadioli M, Angeli ML, Fucci F, Crusco S, et al. Combined pulmonary fibrosis and emphysema: 3D time-resolved MR angiographic evaluation of pulmonary arterial mean transit time and time to peak enhancement. Radiology. 2010;254(2):601-8. http://dx.doi.org/10.1148/radiol.09081546
36. Usui K, Tanai C, Tanaka Y, Noda H, Ishihara T. The prevalence of pulmonary fibrosis combined with emphysema in patients with lung cancer. Respirology. 2011;16(2):326-31. http://dx.doi.org/10.1111/j.1440-1843.2010.01907.x

37. Kwak N, Park CM, Lee J, Park YS, Lee SM, Yim JJ, et al. Lung cancer risk among patients with combined pulmonary fibrosis and emphysema. Respir Med. 2014;108(3):524-30. http://dx.doi.org/10.1016/j.rmed.2013.11.013
38. Chiba S, Ohta H, Abe K, Hisata S, Ohkouchi S, Hoshikawa Y, et al. The diagnostic value of the interstitial biomarkers KL-6 and SP-D for the degree of fibrosis in combined pulmonary fibrosis and emphysema. Pulm Med. 2012;2012:492960. http://dx.doi.org/10.1155/2012/492960
39. Todd NW, Jeudy J, Lavania S, Franks TJ, Galvin JR, Deepak J, et al. Centrilobular emphysema combined with pulmonary fibrosis results in improved survival. Fibrogenesis Tissue Repair. 2011;4(1):6. http://dx.doi.org/10.1186/1755-1536-4-6
40. Todd NW, Atamas SP. Survival in pulmonary fibrosis combined with emphysema: likely defined by characteristics of specific patient subpopulations. Fibrogenesis Tissue Repair. 2011;4:17. http://dx.doi.org/10.1186/1755-1536-4-17
41. Kurashima K, Takayanagi N, Tsuchiya N, Kanauchi T, Ueda M, Hoshi T, et al. The effect of emphysema on lung function and survival in patients with idiopathic pulmonary fibrosis. Respirology. 2010;15(5):843-8. http://dx.doi.org/10.1111/j.1440-1843.2010.01778.x
42. Ando K, Sekiya M, Tobino K, Takahashi K. Relationship between quantitative CT metrics and pulmonary function in combined pulmonary fibrosis and emphysema. Lung. 2013;191(6):585-91. http://dx.doi.org/10.1007/s00408-013-9513-1
43. Jankowich MD, Polsky M, Klein M, Rounds S. Heterogeneity in combined pulmonary fibrosis and emphysema. Respiration. 2008;75(4):411-7. http://dx.doi.org/10.1159/000107048
44. Jankowich MD, Rounds S. Combined pulmonary fibrosis and emphysema alters physiology but has similar mortality to pulmonary fibrosis without emphysema. Lung. 2010;188(5):365-73. http://dx.doi.org/10.1007/s00408-010-9251-6
45. Ryerson CJ, Hartman T, Elicker BM, Ley B, Lee JS, Abbritti M et al. Clinical features and outcomes in combined pulmonary fibrosis and emphysema in idiopathic pulmonary fibrosis. Chest. 2013;144(1):234-40. http://dx.doi.org/10.1378/chest.12-2403

About the authors

Olívia Meira Dias
Attending Physician. Department of Cardiorespiratory Diseases, *Instituto do Coração* – InCor, Heart Institute – University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

Bruno Guedes Baldi
Attending Physician. Department of Cardiorespiratory Diseases, *Instituto do Coração* – InCor, Heart Institute – University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

André Nathan Costa
Attending Physician. Department of Cardiorespiratory Diseases, *Instituto do Coração* – InCor, Heart Institute – University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

Carlos Roberto Ribeiro Carvalho
Full Professor. Department of Cardiorespiratory Diseases, *Instituto do Coração* – InCor, Heart Institute – University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

 http://dx.doi.org/10.1590/S1806-37132014000300014

Simultaneous interstitial pneumonitis and cardiomyopathy induced by venlafaxine*,**

Pneumonite intersticial e miocardiopatia simultâneas induzidas por venlafaxina

Pedro Gonçalo Ferreira, Susana Costa, Nuno Dias, António Jorge Ferreira, Fátima Franco

Abstract

Venlafaxine is a serotonin-norepinephrine reuptake inhibitor used as an antidepressant. Interindividual variability and herb-drug interactions can lead to drug-induced toxicity. We report the case of a 35-year-old female patient diagnosed with synchronous pneumonitis and acute cardiomyopathy attributed to venlafaxine. The patient sought medical attention due to dyspnea and dry cough that started three months after initiating treatment with venlafaxine for depression. The patient was concomitantly taking *Centella asiatica* and *Fucus vesiculosus* as phytotherapeutic agents. Chest CT angiography and chest X-ray revealed parenchymal lung disease (diffuse micronodules and focal ground-glass opacities) and simultaneous dilated cardiomyopathy. Ecocardiography revealed a left ventricular ejection fraction (LVEF) of 21%. A thorough investigation was carried out, including BAL, imaging studies, autoimmune testing, right heart catheterization, and myocardial biopsy. After excluding other etiologies and applying the Naranjo Adverse Drug Reaction Probability Scale, a diagnosis of synchronous pneumonitis/cardiomyopathy associated with venlafaxine was assumed. The herbal supplements taken by the patient have a known potential to inhibit cytochrome P450 enzyme complex, which is responsible for the metabolization of venlafaxine. After venlafaxine discontinuation, there was rapid improvement, with regression of the radiological abnormalities and normalization of the LVEF. This was an important case of drug-induced cardiopulmonary toxicity. The circumstantial intake of inhibitors of the CYP2D6 isoenzyme and the presence of a CYP2D6 slow metabolism phenotype might have resulted in the toxic accumulation of venlafaxine and the subsequent clinical manifestations. Here, we also discuss why macrophage-dominant phospholipidosis was the most likely mechanism of toxicity in this case.

Keywords: Cardiomyopathy, dilated; Lung diseases, interstitial; Antidepressive agents, second-generation/toxicity; Herb-drug interactions.

Resumo

A venlafaxina é um inibidor de recaptação de serotonina e noradrenalina utilizado como antidepressivo. A variabilidade individual ou interações entre fitoterápicos e fármacos podem causar toxicidade induzida por drogas. Relatamos o caso de uma paciente de 35 anos diagnosticada com pneumonite intersticial e miocardiopatia dilatada atribuídas à venlafaxina. A paciente procurou atendimento médico devido a dispneia e tosse seca, que começaram três meses após iniciar tratamento com venlafaxina para depressão. Concomitantemente tomava suplementos fitoterápicos contendo *Centella asiatica* e *Fucus vesiculosus*. A radiografia e a CT de tórax revelaram doença pulmonar parenquimatosa (micronódulos difusos e opacidades em vidro fosco) e, simultaneamente, foi diagnosticada uma miocardiopatia por ecocardiograma, que revelou uma fração de ejeção ventricular esquerda (FEVE) de 21%. Uma investigação ampla foi realizada, incluindo LBA, estudos de imagem, detecção de doenças autoimunes, cateterismo cardíaco direito e biópsia miocárdica. Após a exclusão de outras etiologias e a aplicação da Escala de Probabilidade de Reações Adversas a Medicamentos de Naranjo, foi assumido o diagnóstico de pneumonite/miocardiopatia síncronas associadas à venlafaxina. Já foi demonstrado que os suplementos fitoterápicos utilizados pela paciente podem inibir a isoenzima do complexo enzimático citocromo P450, responsável pelo metabolismo da venlafaxina. Após a descontinuação da venlafaxina, verificou-se uma rápida melhora clínica com regressão das alterações radiológicas e normalização da FEVE. Este é um importante caso de toxicidade cardiopulmonar induzida por droga. A administração circunstancial de inibidores da isoenzima CYP2D6 e a presença de um fenótipo de metabolização lenta de CYP2D6 podem ter resultado na acumulação tóxica da venlafaxina e na manifestação clínica subsequente. Aqui, é discutida a hipótese de a fosfolipidose macrofágica ser o mecanismo de toxicidade.

Descritores: Cardiomiopatia dilatada; Doenças pulmonares intersticiais; Antidepressivos de segunda geração/toxicidade; Toxicidade de Drogas; Interações ervas-drogas.

*Study carried out in the Department of Pulmonology, Department of Cardiology, Advanced Heart Failure Treatment Unit, and Department of Anatomopathology, Coimbra Hospital and University Center, Coimbra, Portugal.
Correspondence to: Pedro Gonçalo Ferreira. Avenida Fernando Namora, 89, 3º esquerdo, 3030-185, Coimbra, Portugal.
Tel. 035 196 6523825. E-mail: p_goncalof@hotmail.com
Financial support: None.
Submitted: 8 May 2013. Accepted, after review: 28 August 2013.
**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

Introduction

Drug-induced lung and heart disease can result from individual drug toxicity or drug-to-drug interactions, with impairment of kinetics and metabolization.[1,2] The causal link between drug intake and an idiosyncratic reaction is usually difficult to recognize, especially in cases of patients treated with multiple medications.[1,3] Therefore, high clinical suspicion and a thorough study are often necessary. The molecular basis of toxic lung injury is still poorly defined for the majority of the presently known offending drugs.[2]

We report the third case of a patient with synchronous interstitial pneumonitis and acute cardiomyopathy induced by venlafaxine, with a new insight regarding the potential lesion mechanism.

Case report

A 35-year-old female patient presented with progressive dyspnea over the previous three months, New York Heart Association (NYHA) functional class III, myalgia, and dry cough. She was hospitalized and submitted to CT angiography of the chest, which excluded pulmonary thromboembolic disease but revealed hazy parenchymal micronodules, thickening of interlobular septa, and subtle bilateral areas of ground-glass attenuation, mainly in the upper lobes, without adenopathies or pleuropericardial effusion (Figure 1). A chest X-ray revealed a mixed (reticular and micronodular) pattern and cardiomegaly (Figure 2). Simultaneously, she was diagnosed with subacute severe heart failure due to a dilated cardiomyopathy.

The patient had a history of depression and was started on a slow-release formulation of venlafaxine three months prior. For the last year, she had been taking *Centella asiatica* and *Fucus vesiculosus* as phytotherapeutic supplements for weight loss. The patient had no history of smoking, alcohol use, or illicit drug use. Previous medical examinations had been negative for heart

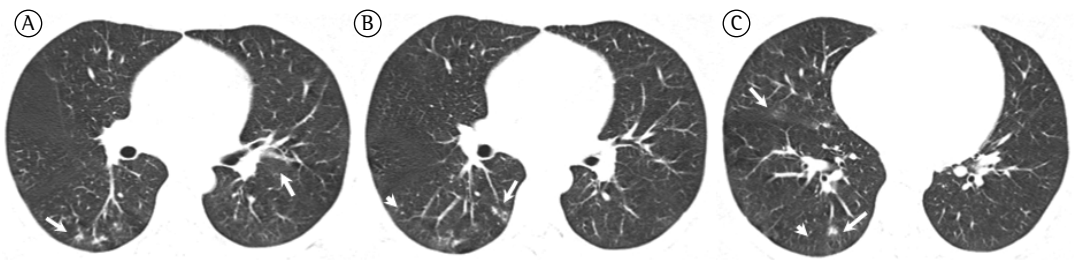


Figure 1 - Chest CT scans at admission revealing hazy parenchymal micronodules (short arrows) with thickening of interlobular septa and subtle diffuse areas of ground glass attenuation (long arrows).

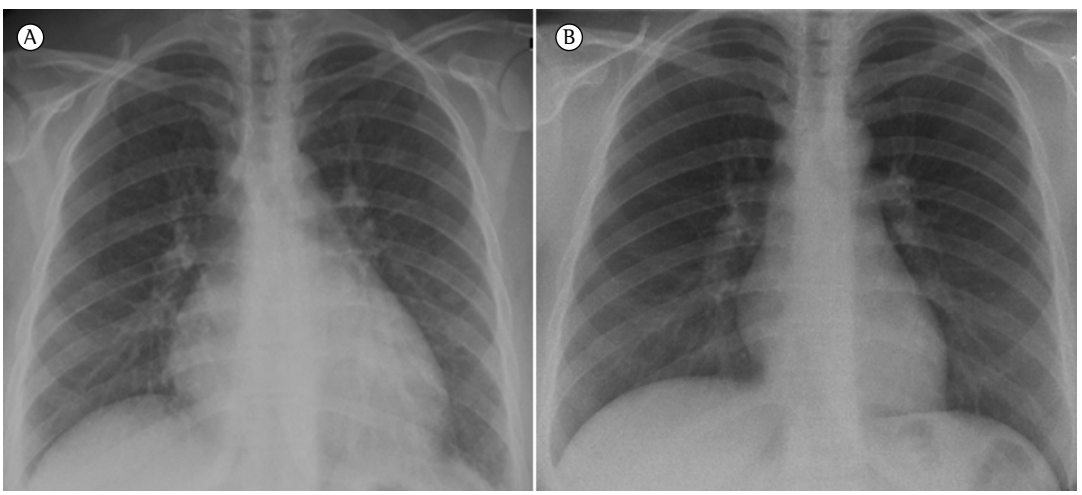


Figure 2 - In A, a chest X-ray taken at admission showing a mixed interstitial pattern (reticular and micronodular) and cardiomegaly. In B, a chest X-ray taken at discharge showing the normalization of the lung fields and of the cardiac silhouette.

 http://dx.doi.org/10.1590/S1806-37132014000300015

disease, and there was neither a relevant family history nor a history of occupational exposure.

Clinical examination revealed apyrexia, mild hypotension, and an SpO_2 of 94%. On auscultation, there were crackles at both lung bases, with a grade II/VI holosystolic murmur typical of mitral regurgitation. The jugular vein was not turgescent, nor was there hepatomegaly or peripheral edema.

Electrocardiography showed a normal sinus rhythm, ventricular premature beats, and T-wave inversion in leads V4-V6, referred to as "strain"; an initial left ventricular ejection fraction (LVEF) of 21% was identified on radionuclide angiography; biatrial dilatation, severe left ventricular enlargement (71/60 mm), and severely impaired global systolic function (LVEF = 20%) were also found; moderate right ventricular enlargement with systolic impairment—tricuspid annular plane systolic excursion of 15 mm (normal value, 15-20 mm), S' velocity of 0.06 m/s (normal value, > 0.15 m/s)—and severe functional mitral and tricuspid regurgitations were found on echocardiography.

Heart catheterization revealed normal coronary arteries and a cardiac index of 2.36 L . min^{-1} . m^{-2} (normal value, 2.6-4.2 L . min^{-1} . m^{-2}). Myocardial biopsies presented mixed cellularity without fibrosis or any other form of infiltration. Tests for DNA detection were negative for herpes simplex virus, human herpesvirus 6 (HHV-6), HHV-8, cytomegalovirus, BK virus, and Epstein-Barr virus.

Pulmonary function test results were normal. However, the diffusion capacity was slightly low (PaO_2/FiO_2 = 320).

Blood workup presented normal inflammatory parameters, euthyroidism, and a brain natriuretic peptide level of 963.6 pg/mL (normal value, < 100 pg/mL). Renal and hepatic parameters, complement proteins, and urinary sediment were all normal. The serological panel was negative for HIV, syphilis, *Mycoplasma* sp., *Coxiella* sp., cytomegalovirus, Epstein-Barr virus, parvovirus B19, and HHV-6. The results of testing for autoimmune disease were unremarkable.

Bronchoscopy results were normal. The BAL fluid presented normal cellularity, a low CD4/CD8 lymphocyte ratio (0.7), and a massive presence of foamy macrophages (Figure 3). The microbiological study on BAL fluid was negative. No transbronchial lung biopsies were obtained, because of ventricular tachycardia during the examination.

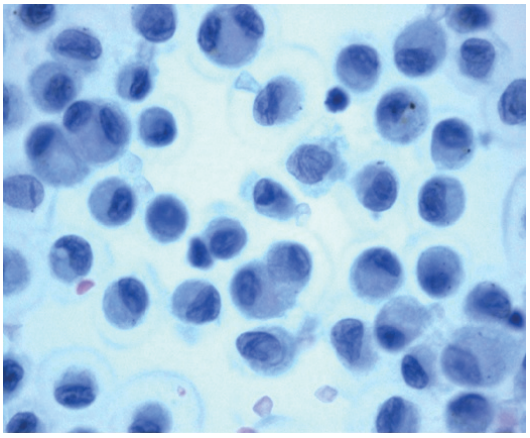


Figure 3 - Photomicrograph showing foamy macrophages in the BAL fluid (May-Grümwald-Giemsa; magnification, ×400).

The patient was started on standard therapy without improvement. Inotropic support was then initiated. However, no improvement was seen over the course of a week. At that time, the decision was made to discontinue venlafaxine. Two weeks later, an HRCT revealed notorious improvement, with only a few centrilobular nodules remaining in the right lower lobe. Together with that radiological upswing, there was an overall improvement, and the LVEF was up to 35% at discharge.

Four months later, the patient presented with an LVEF close to normal (50%), NYHA functional class I, maintaining only mild left ventricle enlargement (60/40 mm), and mild mitral regurgitation. Most of the cardiac medications were therefore discontinued.

Discussion

There have been various reports on adverse effects related to venlafaxine. Acute and subacute interstitial pneumonitis have been reported[4-7] as have cases of eosinophilic pneumonia,[8,9] although the mechanisms involved have yet to be elucidated.

The diagnosis of drug-induced interstitial lung disease is hindered by its clinical nonspecificity, interindividual variations, the confounding effect of comorbidities, and treatment with multiple medications.[4] In addition, cases of venlafaxine-induced acute heart failure have been described, some even in previously healthy patients under standard dosing regimens.[4,10] One group of authors

reported dilated cardiomyopathy and cardiogenic shock in a patient with previously normal heart function, with recovery of the LVEF after the discontinuation of the medication.[10] Using the Naranjo Adverse Drug Reaction Probability Scale,[11] a probable link with venlafaxine was found.[10] Although the mechanism of heart injury is still not fully understood, drug-induced inhibition of myocardial norepinephrine reuptake and blockade of cardiac sodium channels have been reported.[12]

The cytochrome P450 (CYP) superfamily is present in human lung tissue and participates in the enzymatic inactivation of numerous xenobiotics.[13] Metabolic differences related to CYP polymorphisms contribute to interindividual variability reflected in drug responses and unexpected toxicity.[2] One group of authors showed that, among 59 patients with drug-induced interstitial lung disease, 54 (91.5%) had at least one of the studied CYP variant genes.[2] In 87% of those patients, the presence of such genes was found to be relevant to their clinical profile.[2]

Venlafaxine is an antidepressant that is metabolized to O-desmethylvenlafaxine (ODV) by the isoenzyme CYP2D6 and, to a lesser extent, by CYP3A4.[14] For numerous psychotropic drugs, CYP2D6 is a high-affinity/low-capacity enzyme whose polymorphisms can phenotypically determine slow, extended, or rapid metabolization.[15,16] Because a less functional variant might be able to precipitate severe manifestations,[15] the administration of venlafaxine to CYP2D6 slow metabolizers or the coadministration with CYP2D6 inhibitor drugs carries the risk of drug accumulation and subsequent cellular/organic insult.[16] It has been shown that *Centella asiatica* can induce moderate-to-strong inhibition of CYP2D6[17] and that *Fucus vesiculosus* can also inhibit the cytochrome P450 enzyme complex.[18] Accordingly, although the CYP2D6 profile of our patient had not been tested, we hypothesize that she was a CYP2D6 slow metabolizer or that the inhibitory action of the concomitant use of the herbal drugs contributed to venlafaxine accumulation, leading to cardiopulmonary toxicity.

Drug-induced phospholipidosis (DIP) is characterized by intracellular accumulation of phospholipids in various body tissues and formation of lamellar bodies, leading to a foamy macrophage appearance at light microscopy.[19] It can be caused by over 50 drugs sharing a particular molecular structure, with hydrophobic and hydrophilic regions, denominated cationic amphiphilic drugs (CADs).[20] The venlafaxine formulation used by our patient was analyzed by the Department of Organic Chemistry, University of Coimbra, Portugal. The patient was taking a formulation of the racemic mixture of (R/S)-1-[(2-dimethylamino)-1-(4-methoxyphenyl) ethyl]-cyclohexanol hydrochloride. Its structure combines a hydrophilic cationic component (terminal amine and tertiary alcohol groups) with a hydrophobic nonpolar component (aromatic ring methoxylation and cyclohexyl group), conferring amphiphilicity.[21] In addition, it has a cationic property,[22] because ODV presents an acidic phenol group (from the hydrolysis of the methyl ethers connected to the aromatic ring) and the amine group can undergo extensive intramolecular protonation, becoming a CAD by staying in zwitterion form (negative aromatic-O- and positive H+ amine groups). This fact, supported by the clinical context and the massive presence of foamy macrophages observed in the BAL fluid, strongly suggests a mechanism of venlafaxine-induced phospholipidosis.

Although DIP can occur from hours to months after the beginning of treatment,[19] its precise mechanisms have yet to be completely clarified. Lysosomes act as a place of accumulation of CADs and phospholipids because of their direct connection with CADs and the inhibition of phospholipases.[19,20] Because lysosomes participate in a wide variety of cellular processes, there might be ionic transport impairment, oxidative phosphorylation, heterophagy, autophagy, organelle recycling, cell membrane repairing, and cell cycle regulation. Three recognized damage patterns can occur: macrophage-dominant phospholipidosis (the commonest), parenchymal-cell-dominant phospholipidosis, and localized phospholipidosis.[19]

Another typical feature of DIP is its reversibility after drug discontinuation.[1] Because phospholipid levels normalize and drug efflux occurs, organic, functional, and radiological abnormalities usually improve from weeks to months.[19] Reversibility is usually complete, but permanent damage can subside in cases of more severe organic injury.[2]

The present case details the occurrence of cardiopulmonary toxicity, which was probably associated with venlafaxine. The case occurred in a previously healthy 35-year-old patient submitted to exhaustive investigation after the exclusion

 http://dx.doi.org/10.1590/S1806-37132014000300015

of other possible causes. Only two similar cases have been reported in the literature.[4]

We recognized the concomitant intake of two herbal drugs known to inhibit the specific metabolizing isoenzyme of venlafaxine, which would explain the accumulation of venlafaxine and ODV to toxic levels. The strong temporal connection between the drug intake and the clinical manifestations and, conversely, between the drug discontinuation and the rapid improvement in lung abnormalities and heart function, provides additional support for that hypothesis. For ethical reasons, rechallenge with venlafaxine was not carried out. After an objective analysis, considering the Naranjo Adverse Drug Reaction Probability Scale,[11] we made a diagnosis of venlafaxine-induced cardiopulmonary toxicity.

A DIP could be the mechanism of toxicity, because venlafaxine is a potential CAD, as well as because there were striking quantities of foamy macrophages in the BAL fluid, a low CD4/CD8 T lymphocyte ratio in the blood and BAL fluid, and features that were consistent with a "macrophage-dominant phospholipidosis" subtype pattern.

The present case highlights the importance of high clinical suspicion for the timely recognition of drug-induced cardiopulmonary toxicity, especially in cases of initially unexplained disease. Prompt discontinuation of the drug usually results in remarkable clinical and prognostic improvement.

Acknowledgments

The authors would like to thank Professor Vítor Sobral of the Department of Chemistry of the University of Coimbra for his vital contribution in the analysis of the chemical structure of venlafaxine.

References

1. Nemery B, Bast A, Behr J, Borm PJ, Bourke SJ, Camus PH, et al. Interstitial lung disease induced by exogenous agents: factors governing susceptibility. Eur Respir J Suppl. 2001;32:30s-42s.
2. Wijnen PA, Drent M, Nelemans PJ, Kuijpers PM, Koek GH, Neef C, et al. Role of cytochrome P450 polymorphisms in the development of pulmonary drug toxicity. Drug Saf. 2008;31(12):1125-34. http://dx.doi.org/10.2165/0002018-200831120-00008
3. Wijnen P, Bekers O, Drent M. Relationship between drug-induced interstitial lung diseases and cytochrome P450 polymorphisms. Curr Opin Pul Med. 2010;16(5):496-502. http://dx.doi.org/10.1097/MCP.0b013e32833c06f1
4. Drent M, Singh S, Gorgels AP, Hansell DM, Bekers O, Nicholson AG, et al. Drug-induced pneumonitis and heart failure simultaneously associated with venlafaxine. Am J Respir Crit Care Med. 2003;167(7):958-61. http://dx.doi.org/10.1164/rccm.200207-739CR
5. Vázquez MJ, Carretero Quevedo B. Pneumonitis related to venlafaxine. Psychosomatics. 2008;49(1):84-5. http://dx.doi.org/10.1176/appi.psy.49.1.84
6. Turner RC, Nelson JE, Roberts BT, Gillam DM. Venlafaxine-associated interstitial pneumonitis. Pharmacotherapy. 2005;25(4):626-9. http://dx.doi.org/10.1592/phco.25.4.626.61029
7. Borderías Clau L, Marigil Gómez MA, Val Adán P, Marcén Letosa M, Biescas López R, Garrapiz López FJ. Hypersensitivity pneumonitis due to venlafaxine [Article in Spanish]. Arch Bronconeumol. 2008;44(10):571-3. http://dx.doi.org/10.1016/S1579-2129(08)60104-4
8. Fleisch MC, Blauer F, Gubler JG, Kuhn M, Scherer TA. Eosinophilic pneumonia and respiratory failure associated with venlafaxine treatment. Eur Respir J. 2000;15(1):205-8. http://dx.doi.org/10.1183/09031936.00.15120500
9. Priou P, Gagnadoux F, Dehé C, Hureaux J, Pearson C, Urban T, et al. Drug-induced pneumonitis in a patient treated with venlafaxine and propanolol [Article in French]. Rev Mal Respir. 2008:25(5):610-3. http://dx.doi.org/10.1016/S0761-8425(08)71620-6
10. Charniot JC, Vignat N, Monsuez JJ, Kidouche R, Avramova B, Artigou JY, et al. Cardiogenic shock associated with reversible dilated cardiomyopathy during therapy with regular doses of venlafaxine. Am J Emerg Med. 2010;28(2):256.e1-5.
11. Naranjo CA, Busto U, Sellers EM, Sandor P, Ruiz I, Roberts EA, et al. A method for estimating the probability of adverse drug reactions. Clin Pharmacol Ther. 1981;30(2):239-45. http://dx.doi.org/10.1038/clpt.1981.154
12. Khalifa M, Daleau P, Turgeon aJ. Mechanism of sodium channel block by venlafaxine in guinea pig ventricular myocites. J Pharmacol Exp Ther. 1999;291(1):280-4.
13. Zhang JY, Wang Y, Prakash C. Xenobiotic-metabolizing enzymes in human lung. Curr Drug Metab. 2006;7(8):939-48. http://dx.doi.org/10.2174/138920006779010575
14. Veefkind AH, Haffmans PM, Hoencamp E. Venlafaxine serum levels and CYP2D6 genotype. Ther Drug Monit. 2000;22(2):202-8. http://dx.doi.org/10.1097/00007691-200004000-00011
15. Eichelbaum M, Evert B. Influence of pharmacogenetics on drug disposition and response. Clin Exp Pharmacol Physiol. 1996;23(10-11):983-5. http://dx.doi.org/10.1111/j.1440-1681.1996.tb01154.x
16. Shams ME, Arneth B, Hiemke C, Dragicevic A, Müller MJ, Kaiser R, et al. CYP2D6 polymorphism and clinical effect of the antidepressant venlafaxine. J Clin Pharm Ther. 2006;31(5):493-502. http://dx.doi.org/10.1111/j.1365-2710.2006.00763.x
17. Pan Y, Abd-Rashid BA, Ismail Z, Ismail R, Mak JW, Pook PC, et al. In vitro modulatory effects on three major human cytochrome P450 enzymes by multiple active constituents and extracts of Centella asiatica. J Ethnopharmacol. 2010;130(2):275-83. http://dx.doi.org/10.1016/j.jep.2010.05.002
18. Parys S, Kehraus S, Krick A, Glombitza KW, Carmeli S, Klimo K, et al. In vitro chemopreventive potential of fucophlorethols from the brown alga Fucus vesiculosus L. by anti-oxidant activity and inhibition of selected cytochrome P450 enzymes. Phytochemistry. 2010;71(2-3):221-9. http://dx.doi.org/10.1016/j.phytochem.2009.10.020

19. Nonoyama T, Fukuda R. Drug-induced phospholipidosis-pathological aspects and its prediction. J Toxicol Pathol. 2008;21(1):9-24. http://dx.doi.org/10.1293/tox.21.9
20. Anderson N, Borlak J. Drug-induced phospholipidosis. FEBS Lett. 2006;580(23):5533-40. http://dx.doi.org/10.1016/j.febslet.2006.08.061
21. Jamal MA, Bukhari IH, Muneer M, Shahzad K. Influence of external factors on self-aggregation of amphiphilic antidepressant drug: a thermodynamic study. J Chem Soc Pakistan. 2013;35(2):262-6.
22. Balasubramaniam J, Bindu K, Rao VU, Ray D, Haldar R, Brzeczko AW. Effect of superdisintegrants on dissolution of cationic drugs. Dissolut Technol. 2008;15(2):18-25.

About the authors

Pedro Gonçalo Ferreira
Physician. Department of Pulmonology, Coimbra Hospital and University Center, Coimbra, Portugal.

Susana Costa
Physician. Department of Cardiology, Advanced Heart Failure Treatment Unit, Coimbra Hospital and University Center, Coimbra, Portugal.

Nuno Dias
Physician. Department of Anatomopathology, Coimbra Hospital and University Center, Coimbra, Portugal.

António Jorge Ferreira
Physician. Department of Pulmonology Coimbra Hospital and University Center, Coimbra, Portugal.

Fátima Franco
Physician. Department of Cardiology, Advanced Heart Failure Treatment Unit, Coimbra Hospital and University Center, Coimbra, Portugal.

 http://dx.doi.org/10.1590/S1806-37132014000300015

Lung cancer and schwannoma — the pitfalls of positron emission tomography

Câncer de pulmão e schwannoma — as armadilhas da tomografia por emissão de prótons

Fernando Luiz Westphal, Luiz Carlos de Lima, José Correa Lima-Netto, Michel de Araújo Tavares, Felipe de Siqueira Moreira Gil

To the Editor:

Imaging studies play an important role in the tumor-node-metastasis staging of lung cancer, particularly in the evaluation of tumor size/extent and regional lymph node involvement. There is a learning curve for interpreting positron emission tomography (PET) findings; PET allows the estimation of the metabolic activity of a lesion, therefore facilitating the differential diagnosis between benign and malignant disease, as well as the determination of the extent of malignancy. When used in the study of lung cancer, PET can give false-negative results in cases of decompensated hyperglycemia, small lesions, or lesions with low metabolic activity, among others.[1,2] In addition, PET can give false-positive results in cases of inflammatory disease or concomitant tumors.

We report the case of a 61-year-old male patient who, in October of 2011, presented to our emergency room with airway infection. A chest X-ray revealed right upper lobe atelectasis, which was confirmed by chest CT (Figure 1A).

Fiberoptic bronchoscopy showed an exophytic lesion in the right upper lobe orifice. The lesion caused total bronchial lumen occlusion and affected the secondary carina, without tracheal involvement. The histopathological findings were consistent with moderately differentiated squamous cell carcinoma. Blood and kidney function test results were normal, the exception being serum glucose levels, which were elevated.

A PET scan showed a solid, lobulated paramediastinal lesion in the right upper lobe. The lesion measured 3.0 cm × 2.4 cm, and the standardized uptake value (SUV) was 12.2. The lesion occluded the right upper lobe bronchus and therefore caused the atelectasis. In addition, the PET scan showed a right-sided image that was suggestive of lymph node enlargement in the superior mediastinum and measured approximately 2.0 cm, the SUV being 3.8 (Figure 1B).

The patient underwent right upper sleeve lobectomy and radical lymphadenectomy, the affected lymph node being resected (Figure 2A). Histopathology of the lesion in the right lung apex revealed invasive, moderately differentiated squamous cell carcinoma measuring 3.2 cm × 1.6 cm, with angiolymphatic invasion, marked desmoplasia, necrotic foci, and scattered foci of squamous differentiation. Analysis of the nodule in the superior mediastinum revealed, instead of lymph node enlargement, a well-defined, benign encapsulated schwannoma (Figure 2B) without cellular atypia or necrotic areas. There were no postoperative complications, and the patient underwent chemotherapy. Screening tests performed one year later were negative for metastatic disease and recurrence.

Schwannomas (also known as neurilemmomas) and neurofibromas account for 95% of all benign neurogenic mediastinal tumors. A schwannoma is a mesenchymal neoplasm originating from Schwann cells of the nerve sheath. Although schwannomas commonly affect the mediastinum, they can be found in the abdomen, in the pelvis, and, much more rarely, in the chest wall. Schwannomas affect males and females alike, being benign in up to 90% of cases. In general, schwannomas are well defined and asymptomatic, being diagnosed incidentally, usually after the age of 30 years.[3,4] Given that schwannomas show variable 18F-fluorodeoxyglucose (FDG) uptake, with the SUV ranging from 1.9 to 12.0, PET has limited utility in distinguishing between schwannomas and malignant peripheral nerve sheath tumors.[5] In many cases, it is impossible to distinguish between a schwannoma and a malignant tumor before biopsy or surgery precisely because schwannomas can show high FDG uptake.

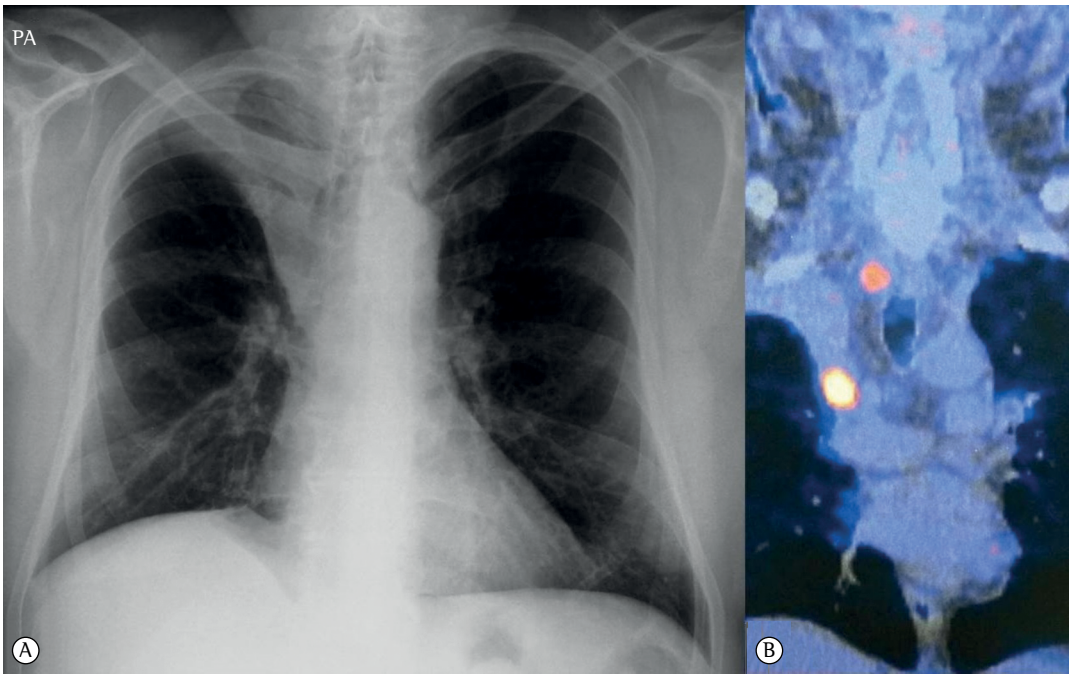


Figure 1 - In A, posteroanterior chest X-ray showing a radiopaque triangular image in the right upper lobe determining cranial, fissural, and hilar retraction, suggestive of atelectasis. In B, coronal proton emission tomography and CT fusion images of two oval nodes with increased radiotracer uptake. One of the images represents a right upper lobe paramediastinal mass, which occluded the right upper lobe bronchus and therefore caused atelectasis. The other image represents a lesion in the right paratracheal lymph node station. Although the image was suggestive of lymph node enlargement, histopathological examination revealed a schwannoma.

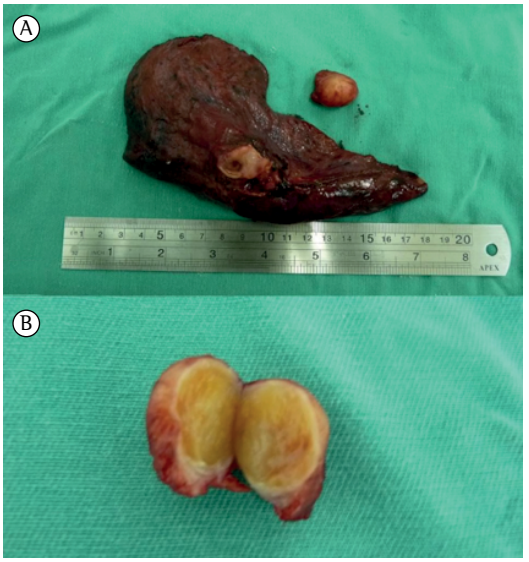


Figure 2 - In A, photograph of the resected right upper lobe and mediastinal lesion. In B, photograph of the mediastinal lesion.

Accurate staging is required for proper treatment of patients with lung cancer, staging being based on tumor size, regional lymph node involvement, and the presence of metastases. Although chest CT is considered the modality of choice for the diagnosis of intrathoracic metastases, there is no consensus regarding the study of metastases. PET-CT was introduced and developed as an integrated modality for accurate nodal staging and for detection of metastatic lesions in the entire body. Commonly, PET-CT is more effective than chest CT for lung cancer staging, assisting in detecting distant metastases that are not detected by traditional methods in 5-20% of patients; in addition, PET-CT can influence the treatment strategies and assist in predicting survival.[6] Although the degree of regional lymph node involvement in our patient was classified as N2, surgery was recommended because only one lymph node was affected, and it was resectable.

Given that schwannomas show highly variable FDG uptake—which is why it is difficult to differentiate between schwannomas and other tumors by means of images alone—PET findings in patients with lung masses should be interpreted carefully, as should lymph nodes with high SUV, which should be biopsied, in order to avoid an incorrect diagnosis or incorrect staging and the hazardous consequences of false-positive results.

 http://dx.doi.org/10.1590/S1806-37132014000300016

Fernando Luiz Westphal
Coordinator of the Teaching and Research Center, Federal University of Amazonas School of Medicine Getúlio Vargas University Hospital, Manaus, Brazil

Luiz Carlos de Lima
Chief, Department of Thoracic Surgery, Federal University of Amazonas School of Medicine Getúlio Vargas University Hospital, Manaus, Brazil

José Correa Lima Netto
Physician, Department of Thoracic Surgery, Federal University of Amazonas School of Medicine Getúlio Vargas University Hospital, Manaus, Brazil

Michel de Araújo Tavares
Assistant Professor of Radiology, Department of Clinical Medicine, Federal University of Amazonas School of Medicine, Manaus, Brazil

Felipe de Siqueira Moreira Gil
Medical Student, Federal University of Amazonas School of Medicine, Manaus, Brazil

References

1. Visioni A, Kim J. Positron emission tomography for benignant and malignant disease. Surg Clin North Am. 2011;91(1) 249-66. PMid:21184913 PMCid:PMC3021752. http://dx.doi.org/10.1016/j.suc.2010.10.012
2. Kostakoglu L, Hardoff R, Mirtcheva R, Goldsmith SJ. PET-CT fusion imaging in differentiating physiologic from pathologic FDG uptake. Radiographics. 2004;24(5):1411-31. PMid:15371617. http://dx.doi.org/10.1148/rg.245035725
3. Bibas BJ, Madeira M, Gavina R, Hoehl-Carneiro L, Sardinha S. A rare case of synchronous malignant thoracic tumors. J Bras Pneumol. 2009;35(2):186-9. PMid:19287923. http://dx.doi.org/10.1590/S1806-37132009000200013
4. Henn LA, Gonzaga RV, Crestani J, Cerski MR. Intercostal schwannoma simulating pulmonary neoplasms [Article in Portuguese]. Rev Assoc Med Bras. 1998;44(2):146-8. PMid:9699334. http://dx.doi.org/10.1590/S0104-42301998000200014
5. Moon H, Park SJ, Kim SR, Park HS, Lee YC. Benign intercostal schwannoma mimicking a solitary metastasis from lung cancer. Thorax. 2010;65(8):753-4. PMid:20530120. http://dx.doi.org/10.1136/thx.2009.129189
6. Yi CA, Shin KM, Lee KS, Kim BT, Kim H, Kwon OJ, et al. Non-small cell lung cancer staging: efficacy comparison of integrated PET/CT versus 3.0-T whole-body MR imaging. Radiology. 2008;248(2):632-42. PMid:18552311. http://dx.doi.org/10.1148/radiol.2482071822

Submitted: 11 October 2013. Accepted, after review: 11 November 2013.

Unilateral pulmonary agenesis

Agenesia pulmonar unilateral

Nulma Souto Jentzsch

To the Editor:

I read with great interest the article by Malcon et al. reporting the occurrence of unilateral pulmonary agenesis in an 8-year-old asymptomatic male child without other associated malformations, and I congratulate the authors on it.[1]

I would like to report that we treated a 3-month-old female infant, from the city of Belo Horizonte, Brazil, who had been born at term and had undergone prenatal testing uneventfully. The infant was taken to the Department of Pediatrics of the São José University Hospital in that same city on March of 2012 with a 4-day-history of cough and fever. Her parents reported that she had no comorbidities or previous hospitalizations. The patient presented with acute respiratory failure and required oxygen by nasal catheter. Examination of the respiratory system revealed diminished breath sounds throughout the left hemithorax, without adventitious sounds. A chest X-ray (Figure 1) showed complete opacification of the left hemithorax, together with deviation of the trachea and mediastinum to the left. The left lung was not seen on chest CT (Figure 2). Doppler echocardiography showed agenesis of the left pulmonary artery, without other cardiac abnormalities, and bronchoscopy revealed complete absence of the left lung and absence of bronchial stump. A diagnosis of left lung agenesis was therefore established. The patient's course was satisfactory, and she is under outpatient follow-up. Congenital malformations of the lung are rare and vary widely in their clinical presentation and severity, depending mostly on the degree of lung involvement and their location in the thoracic cavity.[2] The earliest stage of lung development occurs during the first 50 days of gestation and is called embryonic stage: around the 26th day, the anterior part of the foregut invaginates and forms the laryngotracheal bud; subsequently, the two main bronchi are formed. After 48 days of gestation, the segmental and subsegmental bronchi start forming. The pulmonary arteries form from the sixth aortic arch, and the pulmonary veins form from the invagination of the sinoatrial region of the heart. The development of the conducting airways starts early, whereas the respiratory bronchioles, alveolar ducts, and alveoli form later in gestation, in the stages called pseudoglandular, canalicular, saccular, and alveolar.

In unilateral pulmonary agenesis, the right or left main bronchus does not develop, and there is absence of bronchi, parenchyma, and pulmonary vessels. The origin of pulmonary agenesis is unknown, and its prevalence, including the bilateral and unilateral forms, is 0.5-1.0 per 10,000 live births. The bilateral form is incompatible with life.[3]

In unilateral pulmonary agenesis, the mortality rate in the neonatal period is approximately 50%, especially if there are other associated malformations (especially cardiac malformations).[4] Musculoskeletal, gastrointestinal, and renal abnormalities may also be present. The mortality rate is higher when there is agenesis of the right lung. This difference can be explained by a greater mediastinal shift, leading to tracheal compression.[3] Agenesis of the left lung is more common, causing compensatory growth of the remaining lung and its herniation into the contralateral hemithorax.[5]

Asymptomatic patients do not require intervention, especially in the absence of associated anomalies. However, pulmonary infections or other lung diseases should be treated early, and the patient should have clinical follow-up to detect possible abnormalities, such as pulmonary hypertension. Sometimes, the diagnosis of unilateral pulmonary agenesis is delayed, being made in adulthood in asymptomatic patients. Other associated malformations and recurrent respiratory infections are factors that aid in earlier diagnosis.

The prognosis is better when there is agenesis of the left lung and when there are no cardiac malformations.

 http://dx.doi.org/10.1590/S1806-37132014000300017

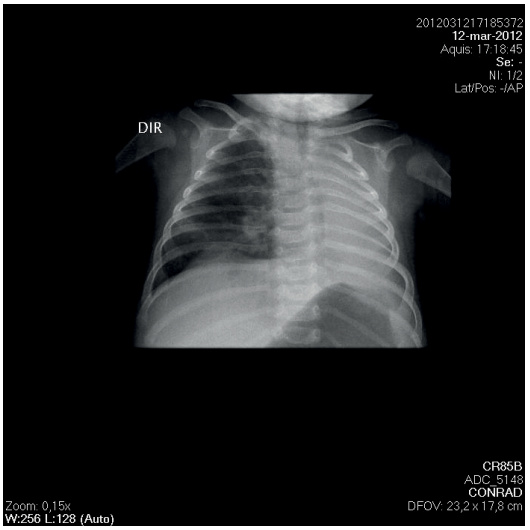


Figure 1 - Chest X-ray showing absence of the left lung.

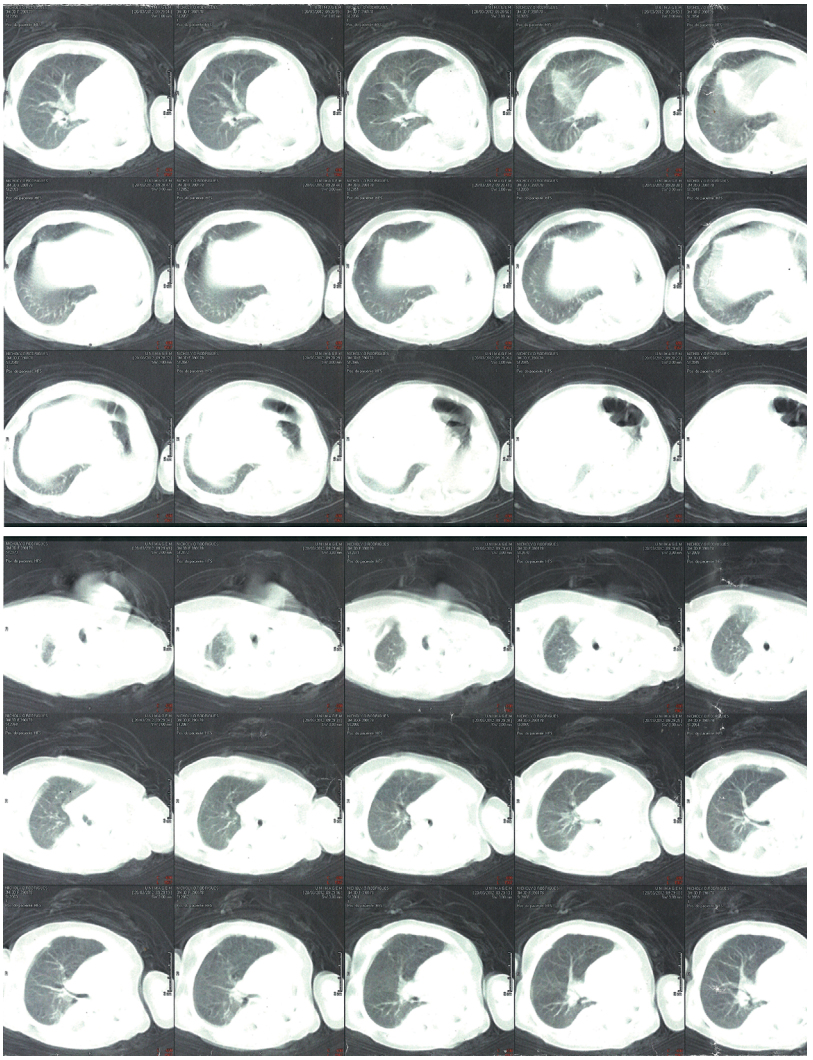


Figure 2 - Chest CT scans showing absence of the left lung.

Nulma Souto Jentzsch
Adjunct Professor of Pediatrics, São José University Hospital, and Researcher, Minas Gerais School of Medical Sciences, Belo Horizonte, Brazil

References

1. Malcon MC, Malcon CM, Cavada MN, Caruso PE, Real LF. Unilateral pulmonary agenesis. J Bras Pneumol. 2012;38(4):526-9. PMid:22964938 http://dx.doi.org/10.1590/S1806-37132012000400016
2. Chou AK, Huang SC, Chen SJ, Huang PM, Wang JK, Wu MH, et al. Unilateral lung agenesis--detrimental roles of surrounding vessels. Pediatr Pulmonol. 2007;42(3):242-8. PMid:17238192 http://dx.doi.org/10.1002/ppul.20561
3. Nandan D, Bhatt GC, Dewan V, Pongener I. Complete right lung agenesis with dextrocardia: an unusual cause of respiratory distress. J Clin Imaging Sci. 2012;2:81. PMid:23393637 PMCid:PMC3551508 http://dx.doi.org/10.4103/2156-7514.105140
4. Eroglu A, Alper F, Turkyilmaz A, Karaoglanoglu N, Okur A. Pulmonary agenesis associated with dextrocardia, sternal defects, and ectopic kidney. Pediatr Pulmonol. 2005;40(6):547-9. PMid:16161099 http://dx.doi.org/10.1002/ppul.20301
5. Alvarez AJ, Vaccaro MI, Verdejo HP, Villarroel CQ, Puentes RR. Unilateral pulmonary agenesis associated with multiple malformations-a case report [Article in Spanish]. Rev Chil Pediatr. 2000;71(1):41-5. http://dx.doi.org/10.4067/S0370-41062000000100008

Submitted: 20 January 2014. Accepted, after review: 31 January 2014.

Edital de Seleção

Brasília, 30 de maio de 2014

No período de 23 de junho a 22 de agosto estarão abertas as inscrições para candidatos a posição de Editor-Chefe do Jornal Brasileiro de Pneumologia com atuação no quadriênio janeiro de 2015 a dezembro de 2018. Os interessados ao posto deverão enviar à administração da SBPT em Brasília, suas propostas de gestão e curriculum vitae na plataforma Lattes. As propostas dos candidatos deverão abranger o campo administrativo, científico e orçamentário, e deverão ser apresentadas em relação aos quatro anos previstos para a duração do mandato. Experiência prévia em publicações científicas será importante critério para a escolha do candidato. Os candidatos deverão conhecer as normas relativas à seleção do Editor-Chefe e o funcionamento do Jornal Brasileiro de Pneumologia, explícitas em seu regulamento, o qual poderá ser obtido por meio de contato com a secretaria do Jornal em Brasília.

Prof. Dr. Jairo Sponholz Araújo
Presidente da SBPT

Prof. Dr. Carlos Roberto Ribeiro Carvalho
Editor-Chefe

Instructions for Authors

The Jornal Brasileiro de Pneumologia (J Bras Pneumol, Brazilian Journal of Pulmonology) ISSN-1806-3713, published once every two months, is the official organ of the *Sociedade Brasileira de Pneumologia e Tisiologia* (Brazilian Thoracic Society) for the publication of scientific papers regarding Pulmonology and related areas.

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

Articles that fail to present merit, have significant errors in methodology or are not in accordance with the editorial policy of the journal will be directly rejected by the Editorial Board, with no recourse. Articles may be written in Portuguese, Spanish or English. In the online version of the Journal (www.jornaldepneumologia.com.br, ISSN-1806-3756), all articles will be made available in Spanish or Portuguese, as well as in English. Authors may submit color figures. However, the cost of printing figures in color, as well as any related costs, will be borne by the authors.

For further clarification, please contact the Journal Secretary by e-mail or by telephone.

The *Jornal Brasileiro de Pneumologia* upholds the World Health Organization (WHO) and International Committee of Medical Journal Editors (ICMJE) policies regarding the registration of clinical trials, recognizing the importance of these initiatives for the registration and international, open-access dissemination of information on clinical trials. Therefore, as of 2007, the Journal only accepts clinical trials that have been given an identification number by one of the clinical trials registries meeting the criteria established by the WHO and the ICMJE. This identification number must be included at the end of the abstract.

Within this context, the *Jornal Brasileiro de Pneumologia* adheres to the definition of a clinical trial as described by the WHO, which can be summarized as "any study that prospectively assigns human beings to be submitted to one or more interventions with the objective of evaluation the effects that those interventions have on health-related outcomes. Such interventions include the administration of drugs, cells and other biological products, as well as surgical procedures, radiological techniques, the use of devices, behavioral therapy, changes in treatment processes, preventive care, etc

Authorship criteria

An individual may be considered an author of an article submitted for publication only if having made a significant intellectual contribution to its execution. It is implicit that the author has participated in at least one of the following phases: 1) conception and planning of the study, as well as the interpretation of the findings; 2) writing or revision of all preliminary drafts, or both, as well as the final revision; and 3) approval of the final version.

Simple data collection or cataloging does not constitute authorship. Likewise, authorship should not be conferred upon technicians performing routine tasks, referring physicians, doctors who interpret routine exams or department heads who are not directly involved in the research. The contributions made by such individuals may be recognized in the acknowledgements.

The accuracy of all concepts presented in the manuscript is the exclusive responsibility of the authors. The number of authors should be limited to six, although exceptions will be made for manuscripts that are considered exceptionally complex. For manuscripts with more than six authors, a letter should be sent to the Journal describing the participation of each.

Presentation and submission of manuscripts

All manuscripts must be submitted online from the home-page of the journal. The instructions for submission are available at: www.jornaldepneumologia.com.br/sgp. Although all manuscripts are submitted online, they must be accompanied by a Copyright Transfer Statement and Conflict of Interest Statement signed by all the authors based on the models available at: www.jornaldepneumologia.com.br.

It is requested that the authors strictly follow the editorial guidelines of the journal, particularly those regarding the maximum number of words, tables and figures permitted, as well as the rules for producing the bibliography. Failure to comply with the author instructions will result in the manuscript being returned to the authors so that the pertinent corrections can be made before it is submitted to the reviewers.

Special instructions apply to the preparation of Special Supplements and Guidelines, and authors should consult the instructions in advance by visiting the homepage of the journal.

The journal reserves the right to make stylistic, grammatical and other alterations to the manuscript.

With the exception of units of measure, abbreviations should be used sparingly and should be limited only to those that are widely accepted. These terms are defined in the List of Abbreviations and Acronyms accepted without definition in the Journal. Click here (List of Abbreviations and Acronyms). All other abbreviations should be defined at their first use. For example, use "C-reactive protein (CRP)", and use "CRP" thereafter. After the definition of an abbreviation, the full term should not appear again. Other than those accepted without definition, abbreviations should not be used in titles, and their use in the abstracts of manuscripts should be avoided if possible.

Whenever the authors mention any substance or uncommon piece of equipment they must include the catalogue model/number, name of manufacturer, city and country of origin. For example:

". . . ergometric treadmill (model ESD-01; FUNBEC, São Paulo, Brazil) . . ."

In the case of products from the USA or Canada, the name of the state or province should also be cited. For example:

". . . guinea pig liver tTg (T5398; Sigma, St. Louis, MO, USA) . . ."

Manuscript preparation

Title Page: The title page should include the title (in Portuguese and in English); the full names, highest academic degrees and institutional affiliations of all authors; complete address, including telephone number, fax number and e-mail address, of the principal author; and a declaration of any and all sources of funding.

Abstract: The abstract should present the information in such a way that the reader can easily understand without referring to the main text. Abstracts should not exceed 250 words. Abstracts should be structured as follows: Objective, Methods, Results and Conclusion. Abstracts for review articles and case reports may be unstructured.

Abstracts for brief communications should not exceed 100 words.

Summary: An abstract in English, corresponding in content to the abstract in Portuguese, should be included.

Keywords: Three to six keywords in Portuguese defining the subject of the study should be included as well as the corresponding keywords in English. Keywords in Portuguese must be based on the Descritores em Ciência da Saúde (DeCS, Health and Science Keywords), published by Bireme and available at: http://decs.bvs.br, whereas keywords in English should be based on the National Library of Medicine Medical Subject Headings (MeSH), available at: http://www.nlm.nih.gov/mesh/MBrowser.html.

Text:

Original articles: For original articles, the text (excluding the title page, abstracts, references, tables, figures and figure legends) should consist of 2000 to 3000 words. Tables and figures should be limited to a total of five. The

number of references should not exceed 30. Original articles should be divided into the following sections: Introduction, Methods, Results, Discussion, Acknowledgments, and References. The Methods section should include a statement attesting to the fact the study has been approved by the ethics in human research committee or the ethics in animal research committee of the governing institution. There should also be a section describing the statistical analysis employed, with the respective references. In the Methods and Results sections, subheadings may be used, provided that they are limited to a reasonable number. Subheadings may not be used in the Introduction or Discussion.

Review and Update articles: Review and Update articles are written at the request of the Editorial Board, which may occasionally accept unsolicited manuscripts that are deemed to be of great interest. The text should not exceed 5000 words, excluding references and illustrations (figures or tables). The total number of illustrations should not exceed eight. The number of references should not exceed 60.

Pictorial essays: Pictorial essays are also submitted only at the request of the Editors or after the authors have consulted and been granted permission by the Editorial Board. The text accompanying such essays should not exceed 3000 words, excluding the references and tables. No more than 12 illustrations (figures and tables) may be used, and the number of references may not exceed 30.

Case Reports: Case Reports should not exceed 1500 words, excluding title page, abstract, references and illustrations. The text should be composed of: Introduction, Case Report, Discussion and References. It is recommended that any and all information that might identify the patient be withheld, and that only those laboratory exams that are important for the diagnosis and discussion be presented. The total number of illustrations (figures or tables) should not exceed three, and the number of references should be limited to 20. When the number of cases presented exceeds three, the manuscript will be classified as a Case Series, and the same rules applicable to an original article will be applied.

Brief Communications: Brief communications should not exceed 1500 words, excluding references and tables. The total number of tables and figures should not exceed two, and the references should be limited to 20. The text should be unstructured.

Letters to the Editor: Letters to the Editor should be succinct original contributions, not exceeding 800 words and containing a maximum of 6 references. Comments and suggestions related to previously published materials or to any medical theme of interest will be considered for publication.

Tables and Figures: All tables and figures should be in black and white, on separate pages, with legends and captions appearing at the foot of each. All tables and figures should be submitted as files in their original format. Tables should be submitted as Microsoft Word files, whereas figures should be submitted as Microsoft Excel, TIFF or JPG files. Photographs depicting surgical procedures, as well as those showing the results of exams or biopsies, in which staining and special techniques were used will be considered for publication in color, at no additional cost to the authors. Dimensions, units and symbols should be based on the corresponding guidelines set forth by the Associação Brasileira de Normas Técnicas (ABNT, Brazilian Association for the Establishment of Technical Norms), available at: http://www.abnt.org.br.

Legends: Legends should accompany the respective figures (graphs, photographs and illustrations) and tables. Each legend should be numbered with an Arabic numeral corresponding to its citation in the text. In addition, all abbreviations, acronyms, and symbols should be defined below each table or figure in which they appear.

References: References should be listed in order of their appearance in the text and should be numbered consecutively with Arabic numerals. The presentation should follow the Vancouver style, updated in October of 2004, according to the examples below. The titles of the journals listed should be abbreviated according to the style presented by the List of Journals Indexed in the Index Medicus of the National Library of Medicine, available at: http://www.ncbi.nlm.nih.gov/entrez/journals/loftext.noprov.html. A total of six authors may be listed. For works with more than six authors, list the first six, followed by 'et al.'

Examples:

Journal Articles

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

Abstracts

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

Chapter in a Book

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

Official Publications

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

Theses

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

Electronic publications

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

Homepages/URLs

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

Other situations:

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

All correspondence to the Jornal Brasileiro de Pneumologia should be addressed to:

Prof. Dr. Carlos Roberto Ribeiro de Carvalho
Editor-Chefe do Jornal Brasileiro de Pneumologia
SCS Quadra 01, Bloco K, Salas 203/204 - Ed. Denasa.
CEP: 70.398-900 - Brasília - DF, Brazil
Telefones/Fax: 0xx61-3245-1030, 0xx61-3245-6218

Jornal Brasileiro de Pneumologia e-mail address:

jpneumo@jornaldepneumologia.com.br
(Editorial assistant: Luana Campos)

Online submission of articles:

www.jornaldepneumologia.com.br

Eventos 2014

NACIONAIS

Simpósio Mineiro de Atualização em Pneumologia
30 e 31 de maio
Belo Horizonte

VIII Fórum Norte Nordeste de DPOC
15 e 16 de agosto
Maceió - AL

1 Jornada de Medicina do Sono
13 de setembro
São Paulo - SP

XXXVI Congresso Brasileiro de Pneumologia e Tisiologiaa
Data: 07 a 11 de outubro de 2014 Local:
Expogramado, Gramado/RS
Informações: Secretaria da SBPT
Portal: www.sbpt.org.br

INTERNACIONAIS

ALAT 2014
31/07 a 02 de agosto
Plaza Mayor, Medellin, Colombia

ERS 2014
06 a 10 de setembro
Munique - Alemanha

Chest 2014
25 a 30 de outubro
Austin - Texas - EUA

XXX Congresso Português de Pneumologia VIII Congresso Luso-Brasileiro de Pneumologia
06 a 09 de novembro
Lisboa - Portugal

IV International Pediatric Sleep Association Congress - IPSA 2014
03 a 05 de dezembro
Porto Alegre/RS - Brasil

SBPT2014

XXXVII Congresso Brasileiro de Pneumologia e Tisiologia
XIII Congresso Brasileiro de Endoscopia Respiratória

De 07 a 11 de outubro de 2014 - Expogramado

Gramado – RS

Nosso Próximo grande encontro já tem data marcada...

Realização

SOCIEDADE BRASILEIRA DE PNEUMOLOGIA E TISIOLOGIA · 1937 ·

Nos vemos em Gramado!

Vannair®
fumarato de formoterol diidratado/budesonida

ÚNICO SPRAY
formoterol/budesonida [1]

FAZBEM
O programa de saúde e bem-estar
0800 014 5578 (opção 2)
www.programafazbem.com.br

- Controle **RÁPIDO** e **SUSTENTADO** da Asma.[1,2]
- **ALCANCE DAS PEQUENAS VIAS AÉREAS,** 50% a 70% de partículas finas.[3]
- **NÃO PRECISA** ser conservado em geladeira.

Referências bibliográficas: 1. Morice AH, Peterson S, Beckman O, Kukova Z. Efficacy and safety of a new pressurised metered-dose inhaler formulation of budesonide/formoterol in children with asthma: a superiority and therapeutic equivalence study. Pulm Pharmacol Ther.2008;21(1):152-9. **2.** Noonan M, Rosenwasser LJ, Martin P, O'Brien CD, O'Dowd L. Efficacy and safety of budesonide and formoterol in one pressurised metered-dose inhaler in adults and adolescents with moderate to severe asthma: a randomised clinical trial. Drugs.2006;66(17):2235-54. **3.** Chambers F, Ludzik A. *In vitro* drug delivery performance of a new budesonide/formoterol pressurized metereddose inhaler. Journal of aerosol medicine and pulmonary drug delivery. 2009 Jun;22(2):113-20.

VANNAIR® 6/100 mcg/inalação e VANNAIR® 6/200 mcg/inalação (fumarato de formoterol di-hidratado/budesonida) VANNAIR® (fumarato de formoterol di-hidratado/budesonida) é composto por substâncias que possuem diferentes modos de ação e que apresentam efeitos aditivos em termos de redução da asma do que outros produtos isoladamente. A budesonida é um glicocorticosteróide que tem uma rápida (dentro de horas) e dose-dependente ação antiinflamatória nas vias aéreas e o formoterol é um agonista beta-2-adrenérgico seletivo de início de ação rápido (1-3 minutos) e de longa duração (pelo menos 12 horas). **Indicações: VANNAIR** está indicado no tratamento da asma nos casos em que o uso de uma associação (corticosteróide inalatório com um beta-2 agonista de ação prolongada) é apropriado. **Contra-indicações: Hipersensibilidade a budesonida, ao formoterol ou a outros componentes da fórmula. Cuidados e Advertências:** Advertências: É recomendado que a dose seja titulada quando o tratamento de longo prazo é descontinuado e este não deve ser interrompido abruptamente. Para minimizar o risco de candidíase orofaríngea, o paciente deve ser instruído a lavar a boca com água após administrar as inalações de **VANNAIR**. Uma deterioração súbita e progressiva do controle da asma é um risco potencial e o paciente deve procurar suporte médico.Pacientes que necessitaram de terapia corticosteróide de alta dose emergencial ou tratamento prolongado de altas doses recomendadas de corticosteróides inalatórios podem exibir sinais e sintomas de insuficiência adrenal quando expostos a situações de estresse grave. Administração de corticosteróide sistêmico adicional deve ser considerada durante situações de estresse ou cirurgia eletiva. **VANNAIR** deve ser administrado com cautela em pacientes com graves alterações cardiovasculares (incluindo anomalias do ritmo cardíaco), *diabetes mellitus*, hipocalemia não tratada ou tireotoxicose. Pacientes com prolongamento do intervalo QTc devem ser cuidadosamente observados (para maiores informações vide bula completa do produto). *Uso durante a gravidez e a lactação:* categoria C de risco de gravidez. Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. A administração de **VANNAIR** em mulheres lactantes deve ser apenas considerada se os benefícios esperados para a mãe superarem qualquer possível risco para a criança (para maiores informações vide bula completa do produto). Interações medicamentosas: o metabolismo da budesonida é mediado principalmente pela CYP3A4, uma subfamília do citocromo P450. Portanto, inibidores desta enzima, como o cetoconazol ou suco de *grapefruit* (pomelo), podem aumentar a exposição sistêmica à budesonida. A cimetidina apresenta um leve efeito inibidor sobre o metabolismo hepático da budesonida. Fármacos como a procainamida, fenotiazina, agentes antihistamínicos (terfenadina), inibidor da monoaminooxidase (MAO) e antidepressivos tricíclicos foram relacionados com um intervalo QTc prolongado e um aumento do risco de arritmia ventricular. **Os bloqueadores beta-adrenérgicos (incluindo os colírios oftálmicos) podem atenuar ou inibir o efeito do formoterol** (para maiores informações vide bula completa do produto). **Reações adversas:** as reações adversas que foram associadas à budesonida ou ao formoterol são apresentadas a seguir. *Comum*: palpitações, candidíase na orofaringe, cefaléia, tremor, leve irritação na garganta, tosse, rouquidão. *Incomum*: taquicardia, náusea, câibras musculares, tontura, agitação, ansiedade, nervosismo eperturbações do sono. (para outras reações adversas, vide bula completa do produto). **Posologia:** a dose de **VANNAIR** deve ser individualizada conforme a gravidade da doença. Quando for obtido o controle da asma, a dose deve ser titulada para a menor dose que permita manter um controle eficaz dos sintomas. **VANNAIR® 6/100 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Crianças (6-11 anos)*: 2 inalações duas vezes ao dia. Dose máxima diária: 4 inalações. **VANNAIR® 6/200 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Instruções de Uso*: vide bula completa do produto. **Superdose:** A superdosagem de formoterol irá provavelmente provocar efeitos típicos dos agonistas beta-2-adrenérgicos: tremor, cefaléia, palpitações e taquicardia. Poderá igualmente ocorrer hipotensão, acidose metabólica, hipocalemia e hiperglicemia. Pode ser indicado um tratamento de suporte e sintomático. A administração de uma dose de 90 mcg durante três horas em pacientes com obstrução brônquica aguda e quando administrada três vezes ao dia como um total de 54 mcg/dia por 3 dias para a estabilidade asmática não suscitou quaisquer problemas de segurança. Não é esperado que uma superdosagem aguda da budesonida, mesmo em doses excessivas, constitua um problema clínico. Quando utilizado cronicamente em doses excessivas, podem ocorrer efeitos glicocorticosteróides sistêmicos (para informações de superdosagem grave vide bula completa do produto). **Apresentações: VANNAIR® 6/100 mcg/inalação:** Aerossol bucal 6/100 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO E PEDIÁTRICO. VANNAIR® 6/200 mcg/inalação:** Aerossol bucal 6/200 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO. USO POR INALAÇÃO ORAL. VENDA SOB PRESCRIÇÃO MÉDICA.** Para maiores informações, consulte a bula completa do produto. (VAN005). AstraZeneca do Brasil Ltda., Rod. Raposo Tavares, Km 26,9 - Cotia - SP - CEP 06707-000 Tel.: 0800-0145578. www.astrazeneca.com.br **Vannair®. MS – 1.1618.0234**

VAN.13.J.214 - Produzido em Outubro/2013

SAC Serviço de Atendimento ao Cliente 0800 014 5578

AstraZeneca do Brasil Ltda.
Rod. Raposo Tavares km 26,9
06707-000 Cotia SP Brasil
Access *net* / SAC 0800 014 55 78
www.astrazeneca.com.br

Access net 0800 014 5577
access@astrazeneca.com

Material destinado ao profissional da saúde.

AstraZeneca RESPIRATÓRIA