ISSN 1806-3713

Jornal Brasileiro de Pneumologia

Published once every two months J Bras Pneumol. v.40, number 2, p. 99-206 March/April 2014

PUBLICAÇÃO OFICIAL DA SOCIEDADE BRASILEIRA DE PNEUMOLOGIA E TISIOLOGIA

HIGHLIGHT

Experimentation with water-pipe tobacco smoking among medical students

Editorial:
Jaqueline Scholz Issa e Gabriel Magalhães Lopes

Jornal Brasileiro de Pneumologia

March/April 2014 volume 40, number 2

p.99-206

ISSN 1806-3713

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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

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ISSN 1806-3713

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Much more than cigarette smoking

Muito além do tabaco

Jaqueline Scholz Issa, Gabriel Magalhães Lopes

The human condition made fragile by fear of death, by affective distress, by diseases, and by aging compels human beings to search for pleasure. The use of licit or illicit drugs is a good example of this fact. Often, this occurs in such an intense way that it condemns individuals to premature death or a subjugated life. To compound this situation, Western society is continuously bombarded by advertisements that encourage the consumption of products. Consumption becomes synonymous with happiness.

The tobacco industry may have been the pioneer in exploiting and addressing this aspect in cigarette advertising,[1] being responsible for inducing billions of users to dependence on the product and, consequently, to premature death and loss of quality of life, placing an unprecedented burden on health care systems worldwide. The consequence of this tobacco epidemic was the adoption of restrictive measures to reduce consumption, and this motivated the tobacco industry to search for products that would maintain nicotine dependence, despite being labeled as "less harmful to health" than typical cigarettes.[2] In this context, products such as hookah or narghile, and, more recently, electronic cigarettes, began to be promoted as harmless, despite there being no scientific support for it. The evidence that there are fewer toxic substances in these alternative forms of tobacco than in conventional cigarettes has been used and exploited as an argument in support of the former being harmless. In fact, however, there are not sufficient data to establish that the use of these substances at lower concentrations, but in a continuous, endless way because of the presence of nicotine vapor, is harmless.

At the epicenter of this discussion, this issue of the Brazilian Journal of Pulmonology features an original article by Martins et al.[3] on experimentation with narghile, the prevalence of its use, and the level of knowledge on the subject among third- and sixth-year medical students at the University of São Paulo School of Medicine, in the city of São Paulo, Brazil, between 2008 and 2013. Although most students recognized the potential health risk from the use of narghile, either because of the risk of contamination with bacteria and viruses or because of the very potential for nicotine dependence resulting from the use of the product, in addition to high exposure to elevated carbon monoxide levels, this knowledge did not prevent more than 40% of the male students from experimenting with it. The low prevalence of cigarette use among third- and sixth-year medical students (9.78% and 5.26%, respectively, among males, and 1.43% and 2.65%, respectively, among females) clearly shows the effectiveness of public policies discouraging smoking initiation, implemented more than 20 years ago in Brazil. On the other hand, it shows the reach of the marketing and advertising campaigns disseminating the use of alternative forms of tobacco.

The reduction in smoking in Brazil in the last 5 years has been gradual and constant among men, with smoking rates ranging from 21% in 2008 to 18% in 2012.[4] Among women, smoking rates decreased and have remained stable since 2008, varying between 12% and 13%. Currently, there are more former smokers than smokers in Brazil, and they represent 22% of the population. In a systematic review of the literature on the use of alcohol and tobacco among adolescents aged 10 to 19 years, Barbosa et al.[5] found that the prevalence of current tobacco use (use in the study period or in the previous month) ranged from 2.4% to 22.0%, with a mean of 9.3%, in Brazil. Although the risk of tobacco use initiation by adolescents is higher when their parents smoke, there are other family-related factors, such as poor parental practices and poor behavioral control of offspring.[6] In addition to the implications of tobacco use on adolescent health, it is of note that the combined use of tobacco and marijuana is high.

The tobacco industry knows that it has factors working in its favor that greatly facilitate experimentation with any product containing nicotine among young people, including, first of all, curiosity, as well as the symbolic power of such experimentation as a rite of passage,

the immaturity of brain structures involved in inhibiting impulsiveness, the influence of peers, and a desire for confrontation, that is, a set of factors that make young people particularly vulnerable to being seduced by the fascination of experimenting with and using drugs.

It is relevant to highlight the importance of cultural and media influence on young people, since adolescence is a crucial stage in the development of personality and individuality. At this stage, adolescents are very influenceable, and marketing and advertising purposely target young people. Studies have shown that cigarette advertising has a strong influence on tobacco use by young people.[7] The release of formerly secret documents of the tobacco industry obtained through litigation in the USA has confirmed the industry's strategy of aiming cigarette advertising primarily at children and adolescents.

The result is that new experiences tend to be lived to the fullest and the use of alcohol, cigarettes, and other drugs is a frequent behavior, which makes this period of life crucial to most studies on and programs for prevention of substance dependence. Approximately 72% of adolescents in the USA report having experimented with alcohol; in Brazil, the prevalence is as high as 84.3% among students aged 17-18 years.[8] With regard to cigarettes, the prevalence of ever smoking among adolescents in the USA is 43.6%, and, in Brazil, the prevalence is 32.1% among 18-year-olds.

In 2009, according to the National School Heath Survey,[9] 24.2% of the ninth graders had experimented with cigarettes and 6.3% smoked regularly. There were no differences between boys and girls. However, tobacco use was higher in public schools (26.4%) than in private schools (18.3%).

Environmental factors are important risk factors for experimentation with and maintenance of alcohol and tobacco use in adolescence, as well as for progression to other drugs.[10] Protective factors have not been studied as extensively as have risk factors. The major protective factors against alcohol and cigarette use include ability to face and overcome problems, especially in females; religiousness; having at least one meal together as a family on most days; and parental or guardian knowledge about what adolescents have been doing in their free time in the last 30 days. Effective parental monitoring seems to be the strongest protective factor against the use of alcohol, tobacco, and other drugs in adolescence and can be the basis for the aforementioned protective factors. Likewise, the family can be one of the most important risk factors for alcohol or tobacco use initiation in adolescence, when adolescents have parents who use alcohol or tobacco, as well as when there is family breakdown and a poor relationship with parents.

In Brazil, cigarette advertising was banned in 2000. This contributed greatly to the reduction in the prevalence of smoking in the country. Studies have shown that adolescents who are widely exposed to these advertisements are the same ones who like them and who end up using these drugs frequently, that is, advertising does encourage consumption and contribute to the increase in the rates of all unfavorable outcomes of this consumption.[7]

There is considerable debate about the role of alternative (smokeless) tobacco products in reducing the harmful health effects of tobacco use. With regard to the use of such products, one should consider the Swedish experience with snus, a sublingual nicotine tablet adopted as an alternative to conventional cigarettes. A review on the use of snus suggests that it is less harmful to health than are cigarettes, reducing the risk of cardiovascular, respiratory and neoplastic diseases.[11]

Some of the global scientific community believes in the adoption of a regulatory policy that would discourage the use of the most harmful nicotine products[12] (tobacco-burning cigarettes or products) in favor of products that are much less harmful, such as snus and, possibly, electronic cigarettes.[13] Electronic cigarettes, because of their similarity to conventional cigarettes, have been shown to be the most attractive form of nicotine delivery to users, considering that worldwide consumption and sales have reached alarming figures in countries where their marketing is permitted. In Brazil, their marketing is prohibited. In the USA, this is already the preferred form of experimentation of 25% of young Americans.

The fact is that human frailty makes us vulnerable to using substances with psychoactive effects and that only cumulative scientific evidence allows the choice of public policies that can protect society from vulnerability and make life

in society a reasonable bargain without it being degraded.

A society built upon a model of reckless and irrational consumption is, of course, vulnerable to drug use and the whole burden of this condition. Only a comprehensive, detailed analysis of the issue will allow the adoption of public policies that can minimize the impact of drug use and that are effective in reducing risk exposure, considering the vulnerability of the human condition.

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Experimentation with and knowledge regarding water-pipe tobacco smoking among medical students at a major university in Brazil*,**

Experimentação de e conhecimento sobre narguilé entre estudantes de medicina de uma importante universidade do Brasil

Stella Regina Martins, Renato Batista Paceli, Marco Antônio Bussacos, Frederico Leon Arrabal Fernandes, Gustavo Faibischew Prado, Elisa Maria Siqueira Lombardi, Mário Terra-Filho, Ubiratan Paula Santos

Abstract

Objective: Water-pipe tobacco smoking is becoming increasingly more common among young people. The objective of this study was to estimate the prevalence of the use of water pipes and other forms of tobacco use, including cigarette smoking, among medical students, as well as to examine the attitudes, beliefs, and knowledge of those students regarding this issue. **Methods:** We administered a questionnaire to students enrolled in the University of São Paulo School of Medicine, in São Paulo, Brazil. The respondents were evaluated in their third and sixth years of medical school, between 2008 and 2013. Comparisons were drawn between the two years. **Results:** We evaluated 586 completed questionnaires. Overall, the prevalence of current cigarette smokers was low, with a decline among males (9.78% vs. 5.26%) and an increase among females (1.43% vs. 2.65%) in the 3rd and 6th year, respectively. All respondents believed that health professionals should advise patients to quit smoking. However, few of the medical students who smoked received physician advice to quit. Experimentation with other forms of tobacco use was more common among males ($p<0.0001$). Despite their knowledge of its harmful effects, students experimented with water-pipe tobacco smoking in high proportions (47.32% and 46.75% of the third- and sixth-year students, respectively). **Conclusions:** The prevalence of experimentation with water-pipe tobacco smoking and other forms of tobacco use is high among aspiring physicians. Our findings highlight the need for better preventive education programs at medical schools, not only to protect the health of aspiring physicians but also to help them meet the challenge posed by this new epidemic.

Keywords: Tobacco products; Smoking/prevention & control; Education, medical, undergraduate; Health knowledge, attitudes, practice.

Resumo

Objetivo: O fumo de narguilé com tabaco está aumentando entre os jovens. O objetivo deste trabalho foi estimar a prevalência do uso de narguilé e outras formas de consumo de tabaco, incluindo o fumo de cigarros, entre estudantes de medicina, assim como as atitudes, crenças e conhecimento desses alunos sobre esse assunto. **Métodos:** Um questionário foi aplicado aos estudantes da Faculdade de Medicina da Universidade de São Paulo. Os entrevistados eram alunos de terceiro e sexto anos entre 2008 e 2013. As respostas foram comparadas entre os dois anos de graduação. **Resultados:** 586 estudantes responderam ao questionário. A prevalência de fumantes foi baixa, com um declínio entre os homens (9,78% contra 5,26%) e um aumento no sexo feminino (1,43% contra 2,65%) no 3º e 6º ano, respectivamente. Todos os entrevistados acreditavam que profissionais de saúde devem aconselhar os pacientes a parar de fumar. No entanto, a maioria dos estudantes de medicina fumantes não recebeu aconselhamento médico para deixar de fumar. A experimentação de outros produtos derivados do tabaco foi maior entre os homens ($p < 0.0001$). Apesar do conhecimento de seus efeitos nocivos à saúde, a experimentação de narguilé foi alta (47,32% e 46,75% entre alunos do terceiro e sexto anos, respectivamente. **Conclusões:** A prevalência da experimentação de narguilé com tabaco e de outras formas de uso de tabaco é alta entre os futuros médicos. Nossos achados enfatizam a necessidade de melhores programas de educação preventiva em universidades médicas para proteger a saúde dos futuros médicos e para ajudá-los a enfrentar esse novo desafio epidêmico.

Descritores: Produtos do tabaco; Hábito de fumar/prevenção & controle; Educação de graduação em medicina; Conhecimentos, atitudes e prática em saúde.

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*Study carried out under the auspices of the Smoking Cessation Program, Pulmonary Division, Heart Institute, University of São Paulo School of Medicine *Hospital das Clínicas*, São Paulo, Brazil.

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Financial support: None
Submitted: 9 December 2013. Accepted, after review: 12 March 2014.

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

Introduction

The water pipe used for smoking tobacco was invented in India during the reign of Emperor Akbar (1556-1605) by a physician named Hakim Abul Fath, who suggested that if tobacco smoke passed through a small receptacle of water before being inhaled it would have fewer ill effects on human health. That historical account might be responsible for the current belief that such a water pipe (now known by various names, including narghile, hookah, shisha, and hubble-bubble) is a less harmful way to smoke tobacco. That belief is reinforced by irresponsible marketing practices. For example, the label of a popular brand of water-pipe tobacco available in southwest Asia and North America states "0% tar and 0.5% nicotine".[1] In addition to that false sense of safety, reasons for the worldwide spread of the use of water pipes might include increased awareness of the negative health effects of cigarette smoking and the pleasing social interaction that comes with water-pipe tobacco smoking sessions. However, water-pipe tobacco smokers usually share the same mouthpiece (passing it from person to person), which can facilitate the spread communicable diseases, such as colds, respiratory infections, tuberculosis, hepatitis, and herpes. There have been reports of drug-resistant tuberculosis being transmitted via water-pipe tobacco smoking.[2-5]

Because of the quick-light charcoal used in water-pipe tobacco smoking, the average carbon monoxide-nicotine ratio in water-pipe smoke is 50:1, compared with 16:1 in cigarette smoke.[6] Among water-pipe tobacco smokers, there have been reports of carbon monoxide poisoning, manifesting as headache, dizziness, nausea, and weakness, followed by syncope.[7] The truth is that water-pipe smokers are exposed to many hazardous substances. In 2010, Akl et al.[8] conducted a systematic review of 24 studies of the health effects of water-pipe tobacco smoking. The authors found that water-pipe tobacco smoking was significantly associated with lung cancer (OR = 2.12; 95% CI: 1.32-3.42) and respiratory illness (OR = 2.3; 95% CI: 1.1-5.1).

One critical point is that the smoking control community will need to counter the current erroneous argument that water-pipe tobacco smoking has fewer ill effects on human health than does cigarette smoking. In 2009, the state of São Paulo, Brazil, enacted Law no. 13779, which prohibited the sale of water pipes to minors (individuals < 18 years of age).[9] Nevertheless, there is a need for additional public health campaigns advising water-pipe tobacco smokers of the health risks to which they are exposing themselves.

As aspiring physicians, medical students might eventually play an important role in shaping smoking control policies. Therefore, it is important that such students are aware of the myths and realities regarding the use of the water pipes. However, there have been few studies of the prevalence of water-pipe tobacco smoking among medical students. In addition, there are few data related to the knowledge, beliefs, and attitudes of such students regarding this subject.

The purpose of this study was to estimate the prevalence of experimentation with water-pipe tobacco smoking, as well as with other forms of tobacco use, including cigarette smoking, cigar/cheroot smoking, pipe smoking, and the use of smokeless tobacco products (chewing tobacco and snuff), among third- and sixth-year medical students. An additional objective was to evaluate attitudes, beliefs, and knowledge of those students regarding the various forms of tobacco use.

Methods

Medical students at the *Faculdade de Medicina da Universidade de São Paulo* (FMUSP, University of São Paulo School of Medicine) were asked to complete a structured questionnaire regarding their smoking habits. The questionnaire was composed of questions from the Global Health Professions Student Survey[10] and additional modules. The respondents were third- and sixth-year students who were present during regular medical school classes. The questionnaire was administered to students in the second semester of their third year and to the same class of students in the second semester of their sixth year. Three classes of students were evaluated: those in their third year in 2008 and in their sixth year in 2011; those in their third year in 2009 and in their sixth year in 2012; and those in their third year in 2010 and in their sixth year in 2013. The questionnaire was completed on a voluntary basis, and all participating students gave written informed consent. The study was approved by the Research Ethics Committee of the FMUSP *Hospital das Clínicas*.

Water-pipe tobacco smoking and other forms of tobacco smoking were defined as ever having

taken at least a few puffs. Students who had smoked 100 or more cigarettes in their lifetime and were currently smoking were classified as cigarette smokers. Chart 1 shows the questionnaire used in the present study.

Descriptive statistics were calculated. We used Pearson's chi-square test or Fisher's exact test for statistical analyses comparing the proportions of positive responses between the two medical school years. Values of $p < 0.05$ were considered statistically significant. Data were analyzed with the Statistical Analysis System, version 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

We evaluated 586 questionnaires, completed by third-year medical students (n = 335) and sixth-year medical students (n = 251). In medical schools in Brazil, the sixth year is the clinical internship year. During that phase, classes are held less often, which could explain the relatively low number of respondents among the sixth-year students.

The mean ages of the third-year and sixth-year students were 22.0 ± 2.76 years and 24.0 ± 1.94 years, respectively. The prevalence of cigarette smoking was significantly higher among male medical students in their third year than among their sixth-year counterparts (Table 1). Table 1 shows that other forms of tobacco use were significantly more common among male students than among female students, in both of the medical school years evaluated ($p < 0.0001$ for both years). Experimentation with water-pipe tobacco smoking was also more common among the male students (Table 1). The overall prevalence of cigarette smoking among the respondents was quite low and was even lower among the female respondents. The form of tobacco use for which the prevalence was highest was water-pipe smoking (47.32% and 46.75% among the third- and sixth-year students, respectively). As can be seen in Table 1, approximately 40% and 53% of the female and male students, respectively, had experimented with water-pipe tobacco smoking by their third year of medical school ($p < 0.005$ between the genders).

According to their responses on the questionnaire, all of the students who were cigarette smokers in their third or sixth year of

Chart 1 - Questionnaire used in the study.

Questionnaire Please answer Yes or No.
1. Do you smoke cigarettes?
2. Have you ever used other tobacco products (cigars, pipe tobacco, cheroots, chewing tobacco, or snuff)?
3. Have you ever smoked tobacco from a water pipe?
4. Should health professionals routinely advise their smoking patients to quit?
5. Does the likelihood that smokers will quit increase if a health professional advises them to do so?
6. Are health professionals who smoke cigarettes less likely to advise their cigarette-smoking patients to quit?
7. Is smoking a pipe, cigar, or cheroot less harmful, because people puff less or don't inhale?
8. Is cigar or pipe smoke less harmful than cigarette smoke, because it has fewer additives?
9. Should health professionals routinely advise patients to avoid other forms of tobacco use?
10. Do health professionals serve as role models for their patients and the public?
11. Does water-pipe tobacco smoking have fewer harmful health effects, because impurities in the smoke are filtered out through the water bowl?

medical school believed that health professionals should advise their patients who smoke to quit smoking. Table 2 shows that the majority of the respondents believe that the likelihood of smokers quitting increases if they are advised to do so by health professionals. However, most of aspiring physicians who were cigarette smokers were not advised to quit by a health professional: 15 (79%) of the 19 smokers evaluated in their third year; and 8 (89%) of the 9 smokers evaluated in their sixth year.

Health care professionals who smoke cigarettes are less likely to advise their cigarette-smoking patients to quit—that was the belief of 64.5% and 71.6% of the non-cigarette smoking medical students in their third and sixth years, respectively. However, among the smoking students, the proportion of who believed that health professionals who smoke cigarettes are less likely to advise their cigarette-smoking patients to quit increased from 30% in the third year to 50% in the sixth year (Table 2).

Table 3 shows that only a minority of the respondents believed that cigar, pipe, and cheroot smoking is less harmful because smokers puff less or do not inhale. Among the third-year students evaluated, the erroneous belief that cigar and pipe smoking is less harmful because the tobacco involved has lower concentrations of additives was held by 8.33% and 19.01% of the ever-users and never-users of tobacco products other than cigarettes and water-pipe tobacco (p

Table 1 - Prevalence of the various forms of tobacco use among medical students in their third year (in 2008, 2009, or 2010) and sixth year (in 2011, 2012, or 2013), by gender.[a]

Form of tobacco use	Third-year medical students		p*	Sixth-year medical students		p*
	Females	Males		Females	Males	
	n = 146	n = 189		n = 114	n = 137	
	n/N (%)	n/N (%)		n/N (%)	n/N (%)	
Cigarette smoking[b]	2/140 (1.4)	18/184 (9.8)	< 0.001	3/113 (2.7)	7/133 (5.3)	ns
Cigar, pipe, or cheroot smoking,[c] together with tobacco chewing or snuff dipping	16/146 (11.0)	56/189 (30.0)	< 0.0001	13/114 (11.4)	46/137 (33.6)	< 0.0001
Water-pipe tobacco smoking[c]	58/146 (40.0)	101/189 (53.4)	< 0.005	46/113 (40.7)	70/137 (51.0)	ns

n/N: positive responses/total respondents; and ns: not significant. [a]Some denominators vary, because of missing data. [b]Defined as lifetime smoking of ≥ 100 cigarettes and currently describing oneself as a cigarette smoker. [c]Defined as having ever taken one or more puffs. *Pearson's chi-square test.

Table 2 - Attitudes, beliefs, and knowledge regarding cigarette smoking held by medical students in their third year (in 2008, 2009, or 2010) and sixth year (in 2011, 2012, or 2013), by cigarette smoking status.[a]

Question	Third-year medical students		p*	Sixth-year medical students		p*
	Smokers[b]	Nonsmokers		Smokers[b]	Nonsmokers	
	n = 20	n = 324		n = 10	n = 237	
	n/N (%)	n/N (%)		n/N (%)	n/N (%)	
Should health professionals routinely advise their smoking patients to quit?	20/20 (100)	292/304 (96.0)	ns	10/10 (100)	234/237 (99.0)	ns
Does the likelihood that smokers will quit increase if a health professional advises them to do so?	18/20 (90.0)	273/300 (91.0)	ns	10/10 (100)	222/236 (94.0)	ns
Are health professionals who smoke cigarettes less likely to advise their cigarette-smoking patients to quit?	6/20 (30.0)	194/301 (64.4)	< 0.005	5/10 (50.0)	166/232 (71.5)	ns

n/N: positive responses/total respondents; and ns: not significant. [a]Some denominators vary, because of missing data. [b]Defined as lifetime smoking of ≥ 100 cigarettes and currently describing oneself as a cigarette smoker. *Pearson's chi-square test.

< 0.05). The majority of the respondents believed that health professionals should routinely advise their patients not to use any tobacco product (smoked or smokeless).

Table 4 shows that more than 80% of the aspiring physicians evaluated agreed that health professionals occupy a position of leadership and are role models for their patients, as well as for the general population. More than 98% of the respondents knew that impurities in water-pipe tobacco smoke are not filtered out through the water bowl.

Discussion

In the present study, the proportions of self-described cigarette smokers among the respondents were lower than those reported for medical students at other universities in Brazil and abroad, as well as being lower than the current estimated prevalence in the general population of Brazil.[11-14] The National Survey of Health and Nutrition conducted in Brazil in 1989 among smokers > 15 years of age and the Telephone-based System for the Surveillance of Risk and Protective Factors

Table 3 - Comparison between ever-users or never-users of tobacco products other than cigarettes and water-pipe tobacco, in terms of their attitudes, beliefs, and knowledge regarding such products, among medical students in their third year (in 2008, 2009, or 2010) and sixth year (in 2011, 2012, or 2013).[a]

Question	Third-year medical students		p*	Sixth-year medical students		p*
	Ever-user[b]	Never-user		Ever-user[b]	Never-user	
	n = 72	n = 263		n = 59	n = 193	
	n/N (%)	n/N (%)		n/N (%)	n/N (%)	
Is smoking a pipe, cigar, or cheroot less harmful, because people puff less or don't inhale?	6/72 (8.3)	20/263 (7.6)	ns	3/57 (5.2)	6/191 (3.1)	ns
Is cigar or pipe smoke less harmful than cigarette smoke, because it has fewer additives?	6/72 (8.3)	50/263 (19.0)	< 0.05	2/57 (3.5)	20/187 (10.7)	ns
Should health professionals routinely advise patients to avoid other forms of tobacco use?	71/72 (99.0)	248/263 (94.3)	ns	56/59 (95.0)	183/193 (95.0)	ns

n/N: positive responses/total respondents; and ns: not significant. [a]Some denominators vary, because of missing data. [b]Defined as having ever taken one or more puffs from a pipe, cigar, or cheroot; ever having chewed tobacco; or ever having dipped snuff. *Pearson's chi-square test.

Table 4 - Comparison between ever-smokers or never-smokers of water-pipe tobacco, in terms of their attitudes, beliefs, and knowledge about water-pipe tobacco smoking, among medical students in their third year (in 2008, 2009, or 2010) and sixth year (in 2011, 2012, or 2013).[a]

Question	Third-year medical students		p*	Sixth-year medical students		p*
	Ever-smoker[b]	Never-smoker		Ever-smoker[b]	Never-smoker	
	n = 159	n = 176		n = 116	n = 135	
	n/N (%)	n/N (%)		n/N (%)	n/N (%)	
Do health professionals serve as role models for their patients and the public?	131/158 (83.0)	148/176 (84.0)	ns	105/116 (90.5)	119/135 (88.1)	ns
Does water-pipe tobacco smoking have fewer harmful health effects, because impurities in the smoke are filtered out through the water bowl?	2/159 (1.2)	1/175 (0.5)	ns	0/113 (0)	3/131 (2.2)	ns

n/N: positive responses/total respondents; and ns: not significant. [a]Some denominators vary, because of missing data. [b]Defined as having ever taken one or more puffs of water-pipe tobacco smoke. *Pearson's chi-square test.

for Chronic Diseases in the population >18 years of age in 27 Brazilian cities showed that public policies for smoking control led to a drop in the prevalence of smokers from 34.8% in 1989 to 12.0% in 2012, corresponding to a 65.51% decrease.[13,15] It is noteworthy that, in the present study, most of the medical students who smoked reported that they were never advised to quit by a health professional. Medical schools have an ethical responsibility not only to educate but also to raise awareness of health hazards and provide treatment to protect the health of their students.

We found it surprising that, among the medical students evaluated here, experimentation with other forms of tobacco use, such as cigar, pipe, and cheroot smoking, was more common than was cigarette smoking. Experimentation with water-pipe tobacco smoking was critically high among the aspiring physicians at FMUSP, males and females alike. A review of studies on the prevalence of experimentation with water-pipe tobacco smoking among medical students showed that our result (47.0%) is similar to those reported for medical schools in England (51.7%),[16] Canada (40%),[17] and South Africa (43.5%),[18] whereas it is higher than that reported for a medical school in Turkey (28.6%).[19]

Water-pipe tobacco smoking is the first new tobacco trend of the 21st century.[20] It is spreading around the world, having become as fashionable as cigars were in the last century, especially among young professionals and college students.[20] According to Morton et al. (2013), the self-reported prevalence of water-pipe tobacco smoking is highest among the male population of Vietnam (13.02%) and the female population of Russia (3.19%), whereas it remains low in Brazil (0.18% and 0.1% among men and women, respectively).[21] Another possible reason for the spread of water-pipe tobacco smoking is the success of programs to prevent initiation of the (cigarette) smoking habit and to encourage (cigarette) smoking cessation, in Brazil and worldwide. As a result of such anti-smoking campaigns, which target cigarette smokers, susceptible individuals have opted for or migrated to other forms of tobacco use, especially water-pipe smoking.[22]

The way in which a water pipe is smoked is completely different from the way in which a cigarette is smoked. With a water pipe, smokers inhale combustion products from the charcoal used in heating the tobacco as well as the tobacco smoke itself; the smoke is cooled and appears smoother and easier to inhale because it passes through the water bowl. The way in which an individual smokes a water-pipe (frequency of puffing, depth of inhalation, and length of the smoking session) affects the concentrations of toxins absorbed by the smoker. For example, in a typical (one hour) water-pipe tobacco smoking session, a smoker can inhale 100-200 times the volume of smoke inhaled from a single cigarette.[1]

Water pipes use a special type of tobacco that is moistened, and there are various flavors and aromas available, such as apple, mint, cherry, chocolate, coconut, licorice, cappuccino, and watermelon.[1,2] These chemical additives are used by tobacco manufacturers to alter the flavor, and some of them reduce the degree of throat irritation, making the tobacco smoke smoother. That has great appeal to that encourages experimentation by young people, the target population of tobacco industry marketing.[6,20]

The Brazilian *Agência Nacional de Vigilância Sanitária* (ANVISA, National Health Surveillance Agency) regulatory standard designated RDC No. 14 (established 15 March, 2012) bans the use of additives in all tobacco products marketed in Brazil after March 2014. This resolution is an important public health policy measure. By reducing the attractiveness of tobacco, the risk of smoking initiation by children and youths is expected to decrease.[23] However, the tobacco industry responded by claiming that 121 additives are essential to the manufacturing process. Consequently, ANVISA created an exception and exempted those additives for a period of 12 months.[24] During the same period, ANVISA enacted Ordinance No. 1980, which established a working group of experts on food additives, toxicology, pharmacy, cancer, and tobacco control, who were charged with analyzing all of the additives on the list.[25]

Our finding that many smoking and nonsmoking medical students believe that health professionals who smoke cigarettes are less likely to advise their cigarette-smoking patients to quit is supported by the results of other studies.[26,27] In our sample, a minority of the ever-users of tobacco products other than cigarettes and water-pipe tobacco among the third-year students wrongly believed that tobacco products such as cigars, pipe tobacco, and cheroots have fewer additives and therefore

are less harmful than are cigarettes. Only a few students mistakenly believed that the impurities of water-pipe tobacco smoke are filtered out through the water bowl. In the study conducted in Canada,[17] medical students (2.5% and 0.6% of the smokers and nonsmokers, respectively) were also found to hold erroneous beliefs, such as the belief that water-pipe tobacco smoke is less harmful than is cigarette smoke. In the study conducted in Turkey,[19] which evaluated medical and non-medical university students, the authors found that 65.2% of the smokers and 31.0% of the nonsmokers wrongly believed that water-pipe tobacco smoke is less addictive than is cigarette smoke. These populations are at risk because of a lack of knowledge. Therefore, this issue needs to be more widely discussed at universities and should be publicized through the dissemination of public policies on tobacco control.

We found it surprising that, although nearly all of the respondents in our study knew that water-pipe tobacco smoking is harmful, nearly half had experimented with it. The misconceptions that there is no harm in smoking a water pipe occasionally, that it is a safe form of tobacco use, and that the risk of dependence is low, are common among water-pipe tobacco smokers. However, there is now considerable evidence to the contrary.[28] A study conducted in Egypt showed that water-pipe tobacco smokers meet the same criteria for nicotine dependence as do cigarette smokers.[29] Another study employed a 10-item version of the Lebanon Waterpipe Dependence Scale to evaluate adult males who were water-pipe smokers in the United Kingdom.[30] The authors demonstrated that, among such smokers, the risk factors for water-pipe tobacco dependence included being of Arab ethnicity; having a low level of education; having been alone in the last session of smoking; the last session of smoking having been in the home, in a café, or with friends; smoking sessions being longer in duration; and smoking on a daily basis. The diagnostic criteria for nicotine dependence were met by 47% of sample studied by those authors.[30]

In the present study, the form of tobacco use for which the prevalence was highest among the FMUSP medical students, regardless of class year and gender, was water-pipe tobacco smoking. However, the prevalence of cigarette smoking was below the national average. The second highest prevalence was found for the use of other tobacco products (smoked and smokeless, excluding cigarettes and water-pipe tobacco), the prevalence of which was higher among the male students than among the female students.

Almost all of our respondents believed that health professionals should advise their smoking patients to quit, and that the likelihood of smokers quitting increases if a health professional provides such advice. More than half of the nonsmoking respondents believed health professionals who smoke cigarettes are less likely to advise their cigarette-smoking patients to quit. Most of the medical students we evaluated were aware of the dangers of using smoked or smokeless tobacco products. They knew that pipes, cigars, and cheroots are no less harmful than are cigarettes because, contrary to popular belief, such products do not in fact have fewer additives and their users do not inhale less smoke. The medical students evaluated also believed that health professionals should routinely advise their patients to quit. In addition, the majority of the respondents believed that health professionals serve as role models, not only for their patients but also for the general population. A minority of the respondents believed that the impurities of water-pipe tobacco smoke are filtered out through the water bowl, which indicates that the majority had an accurate understanding of the harmfulness of water-pipe tobacco smoking. Despite that knowledge, water-pipe tobacco smoking was relatively popular among the FMUSP medical students evaluated.

The data gathered during this study indicate that medical school curricula should focus greater attention on the hazards of (even sporadic) water-pipe tobacco smoking, as well as taking a more effective approach to the myths and realities regarding this form of tobacco use, in order to prevent occasional smokers from becoming regular users. Such measures could be expected to effect a behavioral change among aspiring physicians, reflected in a decrease in the prevalence of water-pipe tobacco smoking. It is also expected that greater knowledge of the issue will make aspiring physicians more confident and motivated to provide routine guidance to their patients, with the objective of preventing all forms of tobacco use and promoting their cessation. Physicians armed with the requisite knowledge will play an important role in controlling the epidemic of water-pipe tobacco smoking.

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Can bronchodilators improve exercise tolerance in COPD patients without dynamic hyperinflation?*

Os broncodilatadores podem melhorar a tolerância ao exercício na ausência de hiperinsuflação dinâmica em pacientes com DPOC?

Maria Enedina Aquino Scuarcialupi, Danilo Cortozi Berton, Priscila Kessar Cordoni, Selma Denis Squassoni, Elie Fiss, José Alberto Neder

Abstract

Objective: To investigate the modulatory effects that dynamic hyperinflation (DH), defined as a reduction in inspiratory capacity (IC), has on exercise tolerance after bronchodilator in patients with COPD. **Methods:** An experimental, randomized study involving 30 COPD patients without severe hypoxemia. At baseline, the patients underwent clinical assessment, spirometry, and incremental cardiopulmonary exercise testing (CPET). On two subsequent visits, the patients were randomized to receive a combination of inhaled fenoterol/ipratropium or placebo. All patients then underwent spirometry and submaximal CPET at constant speed up to the limit of tolerance (Tlim). The patients who showed ΔIC(peak-rest) < 0 were considered to present with DH (DH+). **Results:** In this sample, 21 patients (70%) had DH. The DH+ patients had higher airflow obstruction and lower Tlim than did the patients without DH (DH–). Despite equivalent improvement in FEV_1 after bronchodilator, the DH– group showed higher ΔIC(bronchodilator-placebo) at rest in relation to the DH+ group ($p < 0.05$). However, this was not found in relation to ΔIC at peak exercise between DH+ and DH– groups (0.19 ± 0.17 L vs. 0.17 ± 0.15 L, $p > 0.05$). In addition, both groups showed similar improvements in Tlim after bronchodilator (median [interquartile range]: 22% [3-60%] vs. 10% [3-53%]; $p > 0.05$). **Conclusions:** Improvement in TLim was associated with an increase in IC at rest after bronchodilator in HD– patients with COPD. However, even without that improvement, COPD patients can present with greater exercise tolerance after bronchodilator provided that they develop DH during exercise.

Keywords: Pulmonary disease, chronic obstructive; Bronchodilator agents; Exercise test; Exercise tolerance; Inspiratory capacity.

Resumo

Objetivo: Investigar os efeitos moduladores da hiperinsuflação dinâmica (HD), definida pela redução da capacidade inspiratória (CI), na tolerância ao exercício após broncodilatador em pacientes com DPOC. **Métodos:** Estudo experimental e randomizado com 30 pacientes com DPOC sem hipoxemia grave. Na visita inicial, os pacientes realizaram avaliação clínica, espirometria e teste de exercício cardiopulmonar (TECP) incremental. Em duas visitas subsequentes, os pacientes foram randomizados para receber uma combinação de fenoterol/ipratrópio ou placebo e, em seguida, realizaram espirometria e TECP com velocidade constante até o limite da tolerância (Tlim). Os pacientes com ΔCI(pico-repouso) < 0 foram considerados com HD (HD+). **Resultados:** Nesta amostra, 21 pacientes (70%) apresentaram HD. Os pacientes HD+ apresentaram maior obstrução ao fluxo aéreo e menor Tlim do que os pacientes sem HD (HD–). Apesar de ganhos equivalentes de VEF_1 após broncodilatador, o grupo HD– apresentou maior ΔCI(broncodilatador-placebo) em repouso em relação ao grupo HD+ ($p < 0,05$). Entretanto, isso não ocorreu com a ΔCI no pico do exercício entre os grupos HD+ e HD– ($0,19 \pm 0,17$ L vs. $0,17 \pm 0,15$ L; $p > 0,05$). Similarmente, ambos os grupos apresentaram melhoras equivalentes do Tlim após broncodilatador (mediana [intervalo interquartílico]: 22% [3-60%] e 10% [3-53%]; $p > 0,05$). **Conclusões:** A melhora da CI em repouso após broncodilatador associou-se com ganho de tolerância ao esforço mesmo nos pacientes com DPOC que não apresentem HD. Por outro lado, pacientes sem melhora da CI em repouso ainda podem obter benefício funcional com o broncodilatador desde que apresentem HD no exercício.

Descritores: Doença pulmonar obstrutiva crônica; Broncodilatadores; Teste de esforço; Tolerância ao exercício; Capacidade inspiratória.

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Financial support: None.
Submitted: 25 October 2013. Accepted, after review: 2 December 2013.

Introduction

Lung hyperinflation is a crucial mechanism of dyspnea on exertion in COPD patients.[1-3] Bronchodilator therapy can reduce static and dynamic lung volumes during exercise, increasing exercise tolerance in such patients.[4,5]

The current concept of the mechanisms whereby bronchodilators can improve exercise tolerance in patients with COPD focuses on the ability to reduce the rate of increase in end-expiratory lung volume (EELV) as the exercise progresses, i.e., a reduction in dynamic hyperinflation (DH).[1,6] In practice, DH can be estimated by serial measurements of inspiratory capacity (IC),[6-8] which reflects EELV, given that TLC does not change significantly with exercise.[9] An alternative (or complementary) mechanism of action of bronchodilators is reduction in operating lung volumes at rest, i.e., pre-exercise deflation.[10,11] In this case, patients can benefit from bronchodilator use even in the absence of DH, given that there are "volume reserves" to be consumed during exercise. In any event, with the use of a bronchodilator, all patients can achieve the same EELV at peak exercise, albeit by different mechanisms (i.e., either by a reduced rate of DH or by reduced static hyperinflation).

Our objective was to investigate whether the administration of a bronchodilator results in improvement in exercise capacity in patients with moderate to severe COPD, despite the fact that bronchodilators act predominantly on exercise-related static hyperinflation or DH. The confirmation of this hypothesis would support the notion that measurements of lung hyperinflation at rest and during exercise are complementary in the evaluation of the effects of bronchodilators on exercise tolerance in such patients.

Methods

We studied a convenience sample of 30 patients diagnosed with COPD in accordance with the Global Initiative for Chronic Obstructive Lung Disease criteria.[12] The patients were over 40 years of age and had a post-bronchodilator FEV_1 < 70% of predicted, an FEV_1/FVC ratio < 70%, and a smoking history of more than 20 pack-years. Patients were recruited from among those treated at the COPD outpatient clinic or pulmonary rehabilitation center of our institution. The exclusion criteria were as follows: severe resting hypoxemia (SpO_2 < 90%); comorbidities contributing to dyspnea and exercise limitation; COPD exacerbation or respiratory infection in the previous month; and contraindication to clinical exercise testing. The study project was approved by the local research ethics committee. All participants gave written informed consent.

At the initial visit, all of the patients who remained eligible after their clinical and functional characteristics had been determined by spirometry performed before and after the administration of 400 μg of inhaled albuterol underwent incremental symptom-limited cardiopulmonary exercise testing (CPET). The patients returned for two more experimental visits (3-7 days apart), during which they randomly received placebo or 0.5 mL of fenoterol hydrobromide (0.5% Berotec®; Boehringer Ingelheim do Brasil, São Paulo, Brazil) with 2 mL of ipratropium bromide (0.025% Atrovent®; Boehringer Ingelheim do Brasil) diluted in 5 mL of saline for nebulization. Within 30 min after nebulization, spirometry was performed, being followed by submaximal CPET at constant speed (i.e., at 70-80% of the maximum speed achieved during incremental CPET at the initial visit). During submaximal CPET at constant speed, serial measurements of IC were made every 2 min (from rest to peak exercise) in order to assess operating lung volumes during exercise. The study design is shown in Figure 1.

All spirometric tests were performed with a Koko PFT® spirometer (PDS Instrumentation, Inc., Louisville, CO, USA). The variables measured were FVC, FEV_1, FEV_1/FVC, and IC. Maximal voluntary ventilation was estimated by multiplying FEV_1 by 37.5.[13] Participants completed at least three slow, forced expiratory maneuvers, considered acceptable and reproducible.

CPET was performed with the patients connected to a Vmax 229c™ system (Vyasis, Yorba Linda, CA, USA) via a face mask and walking on an ATL treadmill (Inbrasport, Porto Alegre, Brazil). During incremental CPET, after 2 min at a constant speed of 1.6 km/h without inclination, the speed was increased every 1 min by 0.3 km/h, 0.5 km/h, or 0.8 km/h depending on the functional capacity of the patient, as determined by the examiner prior to the test. During the tests, the patients were instructed to hold the side bars only when needed (dizziness and loss

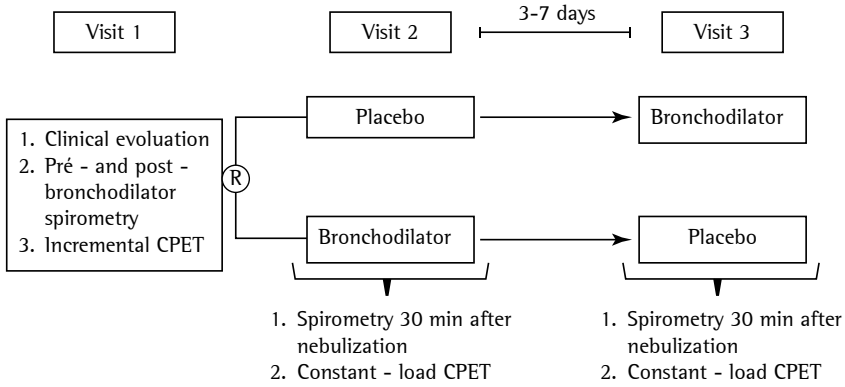

Figure 1 - Study design. BD: bronchodilator; CPET: cardiopulmonary exercise testing; and R: randomization.

of balance, among others). During submaximal CPET at constant speed, after a 2-min warm-up phase, the work rate was suddenly increased to a speed corresponding to 70-80% of the maximum speed achieved during incremental CPET, and the patients were encouraged to walk until they reached their limit of tolerance (Tlim, s). At the end of the initial phase and every 2 min during the tests, the patients were asked about the intensity of dyspnea and leg fatigue, by means of the modified Borg scale.[14]

The following variables were measured (breath by breath) and expressed as mean 15-s time: oxygen consumption, in mL/min, under standard temperature, pressure dry conditions; minute ventilation, in L/min, under body temperature, pressure saturated conditions; tidal volume, in L; and RR, in breaths/min. The R-R interval on a 12-lead electrocardiogram was used in order to determine HR (in bpm), and pulse oximetry with an Onyx 9500™ pulse oximeter (Nonin, Plymouth, MN, USA) was used in order to estimate SpO_2. We evaluated the dynamic changes in operating lung volumes by serial measurements of IC, assuming that TLC remained constant during exercise.[9] During submaximal CPET at constant speed, two IC maneuvers were performed at rest, at the end of the initial period and every 2 min after the beginning of the constant speed test, in order to obtain reproducible values (< 10% difference in relation to the highest value, at each stage). In one of the visits after the administration of placebo (the second or third visit, depending on the randomization), the patients in whom IC at peak exercise was reduced in comparison with IC at rest were included in the DH+ group.[15] A standardized time point near the end of the test marked "isotime", which was defined as the longest exercise duration common to the two submaximal cardiopulmonary exercise tests performed at constant speed by a given individual.

The data are presented as mean and standard deviation for variables with normal distribution and as median (interquartile range) for those with non-normal distribution. Possible differences between groups were analyzed by unpaired t-test, whereas differences between placebo and bronchodilator use were analyzed by paired t-test. Categorical variables were compared by means of Fisher's exact test. Changes in variables after placebo or bronchodilator use and the interaction depending on the presence or absence of DH during exercise were analyzed with the general linear model and multivariate repeated measures ANOVA. The Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA), was used. The level of statistical significance was set at 5% for all tests ($p < 0.05$).

Results

Of the 30 patients studied, 21 (70%) had DH during submaximal CPET at constant speed after placebo administration (the DH+ group) and 9 did not (the DH– group). There were no statistically significant differences between the DH+ group and the DH– group regarding age (67.9 ± 8.4 years vs. 66.1 ± 8.3 years), body mass index (26.6 ± 5.1 kg/m^2 vs. 23.9 ± 4.4 kg/m^2), and maximal exercise capacity, which was determined by measuring oxygen consumption at peak exercise (1,400 ± 382 vs. 1,519 ± 243 mL/min).

After placebo administration, the proportion of patients with FEV_1 < 50% of predicted was

higher in the DH+ group (18/21; 86%) than in the DH– group (4/9; 44%; p = 0.016; Table 1). Surprisingly, however, resting IC tended to be higher in the DH+ group. All of the patients in the DH+ group had resting IC > 40% of predicted, as did 6 (67%) of the 9 patients in the DH– group (p = 0.02). The perception of dyspnea and leg fatigue during exercise was higher in the DH+ group than in the DH– group, whereas Tlim was lower in the former than in the latter (Table 1).

Bronchodilator use resulted in equivalent gains in FEV_1 in the DH+ and DH– groups, with significant increases in flow, which were determined in accordance with the Brazilian Thoracic Association criteria (7/19; 37% vs. 5/9; 56%).[16] However, the variations in resting IC after bronchodilator use were lower in the DH+ group than in the DH– group (Figure 2A). All of the patients in the DH– group showed an increase in resting IC, as did 9 (43%) of the 21 patients in the DH+ group (p < 0.01), resting IC values being therefore equalized (Table 1). Our analysis of operating lung volumes after bronchodilator use showed that IC gains at peak exercise were similar between the two groups (Figure 2B). Although the reduction in dyspnea was greater in the DH+ group than in the DH– group, both groups showed similar improvements in Tlim with the use of placebo (median [interquartile range]: 22% [3-60%] vs. 10% [3-53%]; p > 0.05; Figure 3).

Discussion

The main finding of the present study was a significant increase in Tlim after bronchodilator use, regardless of the previous pattern of DH during exercise (Figure 3). The increase in resting IC after bronchodilator use—reflecting increased static hyperinflation—was associated with increased Tlim in the DH– group. Bronchodilator use improved exercise performance in patients who showed no improvement in resting IC, although only in those who had DH. Therefore, bronchodilator use can improve exercise tolerance in COPD patients by reducing static hyperinflation at

Table 1 - Measurements taken before, during, and after constant-load exercise performed after placebo or bronchodilator use in the groups of patients with and without dynamic hyperinflation during exercise.[a]

Variables	Groups			
	DH+		DH–	
	(n = 21)		(n = 9)	
	PL	BD	PL	BD
Spirometry				
FEV_1, L	1.01 ± 0.26	1.21 ± 0.36	1.32 ± 0.41*	1.55 ± 0.45*
FEV_1, % of predicted	39 ± 11	46 ± 13	49 ± 16*	57 ± 15*
FVC, L	2.18 ± 0.46	2.52 ± 0.59	2.44 ± 0.43	2.77 ± 0.52
FVC, % of predicted	63 ± 11	72 ± 13	70 ± 18	80 ± 19
Resting IC, L	1.83 ± 0.57	1.89 ± 0.52	1.47 ± 0.32*	1.85 ± 0.44**,***
Isotime of exercise				
IC, L	1.50 ± 0.45	1.70 ± 0.51	1.61 ± 0.28	1.78 ± 0.28**
Δ IC isotime-rest, L	–0.32 ± 0.22	–0.19 ± 0.18**	0.14 ± 0.23*	–0.06 ± 0.26**,***
Dyspnea[b]	9.0 (7.0-10)	4.5 (2.0-10)**	4.0 (2.0-7.0)*	3.0 (1.0-7.0)***
Δ dyspnea BD-PL[b]		–3.5 (–6.0 to –1.0)		-1.0 (–3.0 to –4.0)***
Leg fatigue[b]	7 (3-10)	5 (2-10)	5 (3-8)*	5 (1-7)
Δ leg fatigue BD-PL[b]		–1.5 (–7.0 to –5.0)		–2.0 (–3.0 to –4.0)
End of exercise				
Tlim, s	423 ± 170	542 ± 258**	654 ± 255*	783 ± 261*,**
Dyspnea[b]	9.0 (7.0-10)	7.5 (1.0-10)	4.5 (2.0-7.0)*	4.5 (1.0-9.0)
Leg fatigue[b]	7.0 (7.0-10)	7.0 (1.0-10)	5.0 (3.0-8.0)*	5.5 (0.0-9.0)
SpO_2, %	91 ± 6	92 ± 5	87 ± 8	89 ± 9

PL: placebo; BD: bronchodilator; IC: inspiratory capacity; and Tlim: time to the limit of exercise tolerance. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as median (interquartile range). Modified Borg scale. *p < 0.05: intergroup variation at a given time point (PL or BD). **p < 0.05: intraindividual variation (pre- vs. post-BD). ***p < 0.05 intergroup variation (pre- vs. post-BD).

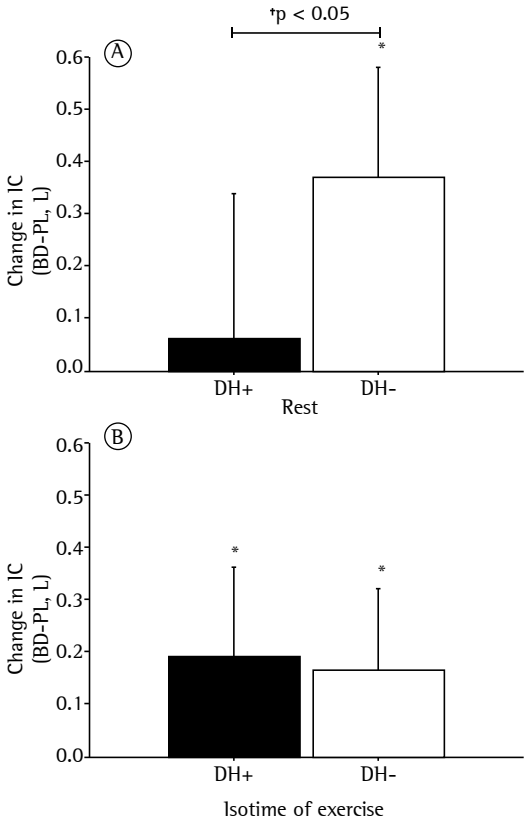

Figure 2 - Change in inspiratory capacity bronchodilator-placebo (BD-PL) at rest (in A) and at isotime of exercise at constant speed (in B) in the groups of patients with dynamic hyperinflation (DH+) and without dynamic hyperinflation (DH–). *p < 0.05; intragroup BD-PL difference. †Intergroup BD-PL difference.

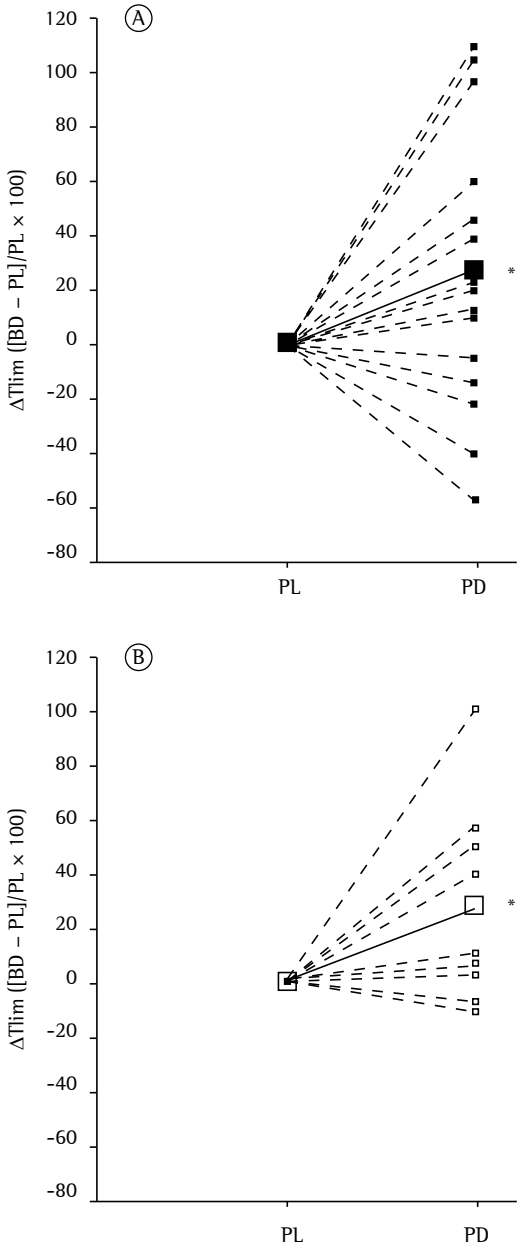

Figure 3 - Bronchodilator/placebo (BD-PL) change in exercise tolerance (Tlim) in the patients (dashed lines) with dynamic hyperinflation (in A) and without dynamic hyperinflation (in B). The solid lines represent the group means. *p < 0.05; intragroup difference.

rest and by reducing the rate of hyperinflation during exercise.

Given that DH plays a central role in limiting exercise in COPD patients,[1-3] a reduction in DH after bronchodilator use[5] (as evidenced by a significant increase in IC at isotime; Figure 2B) was expected to result in increased Tlim in the DH+ patients. Given that reduced DH has consistently been associated with increased endurance time,[6,17-19] the DH+ patients were expected to have a more favorable pathophysiological substrate for bronchodilator activity and show significantly greater increases in Tlim when compared with the DH– patients. However, both groups showed similar improvements in Tlim after bronchodilator use (Figure 3). Although from a conceptual standpoint the DH– patients did not develop DH, the significant increase in resting IC (Figure 2A) seems to have represented an important mechanism to explain improved exercise performance.

Resting IC has been identified as an important modulator of ventilatory capacity, breathing pattern, dyspnea on exertion,[11] and Tlim[10,17] in patients with COPD. This means that static lung volume measurements provide an estimate

of the inspiratory reserve volume available for exercise, delaying a critical limitation in tidal volume expansion.[20] Therefore, the development of ventilatory constraint seems to be the primary component influencing the pattern of respiratory response to exercise in patients with COPD. This important mechanic event during exercise marks the beginning of the progressive disparity between respiratory muscle effort (together with central nervous stimulation) and thoracic movement (neuromechanical dissociation), resulting in intolerable levels of dyspnea and in exercise termination.[20,21] Therefore, a low IC at rest (reflecting static lung hyperinflation) and a critical reduction in IC during exercise (DH) can, in isolation or in combination, limit the ability to increase ventilation or reach a critical inspiratory reserve volume that, limited superiorly by TLC, does not allow a further increase in tidal volume.[11]

Previous studies (including a total of 100 patients) have shown that the pattern of DH influences exercise capacity.[22-25] In contrast, Guenette et al.[26] recently analyzed a total of 130 COPD patients (whose FEV1 values were similar to those observed in previous studies, i.e., ≈ 40-50% of predicted) and reported that the presence or absence of DH during exercise had no influence on the intensity of dyspnea or on exercise tolerance during high-intensity exercise. On the contrary, critical restriction of tidal volume expansion was shown to be the primary mechanism associated with those outcomes, independently of the presence of DH. In addition, the reduction in dyspnea after bronchodilator therapy, hyperoxia, and physical training has been shown to occur independently of the reduction in the rate of DH.[27-29] Therefore, it is likely that other mechanical effects (including an absolute reduction in operating lung volumes with a delay in reaching a critical restriction of tidal volume expansion) occurring after these interventions are more important in explaining the improvement in dyspnea and exercise tolerance than is the small or inconsistent reduction in the rate of development of DH. It is of note that the patients in the DH+ group had higher dyspnea scores at isotime than did those in the DH– group. This finding is consistent with the concept that the magnitude of dyspnea is related to ventilation at increased operating volumes (reduced IC) and the resulting neuromechanical uncoupling.[20,21]

The mechanism whereby the DH– patients in the present study were able to achieve increased IC during exercise (in comparison with reduced IC at rest) after placebo administration remains unexplained. Similar results were obtained in a previous study,[30] in which it was speculated that the abovementioned finding was due to lower expiratory airflow limitation in less severely ill patients, with a respiratory pattern of abdominal muscle recruitment during exercise and, consequently, reduced operating lung volumes. However, unlike the patients in the present study, the patients in that study showed lower Tlim after bronchodilator use than did those who were more severely ill and who had hyperinflation.

The main limitations of the present study include the fact that we evaluated a convenience sample, having recruited patients during a predetermined period (possibly resulting in insufficient statistical power to make certain comparisons), and the fact that we did not measure TLC. This means that the variations in lung volumes were estimated exclusively by IC, rather than by EELV (i.e., TLC/IC). Although this limitation did not allow us to evaluate, in an adequate manner, possible differences in the baseline degree of positioning of operating lung volumes, this was minimized by the crossover design of the study, in which the same individuals were compared after two different interventions. In addition, we did not study patients with severe hypoxemia (resting SpO2 < 90%), in whom the hypoxic drive can modulate the kinetics of DH development and the bronchodilator response. Therefore, our findings should not be extrapolated to such patients.

In conclusion, the heterogeneity of the pattern of development of DH during exercise does not seem to modulate the ability of patients with moderate to severe COPD to improve their exercise capacity after inhaled bronchodilator use. Therefore, increased exercise tolerance in DH– patients seems to be related to a bronchodilator-induced reduction in resting "static" lung hyperinflation. However, patients showing no deflation at rest could still benefit from bronchodilator use, provided that there is a decrease in the rate of development of DH during exercise. Clinically, these data demonstrate that measurements of IC at rest and during exercise are complementary in the evaluation of the mechanisms underlying the beneficial effects of bronchodilators in this population of patients.

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Effect that an educational program for cystic fibrosis patients and caregivers has on the contamination of home nebulizers*

Efeito de um programa de educação para cuidadores e pacientes com fibrose cística na contaminação de nebulizadores de uso domiciliar

Adriana Della Zuana, Doroti de Oliveira Garcia, Regina Célia Turola Passos Juliani, Luiz Vicente Ribeiro Ferreira da Silva Filho

Abstract

Objective: To describe the pathogens found in home nebulizers and in respiratory samples of cystic fibrosis (CF) patients, and to evaluate the effect that a standardized instruction regarding cleaning and disinfection of nebulizers has on the frequency of nebulizer contamination. **Methods:** We included 40 CF patients (22 males), all of whom used the same model of nebulizer. The median patient age was 11.2 ± 3.74 years. We collected samples from the nebulizer mouthpiece and cup, using a sterile swab moistened with sterile saline. Respiratory samples were collected by asking patients to expectorate into a sterile container or with oropharyngeal swabs after cough stimulation. Cultures were performed on selective media, and bacteria were identified by classical biochemical tests. Patients received oral and written instructions regarding the cleaning and disinfection of nebulizers. All determinations were repeated an average of two months later. **Results:** Contamination of the nebulizer (any part) was detected in 23 cases (57.5%). The nebulizer mouthpiece and cup were found to be contaminated in 16 (40.0%) and 19 (47.5%), respectively. After the standardized instruction had been given, there was a significant decrease in the proportion of contaminated nebulizers (43.5%). **Conclusions:** In our sample of CF patients, nebulizer contamination was common, indicating the need for improvement in patient practices regarding the cleaning and disinfection of their nebulizers. A one-time educational intervention could have a significant positive impact.

Keywords: Cystic fibrosis; Nebulizers and vaporizers; Disinfection.

Resumo

Objetivo: Descrever os patógenos encontrados nos nebulizadores de uso domiciliar e nas amostras de trato respiratório de pacientes com fibrose cística (FC) e verificar o efeito de uma instrução padronizada de higiene e desinfecção de nebulizadores na contaminação dos mesmos. **Métodos:** Foram incluídos no estudo 40 pacientes com FC (22 do sexo masculino) que utilizavam um mesmo modelo de nebulizador. A mediana de idade foi de 11,2 ± 3,74 anos. Amostras dos nebulizadores foram coletadas do bocal e do copo reservatório utilizando-se um *swab* estéril umedecido em solução salina estéril. As amostras de trato respiratório dos pacientes foram colhidas por expectoração em coletor estéril ou com *swab* de orofaringe após estímulo de tosse. As culturas foram realizadas em meios seletivos, e a identificação bacteriana foi feita através de provas bioquímicas clássicas. Instruções verbais e escritas sobre higiene e desinfecção dos nebulizadores foram ministradas. Todas as determinações foram repetidas dois meses após, em média. **Resultados:** A contaminação de alguma parte dos nebulizadores foi observada em 23 casos (57,5%). A contaminação do bocal e do copo foi similar, em 16 (40.0%) e 19 casos (47.5%), respectivamente. Houve uma redução significativa da proporção de nebulizadores contaminados (43,5%) após a instrução padronizada. **Conclusões:** Nesta amostra de pacientes com FC, a contaminação dos nebulizadores foi alta, o que indica a necessidade de melhoria nas práticas de higiene e desinfecção dos nebulizadores de pacientes com FC. Uma única intervenção educacional pode ter um impacto positivo significativo.

Descritores: Fibrose cística; Nebulizadores e vaporizadores; Desinfecção.

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Financial support: None.
Submitted: 20 November 2013. Accepted, after review: 6 February 2014.

Introduction

Cystic fibrosis (CF) patients are highly susceptible to colonization by and lung infection with specific bacteria, and the establishment of a chronic bronchopulmonary infection is the leading cause of progressive lung injury.[1] The increasingly frequent need for prescribing inhaled medications to these patients has led to greater use of home nebulizers.[2,3] It is accepted that pathogens are commonly isolated from nebulizers, and there is a concern that nebulizer equipment may be a contributing source of bacterial infection into the lower airways of these patients.[2,4]

According to Rosenfeld et al.,[5] hospitals have developed strict protocols for sterilization of nebulizers. In contrast, there are no guidelines for cleaning home nebulizers or existing guidelines are not well established.[6,7] Hutchinson et al.[8] suggest that contamination of home nebulizers is common and that it may be due to the variety of maintenance practices. Vassal et al.[9] emphasize that, in the absence of cleaning, most nebulizers of CF patients are contaminated with a pathogenic flora.

The risk of contamination of home nebulizer equipment depends on various factors, such as the type of equipment used, including the material the nebulizer is made of; the efficiency of the cleaning and disinfection method recommended to patients; the microbiological quality of tap water (if used); and the quality of patient adherence to recommendations.[10] In addition, Jakobsson et al.[11] are convinced that oral and written instructions given to patients and their caregivers regarding nebulizer cleaning and disinfection practices are important for maintaining high levels of adherence to these practices.

In 2003, a consensus statement on the importance of infection control in CF developed by the Cystic Fibrosis Foundation (CFF) mentioned proper cleaning and disinfection of home nebulizers as one of the relevant principles.[12] In addition, that study pointed out the need for continuing educational programs so that good levels of adherence can be achieved.[12]

The objective of the present study was to describe the pathogens found in home nebulizers of and in respiratory samples from CF patients, and to evaluate the effect that a standardized instruction regarding cleaning and disinfection of nebulizers has on the frequency of nebulizer contamination.

Methods

The study sample consisted of patients diagnosed with CF, in accordance with international standards,[13] who were being treated at the Pediatric Pulmonology Outpatient Clinic of the University of São Paulo School of Medicine *Hospital das Clínicas* Institute for Children, located in the city of São Paulo, Brazil. Patients were selected on the basis of the following inclusion criteria: using a PRONEB® nebulizer and compressor system (PARI Medical Holding GmbH, Starnberg, Germany) and indicating interest in participating in the study upon receiving a telephone call. During a routine hospital visit, the parents or legal guardians of the patients received information about the study and gave written informed consent.

At the study outset, patients were instructed to bring the entire nebulizer system for verification. There was no mention of it being an assessment of contamination. A questionnaire was administered to establish what home method for cleaning and disinfecting nebulizers had been used until then.

At the time, samples were collected from the nebulizer medicine cup and mouthpiece for microbiological culture, by swabbing of the inner surface of the nebulizer medicine cup and mouthpiece with a sterile swab moistened with sterile saline (rotating the swab ten times clockwise).[14]

In addition, sputum samples or oropharyngeal swabs were collected from patients for microbiological culture. Sputum was collected by asking patients to expectorate into a sterile container, and oropharyngeal swabs were collected by rubbing of the retropharynx and pharyngeal pillars with a sterile swab (BD Brasil, São Paulo, Brazil). The samples collected from the patients and from the nebulizers, all of which were properly identified, were placed into an insulated bag with ice packs and sent to the microbiology laboratory within a maximum of three hours.

Cultures were performed at the Bacteriology Laboratory of the Adolfo Lutz Institute, located in the city of São Paulo, Brazil. The sputum samples and the oropharyngeal samples were directly smeared onto selective media. The media used included chocolate Agar, MacConkey agar, and selective media for the *Burkholderia cepacia* complex (B. cepacia selective medium; Oxoid Ltd., Basingstoke, UK), *Stenotrophomonas maltophilia*, and *Staphylococcus aureus*—Baird-Parker agar

and/or mannitol agar (Oxoid)—and all cultures were incubated at 37°C for 16-72 h.[15]

The gram-negative bacilli isolated were identified phenotypically by extensive conventional biochemical tests that are already part of the routine practice of the Adolfo Lutz Institute.

The adopted cleaning and disinfection instructions were adapted from the model recommended by the CFF[12] and from the instructions provided by the manufacturer of the nebulizer system used by the patients. At the end of sample collection, each patient and/or guardian received oral and written instructions regarding a standardized cleaning and disinfection process to be used henceforth that consisted of the following steps:

1. Cleaning: after use, the nebulizer should be disassembled and its parts should be washed inside and outside with mild detergent and tap water (except for the hose and its adapter, which should remain connected to the compressor for two minutes or should be left with the two ends hanging down in order to dry) and should be rinsed with tap water.
2. Disinfection: place the disassembled parts into a container filled with water and let it boil for five minutes. If the parts are disinfected with boiling water, rinsing is not necessary. Do not boil the hose, its adapter, or the mask. Repeat this procedure once a day.
3. Drying: after the final rinse, let the water drain from the material and dry it preferably with paper towels or a clean cloth.
4. Storage: assemble all parts of the nebulizer and store it in a container used for that sole purpose.

Patients were asked to bring their nebulizer equipment again at the next medical visit, and additional samples were collected from the nebulizers and the patients. At the time, the questionnaire was readministered in order to determine adherence to the recommended standardized method.

The study project was approved by the ethics committees of the Institute for Children and the Adolfo Lutz Institute, as well as by the Research Ethics Committee of the University of São Paulo School of Medicine *Hospital das Clínicas* (Protocol no. 0067/08).

For the purposes of the statistical analysis, categorical variables are expressed as frequencies and confidence intervals, and continuous variables are expressed as means, standard deviations, medians, and maximum and minimum values. The association between positive cultures and the remaining categorical variables was investigated by Fisher's exact test or the chi-square test. The difference between the frequencies of nebulizer contamination before and after the cleaning instructions had been given was assessed by McNemar's test. To determine whether the time interval between the first and second assessments would affect the results, we used a generalized estimating equations statistical model with binomial distribution,[16] considering the time interval between the two assessments as a covariate. The sample size was calculated to yield a power of 80% to detect a 50% decrease in the frequency of nebulizer contamination, considering that, according to data in the literature,[14] the rate of nebulizer contamination would be approximately 65% before the application of the proposed technique. For all calculations, the level of significance was set at < 5%. Statistical analyses were performed with PASW Statistics 18 (IBM Corp., Armonk, NY, USA).

The research project was funded entirely by the department and laboratories involved. Interviews and sample collection were performed at the Physical Therapy Outpatient Clinic of the Institute for Children by the principal researcher.

Results

We evaluated 40 CF patients (22 males and 18 females) aged 5 to 18 years (median, 11.2 years). Among the 40 patients evaluated, all (100%) were being treated with inhaled DNase (Pulmozyme®; Roche, São Paulo, Brazil) and 16 (40%) were receiving inhaled antibiotic concomitantly. The median time between the evaluations was 63 days (range, 3-203 days).

The colonization profile of the patients, which was obtained through analysis of medical records, showed a predominance of chronic colonization with *S. aureus* and *Pseudomonas aeruginosa* and a lower frequency of colonization with the *B. cepacia* complex and *S. maltophilia* (Figure 1).

The data obtained from the questionnaire administered to assess patient practices regarding the cleaning and disinfection of their nebulizers showed that, at the time of the first collection, 16

patients (40%) reported having already received instruction on such practices from a professional. Approximately 80% of the patients reported being aware of the importance of proper cleaning, but only 11 (27.5%) considered their cleaning and disinfection practices satisfactory. Patient practices regarding the cleaning, disinfection, drying, and storage of their nebulizer equipment varied widely, and most were considered unsatisfactory; however, there was a marked change after the instructions had been given (Table 1).

Of the 80 respiratory secretion samples collected from the patients at the two assessments, 60 were sputum samples and 20 were oropharyngeal swabs. *S. aureus* predominated (in 68.75%), followed by *P. aeruginosa* (in 43.75%), the *B. cepacia* complex (in 3.75%), and *S. maltophilia* (in 2.75%).

Contamination of the nebulizer (any part) was detected in 23 cases (57.5%), and contamination of the nebulizer mouthpiece and cup was detected in 16 and 19 cases, respectively (Table 2). After standardized instruction regarding the cleaning and disinfection of home nebulizers had been given, the number of contaminated nebulizer cases dropped to 10 (25%), and the number of contaminated nebulizer mouthpiece cases and contaminated nebulizer cup cases dropped to 7 and 5, respectively (Figure 2).

The frequency of contamination decreased by 43.5%, which is significant considering the total number of contaminated nebulizers and the various parts of the nebulizer. However, the time interval between the two assessments had no influence on this decrease in contamination.

The nebulizer sample cultures detected a wide variety of microorganisms, with predominant detection of unidentified gram-negative bacilli (n = 14; Table 3). In 4 cases, the same microorganism was detected in the culture of the respiratory secretion sample from the patient and in the nebulizer (any part) sample culture. In 2 of those cases, the agent was identified as belonging to the genus *Pseudomonas*, and, in the other 2, it was identified as belonging to the genus *Staphylococcus*. Genetic analysis of these isolates (DNA macrorestriction analysis followed by pulsed-field gel electrophoresis) showed that they were unrelated strains (data not shown).

Discussion

Most CF patients use nebulizers routinely,[2] and, in the present study, the prevalence of contamination of home nebulizers was found to be quite significant (57.5%), despite the fact that most patients reported being aware of the importance of nebulizer cleaning and disinfection practices. This indicates the need for improvement in these practices.

The nebulizer cleaning and disinfection methods reported by patients before the standardized instruction had been given were, in most cases, not in line with international recommendations[12], and only 25% of patients boiled the nebulizer parts, which is recommended by the CFF as a disinfection method.

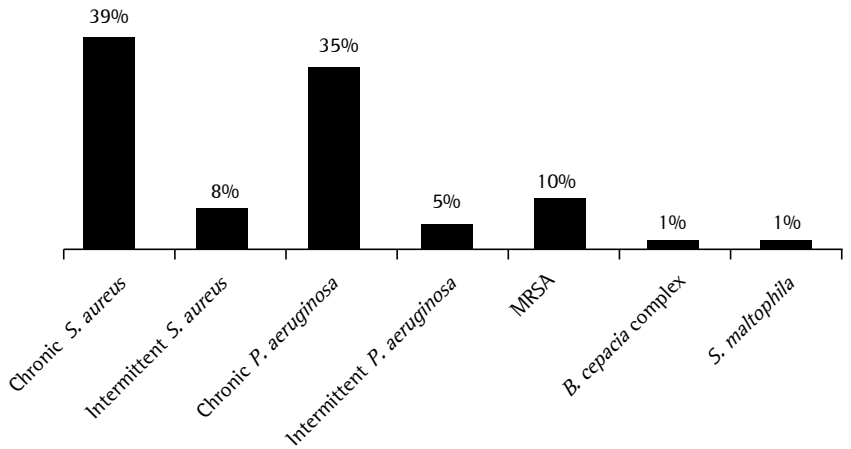

Figure 1 - Prior colonization of the patients included in the study (n = 40). *S. aureus*: *Staphylococcus aureus*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *B. cepacia*: *Burkholderia cepacia*; MRSA: methicillin-resistant *S. aureus*; and *S. maltophilia*: *Stenotrophomonas maltophilia*.

Table 1 - Report of study participants' (n = 40) practices regarding the cleaning, disinfection, drying, and storage of their home nebulizers at the two questionnaire administrations.[a]

Question	First administration	Second administration
You have been instructed on how to clean and disinfect your nebulizer	16 (40.0)	40 (100.0)
The instruction was given by		
A physician	3 (7.5)	0 (0.0)
A nurse	0 (0.0)	0 (0.0)
A physical therapist	8 (20.0)	40 (100.0)
Others	5 (12.5)	0 (0.0)
You are aware of the importance of proper cleaning	32 (80.0)	38 (95.0)
You consider the way you clean your nebulizer equipment		
Satisfactory	11 (27.5)	36 (90.0)
Marginally satisfactory	19 (47.5)	3 (7.5)
Unsatisfactory	0 (0.0)	0 (0.0)
Do not know	10 (25.0)	1 (2.5)
Number of uses per day		
1	27 (67.5)	29 (72.5)
2	0 (0.0)	2 (5.0)
> 2	13 (32.5)	9 (22.5)
Frequency of cleaning per week		
1	1 (2.5)	0 (0.0)
2	3 (7.5)	0 (0.0)
3	1 (2.5)	0 (0.0)
7	10 (25.0)	1 (2.5)
After each inhalation	22 (55.0)	39 (97.5)
Parts that are cleaned		
Cap	40 (100.0)	40 (100.0)
Cup	39 (97.5)	40 (100.0)
Mouthpiece	40 (100.0)	40 (100.0)
Mask	0 (0.0)	0 (0.0)
Inner supply tube	39 (97.5)	40 (100.0)
Hose	29 (72.5)	31 (77.5)
Compressor	24 (60.0)	24 (60.0)
How you clean your nebulizer		
Disassemble it into parts	39 (97.5)	40 (100.0)
Scrubbing with your hands	15 (37.5)	24 (60.0)
Scrubbing with a sponge	17 (42.5)	14 (35.0)
Scrubbing with a cloth	0 (0.0)	1 (2.5)
Detergent	27 (67.6)	40 (100.0)
Tap water	33 (82.5)	40 (100.0)
Boiled water	5 (12.5)	0 (0.0)
Rinsing	32 (80.0)	40 (100.0)
How you disinfect your nebulizer		
Alcohol	3 (7.5)	1 (2.5)
Another product	1 (2.5)	0 (0.0)
Soaking	26 (65.0)	1 (2.5)
Rinsing with hot water	8 (20.0)	1 (2.5)
Boiling of the parts	10 (25.0)	38 (95.0)
How you dry your nebulizer		
Natural air drying	22 (55.0)	8 (20.0)
Paper towel	8 (20.0)	22 (55.0)
Cloth	10 (25.0)	10 (25.0)
How you store your nebulizer		
Bag	7 (17.5)	8 (20.0)
Container	20 (50.0)	31 (77.5)
No specific storage place	13 (32.5)	1 (2.5)

[a]Values expressed as n (%).

Table 2 - Frequency of nebulizer contamination before and after the standardized instruction had been given (n = 40).

Nebulizer contamination	Before the instruction[a]	After the instruction[a]	p*	p after correction by the time interval between assessments**
Any part	23 (57.5)	10 (25.0)	0.002	0.001
Mouthpiece	16 (40.0)	7 (17.5)	0.022	0.011
Cup	19 (47.5)	5 (12.5)	< 0.001	< 0.001

[a]Values expressed as n (%). *McNemar's test. **Generalized estimating equations model.

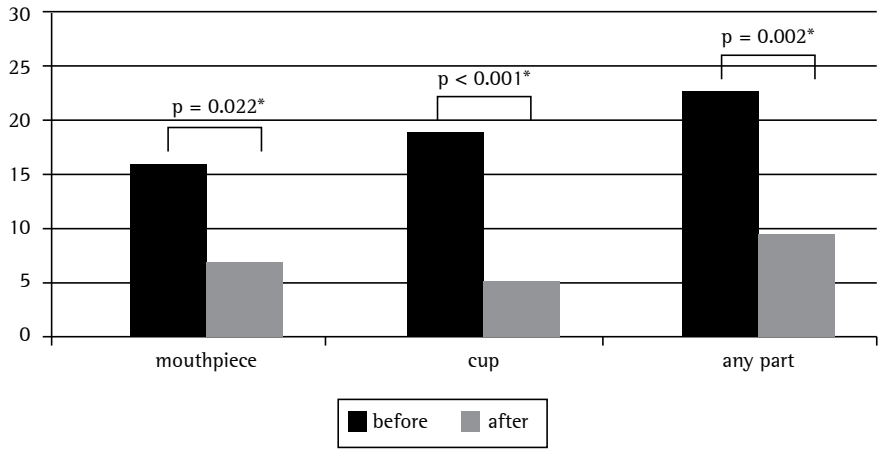

Figure 2 - Frequency of nebulizer contamination before and after the standardized instruction (n = 40). *McNemar's test.

Table 3 - Frequency of identification of microorganisms in the cultures of samples collected from the various parts of the nebulizers before and after the standardized instruction had been given (n = 40).

Microorganism	Before the instruction		After the instruction	
	Mouthpiece	Cup	Mouthpiece	Cup
Non-fermenting gram-negative bacilli	2	8	1	3
Coagulase-negative *Staphylococcus* sp.	6	2	3	2
Acinetobacter sp.	2	3	3	2
Yeasts	8	4	0	0
Pseudomonas putida	1	6	0	0
Enterobacter spp.	1	3	0	1
*Enterobacter*ia spp.	2	3	0	0
Klebsiella sp.	0	2	1	1
Stenotrophomonas maltophilia	1	1	0	1
Gram-positive bacilli	1	2	0	0
Burkolderia cepacia complex	1	1	0	1
P. fluorescens	1	2	0	0
P. aeruginosa	1	2	0	0
Escherichia coli	0	1	1	0
S. aureus	1	0	1	0
Achromobacter xylosoxidans	0	1	0	0

Patients having received one-time standardized oral and written instructions resulted in a 43.5% decrease in contamination within an average of two months between the two assessments, which shows the potential of educational interventions in such a scenario.

Vassal et al.[9] conducted a study in which 44 patients had chronic colonization with *P. aeruginosa*, 30 of whom (68%) had a nebulizer that had been contaminated with bacteria immediately after drug nebulization and did not receive any cleaning. Comparatively, the rate of nebulizer contamination found in the present study was 57.5%. Likewise, Blau et al.,[14] in a study on bacterial contamination of nebulizers in the home treatment of CF patients, evaluated 29 nebulizer systems and found contamination in 19 (65%), *P. aeruginosa* being identified in 10 (35%). In contrast, in a study conducted in Brazil by Brzezinski et al.,[3] only 6 (21%) of 28 nebulizers evaluated were contaminated with bacteria related to CF. The main difference between that study and ours is that, in the former, sample collection occurred at home visits, and it is of note that the samples were left at room temperature before being taken for analysis.[3]

Although in the present study we found a relatively small proportion of microorganisms typical of CF in the nebulizer sample cultures, a significant proportion of these cultures (n = 14) were found to be positive for non-fermenting gram-negative bacilli, which were not characterized phenotypically. These microorganisms can be pathogenic to CF patients, since there are relatively frequent reports of errors in microbiological identification.[17,18]

Rosenfeld et al.[7] reported that the home nebulizer sample cultures from CF patients were frequently positive for *S. aureus* (55%), *P. aeruginosa* (35%), and species of the genus *Klebsiella* (19%). However, the concordance between sputum cultures and nebulizer sample cultures was poor. When studying 35 home nebulizers, Hutchinson et al.[8] found that 3 were contaminated with the *B. cepacia* complex and 4 were contaminated with *S. maltophilia*. Although 34 patients had *P. aeruginosa* in their sputum, none of the nebulizers were positive for this microorganism. In addition, those authors reported that, even after cleaning, 69% of the nebulizers were contaminated with various types of gram-negative bacteria.

Blau et al.[14] stated that the manufacturer's instructions provided with PARI Medical Holding GmbH nebulizer systems were inadequate, since they still recommended soaking the nebulizer in a solution of water and acetic acid for disinfection, which does not ensure disinfection against *S. aureus* or *B. cepacia*.[2] Instructions currently available on that manufacturer's website have been updated in accordance with the CFF recommendations.[19] In addition, Reychler et al.[2] reported no benefits of drying; however, they recognize that this recommendation should be taken into account because pathogens such as *P. aeruginosa* and *B. cepacia* are hydrophilic, and drying should be a step in the cleaning process. The consensus statement published by the CFF[12] states that the practices regarding the cleaning, disinfection, and drying of nebulizer parts are key steps for infection control in CF patients, both at home and in the hospital setting. However, data from questionnaires administered to CF patients regarding their home nebulizer cleaning and disinfection routine show a wide variety of cleaning practices.[20] At our facility, the recommendations regarding the cleaning and disinfection of nebulizers used to be made in an empirical (non-standardized) way; after the results of the present study were made known, the CFF recommendations were adopted. This one-time educational intervention delivered orally and in writing by the same professional resulted in a significant decrease in contamination of the nebulizer equipment, despite the varying time interval between assessments.

The development of recommendations, such as those by the CFF, is only the first step in infection control; it is necessary to disseminate information and educate patients and their caregivers about cleaning and disinfection practices, since there may be cultural and social barriers to their implementation.[21,22] In addition, education about these practices should be offered to undergraduate physical therapists and to all professionals who prescribe inhaled medications.[4] Although various authors have recommended the use of oral and written instructions regarding these practices,[10,11,14] our study unequivocally demonstrates the impact of this type of approach over an average two-month period of reassessment. However, among the limitations of the present study are the lack of a control group and the lack of subsequent sample collections to assess changes in the contamination profile of the nebulizer equipment, since it is possible that adherence to the recommended practices would decrease over time. Regarding the lack of a control group, we consider this to be an appropriate measure to minimize patient exposure to the

theoretical risk of continuing to use contaminated nebulizer equipment, without being provided with correct instructions on how to clean and disinfect it at the first interview. Regarding the possibility of loss of effect, another assessment of contamination of the nebulizer equipment of the same patients would answer this query.

Future directions for studies in this area include determining more effective ways to promote adherence to infection control practices and developing mechanisms to assess the clinical impact of these practices on the basis of the results obtained with patients.

Acknowledgments

We would like to thank Professor Ulysses Dória Filho for his assistance with the sample size calculation and preliminary statistical analyses and statistician Ângela Tavares Paes for her assistance with the statistical model. We would also like to thank the patients and their parents for their commitment to participate in the study.

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Effects of yoga breathing exercises on pulmonary function in patients with Duchenne muscular dystrophy: an exploratory analysis*,**

Efeitos de exercícios respiratórios de ioga na função pulmonar de pacientes com distrofia muscular de Duchenne: uma análise exploratória

Marcos Rojo Rodrigues, Celso Ricardo Fernandes Carvalho, Danilo Forghieri Santaella, Geraldo Lorenzi-Filho, Suely Kazue Nagahashi Marie

Abstract

Objective: Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy in children, and children with DMD die prematurely because of respiratory failure. We sought to determine the efficacy and safety of yoga breathing exercises, as well as the effects of those exercises on respiratory function, in such children. **Methods:** This was a prospective open-label study of patients with a confirmed diagnosis of DMD, recruited from among those followed at the neurology outpatient clinic of a university hospital in the city of São Paulo, Brazil. Participants were taught how to perform hatha yoga breathing exercises and were instructed to perform the exercises three times a day for 10 months. **Results:** Of the 76 patients who entered the study, 35 dropped out and 15 were unable to perform the breathing exercises, 26 having therefore completed the study (mean age, 9.5 ± 2.3 years; body mass index, 18.2 ± 3.8 kg/m^2). The yoga breathing exercises resulted in a significant increase in FVC (% of predicted: 82.3 ± 18.6% at baseline vs. 90.3 ± 22.5% at 10 months later; p = 0.02) and FEV_1 (% of predicted: 83.8 ± 16.6% at baseline vs. 90.1 ± 17.4% at 10 months later; p = 0.04). **Conclusions:** Yoga breathing exercises can improve pulmonary function in patients with DMD.

Keywords: Respiratory therapy; Forced expiratory volume; Vital capacity; Muscular dystrophy, Duchenne; Complementary therapies.

Resumo

Objetivo: A distrofia muscular de Duchenne (DMD) é a forma mais comum de distrofia muscular em crianças, e crianças com DMD morrem prematuramente por causa de insuficiência respiratória. Analisamos a eficácia e segurança de exercícios respiratórios de ioga nessas crianças, bem como os efeitos desses exercícios em sua função respiratória. **Métodos:** Estudo prospectivo aberto envolvendo pacientes com diagnóstico confirmado de DMD recrutados no ambulatório de neurologia de um hospital universitário em São Paulo (SP). Os participantes aprenderam exercícios respiratórios de hatha ioga e foram instruídos a praticá-los três vezes ao dia durante 10 meses. **Resultados:** Dos 76 pacientes incluídos no estudo, 35 o abandonaram e 15 não conseguiram realizar os exercícios respiratórios, de modo que 26 pacientes completaram o estudo (média de idade: 9,5 ± 2,3 anos; índice de massa corporal: 18,2 ± 3,8 kg/m^2). Os exercícios respiratórios de ioga resultaram em um aumento significativo da CVF em porcentagem do previsto (82,3 ± 18,6% antes do início do programa de exercícios vs. 90,3 ± 22,5% 10 meses depois; p = 0,02) e do VEF_1 em porcentagem do previsto (83,8 ± 16,6% antes do início do programa de exercícios vs. 90,1 ± 17,4% 10 meses depois; p = 0,04). **Conclusões:** Os exercícios respiratórios de ioga podem melhorar a função pulmonar de pacientes com DMD.

Descritores: Terapia respiratória; Volume expiratório forçado; Capacidade vital; Distrofia muscular de Duchenne; Terapias complementares.

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Financial support: None.
Submitted: 20 March 2013. Accepted, after review: 18 February 2014.
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Introduction

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy in children, occurring in 1 of every 3,000-3,500 male births.[1,2] There is evidence that corticosteroid therapy has beneficial effects on the health and quality of life of patients with DMD and can decrease the requirement for nocturnal ventilation in such patients.[3] As a result of dystrophin deficiency, patients with DMD (including those who receive optimal treatment) experience a progressive loss of muscle fiber that eventually affects the respiratory muscles.[2] The resulting respiratory failure is the most common cause of premature death in patients with DMD.[1,4-6]

Respiratory muscles are progressively compromised in children with DMD, the expiratory muscles being the most commonly affected.[7] Even short periods of physical inactivity can contribute to muscle weakness and reduced respiratory capacity.[8-10] There is evidence that breathing exercises can improve respiratory function in patients with DMD.[11,12] Hatha yoga is a broad philosophy that encompasses a series of breathing exercises aimed at improving the health of its practitioners. The objective of the present study was to determine whether a 10-month program of yoga breathing exercises that recruit inspiratory and expiratory muscles is safe for children with DMD and can improve their respiratory function.

Methods

The study sample consisted of consecutive patients treated at the neurology outpatient clinic of a university hospital in the city of São Paulo, Brazil. The inclusion criteria were as follows: having been diagnosed with DMD (as confirmed by molecular assessment of the skeletal muscle); being in the 6-14 year age bracket; and using corticosteroids regularly for at least 3 months. Severely ill children who were unable to perform the breathing exercises were excluded from the study. The study protocol was approved by the local research ethics committee, and the parents or legal guardians of all participants gave written informed consent.

The present study was conducted over a 10-month period, with clinical evaluations being performed in the morning at study entry (baseline) and at study termination. During the study period, the patients returned for clinical evaluations at regular intervals (of 1-2 months).

All children were individually taught how to perform the breathing exercises while sitting in a quiet room, practicing each exercise until they were able to perform it without supervision. The children were taught a new exercise at each clinical evaluation. The first breathing exercise that they were taught was kapalabhati, consisting of nasal exhalations produced by fast, vigorous contraction of the abdominal and pelvic muscles, followed by passive inhalations produced by relaxation of the recruited muscles. At 3 months after study entry, the children were taught another breathing exercise, which is known as uddiyana and consists of apnea after forced expiration, followed by thoracic expansion (achieved without inhalation) and voluntary glottic closure. At 6 months after study entry, the children were taught yet another breathing exercise, which is known as agnisara and consists of maximal contraction followed by abdominal projection during apnea after forced expiration. The participants were instructed to perform this sequence of exercises three times a day, every day, as follows: three series of 120 repetitions for kapalabhati; three 10-s repetitions for uddiyana; and three series of five movements for agnisara. Caregivers kept a diary, in which they marked an "x" every time the children performed the home exercises. The diaries were returned to the researchers on a monthly basis. We included only those children whose adherence to the exercise program was at least 75%.

Spirometry was performed with a dry bellows spirometer (Koko Spirometer; PDS Instrumentation, Inc., Louisville, CO, USA) in accordance with the American Thoracic Society/European Respiratory Society Task Force standards for lung function testing.[13] We measured FEV_1 and FVC, predicted normal values being determined by the use of validated equations.[14] We measured MEP and MIP at the mouth using a portable spirometer (microQuark; Cosmed, Rome, Italy) under static conditions, in accordance with a validated method.[15] We measured MEP at TLC and MIP at functional residual capacity, the highest of three valid measurements being recorded. Before these measurements were obtained, patients were allowed to make at least three attempts. The results are expressed as relative values (percentages of the predicted values for age).

Normality was tested with the Kolmogorov-Smirnov test. The Student's t-test for repeated measures (baseline vs. 10 months after study entry) was used in order to evaluate the effects of the intervention on all physiological variables. The level of significance was set at $p < 0.05$. The results were analyzed with the Statistical Package for the Social Sciences, version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

A total of 86 patients with a confirmed diagnosis of DMD were initially considered for inclusion. Of those, 10 were ineligible because they were not receiving corticosteroids. Therefore, 76 patients entered the study. Of those, 35 dropped out and 15 were unable to perform the exercises, being therefore excluded from the study. Many of the patients who dropped out lived in cities that are far from where the present study was conducted and were therefore unable to attend the scheduled clinical evaluations (Figure 1). The demographic characteristics and pulmonary function test results of the 76 patients who entered the study are presented in Table 1.

There were no significant differences between MEP at baseline and MEP at 10 months after study entry (63.9 ± 27.6% of predicted vs. 66.8 ± 27.6% of predicted; $p > 0.05$) or between MIP at baseline and MIP at 10 months after study entry (41.4 ± 13.7% of predicted vs. 43.7 ± 12.8% of predicted; $p > 0.05$). However, FVC at baseline was significantly lower than FVC at 10 months after study entry (82.3 ± 18.6% of predicted vs. 90.3 ± 22.5% of predicted; $p = 0.02$; Figure 2). Likewise, FEV_1 at baseline was significantly lower than FEV_1 at 10 months after study entry (83.8 ± 16.6% of predicted vs. 90.1 ± 17.4% of predicted; $p = 0.04$; Figure 3).

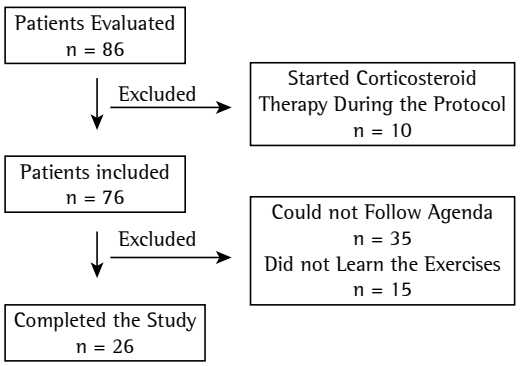

Figure 1 - Flowchart of the study.

Discussion

The present study evaluated children with DMD receiving optimal medical therapy with corticosteroids. In children with DMD, respiratory function is expected to deteriorate over time, at a rate of 5% per year.[7] The novel findings of the present study include the fact that a large

Table 1 - Demographic characteristics and pulmonary function test results of the Duchenne muscular dystrophy patients studied.[a]

Variable	Patients (n = 26)
Demographic data	
Age, years	9.5 ± 2.2
Weight, kg	31.6 ± 11.4
Height, cm	130.3 ± 13+8
Body mass index, kg/m^2	18.2 ± 3.8
Pulmonary function test results	
FVC, % of predicted	82.9 ± 16.8
FEV_1, % of predicted	84.3 ± 16.0
MEP, % of predicted	63.9 ± 27.6
MIP, % of predicted	41.4 ± 13.7

[a]Data expressed as mean ± SD.

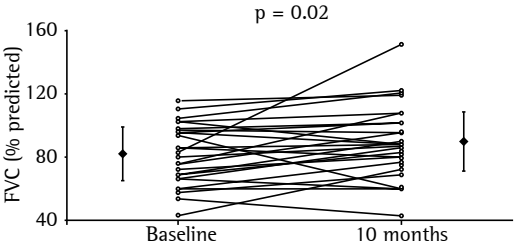

Figure 2 - FVC (% of predicted) at baseline and at 10 months after study entry. Note that FVC was significantly higher at 10 months after study entry. Rhomboids represent mean ± SD.

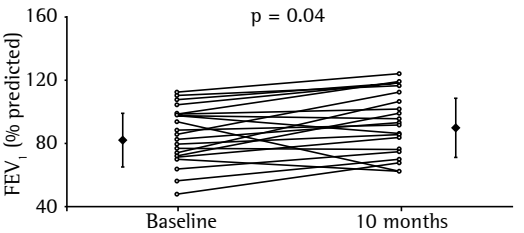

Figure 3 - FEV_1 (% of predicted) at baseline and at 10 months after study entry. Note that FEV_1 was significantly higher at 10 months after study entry. Rhomboids represent mean ± SD.

proportion of patients with DMD (82.6%) were able to learn how to perform yoga breathing exercises and the fact that pulmonary function can improve over a 10-month period, as evidenced by significant improvements in FEV_1 and FVC.

Over the course of DMD, absolute values of lung capacity initially increase, then reach a plateau, and finally decrease. In contrast, relative values (proportional to the values predicted for age and gender) of all pulmonary function variables show a constant decline after the age of 5 years.[12,16,17] For instance, respiratory capacity decreases by approximately 5% per year.[12] Because DMD is a relatively rare and progressive disease with an expected survival of 16-19 years,[18] the best evidence for standard of care and new treatments is primarily from observational studies. One group of authors[19] evaluated 40 DMD patients who were treated with corticosteroids in comparison with 34 who were not and concluded that lung function stabilization constitutes evidence of the beneficial effect of corticosteroids on DMD. Similar findings were recently reported for patients in Brazil. In our study, we included only DMD patients who were using corticosteroids regularly and used predicted values of pulmonary function as primary endpoints. In fact, it has been demonstrated that FVC (in % of predicted) decreases by approximately 4% per year in DMD patients.[20] In our patients, who participated in a 10-month yoga exercise program, lung function stabilized (which is, in and of itself, a positive effect) and there were increases in FEV_1 and FVC (both in % of predicted). Therefore, although our study had no control group, the results should be interpreted as evidence that yoga breathing exercises have an additive positive effect on the lung function of DMD patients undergoing conventional treatment.

One study showed that respiratory muscle training can be beneficial to patients with DMD.[21] Despite the lack of studies addressing the use of yoga breathing exercises as complementary therapy for DMD patients, there have been clinical respiratory studies of the topic, although the results have been controversial. In a recent review of the effects of breathing exercises on COPD,[22] such exercises were found to be effective in increasing the six-minute walk distance. In contrast, another review showed that yoga had no effect on cardiorespiratory variables in asthma patients.[23] However, those two reviews evaluated the effects of yoga in adults. In children with asthma, FEV_1 was significantly increased after yoga training,[24] which included yoga postures and breathing exercises. To our knowledge, the present study is the first to address and confirm the safety and efficacy of yoga breathing exercises in children with DMD. The yoga breathing exercises used in the present study are unique and were chosen because children can learn them after only a few sessions and subsequently practice them by themselves without the need for additional equipment or continuous supervision, the exercise program being therefore accessible to a large number of patients. During the exercise known as kapalabhati, abdominal muscles are kept active, working rapidly and vigorously as expiratory muscles. Exhalation occurs below functional residual capacity and therefore becomes active, whereas inhalation becomes passive. This is in contrast with what occurs physiologically (inhalation being active and exhalation being passive). Kapalabhati therefore allows expiratory muscle training while the inspiratory muscles are at rest. Kapalabhati was the first exercise that the children were taught, being therefore the most practiced exercise and the exercise that probably contributed the most to the results obtained. Both uddiyana and agnisara have conditioning effects on the inspiratory muscles, the former focusing primarily on the intercostal respiratory muscles and the latter focusing primarily on the diaphragm.

Our study has strengths and limitations. The primary objective of the study was to show that yoga breathing exercises can be performed by patients with DMD. This hypothesis had to be carefully tested, particularly because DMD is a progressive, genetic disease, and breathing exercises could have deleterious effects on such patients (i.e., effects similar to those of overtraining in healthy individuals). In fact, the dropout rate was high in our sample. Although this might be due to difficulty breathing during the exercises, we believe that it was probably due to the fact that those patients were unable to attend the exercise sessions; that is, they had no means of transportation. In addition, only a small proportion of patients dropped out because they were unable to perform spirometry (either because they had respiratory problems or because they were unable to learn how to perform the exercises). Furthermore, there were no significant differences among the

patients regarding any of the respiratory variables studied. Our study therefore showed that yoga breathing exercises are feasible, are harmless, and can actually improve respiratory function. Although our results clearly show that pulmonary function improved in terms of the predicted values—meaning that the increase was observed compared to each subject—randomized controlled studies are needed in order to confirm that. It is of note that DMD is a rare, fatal disease, and the best evidence for any form of treatment is from observational studies.

In conclusion, yoga exercises are feasible and can improve lung function in children with DMD. Further studies are needed in order to determine whether yoga exercises can improve quality of life and reduce the number of hospital admissions in such patients.

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Lung function in the absence of respiratory symptoms in overweight children and adolescents*

Função pulmonar de crianças e adolescentes sem sintomas respiratórios e com excesso de peso

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Abstract

Objective: To describe lung function findings in overweight children and adolescents without respiratory disease. **Methods:** This was a cross-sectional study involving male and female overweight children and adolescents in the 8-18 year age bracket, without respiratory disease. All of the participants underwent anthropometric assessment, chest X-ray, pulse oximetry, spirometry, and lung volume measurements. Individuals with respiratory disease were excluded, as were those who were smokers, those with abnormal chest X-rays, and those with an $SpO_2 \leq 92\%$. Waist circumference was measured in centimeters. The body mass index-for-age Z score for boys and girls was used in order to classify the individuals as overweight, obese, or severely obese. Lung function variables were expressed in percentage of the predicted value and were correlated with the anthropometric indices. **Results:** We included 59 individuals (30 males and 29 females). The mean age was 11.7 ± 2.7 years. Lung function was normal in 21 individuals (35.6%). Of the 38 remaining individuals, 19 (32.2%), 15 (25.4%), and 4 (6.7%) presented with obstructive, restrictive, and mixed ventilatory disorder, respectively. The bronchodilator response was positive in 15 individuals (25.4%), and TLC measurements revealed that all of the individuals with reduced VC had restrictive ventilatory disorder. There were significant negative correlations between the anthropometric indices and the Tiffeneau index in the individuals with mixed ventilatory disorder. **Conclusions:** Lung function was abnormal in approximately 65% of the individuals evaluated here, all of whom were overweight. Obstructive ventilatory disorder and positive bronchodilator response predominated.

Keywords: Obesity/complications; Respiratory function tests; Lung diseases/etiology.

Resumo

Objetivo: Descrever os achados de função pulmonar em crianças e adolescentes sem doenças respiratórias e com excesso de peso. **Métodos:** Estudo transversal com crianças e adolescentes de 8 a 18 anos de ambos os sexos, com excesso de peso e sem doença respiratória, submetidos à avaliação antropométrica, radiografia de tórax, oximetria de pulso, espirometria e medidas de volume pulmonar. Indivíduos com patologias respiratórias, tabagistas ativos, radiografia anormal ou $SpO_2 \leq 92\%$ foram excluídos do estudo. A circunferência da cintura foi medida em centímetros. O escore z para índice de massa corpórea/idade e sexo foi utilizado para classificar os indivíduos como com sobrepeso, obesos e obesos graves. As variáveis dos testes de função pulmonar foram expressas em percentual do previsto e correlacionadas com os índices antropométricos. **Resultados:** Foram incluídos 59 indivíduos (30 meninos e 29 meninas). A média de idade foi de 11,7 ± 2,7 anos. Os resultados dos testes de função pulmonar foram normais em 21 indivíduos (35,6%). Dos 38 indivíduos restantes, 19 (32,2%), 15 (25,4%) e 4 (6,7%) apresentaram, respectivamente, distúrbio ventilatório obstrutivo, restritivo e misto. A resposta ao broncodilatador foi positiva em 15 indivíduos (25,4%), e a medida da CPT revelou que todos os indivíduos com CV reduzida apresentavam distúrbio ventilatório restritivo. Houve correlações negativas significantes entre os índices antropométricos e índice de Tiffeneau nos indivíduos com distúrbio ventilatório misto. **Conclusões:** A função pulmonar apresentou-se alterada em aproximadamente 65% dos indivíduos com sobrepeso aqui avaliados, predominando distúrbio ventilatório obstrutivo e resposta positiva ao broncodilatador.

Descritores: Obesidade/complicações; Testes de função respiratória; Pneumopatias/etiologia.

*Study carried out at the Professor Edgard Santos University Hospital, Federal University of Bahia School of Medicine, Salvador, Brazil.
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Financial support: None.
Submitted: 30 September 2013. Accepted, after review: 12 December 2013.

Introduction

Obesity is a multifactorial chronic disease that is epidemic worldwide.[1] Because of the socioeconomic impact, morbidity, and mortality of obesity, there has been growing interest in understanding the disease.[2] Impaired lung function is a possible complication of obesity and is often overlooked, despite occurring in a manner that is similar to that observed in other diseases, such as cancer, cardiovascular disease, and chronic respiratory disease.[3] Lin at al.[4] reported that various obesity-related cytokines can contribute to systemic inflammatory effects in individuals with obstructive airway disease and sleep apnea syndrome.

Although obesity-related lung function changes have been described in adults, there is a lack of data on such changes in children.[5] The increase in childhood obesity is an emerging problem worldwide and directly contributes to obesity in adulthood; as a result, there is an increase in the incidence of fatal diseases such as cardiovascular disease, metabolic syndrome, dyslipidemia, diabetes mellitus, arterial hypertension, and even respiratory changes.[6]

Methodological differences across studies in terms of the diagnostic evaluation of obesity and the selection of a well-characterized population make direct comparisons difficult and underscore the need for further studies. In view of these considerations, the objective of the present study was to describe lung function changes in overweight children and adolescents without respiratory symptoms.

Methods

This was a cross-sectional descriptive study involving male and female overweight patients in the 8-18 year age bracket. Age was recorded in completed whole years, and skin color/ethnicity was classified as black (African), brown (Mulatto), white (Caucasian), yellow (Asian), or red (Indigenous) on the basis of the parameters established by the Brazilian Institute of Geography and Statistics.[7] A convenience sample was recruited between May of 2010 and September of 2011 from among patients treated at the child and adolescent obesity outpatient clinic of the Pediatric Clinical Nutrition Department of the Professor Edgard Santos University Hospital, located in the city of Salvador, Brazil. The participants completed a systematic questionnaire designed for the present study and including data obtained during history taking and physical examination (with emphasis on respiratory findings), ancillary tests being subsequently performed. The inclusion criteria were as follows: having exogenous obesity; having no respiratory symptoms; having normal pulmonary auscultation and chest X-ray findings; and having a baseline SpO_2 > 92 %. Neither smokers nor patients with a history of wheezing, cough, chest pain, or respiratory disease were included in the present study.

Waist circumference was measured with a flexible, nonelastic tape measure at the midpoint between the lower costal margin and the anterior superior iliac crest.[8] Two measurements were performed, and the mean was used. Participants were weighed (in their underwear) on a portable scale (Filizola, São Paulo, Brazil) accurate to within 100 g. Height (in cm) was measured to the nearest 0.1 cm with a stadiometer with a base plate. The body mass index (BMI) was calculated by the formula weight/height^2 (kg/m^2), and the World Health Organization (WHO) BMI-for-age Z score for girls and boys[9] was used to classify the participants as overweight (having a BMI > 1 SD above the WHO growth standard median), obese (having a BMI > 2 SDs above the WHO growth standard median), or severely obese (having a BMI > 3 SDs above the WHO growth standard median).

Lung function was assessed in the Pulmonary Function Laboratory of the Federal University of Bahia, located in the city of Salvador, Brazil. Lung function was assessed by spirometry with pharmacological testing, spirometry being performed with a Koko Digidoser pneumotachograph spirometer (Ferraris Respiratory, Louisville, CO, USA). The tests were performed before and 15 min after the administration of 400 µg of inhaled albuterol. The helium dilution method was used in order to measure RV and TLC, which were measured with a Vmax 21 mass flow sensor (Viasys Healthcare, Palm Springs, CA, USA). Participants were advised to refrain from coffee, tea, medications (such as bronchodilators), and large amounts of food before the tests. Participants performed the tests with their heads in a central position, using a nose clip. All tests were performed at the same time of day (in the afternoon) by the same professional. At least three FVC maneuvers were performed by each

individual; the tests and curves that met the acceptability and reproducibility criteria of the American Thoracic Society[10] and the Brazilian Thoracic Association[11] were selected. The highest of at least three technically acceptable measurements of FEV_1 and FVC were selected. Those were expressed as absolute values and as percentages of predicted values, the latter being defined as the lower limit of normal (LLN) for each individual. The LLN was calculated by the equation described by Pereira,[12] valid for both genders. The reference values for lung volumes (RV and TLC) were those reported by Pennock et al.[13]

On the basis of spirometric values, TLC, and RV, patients were classified as having one of the following:

- normal lung function—FVC ≥ LLN; FEV_1/FVC ≥ LLN; and normal RV and TLC
- obstructive lung disease (OLD)—FEV_1/FVC < LLN; normal or increased RV or TLC; or a positive bronchodilator response
- restrictive lung disease (RLD)—FVC < LLN; FEV_1/FVC ≥ LLN; and reduced RV and TLC
- mixed obstructive and restrictive lung disease (MORLD)—FEV_1/FVC < LLN and reduced TLC

Bronchodilator response testing was performed in accordance with the Brazilian Thoracic Association guidelines,[11] a significant bronchodilator response being characterized by the following:

- abnormal spirometry results showing a post-bronchodilator absolute change in FEV_1 (post-bronchodilator FEV_1 – pre-bronchodilator FEV_1) ≥ 200 mL; an absolute variation in FEV_1 in relation to the predicted value ([post-bronchodilator FEV_1 – pre-bronchodilator FEV_1] × 100/predicted FEV_1) > 7%; and a post-bronchodilator absolute change in FVC (post-bronchodilator FVC – pre-bronchodilator FVC) ≥ 350 mL
- normal spirometry results showing a post-bronchodilator change in FEV_1 (post-bronchodilator FEV_1 – pre-bronchodilator FEV_1) ≥ 10%

Data analysis was performed with the IBM SPSS Statistics software package, version 19.0 (IBM Corporation, Armonk, NY, USA). Quantitative variables were expressed as mean ± standard deviation. Qualitative variables were expressed as absolute and relative frequencies. Pearson's test was used in order to assess correlations among spirometric variables, BMI-for-age Z scores, and waist circumference measurements in the OLD, RLD, and MORLD groups. Values of $p \leq 0.05$ were considered significant. The present study was approved by the local research ethics committee (Ruling no. 80/09), and the parents or legal guardians of all participants gave written informed consent.

Results

The study sample consisted of 59 individuals, the mean age being 11.7 ± 2.7 years. Of those 59 individuals, 30 (50.8%) were male. In addition, 32 (54.2%) were Mulatto, 22 (37.2%) were Black, and 5 (8.4%) were White. The BMI-for-age Z scores ranged from 1.2 SDs to 6.1 SDs, with a mean of 3.1 ± 1.0 (kg/m^2). Therefore, of the 59 individuals studied, 4 (6.7%) were classified as overweight, 28 (47.4%) were classified as obese, and 27 (45.7%) were classified as severely obese. Table 1 shows the lung function measurements in % of predicted.

Of the 59 individuals studied, 17 (30.3%) had TLC values < 80% of predicted, whereas 2 (3.5%) had TLC values > 120%.

Of the 59 individuals studied, 15 (25.4%) had a positive bronchodilator response (as determined by the change in FEV_1), 10 (67.0%) of those 15 individuals being severely obese (Table 2). They all had FEV_1/FVC values below the LLN, being therefore classified as having OLD. The change in FVC (i.e., post-bronchodilator FVC – pre-bronchodilator FVC) characterized a positive bronchodilator response in only 2 individuals, and none of the patients with MORLD had a positive bronchodilator response. One of the individuals with a positive response was unable to undergo measurement of lung volumes.

Considering FEV_1 a predictor of bronchial obstruction, we analyzed the changes occurring in absolute and percent predicted FEV_1 after bronchodilator use. The results are shown in Table 3.

We examined the relationship between lung disease and the degree of obesity in the study sample. The results are shown in Table 4.

Pearson's correlation analysis was performed in order to determine whether the anthropometric measurements (i.e., BMI and waist circumference) correlated with the spirometric variables in the OLD, RLD, and MORLD groups. The results

Table 1 - Lung function measurements (in percentage of predicted values) in 59 overweight children and adolescents with normal lung function, obstructive lung disease, restrictive lung disease, or mixed obstructive and restrictive lung disease.[a]

Lung function	FEV_1		FVC		FEV_1/FVC		$FEF_{25-75\%}$		TLC	RV
	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Pre-Bd
Normal	95.43 ± 10.89	95.10 ± 12.19	93.05 ± 9.51	90.16 ± 11.40	86.81 ± 3.44	88.67 ± 2.79	64.86 ± 14.38	66.86 ± 15.14	91.40 ± 8.70	89.65 ± 27.43
OLD	86.26 ± 13.23	89.74 ± 17.19	93.06 ± 13.51	92.95 ± 15.50	76.42 ± 5.09	80.47 ± 7.40	51.42 ± 14.35	60.63 ± 22.30	99.00 ± 13.55	118.56 ± 47.53
RLD	75.33 ± 13.08	79.27 ± 14.54	72.20 ± 14.30	74.27 ± 15.56	88.80 ± 4.88	89.21 ± 5.80	57.33 ± 12.88	64.20 ± 18.27	69.38 ± 7.95	66.62 ± 42.53
MORLD	73.50 ± 5.91	80.50 ± 12.15	83.25 ± 4.19	86.00 ± 13.24	74.25 ± 3.86	78.75 ± 3.86	45.25 ± 8.57	55.50 ± 14.40	71.75 ± 11.89	37.25 ± 41.99

Bd: bronchodilator; OLD: obstructive lung disease; RLD: restrictive lung disease; and MORLD; mixed obstructive and restrictive lung disease. [a]Values expressed as mean ± SD.

Table 2 - Respiratory function variables (in percentage of predicted values) in 15 children and adolescents showing changes in FEV_1.

Individual	FEV_1		FVC		FEV_1/FVC		$FEF_{25-75\%}$		TLC	RV
	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Post-Bd	Pre-Bd	Pre-Bd
01	85	94	101	102	71	78	53	65	95	62
02	84	91	87	89	79	84	58	75	86	78
03	94	102	100	101	78	83	72	80	94	71
04	101	110	108	112	80	83	70	81	104	99
05	103	110	111	114	78	82	69	82	119	150
06	100	108	101	100	81	89	80	110	89	31
07	70	94	70	95	85	83	33	40		
08	69	78	83	91	70	73	35	43	79	70
09	68	78	75	78	75	80	38	47	84	106
10	92	103	112	108	69	79	48	60	124	171
11	84	96	83	90	84	88	69	69	80	74
12	80	98	89	102	77	81	56	74	78	46
13	86	101	94	92	78	93	51	75	128	235
14	86	97	87	93	82	86	62	78	104	169
15	70	82	68	70	95	100	44	80	73	103

Bd: bronchodilator.

are shown in Table 5. There was a significant negative correlation between the anthropometric measurements and the FEV_1/FVC ratio in the individuals with MORLD.

Discussion

In the present study, we evaluated the lung function of overweight children and adolescents with normal chest X-rays and without respiratory symptoms or a history of respiratory disease. Pulmonary function test results were abnormal in 64.4% of the sample, OLD having predominated (in 32.2%). This suggested that obesity affected respiratory function. In addition, the proportion of normal spirometry results was lower in the individuals who were severely obese than in those who were overweight or obese.

Bronchodilator response was observed in 25.4% of the individuals studied, suggesting the presence of reversible OLD. Although 2 patients had a substantial bronchodilator response, neither had any disease to which airway hyperresponsiveness might have been attributed. The use of exhaled nitric oxide or induced sputum in order to assess airway inflammation might contribute to explaining this finding.

It has been reported that $FEF_{25-75\%}$ is a predictor of small airway obstruction.[14] Pre- and post-bronchodilator $FEF_{25-75\%}$ were below the LLN in 51 (86.4%) and 41 (69.4%), respectively, of the

individuals analyzed in the present study. This suggests that OLD and bronchodilator response occurred mainly at the level of the small airways. The diagnosis of OLD in the present study was primarily based on spirometric data rather than on TLC. This was due to the fact that only 2 of the participants had a TLC of more than 120% of predicted, a finding suggesting that the individuals with OLD had less hyperinflation and, therefore, less severe disease.

Table 3 - Changes in FEV_1 in the 15 overweight children and adolescents with a positive bronchodilator response.

Individual	ΔFEV_1, mL*	ΔFEV_1, %**
01	260	9
02	180	7
03	310	8
04	260	9
05	210	7
06	350	8
07	420	24
08	270	9
09	240	7
10	290	11
11	310	12
12	570	18
13	380	15
14	370	11
15	260	12

*Mean ± SD = 312 ± 96.7 mL. **Mean ± SD = 11.1 ± 4.7%.

Table 4 - Lung function in the study sample, by degree of obesity.[a]

Lung function	Overweight/ obese	Severely obese	Total
Normal	14 (23.7)	07 (11.8)	21 (35.5)
OLD	09 (15.2)	10 (16.9)	19 (32.2)
RLD	07 (11.8)	08 (13.5)	15 (25.4)
MORLD	02 (3.3)	02 (3.3)	04 (6.7)

OLD: obstructive lung disease; RLD: restrictive lung disease; and MORLD: mixed obstructive and restrictive lung disease. [a]Values expressed as n (%).

We found a significant negative correlation between the anthropometric and spirometric variables in the individuals with MORLD. However, this was found in only a small number of participants, further studies being therefore required.

We found studies describing and quantifying lung function changes, with conflicting results. Saxena et al.[15] studied young adults and reported that lung function changes occur in overweight individuals and are proportional to the degree of obesity. According to Ora et al.,[16] reduced lung volumes in adults appear to be associated with obesity and predispose to increased airway resistance and decreased expiratory flow.

Spathopoulos et al.[17] in a cohort study involving children in Greece, investigated the influence of obesity on pulmonary function, as well as a possible association of obesity with atopy and asthma. Teixeira et al.[18] found significant changes in the slow vital capacity and peak expiratory flow of obese children and adolescents. El-Baz et al.[19] investigated the impact of obesity and body fat distribution on lung function in Egyptian children and found that respiratory symptoms were more common in the children who were overweight than in those who were normal weight. The authors also found significant RLD, small airway obstruction, respiratory muscle dysfunction, and increased airway resistance in the overweight children.[19] However, no such association was found in other studies. Bertolini and Koseki[20] found no correlation between lung function and anthropometric measurements in moderately obese children. Pekkarinen et al.[21] investigated individuals over 18 years of age and found no correlation between body composition and spirometric changes; however, the authors

Table 5 - Correlations between anthropometric and spirometric variables in the obstructive lung disease, restrictive lung disease, and mixed obstructive and restrictive lung disease groups.

Spirometric variables	Anthropometric variables											
	Waist circumference						Body mass index					
	OLD		RLD		MORLD		OLD		RLD		MORLD	
	r	p	r	p	r	p	r	p	r	p	r	p
FEV_1	0.064	0.795	0.331	0.228	0.628	0.371	–0.092	0.709	0.146	0.603	0.434	0.566
FVC	0.048	0.845	0.250	0.369	0.645	0.355	–0.127	0.603	0.091	0.747	0.461	0.539
FEV_1/FVC	0.095	0.700	0.113	0.689	–0.996	0.004	–0.219	0.367	0.106	0.708	–0.986	0.014
$FEF_{25-75\%}$	0.055	0.823	0.265	0.341	–0.667	0.333	0.203	0.405	0.358	0.190	–0.527	0.473

OLD: obstructive lung disease; RLD: restrictive lung disease; and MORLD: mixed obstructive and restrictive lung disease.

reported a negative correlation between waist circumference and the FEV_1/FVC ratio. Finally, according to Boran et al.,[22] a likely explanation for these discrepant findings is the fact that most studies investigate extreme levels of obesity or have a small sample size, with no control groups.

The high prevalence of lung disease in the present study is consistent with some of the data from the studies cited herein, given that reduced chest compliance, increased abdominal pressure, early airway collapse, and increased airway resistance are expected in overweight individuals.[23] These changes can explain why overweight individuals have lung disease (OLD, RLD, or MORLD). According to Lopes,[24] inflammatory processes mediated by cytokines produced by adipocytes are responsible for pulmonary changes, characterized by airway hyperresponsiveness, among others. This observation is in agreement with those of El-Baz et al.,[19] who defined fat as a metabolically active tissue.

In a study investigating biomarkers of lung function in overweight adolescents with asthma,[25] weight loss was shown to reduce the concentration of adiponectin (an anti-inflammatory mediator), especially in cases of visceral obesity; to increase FEV_1 and FVC; and to improve asthma symptoms. In a study investigating mildly obese children, Boran et al.[22] reported that 3 of the children had reversible OLD (as determined by pulmonary function test results), despite having no previous respiratory symptoms or atopic diseases; the authors also reported that, given the lack of provocation tests, further studies were needed in order to determine whether obesity caused or increased airway hyperresponsiveness. In the present study, participants were not screened for atopy and no bronchial provocation tests were performed; however, the number of individuals with a bronchodilator response was higher in the present study than in the study conducted by Boran et al.[22]

The pathophysiology of obesity-related lung changes includes changes resulting from restricted lung expansion caused by lipid deposition, with decreased alveolar surface area, and reducing functional residual capacity.[26] Therefore, the etiopathogenesis of OLD in obese individuals appears to involve mechanical and inflammatory processes. Studies have examined the association between asthma and obesity in an attempt to determine whether it is due to reduced lung volumes or increased airway resistance resulting in asthma-like symptoms.[27]

Camilo et al.[28] reported that obesity is not the only factor responsible for the development and increased prevalence of asthma. Other important factors, such as genetic factors, immunological factors, and environmental factors, should be considered in future studies. Story[29] reported that airway inflammation, mechanical changes secondary to obesity, and airway hyperresponsiveness, as well as changes in diet and physical activity, are related to the development of asthma in overweight individuals. Story[29] also reported that obesity increases the severity of asthma and reduces the quality of life in obese children, having reported that further studies are needed in order to clarify the relationship between asthma and obesity. In our study, this finding was considered relevant, given that the study sample consisted of patients without respiratory disease or symptoms.

The present study has limitations that should be considered. One limitation is the number of participants, which can be partly explained by the difficulty in finding individuals meeting the inclusion criteria. Another limitation is related to the use of the helium dilution method for measuring lung volumes. The method is known to underestimate lung volumes by not measuring the volume of air in areas of air trapping excluded from ventilation. This, however, is of little or no significance, given that the study sample consisted of young patients with normal chest X-rays and no history of respiratory disease. Yet another limitation is the difficulty in choosing reference values for the age group under study. Because there are no universally recommended values, we used parameters that are generally accepted in the literature. Finally, there was no control group in the present study; Pereira[12] established reference values, and those were used here. Therefore, lung function should be further investigated in longitudinal studies involving samples that are similar to our study sample, in order to determine the risk of lung disease by means of bronchial provocation tests, measurement of DLCO, and determination of immune, hormonal, and inflammatory mediators.

Because pulmonary function tests provide objective data on lung function,[3] they are essential in the management of individuals with respiratory dysfunction and in individuals at risk

of developing respiratory dysfunction, as is the case of overweight individuals. The combination of spirometry and lung volume measurements should be the method of choice for the evaluation of lung function, given that it is the best and most comprehensive method for measuring lung function, allowing a reliable diagnosis of lung disease. This combination should therefore be part of the routine care of such patients.

In conclusion, the prevalence of lung disease in overweight children and adolescents was high in the present study, OLD having predominated. The bronchodilator response observed in the present study was greater than was that reported in the literature, a positive bronchodilator response being more common in the individuals who were severely obese than in those who were obese or overweight.

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Mycobacterium tuberculosis resistance to antituberculosis drugs in Mozambique*,**

Resistência de *Mycobacterium tuberculosis* aos tuberculostáticos em Moçambique

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Abstract

Objective: To determine the drug resistance profile of *Mycobacterium tuberculosis* in Mozambique. **Methods:** We analyzed secondary data from the National Tuberculosis Referral Laboratory, in the city of Maputo, Mozambique, and from the Beira Regional Tuberculosis Referral Laboratory, in the city of Beira, Mozambique. The data were based on culture-positive samples submitted to first-line drug susceptibility testing (DST) between January and December of 2011. We attempted to determine whether the frequency of DST positivity was associated with patient type or provenance. **Results:** During the study period, 641 strains were isolated in culture and submitted to DST. We found that 374 (58.3%) were resistant to at least one antituberculosis drug and 280 (43.7%) were resistant to multiple antituberculosis drugs. Of the 280 multidrug-resistant tuberculosis cases, 184 (65.7%) were in previously treated patients, most of whom were from southern Mozambique. Two (0.71%) of the cases of multidrug-resistant tuberculosis were confirmed to be cases of extensively drug-resistant tuberculosis. Multidrug-resistant tuberculosis was most common in males, particularly those in the 21-40 year age bracket. **Conclusions:** *M. tuberculosis* resistance to antituberculosis drugs is high in Mozambique, especially in previously treated patients. The frequency of *M. tuberculosis* strains that were resistant to isoniazid, rifampin, and streptomycin in combination was found to be high, particularly in samples from previously treated patients.

Keywords: Extensively drug-resistant tuberculosis; Tuberculosis; Tuberculosis, multidrug-resistant.

Resumo

Objetivo: Avaliar o perfil de resistência de *Mycobacterium tuberculosis* aos tuberculostáticos em Moçambique. **Métodos:** Foram analisados dados secundários do Laboratório Nacional de Referência da Tuberculose, em Maputo, Moçambique, e do Laboratório Regional de Referência da Tuberculose, na Beira, Moçambique. Os dados foram relativos a amostras positivas à cultura e submetidas ao teste de sensibilidade aos tuberculostáticos de primeira linha durante o período de janeiro a dezembro de 2011. Os resultados do teste de sensibilidade foram analisados, e sua frequência foi comparada com o tipo de paciente e sua proveniência. **Resultados:** Foram analisadas 641 cepas, isoladas em cultura e submetidas ao teste de sensibilidade. Das 641 cepas, 374 (58,3%) foram resistentes a pelo menos um tuberculostático e 280 (43,7%) revelaram-se multirresistentes. Dos 280 casos de tuberculose multirresistente, 184 (65,7%) eram pacientes com tratamento prévio, a maioria dos quais era oriunda da zona sul do país. Confirmou-se que 2 (0,71%) dos casos de tuberculose multirresistente eram casos de tuberculose extensivamente resistente a drogas. O sexo masculino foi o mais afetado, particularmente na faixa etária de 21 a 40 anos. **Conclusões:** A resistência de *M. tuberculosis* aos tuberculostáticos é elevada em Moçambique, especialmente em indivíduos com tratamento prévio. A resistência de *M. tuberculosis* à combinação de isoniazida, rifampicina e estreptomicina foi elevada, especialmente em amostras provenientes de indivíduos com tratamento prévio.

Descritores: Tuberculose extensivamente resistente a drogas; Tuberculose; Tuberculose resistente a múltiplos medicamentos.

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Financial support: This study received financial support from the Field Epidemiology and Laboratory Training Program of Mozambique.
Submitted: 21 January 2013. Accepted, after review: 20 January 2014.

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

Introduction

Tuberculosis (TB) remains a serious public health problem in many low- and middle-income countries in Africa, Asia, and the former Soviet Union.[1] According to the World Health Organization, nearly 9 million new TB cases are recorded globally each year, 4 million of which are infectious TB cases. In many countries, the number of TB cases has quadrupled, despite the implementation of effective strategies to combat the disease.[2]

Not only has the number of studies of antituberculosis drug resistance increased (from 1 in 2008 to 10 in 2011), but the number of countries providing representative drug resistance data has also increased (from 19 to 22), Mozambique being one such country.[3] Treatment failure, poor adherence to treatment, and spontaneous mutations in *Mycobacterium tuberculosis* strains have contributed to the emergence of new multidrug-resistant TB (MDR-TB) cases, which can later develop into extensively drug-resistant TB (XDR-TB) cases.[4-6] Recent drug resistance studies have identified high rates of MDR-TB in southern Africa, and 69 countries (including Mozambique) had reported at least one case of XDR-TB by the end of 2010.[2,7,8] In Mozambique, drug-resistant TB is thought to be a major problem. The 2008 Mozambican national antituberculosis drug resistance survey showed that, of all MDR-TB cases, 3.5% were newly diagnosed TB cases and 11.2% occurred among individuals who had previously been treated for TB; in 2011, 47,452 cases of all forms of TB were detected,[3] although none were reported to be cases of XDR-TB.

Because of the increasing number of cases of MDR-TB[4] and the emergence of XDR-TB in Mozambique, we sought to evaluate M. tuberculosis resistance to antituberculosis drugs in previously treated and untreated TB patients in Mozambique. We also sought to determine the magnitude of antituberculosis drug resistance in the country, in order to inform the National Tuberculosis Control Program of the efficacy of TB control measures and treatment, as well as to design effective treatment regimens and strategies for all TB patients in the country.

Methods

This was a cross-sectional study based on secondary laboratory data for the period of January to December of 2011. The data were based on 641 positive TB cultures from the National Tuberculosis Referral Laboratory, located in the city of Maputo, Mozambique, and the Beira Regional Tuberculosis Referral Laboratory, located in the city of Beira, Mozambique. These two laboratories serve all 11 of the provinces of Mozambique.

An MDR-TB case was defined as an individual infected with an isolate resistant to at least isoniazid and rifampin, confirmed cases of XDR-TB being excluded.[9-11] Cases of MDR-TB resistant to a fluoroquinolone and a second-line injectable drug other than streptomycin were defined as XDR-TB cases.[12-15] If an MDR-TB or XDR-TB patient was registered for treatment and had never received TB treatment for longer than 4 weeks, the patient was considered to have primary drug resistance. Patients with MDR-TB/XDR-TB undergoing retreatment and having had the first episode of TB before 2011 were assumed to have acquired drug resistance.

All samples underwent smear microscopy and culture. All culture-positive samples underwent first-line drug susceptibility testing (DST), which was performed by means of the ratio method. Of the MDR-TB samples, 71 were sent to the Supranational TB Referral Laboratory in Milan, Italy, for second-line DST.

Secondary data from the National Tuberculosis Referral Laboratory (demographic data and DST results) were collected from a WixDisa database (version 04.16.04.652; Disa, South Africa) and the laboratory record book, whereas those from the Beira Regional Tuberculosis Referral Laboratory were retrieved from a Microsoft Excel database. All positive culture results and DST results were entered into an Epi Info 3.5.1 database and analyzed. We calculated ORs and their 95% CIs in order to determine the association of previously treated and untreated patients with the results of DST.

The study was reviewed and approved by the Research Ethics Committee of the National Institute of Health, in Maputo, Mozambique. Informed consent was not required, because the study was based on secondary data and we had no access to any identifying patient information.

Results

A total of 641 TB culture-positive samples (561 samples from the National Tuberculosis

Referral Laboratory and 80 from the Beira Regional Tuberculosis Referral Laboratory) were analyzed during the study period. Table 1 shows the distribution of MDR-TB and XDR-TB patients by gender and age bracket.

Of the 641 samples, 430 (67.1%) were from previously treated TB patients and 280 (43.7%) were from MDR-TB patients (Table 2). Of those 280 samples, 148 (53%) were from males and 191 (68.2%) were from individuals in the 21-40 year age bracket. There were 2 XDR-TB patients, both in the 21-40 year age bracket.

Of the MDR-TB samples that were sent to the Supranational TB Referral Laboratory for second-line DST, 2 were confirmed to be XDR-TB samples; of those, 1 was from a previously treated patient, and 1 was from a previously untreated patient (Figure 1).

Among of the 641 samples evaluated, the most common drug resistance pattern was monoresistance to isoniazid (in 5.8%), followed by monoresistance to rifampin (in 3.4%). Table 3 shows the distribution of specific drug resistance patterns by history of TB treatment.

Table 4 shows the distribution of multidrug resistance patterns by history of TB treatment. Most of the *M. tuberculosis* samples, particularly those from previously treated patients, were found to be resistant to isoniazid, rifampin, and streptomycin in combination.

Discussion

Most of the MDR-TB patients were male and in the 21-40 year age bracket (Table 1). The high occurrence of MDR-TB among males and working-age individuals is probably due to the high number of Mozambican males working in South African mines, which constitute a high-risk environment for TB and other infectious diseases. These males often return to their home country whenever they become ill, thereby increasing the risk of infection among their wives and close contacts. The fact that working-age individuals constitute the most commonly affected age group is due to the fact that many such individuals, in search of better pay and, consequently, better living conditions, work in the close quarters of the aforementioned mines. Our results are consistent with those reported in other studies.[3,16-21]

The proportion of MDR-TB samples was higher than was that of non-MDR-TB samples, the difference being statically significant (p <

Table 1 - Distribution of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis patients, by gender and age bracket.

Age bracket (years)	MDR-TB patients (n = 280)			XDR-TB patients (n = 2)		
	Gender		Total	Gender		Total
	Female	Male		Female	Male	
	(n)	(n)		(n)	(n)	
0-20	16	3	19			
21-40	92	99	191	1	1	2
41-60	20	36	56			
> 60	1	4	5			
Missing data	3	6	9			
Total	132	148	280	1	1	2

MDR-TB: multidrug-resistant tuberculosis; and XDR-TB: extensively drug-resistant tuberculosis.

Table 2 - Distribution of multidrug-resistant tuberculosis and non-multidrug-resistant tuberculosis patients, by history of tuberculosis treatment.

Treatment history	MDR-TB patients	Non-MDR-TB patients	OR	p
	n (%)	n (%)		
Previous treatment	184 (65.7)	172 (47.6)	2.06 (1.47-2.88)	< 0.001
No previous treatment	96 (34.3)	185 (51.2)		
Missing data		4 (1.1)		
Total	280 (100)	361 (100)		

MDR-TB: multidrug-resistant tuberculosis.

0.001). Most of the MDR-TB samples were from previously treated patients, and the fact that there were 2 XDR-TB samples shows that there might be more cases of XDR-TB not diagnosed as such. Therefore, further efforts are needed in order to improve diagnosis and treatment.

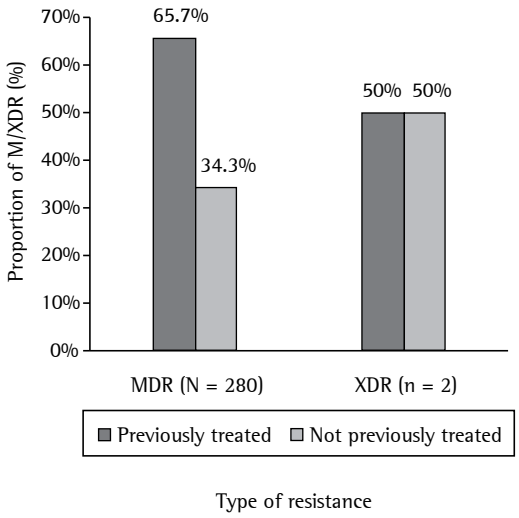

Figure 1 - Proportion of multidrug-resistant tuberculosis (MDR) and extensively drug-resistant tuberculosis (XDR) cases by patient treatment history.

The results of the present study show that M. tuberculosis monoresistance to isoniazid and rifampin was most common in samples from previously treated patients, as was M. tuberculosis resistance to isoniazid, rifampin, and streptomycin in combination. Although previous treatment can influence the onset of resistance to isoniazid, rifampin, and streptomycin in combination, we found no statistically significant difference between previously treated and untreated patients regarding resistance to this drug combination. This finding is consistent with those of similar studies conducted in Brazil, Portugal, and Turkey,[21-24] as well as with those of studies conducted in Mozambique, South Africa, Tanzania, Iran, and New Delhi.[6,13,25-27]

Treatment failure, poor adherence to treatment, and spontaneous mutations in *M. tuberculosis* strains probably played a major role in the emergence of MDR-TB, which can progress to XDR-TB.[4,5,14,15,28] This could explain why the number of MDR-TB cases was higher among previously treated patients and shows

Table 3 - Distribution of specific drug resistance patterns, by history of tuberculosis treatment.

Monoresistance			Treatment history				OR (95% CI)	p
			No previous treatment		Previous treatment			
			(n = 211)		(n = 430)			
Drug	n	%	n	%	n	%		
H	37	56.9	17	53.1	20	60.6	1.36 (0.45-4.09)	0.720
R	22	33.8	10	31.3	12	36.4	1.26 (0.40-4.00)	0.862
S	2	3.1	1	3.1	1	3.0	0.97 (0.03-37.42)	1.000
E	4	6.2	4	12.5	0	0.0	-	0.053
Total	65	100.0	32	100.0	33	100.0		

H: isoniazid; R: rifampin; S: streptomycin; and E: ethambutol.

Table 4 - Distribution of multidrug resistance patterns, by history of tuberculosis treatment.

Drug combination	Multidrug resistance		History of tuberculosis treatment				OR (95% CI)	p
			No previous treatment		Previous treatment			
			(n = 211)		(n = 430)			
	n	%	n	%	n	%		
R+S	3	1.0	1	0.9	2	1.0	0.26 (0.0.8-0.78)	0.011
H+S	18	6.0	12	11.0	6	3.1	1.14 (0.08-32.04)	1.000
H+R	58	19.3	19	17.4	39	20.3	1.21 (0.63-2.32)	0.647
H+R+S	139	46.2	50	45.9	89	46.4	1.02 (0.62-1.68)	0.968
H+R+E	19	6.3	7	6.4	12	6.3	0.97 (0.34-2.83)	0.851
H+R+S+E	64	21.3	20	18.3	44	22.9	1.32 (0.71-2.49)	0.432
Total	301	100.0	109	100.0	192	100.0		

H: isoniazid; R: rifampin; S: streptomycin; and E: ethambutol.

that further efforts are needed to ensure rapid diagnosis of MDR-TB/XDR-TB and access to treatment with second-line drugs in Mozambique. In order to manage drug-resistant forms of TB and prevent further cases of MDR-TB and XDR-TB, a comprehensive approach similar to that used in cases of drug-susceptible TB is needed to ensure rapid detection and appropriate treatment, as are public health measures to cure patients and prevent further transmission of the disease,[12,13,29,30] given that the epidemic of drug-resistant TB has spread.[13,15,28]

The classification of drug resistance as primary or acquired is used as an indicator of the efficacy of national tuberculosis control programs and in the adjustment and development of such programs. Unsupervised treatment can lead to an increase in the number of MDR-TB cases among previously treated patients. Although the directly observed treatment, short-course strategy has been adopted in Mozambique, the efficacy of this strategy needs to be evaluated. Cases of MDR-TB and XDR-TB must be effectively managed, second-line drugs being carefully used in order to reduce the morbidity, mortality, and transmission of MDR-TB and prevent the development of XDR-TB.[12,15,29] In addition, better integration of the National Tuberculosis Control Program activities and activities such as counseling and home-based care could assist in controlling TB in the country.

In conclusion, antituberculosis drug resistance is high in laboratory-confirmed cases of TB in Mozambique, especially among previously treated patients. It is possible that XDR-TB strains are circulating in the population, given that we identified 2 XDR-TB cases in the present study (1 being in a previously treated patient and 1 being in a previously untreated patient). Resistance to isoniazid, rifampin, and streptomycin in combination was found to be high, particularly in previously treated patients.

Acknowledgments

We would like to thank the staff of the National Tuberculosis Referral Laboratory and the Beira Regional Tuberculosis Referral Laboratory, for providing the data for analysis.

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Use of amplified *Mycobacterium tuberculosis* direct test in respiratory samples from HIV-infected patients in Brazil*

Utilização do *amplified Mycobacterium tuberculosis direct test* em amostras respiratórias de pacientes HIV positivos no Brasil

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Abstract

Objective: To compare the accuracy of the amplified *Mycobacterium tuberculosis* direct (AMTD) test with reference methods for the laboratory diagnosis of tuberculosis in HIV-infected patients. **Methods:** This was a study of diagnostic accuracy comparing AMTD test results with those obtained by culture on Löwenstein-Jensen (LJ) medium and by the BACTEC Mycobacteria Growth Indicator Tube 960 (BACTEC MGIT 960) system in respiratory samples analyzed at the Bioassay and Bacteriology Laboratory of the Oswaldo Cruz Foundation Evandro Chagas Clinical Research Institute in the city of Rio de Janeiro, Brazil. **Results:** We analyzed respiratory samples collected from 118 patients, of whom 88 (74.4%) were male. The mean age was 36.6 ± 10.6 years. Using the AMTD test, the BACTEC MGIT 960 system, and LJ culture, we identified *M. tuberculosis* complex in 31.0%, 29.7%, and 27.1% of the samples, respectively. In comparison with LJ culture, the AMTD test had a sensitivity, specificity, positive predictive value, and negative predictive value of 87.5%, 89.4%, 75.7%, and 95.0%, respectively, for LJ culture, whereas, in comparison with the BACTEC MGIT 960 system, it showed values of 88.6%, 92.4%, 83.8%, and 94.8%, respectively. **Conclusions:** The AMTD test showed good sensitivity and specificity in the population studied, enabling the laboratory detection of *M. tuberculosis* complex in paucibacillary respiratory specimens.

Keywords: Molecular diagnostic techniques; Tuberculosis; HIV; Molecular probe techniques.

Resumo

Objetivo: Comparar a acurácia do teste *amplified Mycobacterium tuberculosis direct* (AMTD) com métodos de referência para o diagnóstico laboratorial de tuberculose em pacientes HIV positivos. **Métodos:** Estudo de acurácia diagnóstica comparando os resultados do teste AMTD com os de cultura em Löwenstein-Jensen (LJ) e de BACTEC *Mycobacteria Growth Indicator Tube* 960 (sistema BACTEC MGIT 960) em amostras respiratórias analisadas no Laboratório de Bacteriologia e Bioensaios do Instituto de Pesquisa Clínica Evandro Chagas da Fundação Oswaldo Cruz, no Rio de Janeiro (RJ). **Resultados:** Foram analisadas amostras respiratórias de 118 pacientes, dos quais 88 (74,4%) eram do sexo masculino. A média de idade foi de 36,6 ± 10,6 anos. O complexo *M. tuberculosis* foi identificado em 31,0%, 29,7% e 27,1% das amostras através do teste AMTD, sistema BACTEC MGIT 960 e LJ, respectivamente. Na comparação com a cultura em LJ, o teste AMTD apresentou sensibilidade, especificidade, valor preditivo positivo e valor preditivo negativo de 87,5%, 89,4%, 75,7% e 95,0%, respectivamente, enquanto na comparação com o sistema BACTEC MGIT 960, os valores foram de 88,6%, 92,4%, 83,8% e 94,8%, respectivamente. **Conclusões:** O teste AMTD mostrou boa sensibilidade e especificidade na população estudada, possibilitando a detecção laboratorial do complexo *M. tuberculosis* em espécimes respiratórios paucibacilares.

Descritores: Técnicas de diagnóstico molecular; Tuberculose; HIV; Técnicas de sonda molecular.

*Study carried out at the Evandro Chagas Clinical Research Institute and at the National Institute of Quality Control in Health, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.

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Financial support: None.
Submitted: 9 July 2013. Accepted, after review: 17 March 2014.

Introduction

Even though more than a century has passed since the discovery of the etiologic agent of tuberculosis, i.e., *Mycobacterium tuberculosis*, the disease remains a public health problem worldwide. Each person with active tuberculosis will infect between 10 and 15 people every year.[1] It is estimated that at least one of every 10 people who have come in contact with the tuberculosis bacillus will develop the disease and that, in HIV-infected patients, this risk is 20 to 40 times higher.[2] Studies evaluating survival in patients with tuberculosis/HIV co-infection have shown that the risk of death is higher in these patients than in HIV-infected patients without tuberculosis.[3-6]

Mycobacterial culture on solid Lowenstein-Jensen (LJ) medium is considered the gold standard isolation method.[7] Although the limitation of this method is the long incubation period (2-8 weeks), it is used by most developing countries because of its low cost. Techniques such as nucleic acid amplification and automated liquid culture systems are costly and depend on sophisticated tools, which prevents their routine use in poor countries.

In the last decade, laboratory tests for detection of *M. tuberculosis* have evolved considerably.[8] Today we have new methods, such as GeneXpert (Cepheid, Sunnyvale, CA, USA), which can yield results in 2 h, detecting *M. tuberculosis* complex and determining whether the strains are rifampin resistant; however, this method remains costly and has just begun to be used and validated for use in Brazil. The amplified *Mycobacterium tuberculosis* direct (AMTD) test (Gen-Probe, San Diego, CA, USA) can detect *M. tuberculosis* complex rRNA in approximately 3 h. This test was approved by the Food and Drug Administration for use in smear microscopy-positive respiratory samples in 1995, and, after it was improved in 1999, it was approved for use in smear microscopy-negative samples.[9] There is still need for a better understanding of the performance of this test for paucibacillary patients, such as HIV-infected patients in Brazil, since the quality of their samples makes it difficult to establish a laboratory diagnosis, even by gold standard methods, such as liquid culture. The objective of the present study was to compare the diagnostic accuracy of the AMTD test with other culture methods in respiratory samples collected from HIV-infected patients, by means of a study under real-life routine conditions in a mycobacteriology laboratory.

Methods

This was a study of diagnostic accuracy, conducted under routine conditions at the bacteriology laboratory of the Evandro Chagas Clinical Research Institute, which is a referral center for the treatment of infectious diseases, located in the city of Rio de Janeiro, Brazil. All respiratory samples provided by HIV-infected patients suspected of having pulmonary tuberculosis and sent to the laboratory between January of 2008 and June of 2009 were included in the study. All samples collected from the same patient subsequent to the first sample were excluded from the study. Respiratory samples included sputum, induced sputum, and bronchoalveolar lavage samples.

The clinical specimens were processed as shown in Figure 1. The samples were analyzed by smear microscopy, LJ culture, and the BACTEC Mycobacteria Growth Indicator Tube 960 (BACTEC MGIT 960) system (Becton Dickinson, Sparks, MD, USA). Smear microscopy was performed on the same day the clinical specimen was received at the laboratory. In contrast, cultures were performed over the course of 2 days at most. The samples showing growth on LJ medium, through culture or through subculture of positive BACTEC MGIT 960 cultures, were sent for biochemical identification of *M. tuberculosis* complex (detection of niacin production, nitrate reduction, and thermal inactivation of catalase).[10] In the present study, cultures that produced niacin, reduced nitrate to nitrite, and showed inactivation of catalase at 68°C were identified as positive for *M. tuberculosis* complex. Different results from those described above were analyzed and defined as positive for mycobacteria other than tuberculosis (MOTT). Part of the pellet obtained from decontamination of the samples was sent for AMTD tests and subsequent interpretation of results and for incubation in the BACTEC MGIT 960 system. Both methods were carried out as described by the respective manufacturers.[11,12] A positive result was defined as the presence of *M. tuberculosis* complex in the sample, and a negative result was defined as the absence of *M. tuberculosis* complex. The AMTD test was performed weekly, and biochemical identification was obtained in the same week the cultures or subcultures yielded

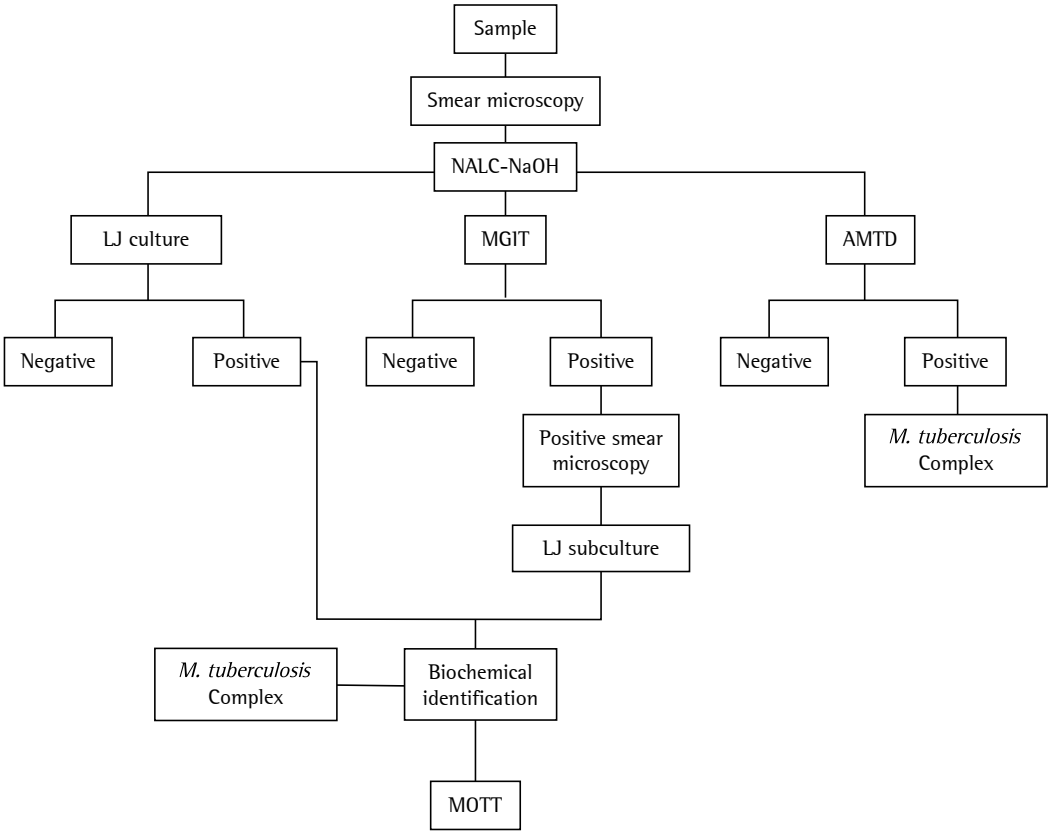

Figure 1 - Sample processing flowchart. NALC-NaOH: N-acetyl-L-cysteine-sodium hydroxide; LJ: Löwenstein-Jensen; MGIT: Mycobacteria Growth Indicator Tube; AMTD: amplified *Mycobacterium tuberculosis* direct (test); and MOTT: mycobacteria other than tuberculosis.

positive results. All collaborators who performed the tests mentioned above are regularly trained and evaluated on these procedures. There was no blinding of the collaborators, since they were performing routine tests.

The outcomes of interest were sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), accuracy, likelihood ratio (LR), and respective 95% CIs. These measures were calculated using the Statistical Package for the Social Sciences, version 17.0 (SPSS, Chicago, IL, USA) and WINPEPI, version 11.15 (http://www.brixtonhealth.com/pepi4windows.html).

The present study was approved by the Research Ethics Committee of the Evandro Chagas Clinical Research Institute (Protocol no. 0002.0.009.000-11) and was developed in accordance with the recommendations of the Standards for Reporting Diagnostic Accuracy.[13]

A letter about the present study has been published.[14]

Results

Of the 175 samples eligible for the study, 57 were excluded because they were subsequent samples from the same patient. Therefore, we analyzed the first respiratory samples collected from 118 patients, of whom 88 (74.4%) were male. The mean age was 36.6 ± 10.6 years. The results of all tests performed were conclusive. Figure 2 shows the study processing flowchart.

Of the 118 samples analyzed, 16 (13.6%) had positive results by smear microscopy. Of those 118 samples, 33 (27.9%) were positive by LJ culture, 1 of which was identified as MOTT, whereas (33.1%) were positive by the BACTEC MGIT 960 system, 3 of which were identified as MOTT and 1 of which was identified as *Rhodococcus* spp. The AMTD test detected 37 positive samples (31.4%) for *M. tuberculosis* complex. The isolated MOTT and *Rhodococcus* spp. strains were excluded from the main analysis because they are not targeted by the method under analysis.

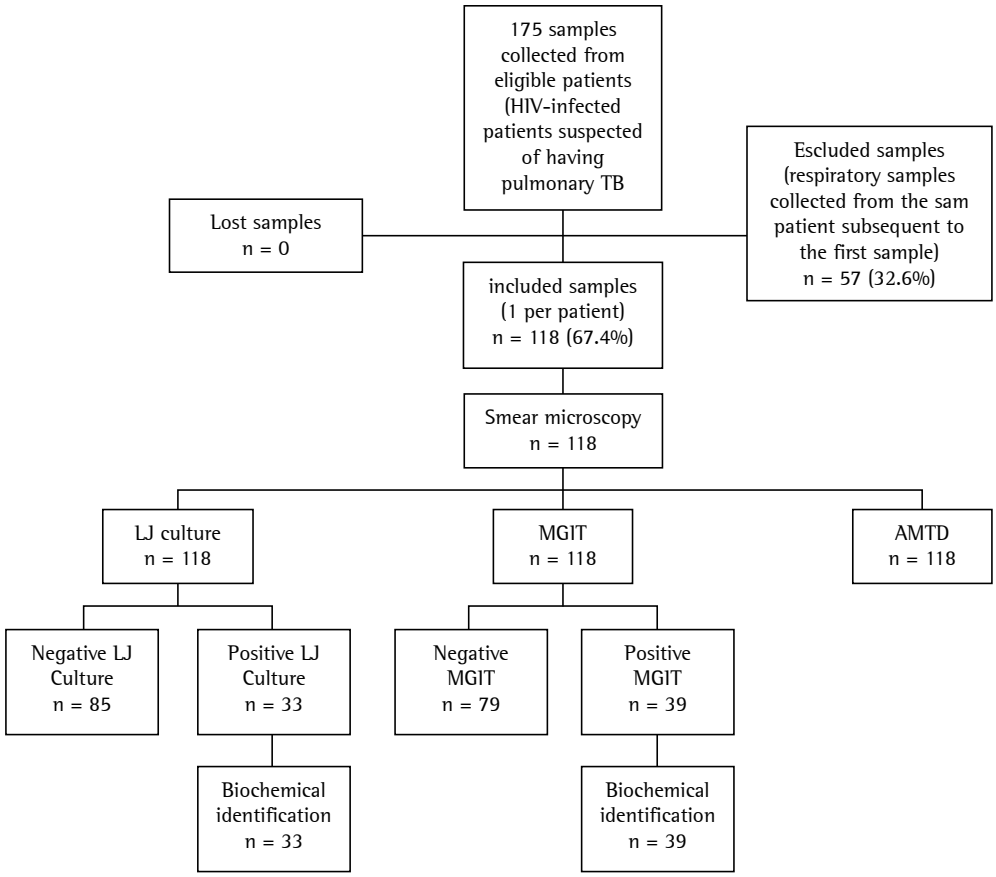

Figure 2 - Study design diagram. TB: tuberculosis; LJ: Löwenstein-Jensen; MGIT: Mycobacteria Growth Indicator Tube; and AMTD: amplified *Mycobacterium tuberculosis* direct (test).

After exclusion of those 5 samples, the comparison of the AMTD test results with those obtained by LJ culture showed that there were four false-negative results and nine false-positive results, whereas the comparison of the AMTD test results with those obtained by the BACTEC MGIT 960 system showed that there were six false-positive results and four false-negative results. Table 1 shows the diagnostic accuracy of the AMTD test in comparison with LJ culture and with the BACTEC MGIT 960 system.

In comparison with the BACTEC MGIT 960 system, the AMTD test had a sensitivity, specificity, PPV, and NPP of 88.6%, 92.4%, 83.8%, and 94.8%, respectively, whereas, in comparison with LJ culture, it showed values of 87.5%, 89.4%, 75.7%, and 95.0%, respectively. These results, together with their 95% CIs, the LRs, and the accuracy values, are shown in Table 1.

The same parameters were calculated for a subgroup of smear microscopy-negative samples, although that was not part of the initial analysis plan. The following results were obtained: sensitivity, 70.8% (95% CI: 48.6-87.3); specificity, 94.8% (95% CI: 87.2-98.6); PPV, 81.0% (95% CI: 58.1-94.6); and NPV, 91.3% (95% CI: 82.8-96.4).

Discussion

In our study, regardless of the smear microscopy results, the AMTD test results showed sensitivity and specificity comparable to those in the literature. A systematic review of 125 studies not exclusively of patients with paucibacillary disease estimated a sensitivity of 85% and a specificity of 96.8% for commercial nucleic-acid amplification tests.[15] A study not exclusively of patients with paucibacillary disease that compared the AMTD test with GeneXpert found a sensitivity of 96.8% and a specificity of 91.2% for the AMTD test.[16] It is possible that the difference observed relative to the values estimated in our study is due to the sample composition: whereas the

Table 1 - Accuracy of the amplified *Mycobacterium tuberculosis* direct test relative to culture on Löwenstein-Jensen medium and to the BACTEC Mycobacteria Growth Indicator Tube 960 system.[a]

Variable	AMTD vs. LJ	AMTD vs. MGIT
Sensitivity	87.5 (71.0-96.5)	88.6 (73.3-96.8)
Specificity	89.4 (80.8-95.0)	92.4 (84.2-97.2)
Positive predictive value	75.7 (58.8-88.2)	83.8 (68.0-93.8)
Negative predictive value	95.0 (87.7-98.6)	94.8 (87.2-98.6)
Positive likelihood ratio	8.25 (4.39-15.54)	11.66 (5.35-25.40)
Negative likelihood ratio	0.14 (0.06-0.35)	0.12 (0.05-0.31)
Accuracy	88.9 (81.7-93.9)	91.2 (84.5-95.7)

AMTD: amplified *Mycobacterium tuberculosis* direct (test); LJ: Löwenstein-Jensen; and MGIT: Mycobacteria Growth Indicator Tube. [a]Values expressed as % (95% CI).

eligibility criteria of the aforementioned study were too restrictive, our patients were selected only because they were seropositive for HIV.

The observed discrepant results, i.e., results that were positive by the AMTD test and negative by culture, may be due to laboratory contamination or to characteristics of the method used. The AMTD test can detect non-viable or dead bacilli, which are hardly to grow in culture. The opposite, i.e., results that were negative by the AMTD test and positive by culture, may indicate the presence of inhibitory substances, which were not examined in the present study.

A feature of nucleic-acid amplification tests is that sensitivity is compromised at the expense of specificity.[15] Other factors that contribute to decreased sensitivity are poor, paucibacillary, or negative samples (in HIV-infected patients) and the presence of inhibitory substances.

The present study showed that, in tuberculosis/HIV-infected patients, the AMTD test was able to detect *M. tuberculosis* complex in a greater number of samples than culture. However, culture is not 100% sensitive and can yield false-negative results, such as when samples contain dead bacilli, non-viable bacilli (because of decontamination of samples), or less than the minimum detectable amount for culture (approximately 10^2 bacilli/mL). Therefore, the study results may have been influenced by the chosen reference test.

Although it was not the purpose of our study, analysis of the results of direct examination showed that only one smear microscopy-positive sample was not detected by the AMTD test, possibly because of inhibitors, given that *M. tuberculosis* was isolated by the two culture methods. Smear microscopy did not detect AFB in approximately 21% of the samples in which the AMTD test was positive. This shows the weakness of smear microscopy in detecting mycobacteria in patients who are seropositive for HIV. Several factors, such as the expertise of the technician; the quality of the sample, which needs to contain between 5,000 and 10,000 bacilli/mL in order to prevent false-negative results[17,18]; and particular conditions, such as HIV co-infection,[18-20] directly influence smear microscopy results. However, smear microscopy remains an important tool for resource-poor countries, since it is the most rapid and inexpensive method available in all countries.

Studies have reported that the sensitivity of the AMTD test varies depending of the prevalence of HIV. However, they have shown the effectiveness of the method in identifying strains in smear microscopy-negative samples.[21-23]

The AMTD test is approved for use in respiratory samples regardless of smear microscopy results. It provides the greatest benefit to patients when used in smear microscopy-negative samples, given that it enables early diagnosis and the initiation of specific treatment. In our study, according to the reference method used, we obtained sensitivity and specificity similar to that reported by other studies not exclusively of HIV-infected patients.[24-28]

The main advantage of the routine use of nucleic-acid amplification tests in laboratories is the speed at which results are obtained, enabling early intervention when necessary. However, these tests should not replace culture, since they are able to detect non-viable microorganisms. For the same reason, they are also not useful for monitoring treatment, given that they provide non-quantitative results, which should be interpreted together with results of the conventional tests and with clinical data. However, they are useful in distinguishing between *M. tuberculosis* and MOTT, becoming

an important tool in patients with heavy MOTT colonization/MOTT disease, as is the case of HIV-infected patients.

In conclusion, the AMTD test showed good sensitivity and specificity in the population studied, enabling the laboratory detection of *M. tuberculosis* complex in paucibacillary respiratory specimens.

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Drug-resistant tuberculosis in subjects included in the Second National Survey on Antituberculosis Drug Resistance in Porto Alegre, Brazil*,**

Tuberculose resistente em pacientes incluídos no II Inquérito Nacional de Resistência aos Fármacos Antituberculose realizado em Porto Alegre, Brasil*

Vania Celina Dezoti Micheletti, José da Silva Moreira, Marta Osório Ribeiro, Afranio Lineu Kritski, José Ueleres Braga

Abstract

Objective: To describe the prevalence of multidrug-resistant tuberculosis (MDR-TB) among tuberculosis patients in a major Brazilian city, evaluated via the Second National Survey on Antituberculosis Drug Resistance, as well as the social, demographic, and clinical characteristics of those patients. **Methods:** Clinical samples were collected from tuberculosis patients seen between 2006 to 2007 at three hospitals and five primary health care clinics participating in the survey in the city of Porto Alegre, Brazil. The samples were subjected to drug susceptibility testing. The species of mycobacteria was confirmed using biochemical methods. **Results:** Of the 299 patients included, 221 (73.9%) were men and 77 (27.3%) had a history of tuberculosis. The mean age was 36 years. Of the 252 patients who underwent HIV testing, 66 (26.2%) tested positive. The prevalence of MDR-TB in the sample as a whole was 4.7% (95% CI: 2.3-7.1), whereas it was 2.2% (95% CI: 0.3-4.2) among the new cases of tuberculosis and 12.0% (95% CI: 4.5-19.5) among the patients with a history of tuberculosis treatment. The multivariate analysis showed that a history of tuberculosis and a longer time to diagnosis were both associated with MDR-TB. **Conclusions:** If our results are corroborated by other studies conducted in Brazil, a history of tuberculosis treatment and a longer time to diagnosis could be used as predictors of MDR-TB.

Keywords: Tuberculosis/diagnosis; Drug resistance; HIV.

Resumo

Objetivo: Descrever a prevalência de tuberculose multirresistente (TBMR) em pacientes com tuberculose em uma importante cidade brasileira através do II Inquérito Nacional de Resistência aos Fármacos Antituberculose, assim como as características sociais, demográficas e clínicas desses pacientes. **Métodos:** De 2006 a 2007, amostras clínicas de pacientes de três hospitais e das cinco unidades básicas de saúde participantes do inquérito realizado em Porto Alegre foram coletadas e submetidas ao teste de sensibilidade aos fármacos. A confirmação das espécies de micobactérias ocorreu por métodos bioquímicos. **Resultados:** Foram incluídos 299 pacientes. Desses, 221 (73,9%) eram homens e 77 (27,3%) tinham história de tuberculose. A idade média foi de 36 anos. Dos 252 pacientes testados para HIV, 66 (26,2%) estavam infectados. A prevalência da TBMR na amostra geral foi de 4,7% (IC95%: 2,3-7,1); enquanto essa foi de 2,2% (IC95%: 0,3-4,2) nos pacientes virgens de tratamento e de 12,0% (IC 95%: 4,5-19,5) naqueles com história de tratamento antituberculose. A análise multivariada mostrou que história de tuberculose e maior tempo para o diagnóstico associaram-se a TBMR. **Conclusões:** Caso esses resultados sejam confirmados em outros estudos no Brasil, a história de tratamento antituberculose e o maior tempo para o diagnóstico poderão ser utilizados como preditores de TBMR.

Descritores: Tuberculose/diagnóstico; Resistência a medicamentos; HIV.

*Study carried out in the Department of Health of the City of Porto Alegre, Porto Alegre, Brazil; at the Federal University of Rio Grande do Sul, Porto Alegre, Brazil; and at the Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

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Financial support: This study received financial support from the Brazilian *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES, Office for the Advancement of Higher Education).
Submitted: 15 July 2013. Accepted, after review: 5 March 2014.

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

Introduction

There was a recrudescence of tuberculosis in the late 1980s, which led the World Health Organization (WHO) to declare it a public health emergency in 1993.[1] In early 1994, the WHO also initiated the Global Project on Anti-Tuberculosis Drug Resistance Surveillance, in collaboration with the International Union against Tuberculosis and Lung Disease (IUATLD).[2] Between 1994 and 1999, the WHO and the IUATLD compiled drug resistance data from surveys carried out in 58 countries.[3] They found that the mean prevalence of primary multidrug-resistant tuberculosis (MDR-TB), in patients with no history of tuberculosis treatment was 1.0% (range, 0-14.1%), and that the mean prevalence of acquired MDR-TB was 9.3% (range, 0-48.2%).[3]

Studies conducted between 2002 and 2006, collectively involving 90,000 patients in 81 countries, demonstrated an increase in the estimated prevalence of drug-resistant tuberculosis (DR-TB).[4,5] In 2005, there were 500,000 new cases of MDR-TB worldwide, corresponding to 5% of the total number of cases of tuberculosis. In that same year, the prevalence of primary MDR-TB was 2.9% (range, 2.2-3.6%), whereas that of acquired MDR-TB was 15.3% (range, 9.6-21.1%), respectively.[4]

In 2006, cases of extensively drug-resistant tuberculosis (XDR-TB) were reported in South Africa, mostly in HIV-infected hospitalized patients. By 2009, cases of XDR-TB had been reported in various other regions of the world.[6] Research also showed that death rates were higher in countries with an elevated prevalence of tuberculosis/HIV co-infection (which included cases of MDR-TB or XDR-TB in HIV-infected individuals), underscoring the need for effective interventions for the prevention and treatment of infection with resistant strains of *Mycobacterium tuberculosis*.[7]

In Brazil, reductions in incidence and mortality rates suggest that the tuberculosis situation has improved over the last ten years. However, in certain metropolitan regions of the country, there has been no improvement at all.[2] For instance, in the southern Brazilian city of Porto Alegre, the incidence of tuberculosis increased from 97/100,000 population to 116/100,000 population between 2001 and 2009, 30% of all tuberculosis cases reported for the city being diagnosed in hospitals. That increase was accompanied by a high prevalence of tuberculosis/HIV co-infection, a decrease in the tuberculosis cure rate (from 69% to 65% of all treated cases) and an increase in the rate of default from treatment (from 15% to 20%).[8]

In 1996, the First National Survey on Antituberculosis Drug Resistance was conducted in Brazil.[9] Participants were recruited from 13 health care facilities throughout the country, and approximately 6,000 strains of M. tuberculosis were identified.[9] The prevalence rates of primary and acquired MDR-TB were 1.1% and 7.9%, respectively.[10] However, the survey did not assess the prevalence of HIV infection and was limited to patients treated at primary health care clinics.[10] In southern Brazil, the prevalence rates of primary and acquired MDR-TB (0.8% and 5.8%, respectively) were lower than the nationwide prevalence.[10] Since then, no other epidemiological (population-based) studies of antituberculosis drug resistance have been conducted in any of the major cities of southern Brazil. Therefore, the present study aimed to characterize the prevalence of DR-TB and MDR-TB in the city of Porto Alegre, where the efficacy of tuberculosis control programs has decreased significantly in recent years. The present study was also aimed at identifying the prevalence of HIV infection and any demographic or clinical characteristics associated with antituberculosis drug resistance in a population recruited from primary health care clinics and hospitals.

Methods

The data analyzed in the present study were collected in the city of Porto Alegre as part of the Second National Survey on Antituberculosis Drug Resistance, conducted between 2006 and 2007. Between March of 2006 and December of 2007, patients were recruited from five primary health care clinics and three public hospitals. All patients provided sputum samples for smear microscopy and mycobacterial culture. The samples were also tested for resistance to rifampin, streptomycin, ethambutol, and isoniazid. However, due to the poor reproducibility of tests for resistance to streptomycin and ethambutol, those results were not considered in the present study.

On the basis of the results of the bacteriological examination, we defined DR-TB as resistance to any antituberculosis drug and MDR-TB as resistance to (at least) the combination of isoniazid and rifampin. The presence of organisms

resistant to one or more drugs in patients with no history of tuberculosis treatment, or with prior treatments lasting one month or less, was classified as primary drug resistance. The presence of resistant microorganisms in patients with a history of tuberculosis treatments lasting over a month was classified as acquired drug resistance.

Given the differences in the expected prevalence of rifampin resistance in new patients (primary resistance) and re-treated patients (acquired resistance), minimum sample sizes were calculated for these two groups. These calculations were performed using a proportional-to-population-size cluster sampling method, taking into account the size of the tuberculosis diagnostic facilities and consequently the number of patients admitted for diagnosis and treatment at each health care facility.[11,12]

Participants were recruited from five primary health care clinics (Modelo; Navegantes; Institute for Childhood Protection and Assistance; Vila dos Comerciários; and Sanatório), as well as from three hospitals (the Nossa Senhora da Conceição Hospital of Porto Alegre; the Sanatório Partenon Hospital; and the Porto Alegre Hospital de Clínicas of the Federal University of Rio Grande do Sul School of Medicine), all located in the city of Porto Alegre. All patients who visited any of these health care centers during the recruitment period and were suspected of having pulmonary tuberculosis were eligible for participation. Suspected pulmonary tuberculosis was defined as the presence of respiratory symptoms or clinical or radiological signs of tuberculosis, as per the Brazilian National Guidelines for the Control of Tuberculosis.[13] Mycobacterial cultures were carried out for all clinical samples, regardless of the sputum smear test results.

Eligible patients were included in the study if they met one of the two following criteria: being classified as a new case (no history of tuberculosis treatment) with culture-positive pulmonary tuberculosis (regardless of smear test results); and having a history of tuberculosis treatment (relapse or history of default from tuberculosis treatment), presenting with culture-positive pulmonary tuberculosis, or having used antituberculosis drugs in the 30 days prior to survey participation and sputum sample collection. We applied the following exclusion criteria: being under 18 years of age; being pregnant; and having negative culture results (regardless of the smear microscopy results) or no drug susceptibility testing (DST) results (i.e., DST not carried out in accordance with the Brazilian National Guidelines for the Control of Tuberculosis).[13] Sputum samples were collected prior to the beginning of treatment. Patients were not required to consent to HIV testing in order to participate in the survey.

Patients were interviewed at the health care facilities involved, in rooms reserved specifically for that purpose, by researchers trained in data collection via an instrument with pre-coded closed questions. The instrument was designed to assess the following variables: sociodemographic data (gender, age, and place of residence); willingness to undergo HIV testing; time to diagnosis (hereafter time to diagnosis); history of hemoptysis; history of tuberculosis (for this variable, the self-reported answers—"yes", "no", or "don't know"—were verified against the patient records available at the primary health care clinics or in other patient record systems); use of antituberculosis drugs; cough with expectoration for more than 3 weeks; previous chest X-ray; previous sputum testing; previous use of antituberculosis drugs; and case type (new case, re-treatment after cure, re-treatment after default from treatment, chronic treatment failure, or unknown). All patients were informed that HIV testing is a routine assessment procedure and were invited to undergo said testing. Researchers were trained in the provision of pre- and post-HIV test counseling. The samples collected for HIV testing were sent to a laboratory for diagnostic testing with ELISA. Patients were informed of the HIV test results and, when necessary, were offered counseling and directed to the AIDS treatment facility nearest to their place of residence.

Two sputum samples were collected from each patient at the respective health care facilities. Sputum smears were then examined using Ziehl-Neelsen staining. Procedures for smear microscopy preparation, staining and reading were conducted according to international guidelines.[14,15] Clinical samples were sent to the Rio Grande do Sul State Referral Laboratory for processing. After decontamination, material was inoculated into two tubes containing Löwenstein-Jensen (LJ) medium. Cultures were incubated at 37°C for up to 6 weeks, until colony growth was observed. Cultures were inspected 48 h after inoculation and weekly until day 42 of incubation. Strain

morphology and pigmentation were observed, and the date on which colonies appeared was recorded. These procedures were conducted according to the tuberculosis guidelines established by the Brazilian National Ministry of Health.[16] We identified strains of M. tuberculosis by growth inhibition test, using p-nitrobenzoic acid at a concentration of 500 µg per 1 mL of LJ medium, as well as niacin and nitrate tests.[15]

Indirect susceptibility testing was performed on the samples obtained from the participants. Culture growth on day 28 of incubation determined the final results, which were interpreted in relation to the resistance criteria recommended in the WHO guidelines (i.e., 1%).[14] For each lot of LJ medium and each antituberculosis drug tested, DST was also conducted on the reference strain of *M. tuberculosis* (H37Rv), which was thus used as a susceptible control. All laboratories involved in testing used a double-blind method for internal quality control. In addition, 100% of the samples identified as drug-resistant were retested by another referral laboratory, as were 15% of those identified as susceptible.

A database was created using the EpiData® program (EpiData Association, Odense, Denmark). Data analyses comprised prevalence estimates, confidence intervals (considered significant at 5%) and group comparisons. Chi-square tests were used in comparisons between individuals infected with resistant strains and those infected with susceptible strains. Measures of association, such as prevalence ratios, were calculated using STATA software, version 10.

The present study was approved by the Research Ethics Committee of the Porto Alegre Municipal Health Department (Protocol no. 001.053413.05.9; approved 16 December, 2005). The nationwide project (the Second National Survey on Antituberculosis Drug Resistance) was approved by the National Committee for Research Ethics (Protocol no. 25000.178623/2004-80; approved 24 May, 2005). All participating patients (or their legal guardians) gave written informed consent.

Results

Of all patients with suspected pulmonary tuberculosis seen at the participating health care facilities, 714 were eligible for participation in the present study. Of those 714 patients, 208 (29.1%) and 96 (13.4%) were found to be smear positive-culture positive and smear negative-culture positive, respectively, 299 (41.9%) subsequently undergoing DST (Figure 1).

Table 1 displays the demographic and clinical characteristics of the survey participants. The majority of participants were young adults, and the male-to-female ratio was 3:1. There were no gender or age differences between the patients with a history of treatment for tuberculosis and those without.

One fifth of patients reported having undergone prior HIV testing. The frequency of HIV testing in the two months prior to the survey was higher in patients with a history of tuberculosis treatment than in those without. Although the mean time to diagnosis was longer in the patients with a history of tuberculosis than in those without, it was greater than three months in both groups.

Resistance to at least one antituberculosis drug (DR-TB) and combined resistance to at least isoniazid and rifampin (MDR-TB) were observed in 14.0% and 4.7% of the patients, respectively. Drug resistance was eight times greater in the patients with a history of tuberculosis ($p = 0.01$). Isoniazid monoresistance was more common than was rifampin monoresistance. The prevalence rates of primary and acquired MDR-TB were 2.2% and 12.0%, respectively. Positive HIV test results were seen in 26% of the patients, and the frequency of such results was higher in the patients with a history of tuberculosis (Table 2). As can be seen in Table 3, HIV infection was not found to be associated with DR-TB or MDR-TB. However, the time to diagnosis was associated with DR-TB and MDR-TB. In patients with a history of hemoptysis, there was a higher prevalence of DR-TB but not of MDR-TB.

In summary, the bivariate analyses indicated that the following variables were associated with DR-TB: tuberculosis re-treatment; time to diagnosis; and history of hemoptysis. We also found that re-treatment was associated with MDR-TB, as was the time to diagnosis. Multivariate analyses revealed that DR-TB was independently associated with tuberculosis re-treatment and with the time to diagnosis. When this calculation was adjusted for the influence of other variables (Table 3), only the time to diagnosis was associated with MDR-TB.

Discussion

The prevalence rates of primary and acquired MDR-TB observed in the present study (2.2%

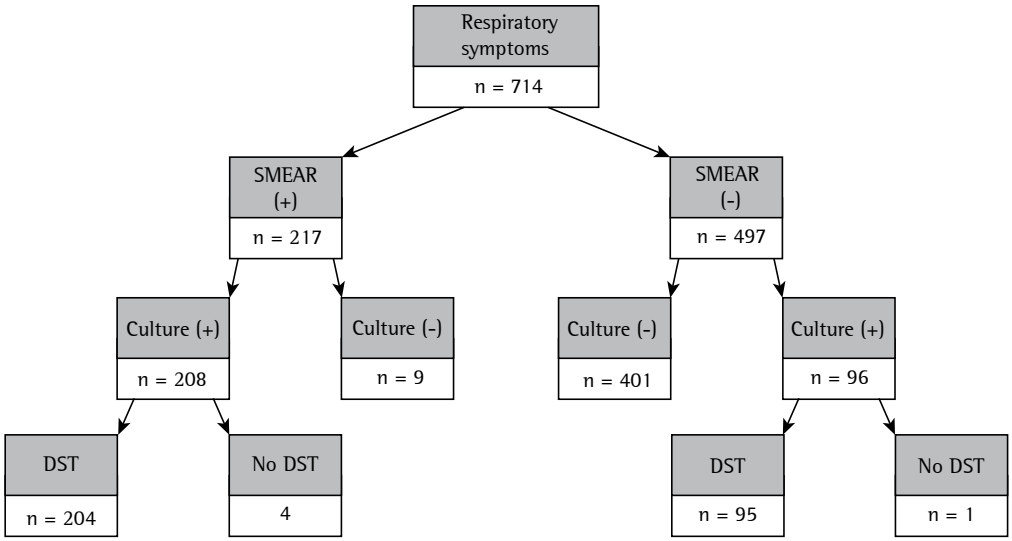

Figure 1 - Patient distribution according to laboratory tests conducted in Porto Alegre, 2006-2007. DST: drug susceptibility testing

and 12.0%, respectively) were higher than those reported in the First National Survey on Antituberculosis Drug Resistance (1.1% and 7.9%, respectively), which was carried out in Brazil in 1996, and in the International WHO-IUATLD report, which was conducted in 58 countries between 1994 and 1999 (1.0% and 9.3%, respectively).[3] However, the present estimates of primary and acquired MDR-TB prevalence were lower than the respective rates of 2.9% and 15.3% reported in the WHO-IUATLD survey conducted between 2002 and 2007.[3] The prevalence rates of MDR-TB in Lithuania and Azerbaijan, for instance, were 14.4% and 22.3%, respectively.[3,10] The high prevalence of primary MDR-TB found in the present study (2.2%) might be attributable to the increase in the rate of default from treatment observed over the last 10 years in the city of Porto Alegre.[8]

Our results suggest that DR-TB is associated with re-treatment and with a longer time to diagnosis. These conditions, in turn, might represent the consequences of delayed diagnosis and lack of prompt treatment in cases of tuberculosis, as has been suggested in previous studies.[17-19] Although difficulties in the diagnosis of tuberculosis and the detection of resistance to antituberculosis drugs—even after the implementation of the directly observed treatment, short-course (DOTS) strategy or the DOTS-plus strategy—have been reported in a number of countries, few studies have evaluated the variables associated with delayed detection of DR-TB.[20] A recent analysis of tuberculosis transmission and delayed diagnosis suggested that the duration of this delay is the main obstacle in controlling the tuberculosis epidemic.[17] Storla et al.[19] also suggested that repeated attempts by patients to seek treatment at the same level of health care and the inconclusive test results obtained at that level are responsible for delaying the diagnosis of tuberculosis.

In the present sample, the mean time from symptom onset to a diagnosis of tuberculosis was 110.9 days, which is longer than the delays reported for other developing countries (61.3 days) and for developed countries (67.8 days).[21] This figure is also higher than (or comparable to) that reported in surveys conducted in other major Brazilian cities: 68 days in Rio de Janeiro; 110 days in Vitória; and 90 days in Recife.[7,22,23]

Among our sample of patients in the city of Porto Alegre, the association found between a history of tuberculosis treatment and the time to diagnosis, which was 184.8 days for those with such a history, has not been observed in surveys conducted in other Brazilian cities, such as Rio de Janeiro.[23] One of the risk factors for delayed detection of DR-TB is an increased probability of transmission to individuals at home or in hospital environments, or even in prisons or shelters. The chain of transmission continues and leads to further contamination and aggravation of existing cases of tuberculosis, contributing to

Table 1 - Clinical and demographic characteristics of participants in the Second National Survey on Antituberculosis Drug Resistance, in Porto Alegre, Brazil. 2006-2007.

Variable	History of tuberculosis treatment		Total
	No	Yes	
	(n = 224[a])	(n = 75[a])	(n = 299[a])
Age (years), mean	35.0	38.0	36.0
Gender, n (%)			
Male	165 (73.6)	56 (74.7)	221 (73.9)
Female	59 (26.4)	19 (25.3)	78 (26.1)
HIV testing in the past 2 months, n (%)			
Yes	37 (18.7)	17 (27.9)	54 (20.8)
No	161 (81.3)	44 (72.1)	205 (79.2)
Consented to HIV testing, n (%)	123 (63.4)	34 (57.6)	157 (62.1)
Time to diagnosis (days), mean	86.6	184.8	110.9
Self-reported history of tuberculosis, n (%)			
Yes	5 (2.4)	72 (100.0)	77 (27.3)
No	202 (97.6)		202 (72.7)
Productive cough for > 3 weeks, n (%)			
Yes	93 (45.4)	67 (91.8)	160 (57.5)
No	112 (54.6)	6 (8.2)	118 (42.5)
Hemoptysis, chest pain, or other symptom of lung disease, n (%)			
Yes	70 (34.1)	55 (73.3)	125 (45.0)
No	135 (65.9)	18 (26.7)	153 (55.0)
Chest X-ray, n (%)			
Yes	125 (61.0)	70 (95.9)	195 (70.1)
No	80 (39.0)	3 (4.1)	83 (29.9)
Previous sputum testing, n (%)			
Yes	70 (34.8)	73 (98.6)	143 (52.0)
No	131 (65.2)	1 (1.4)	132 (48.0)
Antituberculosis medication for > 1 month, n (%)			
Yes	6 (3.1)	73 (98.6)	79 (29.6)
No	187 (96.9)	1 (1.4)	188 (70.4)

[a]The maximum possible numbers of patients; because of missing data, the values for some variables are based on smaller numbers.

the worldwide epidemic. In Porto Alegre, delayed detection of DR-TB is one of the main aggravating factors of the epidemiological situation. This might be attributable to flaws in the health care system, because patients often continue to visit health care facilities until receiving a diagnosis. Therefore, variables related to patient behavior and to the health care system contribute to delays in the detection of DR-TB.

The mean age and the male-to-female ratio observed in the present study were similar to those described by the Porto Alegre Municipal Health Department, as well as in the national and international literature.[20,24-26] A high number of HIV-infected patients were also found in the sample. That might be explained by the type of health care facilities investigated in the current study. It is possible that some of those facilities had multidisciplinary teams and treated patients who were referred from other health care facilities. We also found that patients with a history of tuberculosis treatment were more likely to have undergone HIV testing, probably because they sought diagnostic and treatment services via tuberculosis control programs within which HIV testing has become a routine requirement.

The responses to the screening questions for a history of tuberculosis treatment indicated that 61% of previously untreated patients had previously undergone chest X-ray, even though the Brazilian National Ministry of Health does not recommend X-ray screening in patients with a productive cough and suspected tuberculosis.[13] In the present study, a history of tuberculosis

Table 2 - Prevalence of combined, primary, and acquired resistance to antituberculosis drugs and HIV infection among participants in the Second National Survey on Antituberculosis Drug Resistance, in Porto Alegre, Brazil. 2006-2007.

Variable	No history of TB treatment (primary resistance)			History of TB treatment (acquired resistance)			Combined resistance		
	n	Prevalence, %	95% CI	n	Prevalence, %	95% CI	n	Prevalence, %	95% CI
Drug susceptibility	224	91.5	87.9-95.2	75	68.0	57.2-78.8	299	85.6	81.7-89.7
Any resistance	224	8.5	4.8-12.1	75	32.0	21.2- 42.8	299	14.4	10.4-18.4
INH	224	7.1	3.7-10.5	75	29.3	18.8-39.9	299	12.7	8.9-16.5
RIF	224	2.2	0.3-4.2	75	13.3	5.4-21.2	299	5.0	2.5-7.5
Monoresistance	224	4.9	2.0-7.8	75	18.7	9.6-27.7	299	8.4	5.2-11.5
INH	224	4.9	2.0-7.8	75	17.3	8.6-26.1	299	8.0	4.9-11.1
RIF	224	0.0	0.0-0.0	75	1.3	0.0-3.9	299	0.3	0.0-0.9
Multidrug resistance									
INH+RIF	224	2.2	0.3-4.2	75	12.0	4.5-19.5	299	4.7	2.3-7.1
Resistance to 1 drug	224	4.9	2.0-7.8	75	18.7	9.6-27.7	299	8.4	5.2-11.5
Resistance to 2 drugs	224	2.2	0.3-4.2	75	12.0	4.5-9.5	299	4.7	2.3-7.1
HIV infection	185	23.8	17.6-30.0	67	32.8	23.1-44.4	252	26.2	20.7-31.6

INH: isoniazid; and RIF: rifampin.

Table 3 - Variables associated with drug resistance and multidrug resistance, in bivariate and multivariate analyses, among participants in the Second National Survey on Antituberculosis Drug Resistance, in Porto Alegre, Brazil. 2006-2007.

Variable	n	Bivariate analysis				Multivariate analysis			
		Resistance		Multidrug resistance		Resistance		Multidrug resistance	
		PR	95% CI	PR	95% CI	PR	95% CI	PR	95% CI
Re-treatment									
No	224	1.00		1.00		1.00			
Yes	75	5.08	2.58-9.98	5.97	1.93-18.44	4.10	1.61-10.41	4.96	0.87-28.44
HIV infection									
No	186	1.00		1.00		1.00		1.00	
Yes	66	0.72	0.31-1.65	1.22	0.30-4.85	0.31	0.09-1.08	0.20	0.01-2.63
Time to diagnosis (days)	258	1.00[a]	1.00-1.00[b]	1.00[c]	1.00-1.00[d]	1.00[e]	1.00-1.00[f]	1.00[g]	1.00-1.00[h]
History of hemoptysis									
No	153	1.00		1.00		1.00		1.00	
Yes	125	2.03	1.03-4.03	2.30	0.75-7.04	0.94	0.37-2.37	0.50	0.09-2.62

PR: prevalence ratio. [a]observed value: 1.001; [b]observed value: 1.0003-1.002; [c]observed value: 1.001; [d]observed value: 1.0004-1.003; [e]observed value: 1.001; [f]observed value: 1.00002-1.002; [g]observed value: 1.001; [h]observed value: 1.0001-1.003.

symptoms was investigated through questions related to hemoptysis, chest pain, and other symptoms of pulmonary tuberculosis. Such symptoms were identified in 45% of the study sample and were more common in patients with a history of tuberculosis treatment, as would be expected. It is of note that we also investigated hemoptysis, which is a less common symptom that presents later in the course of illness.[27]

The frequency of HIV infection among our study subjects was elevated but lower than that reported in the Brazilian National Case Registry Database for Porto Alegre.[8] Our results differed from those in the literature in that the incidence

of DR-TB in HIV-infected patients with a history of tuberculosis treatment was higher in our sample (32.8%). A study conducted in the state of Santa Catarina (also in southern Brazil) found that the prevalence of HIV infection was higher in patients who had never been treated for tuberculosis than in those with a history of tuberculosis treatment (20% vs. 9%).[28] Our findings also support the hypothesis that the frequency of DR-TB is higher in regions where there are high rates of default from treatment.

The results of the present study call for awareness of tuberculosis control strategies by health care authorities, managers, and workers, in order to improve the health situation in the region studied. There is an urgent need to increase treatment coverage, reduce the rate of default from treatment, and identify strategies for early diagnosis of DR-TB and MDR-TB at primary health care clinics and hospitals in Porto Alegre. Effective strategies could include new diagnostic tests (liquid culture or molecular testing) or the use of clinical prediction rules. The latter method was suggested by researchers in Peru, a country with a high prevalence of DR-TB, where significant technical and political efforts have been made toward the implementation of programs for the control of DR-TB and MDR-TB.[29]

In the present study, it was possible to analyze the epidemiological behavior of DR-TB and the variables associated with this condition in a group of patients included in the Second National Survey on Antituberculosis Drug Resistance, which was conducted in the city of Porto Alegre. A longer time from symptom onset to a diagnosis of tuberculosis and history of tuberculosis treatment were found to be associated with the occurrence of DR-TB and MDR-TB. If our results are corroborated by other studies conducted in Brazil, these variables could be used as predictors of MDR-TB, thus contributing to the investigation and implementation of appropriate drug therapy. In addition, these findings could promote lower morbidity and mortality rates, as well as lowering the risk of tuberculosis transmission within the community.

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The role of intercostal nerve preservation in acute pain control after thoracotomy*

O papel da preservação do nervo intercostal no controle da dor aguda pós-toracotomia

Marco Aurélio Marchetti-Filho, Luiz Eduardo Villaça Leão, Altair da Silva Costa-Junior

Abstract

Objective: To evaluate whether the acute pain experienced during in-hospital recovery from thoracotomy can be effectively reduced by the use of intraoperative measures (dissection of the neurovascular bundle prior to the positioning of the Finochietto retractor and preservation of the intercostal nerve during closure). **Methods:** We selected 40 patients who were candidates for elective thoracotomy in the Thoracic Surgery Department of the Federal University of São Paulo/Paulista School of Medicine, in the city of São Paulo, Brazil. The patients were randomized into two groups: conventional thoracotomy (CT, n = 20) and neurovascular bundle preservation (NBP, n = 20). All of the patients underwent thoracic epidural anesthesia and muscle-sparing thoracotomy. Pain intensity was assessed with a visual analog scale on postoperative days 1, 3, and 5, as well as by monitoring patient requests for/consumption of analgesics. **Results:** On postoperative day 5, the self-reported pain intensity was significantly lower in the NBP group than in the CT group (visual analog scale score, 1.50 vs. 3.29; p = 0.04). No significant differences were found between the groups regarding the number of requests for/consumption of analgesics. **Conclusions:** In patients undergoing thoracotomy, protecting the neurovascular bundle prior to positioning the retractor and preserving the intercostal nerve during closure can minimize pain during in-hospital recovery.

Keywords: Pain, postoperative; Analgesia; Thoracotomy.

Resumo

Objetivo: Avaliar se a dor aguda na recuperação intra-hospitalar devido a toracotomia pode ser efetivamente reduzida pelo uso de medidas intraoperatórias (dissecção do feixe neurovascular antes da colocação do afastador de Finochietto e preservação do nervo intercostal durante o fechamento). **Métodos:** Foram selecionados 40 pacientes candidatos à toracotomia eletiva na Disciplina de Cirurgia Torácica, Universidade Federal de São Paulo/Escola Paulista de Medicina, em São Paulo (SP), os quais foram randomizados em dois grupos de 20 pacientes: grupo toracotomia convencional (TC) e grupo de preservação do feixe (PF) neurovascular. Todos os pacientes foram submetidos a anestesia peridural torácica e técnica de toracotomia poupadora da musculatura. A intensidade da dor foi determinada utilizando-se uma escala visual analógica no 1º, 3º e 5º dias pós-operatórios, assim como a medida do consumo de analgésicos por demanda do paciente. **Resultados:** Houve uma diminuição significativa da intensidade da dor relatada somente no 5º dia pós-operatório no grupo PF quando comparado ao grupo TC (escore da escala analógica visual, 1,50 vs. 3,29; p = 0,04). Não houve diferenças significativas no consumo de analgésicos por demanda nos dois grupos. **Conclusões:** Em pacientes submetidos à toracotomia, a proteção do feixe neurovascular antes da colocação do afastador e a preservação do nervo intercostal no fechamento da toracotomia podem minimizar a dor no período intra-hospitalar.

Descritores: Dor pós-operatória; Analgesia; Toracotomia.

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Financial support: None.
Submitted: 22 November 2013. Accepted, after review: 26 March 2014

Introduction

A thoracotomy is one of the most painful procedures in surgical practice. The pain that patients experience in the immediate postoperative period and in the late postoperative period constitutes a constant concern among thoracic surgeons, because it is well established that patients with severe postoperative pain are at an increased risk of developing complications, including atelectasis and pulmonary infection.[1,2] In addition, chronic pain is a common cause of prolonged work absenteeism because it often prevents patients from performing their regular activities for months after the surgical procedure. Many studies have shown that the presence of severe pain in the immediate postoperative period is associated with a higher occurrence of chronic pain.[3-5]

Postoperative pain assessment is based on individual perception, the subjective nature of pain and the difficulty in measuring pain intensity making it difficult to standardize studies addressing the issue of postoperative pain.

Pain resulting from the stimulation of receptors is designated nociceptive (myofascial) pain. Nociceptive pain after thoracotomy might be due to any of the following: skin incision; muscle retraction; rib spreading; trauma to sternocostal and costovertebral joints; intercostal nerve compression; damage to the lung parenchyma; and damage to the parietal pleura. Intercostal nerve injury can lead to the formation of a localized neuroma that can cause persistent stimulation and, consequently, hyperalgesia (pain resulting from noxious stimuli) and allodynia (pain resulting from typically painless stimuli). In such cases, pain is designated neuropathic pain. Post-thoracotomy pain syndrome can therefore be defined as a combination of nociceptive and neuropathic stimuli.[6,7]

Many thoracic surgical procedures are currently performed as video-assisted thoracoscopic procedures; however, pulmonary resections, including those for the treatment of lung cancer, are not, despite numerous studies showing the advantages of video-assisted thoracoscopy. According to the American Association for Thoracic Surgery, video-assisted procedures account for less than 20% of all major pulmonary resections recently performed in the USA and less than 10% of all major pulmonary resections recently performed in Europe.[8]

During conventional thoracotomy, two intercostal nerves can be injured unless the neurovascular bundle is preserved: one by the Finochietto retractor and one by thoracotomy closure. On the basis of the assumption that post-thoracotomy pain is primarily due to intercostal nerve injury, postoperative pain can be reduced by avoiding intercostal nerve crushing.

There are currently three thoracotomy closure techniques: intracostal suture closure (whereby stitches perforate the rib); subperiosteal suture closure (whereby stitches are placed between the periosteum and the neurovascular bundle at the lower rib; Figure 1); and pericostal suture closure (whereby stitches are placed in the middle of the intercostal muscles, thus crushing the intercostal nerve against the rib).

Cerfolio et al.[9] compared post-thoracotomy pain between patients undergoing pericostal suture closure and those undergoing intracostal suture closure. The authors reported that intracostal sutures resulted in significantly less postoperative pain. In another study, postoperative pain was evaluated in patients in whom the neurovascular bundle had been dissected before retractor placement in order to protect it from being compressed by the retractor.[10] Many groups of authors have attempted to reproduce the results of the two aforementioned studies,[11,12] having reached similar conclusions; some have attributed the reduction in pain to the protection provided by the muscle flap containing the bundle, whereas others have attributed it to the thoracotomy closure technique.

The present study was designed to compare, in a systematic manner, different techniques for neurovascular bundle preservation (NBP), in an attempt to determine their efficacy in reducing

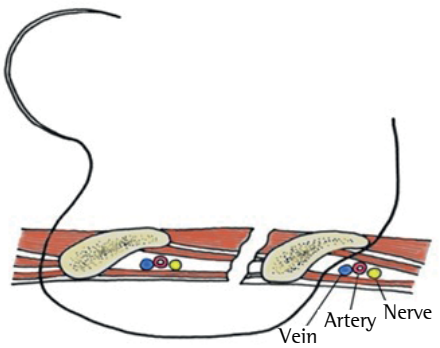

Figure 1 - Schematic illustration of subperiosteal suture placement.

pain in the immediate postoperative period and during the in-hospital postoperative period.

Methods

This was a prospective randomized clinical trial of candidates for elective thoracotomy in the Thoracic Surgery Department of the Federal University of São Paulo/Paulista School of Medicine, located in the city of São Paulo, Brazil. The trial was conducted between January of 2009 and January of 2010. All patients were blinded to the technique used. We initially selected 142 patients requiring thoracotomy. The inclusion criteria were being 18 years of age or older and agreeing to participate in the study. Most of the patients who were initially selected were excluded during the perioperative period on the basis of the following criteria: need for thoracoplasty or pleurectomy; occurrence of rib fractures caused by retractor placement; impossibility of performing epidural anesthesia; impossibility of using a visual analog scale in the postoperative period (because of prolonged intubation); and need for reintervention.

The study was approved by the Research Ethics Committee of the Federal University of São Paulo (Protocol no. 1323/09).

Before the surgical procedure, all patients underwent thoracic epidural anesthesia in a sitting position (puncture at the level of T5-T6), followed by local anesthetic infusion and epidural catheter placement. The incision was 8-10 cm in length. The latissimus dorsi was dissected and reflected posteriorly. The serratus anterior was identified, dissected, and reflected medially.

The intercostal muscles were separated midway between the upper and lower rib edges. A medium-sized Finochietto retractor with 4-cm blades was used in all procedures.

The patients were randomized into two groups of 20 patients: the conventional thoracotomy group and the NBP group. In the group of patients undergoing conventional thoracotomy, the retractor was placed immediately after separation of the intercostal muscle. After the surgical procedure, intercostal space closure was achieved with pericostal sutures (absorbable polyglactin 1 suture material). In the NBP group, the intercostal muscle was dissected together with the neurovascular bundle, over a length of 5 cm, with the use of an osteotome and electrocautery, being therefore freed from the upper rib; the muscle flap was subsequently tied by a Penrose drain (Figure 2A) and retracted for the placement of the Finochietto retractor. After the surgical procedure, intercostal space closure was achieved with three stitches with subperiosteal sutures (a single layer of absorbable polyglactin 910 suture material; Figure 2B).

A visual analog scale ranging from zero to ten (i.e., from "no pain at all" to "the worst pain I have ever felt") was used for postoperative pain assessment. Patients used the visual analog scale to rate their pain on postoperative days 1, 3, and 5. All ratings were performed at 8:00 a.m. before any manual handling of patients, including bathing, physical therapy, and radiological examinations.

Objective postoperative pain assessment consisted of measuring the consumption of analgesics by patient demand (analgesics being prescribed and administered on the basis of self-reported pain).

Analgesia was achieved by the administration of 0.25% bupivacaine with 2 mg of morphine via an epidural catheter between 10:00 a.m. and 12:00 p.m. on postoperative days 1, 2, and, 3, the catheter being then removed. We analyzed the following parameters: duration of surgery; length

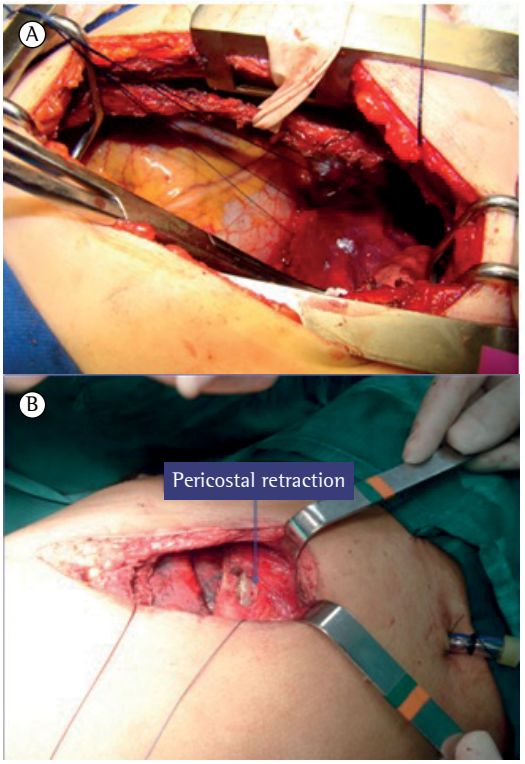

Figure 2 - In A, intercostal muscle flap tied by a Penrose drain. In B, periosteal retraction.

of hospital stay; duration of chest tube drainage; pain intensity; consumption of analgesics by patient demand; and complications. Complications included segmental or lobar atelectasis, pneumonia, prolonged air leak (i.e., air leak for more than 3 days), and surgical wound complications, including seroma, hematoma, and infection.

Statistical analysis was performed with the chi-square test and the Student's t-test. Sample size was calculated by comparison with similar studies,[11,13] the mean and standard deviation being taken into consideration; the expected response was 30%, with a value of $p < 0.05$. A descriptive analysis was performed with calculations of arithmetic means and standard deviations.

Results

Of the 142 patients requiring elective thoracotomy during the study period, 40 (28%) were included in the study. Patients were excluded for the following reasons: lack of thoracic epidural anesthesia, in 38 patients; rib fracture during the intraoperative period, in 24; impossibility of assessing pain intensity (because of prolonged intubation), in 15; history of chronic analgesic use, in 12; chest wall invasion by lung cancer, in 8; and need for reintervention, in 5.

The 40 patients included in the present study were randomized into two groups of 20 patients: the conventional thoracotomy group and the NBP group. The mean age was 48.3 ± 14.5 years in the conventional thoracotomy group and 48.8 ± 17.3 years in the NBP group. The body mass index was 24.6 ± 3.7 kg/m^2 in the former and 22.8 ± 4.5 kg/m^2 in the latter. Of the 20 patients in the conventional thoracotomy group, 11 were male, and 7 were smokers. Of the 20 patients in the NBP group, 10 were male, and 8 were smokers. Table 1 shows the surgical procedures performed in each group of patients.

We analyzed the duration of surgery, length of hospital stay, and duration of pleural drainage in the two groups of patients (Table 2). We found no significant differences between the two groups regarding any of the aforementioned parameters.

Regarding the subjective assessment of postoperative pain, mean visual analog scale scores were higher in the conventional thoracotomy group than in the NBP group ($p = 0.04$). Although postoperative pain intensity on postoperative days 1, 3, and 5 was lower in the NBP group than in the conventional thoracotomy group, the difference was significant only on postoperative day 5 ($p = 0.04$; Table 3).

Although the consumption of analgesics (tramadol hydrochloride and dipyrone) by patient demand was lower in the NBP group than in the conventional thoracotomy group, the difference was not significant. The mean consumption of tramadol hydrochloride was 1,025 ± 464 mg in the conventional thoracotomy group and 834 ± 568 mg in the NBP group ($p = 0.22$). The mean consumption of dipyrone was 16.67 ± 12.06 g in the former and 15.71 ± 11.73 g in the latter ($p = 0.98$).

Regarding the occurrence of postoperative complications, there were no significant differences between the conventional thoracotomy and NBP groups (28.18% vs. 30.77%; $p = 0.58$). In addition, none of the complications were attributable to the intervention (dissection of the neurovascular bundle and subperiosteal suture closure).

Table 1 - Surgical procedures in the patients undergoing conventional thoracotomy (n = 20) and in those undergoing thoracotomy with neurovascular bundle preservation (n = 20).[a]

Surgical procedures	Groups	
	CT	NBP
Segmentectomy	7	9
Lobectomy	6	8
Mediastinal tumor resection	2	1
Metastasectomy	2	1
Diaphragmatic defect repair	1	
Pneumonectomy	1	1
Pleural tumor resection	1	

CT: conventional thoracotomy; and NBP: neurovascular bundle preservation. [a]Values expressed as n of patients.

Table 2 - Duration of surgery, length of hospital stay, and duration of chest tube drainage in the groups studied.[a]

Variables	Groups		p*
	CT	NBP	
Duration of surgery, min	206.00 ± 112.96	190.32 ± 86.08	0.53
Length of hospital stay, days	6.0 ± 5.3	6.3 ± 3.9	0.85
Duration of chest tube drainage, days	4.6 ± 2.7	4.3 ± 2.6	0.21

CT: conventional thoracotomy; and NBP: neurovascular bundle preservation. [a]Values expressed as mean ± SD. *Student's t-test.

Table 3 - Pain intensity, as assessed by visual analog scale scores, in the groups studied.[a]

Results	Groups		p*
	CT	NBP	
Highest score	6.14 ± 3.38	4.12 ± 2.63	0.04
Postoperative day 1	5.29 ± 3.94	3.58 ± 2.30	0.13
Postoperative day 3	2.86 ± 2.47	2.65 ± 1.83	0.51
Postoperative day 5	3.29 ± 2.36	1.50 ± 1.82	0.04

CT: conventional thoracotomy; and NBP: eurovascular bundle preservation. [a]Values expressed as mean ± SD. *Student's t-test.

Discussion

Although post-thoracotomy pain is a topic of great interest to thoracic surgeons, few studies have examined it. This might be due to the lack of objective data to quantify post-thoracotomy pain. In the present study, we sought to answer a simple question: what can surgeons do to minimize the pain of patients undergoing thoracotomy?

Although thoracic epidural anesthesia is still considered the gold standard for postoperative analgesia in thoracic surgery, it can cause nausea, vomiting, dizziness, and torpor (all of which are due to hypotension), as well as muscle weakness and urinary retention, in 15-20% of cases.[14]

Thoracic epidural anesthesia is contraindicated in patients with coagulation disorders and depends on the skill and experience of the anesthesiologist. Thirty-eight patients were excluded from the present study because of the impossibility of performing epidural anesthesia (either because it was medically contraindicated or because of technical difficulties). We do not question the benefits of thoracic epidural anesthesia, which is routinely used at our facility. However, it is sometimes impossible to use it.

In such cases, one alternative is intercostal nerve block under direct vision, covering one intercostal space above the incision and one below it, which can be beneficial within the first 24 h after surgery.

Muscle-sparing thoracotomy is a variant of posterolateral thoracotomy, which is an open procedure rather than a laparoscopic procedure. During muscle-sparing thoracotomy, the latissimus dorsi and serratus anterior muscle fibers are separated rather than sectioned. Although most pulmonary resections can be performed via a muscle-sparing thoracotomy, the efficacy of this approach in reducing postoperative pain remains controversial; some authors have suggested that it is ineffective in reducing postoperative pain,[15] whereas others have reported that it significantly reduces postoperative pain.[14] In our study, muscle-sparing thoracotomy was used in both groups, which were therefore not compared in terms of the technique.

The purpose of our study was to determine the extent to which the techniques that protect the neurovascular bundle from being compressed by the Finochietto retractor and the modified intercostal space closure technique can reduce postoperative pain.

Cerfolio et al.[9] demonstrated the advantages of intracostal suture closure over conventional (pericostal) suture closure.

Few studies have examined the issue of intercostal nerve compression by the Finochietto retractor. Retractor-related factors contributing to the severity of post-thoracotomy pain include the amount of rib spreading, the size of the blades, and the area of contact between the retractor and the intercostal nerve.

In a study conducted in 2005, Cerfolio et al.[10] proposed that an intercostal muscle flap containing the neurovascular bundle be harvested before placement of the retractor. The study included 114 patients, who were randomized to conventional thoracotomy or thoracotomy with intercostal muscle flap to protect the intercostal nerve. The authors found that the latter technique reduced postoperative pain.

The chest tube is known to play a role in postoperative pain, chest tube removal being often associated with a reduction in pain. In the present study, we found no significant difference between the two groups in terms of the duration of chest tube drainage. The results might have been different if we had.

In a prospective randomized study of 144 patients undergoing pulmonary resection, Wu et al.[12] sought to determine whether the combination of intracostal suture closure with intercostal muscle flap provided better pain relief than did intracostal suture closure alone. Pain intensity was assessed by a visual analog scale in the period between postoperative day 1 and postoperative day 7, as well as in the period between postoperative week 2 and postoperative week 12. The combination of intracostal suture closure with intercostal muscle flap did not reduce postoperative pain when compared with intracostal suture closure alone.

In a prospective randomized study that involved 120 patients[11] and that is similar to the present study, 60 patients underwent intercostal muscle flap and intracostal suture closure (for intercostal nerve protection) and 60 underwent conventional thoracotomy. Postoperative pain intensity was assessed by a visual analog scale and by analgesic consumption and was found to be lower at postoperative week 1 and at postoperative month 1 in the group of patients who underwent intercostal muscle flap and intracostal suture closure.

In the present study, the two groups of patients were found to be similar in terms of the length of hospital stay. This finding shows that the intervention reduces postoperative pain but not the length of hospital stay. This was not taken into consideration in studies similar to ours.[10-12] We believe that this is due to the fact that postoperative pain is quite common and therefore a factor that is not relevant to discharge planning.

In the present study, the two groups were similar in terms of complication rates, and the intervention was ineffective in preventing the most common post-thoracotomy complications. Atelectasis and pneumonia are chief among the complications on which a reduction in pain might have any impact. In the present study, atelectasis occurred in 3 of the patients in the conventional thoracotomy group and in 2 of those in the NBP group. This difference might have reached statistical significance had our study involved a larger sample size. None of the complications observed in the present study were attributable to the intervention performed.

We conclude that the harvesting of an intercostal muscle flap before placement of the retractor and thoracotomy closure with subperiosteal sutures are measures that are not associated with additional morbidity or longer duration of surgery and can reduce in-hospital postoperative pain in patients undergoing thoracotomy.

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Utility of Asthma Control Questionnaire 7 in the assessment of asthma control*

Utilidade do instrumento *Asthma Control Questionnaire* 7 na avaliação do controle da asma

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Abstract

Our objective was to evaluate the reproducibility of Asthma Control Questionnaire 7 (ACQ-7) in asthma patients, comparing our results against those obtained with the Global Initiative for Asthma (GINA) criteria. We evaluated 52 patients. Patients completed the ACQ-7, underwent spirometry, and were clinically assessed to determine the level of asthma control according to the GINA criteria, in two visits, 15 days apart. The ACQ-7 cutoff for uncontrolled asthma was a score of 1.5. The ACQ-7 showed good reproducibility, with a correlation coefficient of 0.73. The ACQ-7 identified a greater number of patients with uncontrolled asthma than did the GINA criteria; according to the GINA criteria, 47 patients (90.4%) presented with partially controlled asthma.

Keywords: Asthma/prevention and control; Asthma/classification; Questionnaires.

Resumo

Nosso objetivo foi avaliar a reprodutibilidade do *Asthma Control Questionnaire 7* (ACQ-7) em asmáticos e comparar os resultados com os critérios de controle da *Global Initiative for Asthma* (GINA). Foram avaliados 52 pacientes em duas visitas com intervalo de 15 dias entre si. Os pacientes responderam o ACQ-7, realizaram espirometria e foram avaliados clinicamente para verificar o controle da asma de acordo com a GINA nas duas visitas. Em relação ao ACQ-7, o ponto de corte para asma não controlada foi definido em 1,5. Os resultados de ACQ-7 demonstraram boa reprodutibilidade, com coeficiente de correlação de 0,73. O ACQ-7 identificou um maior número de pacientes com asma não controlada em relação aos critérios da GINA; segundo os critérios GINA, 47 pacientes (90,4%) tinham asma parcialmente controlada.

Descritores: Asma/prevenção e controle; Asma/classificação; Questionários

The objectives of asthma treatment are to control symptoms, prevent exacerbations, achieve the best possible lung function, allow patients to perform their regular activities, and prevent irreversible airway obstruction and death from asthma.[1] Asthma control can be monitored in a variety of ways. Spirometry is a noninvasive technique to evaluate lung function in children who have asthma and are over 5 years of age. However, spirometry has limitations, which include the need for a professional trained in performing the test and the need for patient understanding and cooperation. Other noninvasive methods for monitoring asthma include measurement of peak expiratory flow, measurement of exhaled nitric oxide, and sputum examination for inflammatory cells.[2] Asthma control questionnaires and quality of life questionnaires can be used in order to assess asthma control.[1]

In clinical practice, incorrect assessment of asthma control can result in inappropriate treatment. Therefore, efforts to provide physicians and patients with simple, rapid, and inexpensive instruments for accurate assessment of symptom control are warranted. The ideal tool should have good reproducibility and responsiveness, should provide cutoffs for uncontrolled asthma, should be practical, and should not pose health risks.[3] Asthma control questionnaires are therefore important for the evaluation of disease control.

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Financial support: None.
Submitted: 17 January 2014. Accepted, after review: 17 March 2014.

There are currently 17 previously validated questionnaires, all of which include questions regarding nocturnal symptoms and sleep disturbances; most assess the frequency of symptoms, the use of short-acting β_2 agonists, and how asthma symptoms affect the performance of activities of daily living and physical exercise.[3]

One useful instrument is the Asthma Control Questionnaire (ACQ), which can be administered to asthma patients who are 12 years of age or older; the Spanish version and, more recently, the Brazilian Portuguese version of the ACQ have been validated for use.[4] However, the reproducibility and responsiveness of ACQ-7, which includes six questions and one lung function parameter, have yet to be evaluated in Brazil.

The objectives of the present study were to evaluate the reproducibility of ACQ-7 and to compare ACQ-7 with the Global Initiative for Asthma (GINA) criteria in terms of their utility in identifying controlled and uncontrolled asthma.

The inclusion criteria were as follows: being 12 years of age or older; being under follow-up at one of the specialized clinics of the Federal University of Paraná School of Medicine *Hospital de Clínicas*, located in the city of Curitiba, Brazil; having been diagnosed with asthma and having received a diagnosis of asthma severity in accordance with the GINA criteria[5]; having received treatment with 800 µg/day of inhaled beclomethasone or equivalent, with or without long-acting β_2 agonists, in the last six months. The exclusion criteria were as follows: need for systemic corticosteroids in the last three months; history of smoking in the last three months; current pregnancy; and presence of severe comorbidities.

Patients were assessed in two visits, the second occurring 15 days after the first. In the two visits, patients completed ACQ-7 and were evaluated by a specialist, who determined the level of asthma control on the basis of the GINA criteria.[5]

Patients completed the Brazilian Portuguese version of ACQ-7, which had previously been validated. The ACQ-7 cutoff for controlled asthma was a score ≤ 0.75, and the ACQ-7 cutoff for uncontrolled asthma was a score ≥ 1.5.[6]

Spirometry was performed with a portable spirometer (Microlab; Micro Medical Ltd., Rochester, UK), the Spida 5 software (Micro Medical Ltd.) and previously established reference values being used.[7] In the two visits, spirometry was performed by the same professional, who was trained and qualified to do so. Values of $FEV_1 \geq 80\%$ were considered normal.[8] The level of asthma control was determined by a specialist, on the basis of the GINA criteria. However, because the specialist had no access to the spirometry results or ACQ-7 scores during the consultation, FEV_1 was not taken into consideration.

Categorical variables are presented as frequency distributions, and continuous variables are presented as the mean percentage of absolute values. Statistical analysis was performed with the GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA). Pearson's correlation test was used in order to determine the ACQ-7 interclass correlation coefficient, the Wilcoxon test was used in order to determine the differences in FEV_1 between the two visits, and the chi-square test was used in order to compare the variables. A convenience sample was used.

The study was approved by the Human Research Ethics Committee of the Federal University of Paraná *Hospital de Clínicas*. All patients gave written informed assent, consent, or both.

A total of 52 patients were included in the present study. The median age was 16.5 years (range, 12-84 years), and 65% of the patients were female. The mean height was 160.3 ± 7.5 cm, and the mean body mass index was 25.5 ± 6.3 kg/m^2. Regarding asthma severity before treatment initiation, half of the patients were classified as having mild persistent asthma and half were classified as having moderate persistent asthma.

In order to evaluate the reproducibility of ACQ-7, we used the interclass correlation test for the ACQ-7 scores obtained in the initial visit and those obtained 15 days later (Figure 1).

A correlation coefficient of 0.73 was found, showing that the ACQ-7 scores obtained in the two visits correlated well. In order to determine whether there were any differences between asthma severity as assessed in the first visit and asthma severity as assessed in the second visit, we evaluated the variable FEV_1 in isolation. No significant differences were found ($p = 0.15$).

We found no correlation between ACQ-7 scores and the level of asthma control by the GINA criteria (Table 1).

Although 23 patients had ACQ-7 scores ≥ 1.5 (i.e., uncontrolled asthma), only 2 were classified as having uncontrolled asthma on the basis of the

GINA criteria. Of the 47 patients with partially controlled asthma by the GINA criteria, 22 were classified as having uncontrolled asthma on the basis of their ACQ-7 scores (i.e., ≥ 1.5).

We found no correlation between FEV_1 and the level of asthma control as determined by the GINA criteria. Most (90.3%) of the 52 patients included in the study were classified as having partially controlled asthma on the basis of the GINA criteria. Of those, 21 (45%) had FEV_1 > 80% and 26 (55%) had FEV_1 < 80%.

Of the 17 previously validated asthma control and quality of life questionnaires for monitoring symptoms in asthma patients, only 2 include items on lung function parameters: ACQ-7 and the Asthma Control Scoring System.[3] The confirmation of the reproducibility of the Brazilian Portuguese version of ACQ-7 provides an instrument that includes items covering subjective symptoms and one lung function parameter and that can be used in clinical practice and research, its validity having been confirmed.[4]

We found no significant differences between the ACQ-7 scores obtained in the first visit and those obtained in the second. Because FEV_1 values were similar between the two visits, ACQ-7 scores were expected to be similar as well. The fact that they were demonstrates the good reproducibility of ACQ-7.

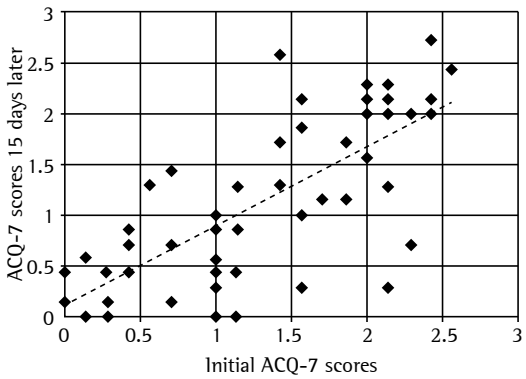

Figure 1 - Correlation between the Asthma Control Questionnaire 7 (ACQ-7) scores obtained in the initial visit and those obtained 15 days later in the 52 patients studied. r = 0.73; 95% CI: 0.58-0.83; p < 0.0001.

In the present study, the level of asthma control as determined by ACQ-7 scores differed from the level of asthma control as determined by the GINA criteria. We found that ACQ-7 was more effective in identifying patients with uncontrolled asthma. These data show that the use of an instrument that includes items covering clinical symptoms and lung function parameters on an objective point scale can provide information to facilitate the clinical management of patients, given that asthma treatment progression is based primarily on the level of asthma control.

In a study similar to ours, the GINA criteria were compared with the Asthma Control Test (ACT), which is a 5-item questionnaire that does not include items on lung function parameters. It was concluded that ACT scores ≤ 19 were useful in identifying patients classified as having uncontrolled or partially controlled asthma on the basis of the GINA criteria.[9] Although the ACT does not include items on lung function parameters, the ACT cutoff for uncontrolled asthma correlates well with the ACQ.[10] Therefore, the GINA criteria were expected to correlate well with ACQ scores. However, we found no such correlation in the present study.

We found that some of the patients who were classified as having partially controlled asthma on the basis of the GINA criteria had normal FEV_1, whereas others had reduced FEV_1. This finding suggests that the definition of partially controlled asthma does not accurately reflect lung function. When a patient is classified as having partially controlled asthma on the basis of the GINA criteria, the significance of this classification should be questioned, given that the patient might or might not have normal pulmonary function test results. In such patients, FEV_1 should be measured in order to aid in making treatment decisions, given that it provides complementary information and is weakly associated with symptoms.[11]

Table 1 - Identification of patients with controlled asthma on the basis of the Global Initiative for Asthma criteria and Asthma Control Questionnaire 7 scores.

ACQ-7 score[a]	GINA criteria		
	Controlled asthma	Partially controlled asthma	Uncontrolled asthma
< 1.5	3	25	1
≥ 1.5	0	22	1

ACQ-7: Asthma Control Questionnaire 7; and GINA: Global Initiative for Asthma. [a]Cutoff for uncontrolled asthma = 1.5. *p = 0.06, chi-square test.

The present study has some limitations. First, the ACQ-7 cutoffs aid in distinguishing between controlled and uncontrolled asthma; that is, they do not aid in assessing partially controlled asthma. Second, the GINA criteria and the National Asthma Education and Prevention Program lack a clear definition of asthma control.[12]

In conclusion, ACQ-7 showed good reproducibility in the present study. In addition, in the patients over 12 years of age in our sample, the level of asthma control by the GINA criteria differed from the level of asthma control as assessed by ACQ-7 scores, and ACQ-7 identified a greater number of patients with uncontrolled asthma than did the GINA criteria.

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Musculoskeletal involvement in sarcoidosis*,**

Acometimento músculo-esquelético na sarcoidose

Akasbi Nessrine, Abourazzak Fatima Zahra, Harzy Taoufik

Abstract

Sarcoidosis is a multisystem inflammatory disorder of unknown cause. It most commonly affects the pulmonary system but can also affect the musculoskeletal system, albeit less frequently. In patients with sarcoidosis, rheumatic involvement is polymorphic. It can be the presenting symptom of the disease or can appear during its progression. Articular involvement is dominated by nonspecific arthralgia, polyarthritis, and Löfgren's syndrome, which is defined as the presence of lung adenopathy, arthralgia (or arthritis), and erythema nodosum. Skeletal manifestations, especially dactylitis, appear mainly as complications of chronic, multiorgan sarcoidosis. Muscle involvement in sarcoidosis is rare and usually asymptomatic. The diagnosis of rheumatic sarcoidosis is based on X-ray findings and magnetic resonance imaging findings, although the definitive diagnosis is made by anatomopathological study of biopsy samples. Musculoskeletal involvement in sarcoidosis is generally relieved with nonsteroidal anti-inflammatory drugs or corticosteroids. In corticosteroid-resistant or -dependent forms of the disease, immunosuppressive therapy, such as treatment with methotrexate or anti-TNF-α, is employed. The aim of this review was to present an overview of the various types of osteoarticular and muscle involvement in sarcoidosis, focusing on their diagnosis and management.

Keywords: Sarcoidosis; Joints; Muscles; Bone and Bones.

Resumo

A sarcoidose é um distúrbio inflamatório multissistêmico de causa desconhecida, frequentemente afetando o sistema pulmonar e também o sistema músculo-esquelético, mas de forma menos frequente. Em pacientes com sarcoidose, o acometimento reumático é polimórfico, podendo ser o sintoma de apresentação da doença ou aparecer durante sua progressão. O acometimento articular é dominado por artralgia inespecífica, poliartrite e síndrome de Löfgren, que é definida como a presença de adenopatia pulmonar, artralgia (ou artrite) e eritema nodoso. Manifestações esqueléticas, especialmente dactilite, aparecem principalmente como complicações de sarcoidose crônica e em vários órgãos. O acometimento muscular na sarcoidose é raro e geralmente assintomático. O diagnóstico de sarcoidose reumática baseia-se em achados radiográficos e de ressonância magnética, embora o diagnóstico definitivo seja feito pelo estudo anatomopatológico de amostras de biópsia. O acometimento músculo-esquelético na sarcoidose é geralmente aliviado com o uso de anti-inflamatórios não esteroidais ou corticosteroides. Em formas da doença resistentes ao corticosteroide ou corticosteroide dependentes, a terapia de imunossupressão, como o tratamento com metotrexato ou anti-TNF-α, é utilizada. O objetivo desta revisão foi apresentar uma visão geral dos vários tipos de acometimento osteoarticular e muscular na sarcoidose, com foco no diagnóstico e manejo.

Descritores: Sarcoidose; Articulações; Músculos; Osso e Ossos.

Introduction

Sarcoidosis is a granulomatous disease of unknown etiology that involves multiple systems. It most commonly affects the lungs, lymph nodes, skin, and eyes but can also affect other organs and systems, including the musculoskeletal system.[1] Rheumatic manifestations of sarcoidosis, although rare, include inflammatory arthritis, periarticular soft tissue swelling, tenosynovitis, dactylitis, bone involvement, sarcoid myopathy, and bone loss. The primary types of articular involvement are Löfgren's syndrome and acute polyarthritis, whereas bone involvement is dominated by sarcoid dactylitis and osteolysis. Muscle involvement often goes unrecognized and can appear as chronic myopathy, acute myositis, or pseudotumor. Sarcoidosis can also manifest as calcium balance

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Financial support: None.
Submitted: 21 September 2013. Accepted, after review: 21 February 2014.
**A versão completa em português deste artigo está disponível em www.jornaldepneumologia.com.br

disturbances, principally hypercalcemia, which is often asymptomatic but can occasionally be the presenting clinical symptom of the sarcoidosis.[2]

The diagnosis of sarcoidosis is based on clinical and radiological findings, together with evidence of noncaseating granulomas in biopsy specimens, after other granulomatous disorders, such as tuberculosis, have been excluded.[2] Treatment of rheumatic involvement often requires the use of nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, or methotrexate. Biological therapies such as the use of anti-TNF-α agents and anti-CD20 monoclonal antibodies have been shown to be effective in some cases of severe or refractory sarcoidosis.[3] The aim of this review was to present an overview of the various types of musculoskeletal involvement in sarcoidosis, focusing on their diagnosis and management.

Physiopathology

The exact cause of sarcoidosis remains unknown. The Th1-type of inflammation is present in the sarcoid granuloma which expresses and produces a variety of inflammatory cytokines, such as IL-2, IL-12, IL-6, and IFN-γ, as well as TNF-α, which is the central mediator of this inflammatory process.[4] Because of clinical and histological similarities with mycobacterial and fungal diseases, infectious causes have been investigated. However, such studies are controversial.[5] Recent evidence suggests that a genetic component is implicated in susceptibility to sarcoidosis. There is a strong link between sarcoidosis and variants in the class I and II HLA locus. A recent study identified annexin A11 as a novel non-HLA susceptibility locus for sarcoidosis.[6] Many other loci encoding TNF-α and co-stimulatory molecules on antigen-presenting cells such as CD80 and CD86, as well as the chemokine receptors CCR2 and CCR5, have been found to increase susceptibility to sarcoidosis.[7]

Articular involvement

The reported prevalence of arthritis in sarcoidosis ranges from 10% to 38%.[8] Nonspecific arthralgia affects the majority of sarcoidosis patients, especially females. With the exception of Löfgren's syndrome, joint manifestations are rarely seen at symptom onset in sarcoidosis. Two types of arthritis, differing in their clinical course and prognosis, have been identified. The first is acute polyarthritis, which is typically accompanied by erythema nodosum and occasionally by acute uveitis. Acute polyarthritis resolves without permanent sequelae. The second type is chronic sarcoid arthritis, which, although less common, can progress to joint deformity. Other forms of articular manifestations, such as periarticular soft tissue swelling and tenosynovitis, can also be seen.[9]

Acute arthropathy

Acute polyarthritis occurs in 40% of patients with sarcoidosis, particularly in the earlier stages of the disease, and can be the presenting feature. It is self-limiting, is usually symmetric, and resolves without permanent sequelae.[10] The most common form of acute arthropathy in sarcoidosis is Löfgren's syndrome, which occurs in acute onset sarcoidosis and typically manifests as bilateral hilar lymphadenopathy, arthritis, and erythema nodosum. Löfgren's syndrome is associated with a good prognosis and spontaneous remission.[11,12] In patients with sarcoidosis, acute polyarthritis most commonly involves the ankles (in > 90% of cases), often bilaterally, followed by other large joints of the lower limbs, only occasionally involving the small joints of hands and feet. This type of polyarthritis is only mildly painful, migratory and transient. Oligoarthritis or monoarthritis are relatively rare forms of acute sarcoid arthropathy.

Chronic arthropathy

Chronic arthropathy is rare in sarcoidosis, occurring in only 0.2% of cases. It most often affects black males and is usually accompanied by other systemic disorders, mainly those of the lungs and eyes.[4] Various forms of chronic arthritis can occur in patients with sarcoidosis: nondeforming arthritis with granulomatous synovitis; Jaccoud's arthropathy; and joint swelling adjacent to a sarcoid bone lesion. Among such patients, the arthritis is rheumatoid factor-positive in 10-47% of cases. That nonspecific reactivity is due to increased circulating polyclonal IgG. Therefore, sarcoidosis-related arthritis can mimic rheumatoid arthritis, especially when accompanied by joint deformities. The differential diagnosis is usually made on the basis of clinical criteria, including negative serology for anti-cyclic citrullinated peptide antibodies and antinuclear antibodies, as well as the absence of the specific erosive

joint deformity seen in rheumatoid arthritis.[13] A finding of granuloma on synovial biopsy helps in establishing the diagnosis of sarcoidosis. Although all joints can be affected, affected ankles strongly indicate the diagnosis of sarcoidosis. In some cases, X-rays show soft tissue swelling. Magnetic resonance imaging (MRI) can depict lesions that cannot be visualized on X-rays.

Involvement of periarticular structures

Among patients with sarcoidosis, tenosynovitis is common in the tendons of the ankles and wrist, occasionally accompanied by carpal tunnel syndrome in the latter case.[14] Although tenosynovitis, tendinitis, bursitis, and synovitis can be demonstrated on MRI scans, they are nonspecific findings and biopsy is therefore required in order to confirm the diagnosis of sarcoidosis.[15]

Sacroiliitis in sarcoidosis

Sacroiliac involvement in sarcoidosis is rare and generally unilateral. Sarcoidosis cannot be established without a biopsy to rule out tuberculosis or other infectious process of that joint.[16] Sacroiliitis can reveal ankylosing spondylitis that can be associated with sarcoidosis, especially in patients testing positive for HLA-B27.

Treatment of sarcoid arthropathy

In 90% of cases, acute polyarthritis resolves spontaneously. In others, it requires treatment with NSAIDs, corticosteroid injections into the joint, or a short course of corticosteroids at 10-15 mg/day. Hydroxychloroquine and colchicine can be used in some cases, especially in those of Löfgren's syndrome.[17] The use of other immunosuppressive agents should be reserved for patients with progressive chronic sarcoid arthropathy that is refractory to treatment with systemic corticosteroids or in whom steroids have generated side effects. According to the Brazilian Thoracic Association Guidelines for Interstitial Lung Diseases,[18] there are alternative treatments, such as methotrexate, azathioprine, leflunomide and hydroxychloroquine. Methotrexate is an efficient and corticosteroid-sparing therapeutic agent for the treatment of musculoskeletal manifestations of sarcoidosis.[19] Many studies have shown the importance of TNF-α in sarcoid granuloma development, which makes TNF-α a potential target in the treatment of sarcoidosis. Many interesting reports suggest some efficacy of TNF-α antagonists (infliximab, etanercept, and adalimumab) in refractory sarcoidosis with musculoskeletal involvement.[20-23] Paradoxical cases of proven sarcoidosis have been reported in patients receiving anti-TNF-α agents for other chronic inflammatory rheumatic diseases. This paradoxical effect of anti-TNF-α agents must be known by the clinician.[24,25] The use of B-cell-depleting agents might also be of benefit in sarcoid arthritis. A recent case report of a patient with sarcoidosis of the lungs and joints showed that rituximab is effective in treating sarcoidosis without major side effects.[26] Based on the success of rituximab in this disease, other B-cell therapies, such as ocrelizumab, need to be evaluated in systemic sarcoidosis.[27]

Osseous sarcoidosis

Bone involvement is reported in 1-15% of sarcoidosis patients. It is more common in black patients and is usually accompanied by infiltrative skin lesions, especially lupus pernio.[28]

Involvement of small bones

Although bone lesions are frequently asymptomatic, some sarcoidosis patients present with symptomatic dactylitis. Bone, skin and soft tissue are involved, especially in the second and third phalanges, resulting in sausage-like fingers resembling those seen in the spondyloarthropathies.[29]

The bony lesions are usually cystic, sclerotic lesions rarely being reported. Multiple cystic lesions sometimes result in a "lacy" pattern, which is typical of sarcoid bone disease. The classic lesions in the small bones of the hands and feet are known as Perthes disease and Jüngling's disease. They are well characterized on standard X-rays.

There are three radiological types of sarcoid bone disease: type I, characterized by big cystic lesions (Figure 1), which is quite rare and can be associated with a stress fracture from a pathological fracture; type II, characterized by multiple, small circumscribed cysts, occasionally conflicting, and polycyclic (Figure 2); and type III, characterized by tunneling of the cortex of the phalanx, which leads to remodeling of the cortical and trabecular architecture. All three forms can coexist in the same bone. Acro-osteolysis,

presenting as nodular densities in the terminal phalanges, can also occur.[17]

Involvement of long bones

Involvement of the axial skeleton and long bones is uncommon in sarcoidosis. Vertebral sarcoidosis can present as purely lytic lesions, as purely sclerotic lesions (in rare cases) mimicking blastic metastases, or as a mixture of the two. The lower dorsal and upper lumbar vertebrae are mostly involved. Because it can guide the selection of biopsy sites, MRI has gained attention as a modality that facilitates the histopathological confirmation of the diagnosis and can be used in evaluating the efficacy of the treatment of bone lesions. The MRI findings are nonspecific; showing multifocal lesions that are hypointense on T1-weighted images and hyperintense on T2-weighted images.[30]

Any bone, including the skull, ribs, nasal bone, and long bones, can be affected in sarcoidosis. In the skull, sarcoidosis manifests as asymmetrical, asymptomatic lytic lesions of variable size. Positron emission tomography/computed tomography (PET/CT) imaging can be useful in the assessment of bone involvement in sarcoidosis patients.[31]

Treatment of osseous sarcoidosis

Asymptomatic osseous sarcoidosis generally does not require therapy, although the indications for therapeutic intervention are not well defined. However, treatment is usually indicated when the symptoms include uncontrolled pain, stiffness, or bony destruction. Therapy generally consists of oral corticosteroids at 15-20 mg/day. The dosage is adjusted according to the clinical response.[32] Methotrexate and hydroxychloroquine can also be used. Although there is some evidence that anti-TNF-α agents are efficacious in sarcoid bone lesions, this effect needs to be verified.[33]

Muscle sarcoidosis

Muscle sarcoidosis is a rare entity and is usually asymptomatic. It is symptomatic in only 1% of cases. It typically appears as a complication of systemic sarcoidosis.[34] The histological pattern of sarcoid myopathy is as a noncaseating granuloma in the perimysial connective tissue. Large granulomas compress and destroy adjacent muscle fibers, resulting in degeneration and focal lymphocyte infiltration, and foci of necrosis or fibrosis can also be observed.[34] Fiber destruction in this disease is caused mainly by fiber infiltration rather than by mechanical compression or ischemia.

In a patient with sarcoidosis, the presence of muscle weakness, muscle pain, or muscle nodules is suggestive of sarcoid myopathy. Fatigue and general weakness are common, which could

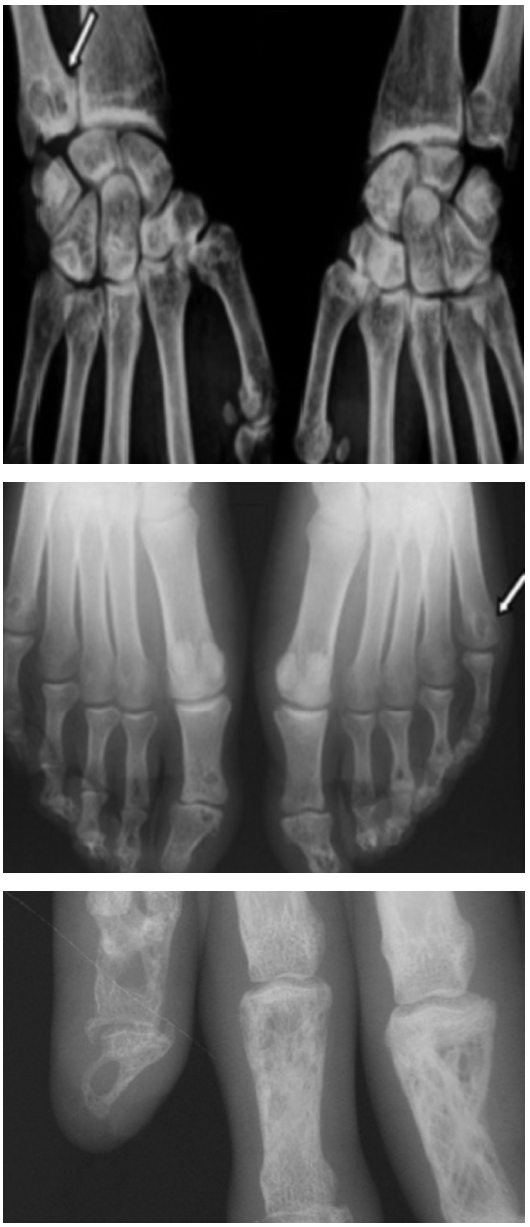

Figure 1 - Pattern of bone involvement found on X-rays in a patient with sarcoidosis: multiple, large cysts (type 1). From the collection of Professor Yannick Allanore, of the Department of Rheumatology A, Descartes University, Medical School, Cochin Hospital, Paris, France. Used with the permission of Professor Allanore.

explain why patients with sarcoidosis frequently experience exercise intolerance.[35,36] The cause is not only sarcoid myopathy but also the high circulating levels of inflammatory cytokines such as TNF-α, IL-6, and IFN-γ.

In sarcoid myopathy, three clinical patterns are generally recognized[37]: chronic myopathy (seen in 86% of cases), which is the most common form, characterized by an insidious onset of proximal muscle weakness with normal or elevated serum levels of muscle enzymes; acute myositis (seen in 11% of cases); and nodular or tumor-like myositis (seen in only 3% of cases). Nodular myopathy manifests as multiple, tumor-like, palpable nodules in the muscles.[37,38] The use of MRI and PET/CT facilitates the diagnosis of muscle sarcoidosis.

The mainstay of the treatment of patients with muscle sarcoidosis is 8-12 weeks of systemic glucocorticoid therapy at an initial daily dose of 0.5-1 mg/kg with progressive tapering. Methotrexate, chloroquine and azathioprine have been used in corticosteroid-resistant and corticosteroid-dependent forms.[39] Thalidomide and infliximab have been found to be beneficial in some cases of sarcoid myopathy. The effectiveness of these medications seems related to TNF-α inhibition. Corticosteroid-induced myopathy can also occur as a complication of the treatment of sarcoidosis. Affected patients typically develop proximal muscle weakness that has a gradual onset (over several weeks) and is accompanied by muscle wasting. A common manifestation is difficulty getting up from a chair or climbing stairs. Myalgia and muscle tenderness are not observed.[40]

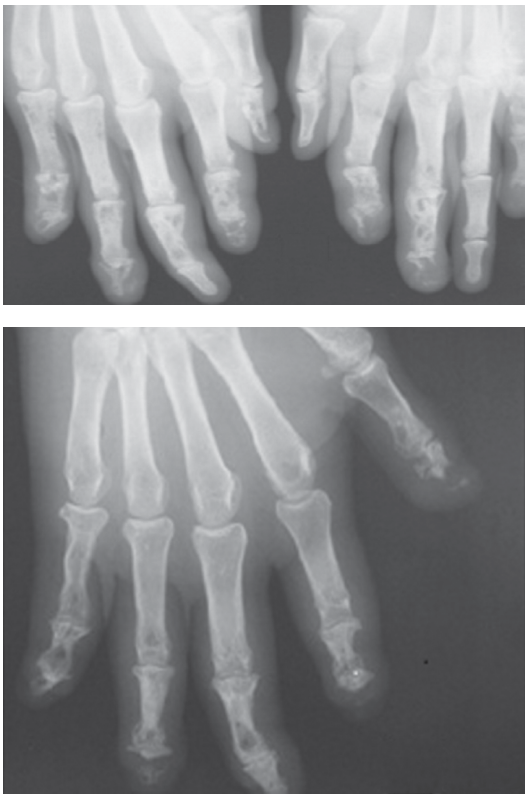

Figure 2 - X-ray of hands showing cysts and acro-osteolysis (type II). From the collection of Professor Yannick Allanore, of the Department of Rheumatology A, Descartes University, Medical School, Cochin Hospital, Paris, France. Used with the permission of Professor Allanore.

Combination of sarcoidosis and rheumatic disease

Sarcoidosis can be associated with other chronic inflammatory disease like systemic lupus erythematosus (SLE), Sjögren's syndrome or psoriatic arthritis. In sarcoidosis, suspicion of SLE is raised when the patient develops a butterfly rash or discoid lesions. The treatment of sarcoidosis patients with SLE is challenging and should be individualized.[41] The use of anti-TNF-α agents should be avoided in patients who have active SLE. In rare cases, sarcoidosis and Sjögren's syndrome can both affect the salivary glands. Dryness and diffuse swelling of oral mucosal tissues can be the presenting symptom of sarcoidosis.[42]

Approximately 6% of all patients with sarcoidosis develop a psoriatic form of arthritis.[43] Although anti-TNF-α therapy is helpful in psoriasis, it can also paradoxically induce progressive psoriasis, and patients treated with anti-TNF-α agents should be closely monitored.

Changes in calcium metabolism

In sarcoidosis, hypercalciuria is more common than is hypercalcemia. Either can be caused by nephrocalcinosis, kidney stones, or renal failure. Granulomatous macrophages increase conversion of 25-hydroxyvitamin D to active 1,25-dihydroxyvitamin D (calcitriol), leading to increased calcium absorption by the intestine.[44] The high levels of calcitriol induce osteoclast activation and bone resorption. In addition, corticosteroid-treated patients are at a higher

risk of osteoporosis. Bone loss could be also increased by high levels of parathyroid hormone-related peptide identified in sarcoid tissue.[45] Corticosteroids have been successfully used to improve disorders of calcium metabolism.[46] Mycophenolate mofetil and infliximab have been used in select cases.[47,48]

Bone loss in sarcoidosis

The bone loss in sarcoidosis can be caused by multiple factors, including diffuse skeletal granulomatosis, calcitriol, osteoclast activating factor, and glucocorticoid therapy, particularly in postmenopausal patients. In one study of corticosteroid therapy in patients with sarcoidosis, the authors found that the rate of bone loss in corticosteroid-treated postmenopausal patients with sarcoidosis was greater than that reported for corticosteroid-treated patients with rheumatoid arthritis or asthma.[49]

The prevention and treatment of bone loss in patients with sarcoidosis is difficult. Calcium and vitamin D, both commonly administered to patients at risk for osteoporosis, should be considered with caution in patients with hypercalcemia, hypercalciuria, high levels of parathormone, and kidney stones.[50] Guidelines published by the American College of Rheumatology recommend that patients receiving corticosteroids should undergo bone mineral density testing.[51] The World Health Organization fracture assessment risk tool can be used in order to calculate patient risk of fracture.

For patients with sarcoidosis at risk of developing osteoporosis because of prolonged use of corticosteroids, bisphosphonates are effective in preventing glucocorticoid-induced bone loss. The American College of Rheumatology recommends using bisphosphonates in low-risk patients receiving corticosteroids at doses of $\geq$ 7.5 mg/day, as well as in all medium- or high-risk patients receiving corticosteroids. Of the available bisphosphonates, alendronate, risedronate, zoledronic acid, and teriparatide effectively reduce bone loss and can thus diminish fracture risk.[51] Further studies on bisphosphonate use in osteoporosis during sarcoidosis are needed.

Final considerations

Although sarcoidosis can affect any organ, sarcoidosis involving the musculoskeletal system is rare. The disease can affect the muscles, joints and the bones. Those conditions, which are polymorphic, can be the presenting symptoms the disease or can appear during the course of its progression. Corticosteroids are the cornerstone of sarcoidosis treatment but only have a postponing effect. Prospective, randomized, controlled trials assessing anti-TNF-α agents are needed in order to evaluate their efficacy in cases of sarcoidosis with rheumatic complications.

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The value of family history in the diagnosis of hypersensitivity pneumonitis in children*

O valor da história familiar no diagnóstico de pneumonite de hipersensibilidade em crianças

Joana Cardoso, Isabel Carvalho

Abstract

Hypersensitivity pneumonitis (HP), or extrinsic allergic alveolitis, is an immunologically mediated disease resulting from the inhalation of organic substances that trigger an inflammatory response in the alveolar wall, bronchioles, and interstitium in susceptible individuals. Although HP is predominantly an occupational disease, seen in adulthood, cases in children have been described. The diagnosis of HP requires a high degree of suspicion. The treatment consists in avoiding contact with the antigen, and, in some cases, systemic corticosteroids might be necessary in order to prevent its progression to pulmonary fibrosis. We report the clinical cases of three children with a history of contact with birds and a family history of HP. All three patients presented with cough and dyspnea on exertion. The disease was diagnosed on the basis of the clinical history and ancillary diagnostic test results consistent with the diagnosis, including a predominance of lymphocytes (> 60%, CD8+ T lymphocytes in particular) in bronchoalveolar lavage fluid and a ground-glass pattern seen on HRCT of the chest. Early diagnosis is crucial in order to prevent HP from progressing to pulmonary fibrosis. Hereditary factors seem to influence the onset of the disease.

Keywords: Alveolitis, extrinsic allergic; Bronchoalveolar lavage; Glucocorticoids.

Resumo

A pneumonite de hipersensibilidade (PH), ou alveolite alérgica extrínseca, é uma doença imunologicamente mediada, resultante da inalação de substâncias orgânicas que desencadeiam uma reação inflamatória na parede dos alvéolos, bronquíolos e interstício em indivíduos susceptíveis. Apesar de ser uma doença ocupacional de predomínio na idade adulta, estão descritos casos em crianças. O diagnóstico de PH requer grande suspeição, e seu tratamento consiste na ausência de contato com o antígeno e, em alguns casos, pode ser necessária corticoterapia sistêmica, evitando-se a progressão para fibrose pulmonar. Relatamos três casos clínicos de crianças com história de contato com aves e história familiar de PH. Todos os casos se apresentaram com tosse e dispneia aos esforços. O diagnóstico foi possível por história clínica e exames auxiliares de diagnóstico compatíveis, incluindo lavado broncoalveolar com predomínio de linfócitos (> 60%, especialmente linfócitos T CD8+) e TCAR de tórax com padrão em vidro fosco. O diagnóstico precoce é fundamental na PH para se prevenir a evolução para fibrose pulmonar. Fatores hereditários parecem influenciar seu aparecimento.

Descritores: Alveolite alérgica extrínseca; Lavagem broncoalveolar; Glucocorticoides.

Introduction

Hypersensitivity pneumonitis (HP), or extrinsic allergic alveolitis, is an immunologically mediated disease caused by the inhalation of organic substances that, in susceptible individuals, trigger an inflammatory response in the alveolar wall, bronchioles, and interstitium.[1-8] Although HP is predominantly an occupational disease, seen in adulthood, cases in children have been described, most of which occurred after exposure to avian proteins.[4,6]

The prevalence of HP ranges from 5-15% in individuals exposed to one allergen,[1-3] and the frequency of the disease is related to several factors (the amount of allergen inhaled, the duration of exposure, the nature of the antigen, and the host immune response).[1,2] Heredity may

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Financial support: None.
Submitted: 5 December 2012. Accepted, after review: 16 May 2013.

play an important role, with families positive for HLA-DR7, HLA-B8, and HLA-DQw3 showing a stronger predisposition.[2,6]

HP can be classified on the basis of symptoms (as acute, subacute, or chronic) or on the basis of the dynamic nature of the disease (as acute progressive, acute intermittent non-progressive, or recurrent non-acute disease).[1-3,5-6,8] The acute form corresponds to intermittent, intense allergen exposure, and it is similar to a respiratory infection of viral etiology that resolves within 24-48 h.[1-3,5-7] The subacute form is characterized by dyspnea on exertion, asthenia, and weight loss, and it corresponds to continued but less intense allergen exposure.[1-3,5-6] The chronic form is characterized by progression to irreversible lung injury, with pulmonary fibrosis,[1,3] and it corresponds to prolonged insidious inhalation of low concentrations of antigen, with no history of acute disease.[1-3,5-7]

There is no pathognomonic diagnostic test, and a presumptive diagnosis is made on the basis of a high index of suspicion, clinical history, physical examination, laboratory test results, pulmonary function testing (PFT), and imaging study results.[1-4,6]

Laboratory data are nonspecific—leukocytosis, increased ESR, and increased levels of C-reactive protein (CRP) and immunoglobulin.[1,2,4] Skin tests and the presence of precipitating antibodies to the antigen are markers of exposure, but negative results do not exclude the diagnosis.[1-4,6] Inhalation challenge testing with the predisposing antigen is the best diagnostic method when diagnostic questions persist, but it must be performed in a hospital setting.[1-4,6]

Usually, PFT shows a restrictive pattern, characterized by a decrease in FVC and TLC. In the chronic phase, it is possible to find an obstructive pattern, and DLCO is usually decreased.[1-4,6]

Bronchoalveolar lavage (BAL) fluid shows a predominance of lymphocytes (> 60%), namely suppressor T lymphocytes (CD8+), with a decrease in the CD4/CD8 index < 1% (rarely found in children).[1-4,6]

Chest X-ray changes appear as a function of the degree of disease and are little related to symptom severity, ranging from normal to a nodular/reticulonodular pattern (in acute and subacute forms).[1-6] Chest HRCT can show a diffuse micronodular pattern (acute phase) or obstructive emphysema, interstitial fibrotic lesions, and ground-glass areas (chronic phase),[1-7] being the most useful test for diagnosis.

The treatment consists in avoiding contact with the antigen, which can be the sole treatment in acute forms. Systemic corticosteroids are the treatment of choice in subacute and chronic forms.[1-4,6] The prognosis varies from full recovery in acute and non-progressive forms to pulmonary fibrosis in chronic forms. The degree of pulmonary fibrosis at diagnosis is the major prognostic factor.[1,2]

Case reports

Case 1

We report the case of a 12-year-old boy with a history of asthma and a family history of HP. Living in a rural area, the boy had contact with pigeons and canaries, which aggravated the symptoms. He presented to the emergency room with a 2-day history of productive cough and fever, accompanied by anorexia and weight loss. A chest X-ray showed a bilateral perihilar infiltrate, and the patient was discharged after being treated with clarithromycin, which was replaced by a combination of amoxicillin and clavulanic acid 5 days later because of persistence of symptoms. One month later, because the patient continued to have an intermittent cough with periods of worsening and dyspnea on minimal exertion, he again sought medical attention, presenting with perioral cyanosis, pallor, hypoxemia (SpO_2 = 85% on room air), overall retraction, and an overall decrease in breath sounds, with crackles at the lung bases. Ancillary test results were as follows: ESR, 36 mm/h; PCR, 22.8 mg/L; IgA and IgG levels, increased; Phadiatop® test (Phadia, Uppsala, Sweden) for inhalation and food allergy, negative; PPD, 0 mm induration; sweat test, 34 mEq/L; chest X-ray findings, bilateral perihilar and basilar reticulonodular infiltrate (Figure 1); echocardiogram, unremarkable; chest HRCT findings, air-space consolidation and air bronchogram in both lower lobes, accompanied by a diffusely distributed bilateral interstitial pattern, a centrilobular pattern with some micronodules, and ground-glass areas; serology for *Chlamydophila psittaci*, negative; PFT, indicative of mild obstructive lung disease; DLCO, moderate defect; BAL fluid findings, an intense lymphocytic alveolitis, with a marked predominance of CD8+ (CD4/CD8 ratio < 0.3%).

The patient received oral corticosteroids for a year, which resulted in complete resolution of the symptoms and complete resolution of the lesions seen on HRCT of the chest, as well as in improvement in PFT results by the end of a three-year follow-up period.

Case 2

A 14-year-old girl who lived in a rural area and had birds at home presented with a family history of HP. The patient had been healthy until November of 2002, when she sought emergency room treatment due to fever and cough. Physical examination revealed crackles and respiratory distress. A chest X-ray showed an interstitial infiltrate. The patient was discharged on clarithromycin and prednisolone. She returned to the outpatient clinic one week later, reporting dyspnea on minimal exertion. Ancillary test results were as follows: ESR, 24 mm/h; IgG level, increased; levels of other immunoglobulins, no abnormalities; serologies for *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*, negative; IgG and IgM, negative; chest X-ray findings, increased bronchovascular markings bilaterally and bilateral, perihilar predominant diffuse interstitial infiltrate; chest HRCT findings, mild, slightly heterogeneously distributed parenchymal thickening in both lungs, small nodules with ill-defined borders, and ground-glass changes (Figure 2); echocardiogram and electrocardiogram, unremarkable; BAL fluid findings, an intense lymphocytic alveolitis and a mild neutrophilic alveolitis, with a marked predominance of CD8+ (CD4/CD8 ratio < 0.1%). The patient was maintained on inhaled corticosteroids for 2 years, which resulted in progressive clinical improvement.

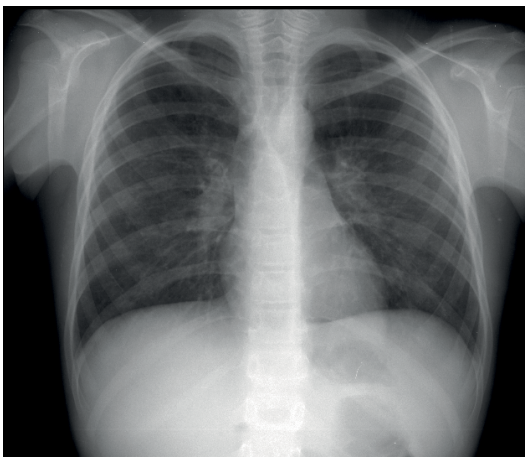

Figure 1 - Chest X-ray showing bilateral perihilar and basilar reticulonodular infiltrate.

Case 3

A 7-year-old boy, whose father and paternal grandmother were under investigation for suspected HP, presented with a personal history of *situs inversus totalis* and recurrent respiratory infection. The boy was admitted to the emergency room with a 5-month history of productive cough, accompanied by progressively worsening dyspnea on exertion, with no improvement with bronchodilators and inhaled corticosteroids. It was reported that there had been a parakeet at home since the onset of the symptoms, and that the home also had a henhouse and a cooperage. The initial examination revealed an SpO_2 of 84% on room air, subcostal retraction, and bilateral crackles. Ancillary test results were as follows: ESR, 30 mm/h; PCR, 1.41 mg/dL; IgG, IgA, and IgM levels, increased; serologies for *Mycoplasma pneumoniae, Legionella pneumophila*, and *Chlamydia pneumoniae* (IgG and IgM), negative; chest X-ray findings, bilateral diffuse perihilar interstitial infiltrate; sweat test, 32 mEq/L; PPD, 0 mm induration; serum precipitins to birds droppings and feathers, negative; echocardiogram, *situs inversus totalis*; chest HRCT findings, pronounced ground-glass changes in both

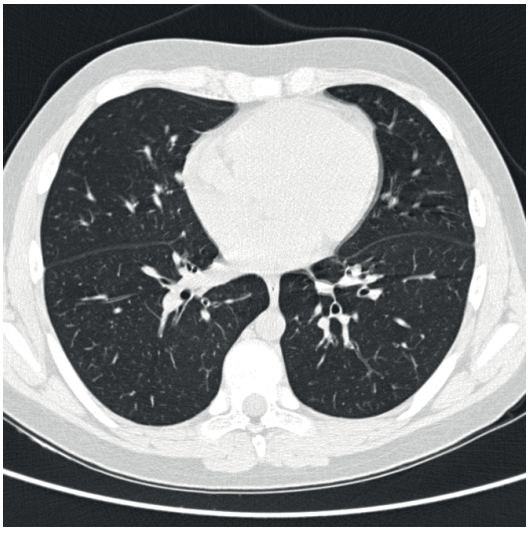

Figure 2 - HRCT scan of the chest showing mild, slightly heterogeneously distributed parenchymal thickening in both lungs; small nodules with ill-defined borders; and ground-glass changes.

lung fields, consistent with extrinsic allergic alveolitis (Figure 3); PFT, indicative of severe obstructive lung disease that was unresponsive to bronchodilators; BAL fluid findings, an intense lymphocytic alveolitis and neutrophilia, with a marked predominance of CD8+ (CD4/CD8 ratio < 0.1%). The patient received inhaled corticosteroids for 2 years, which resulted in progressive clinical and radiological improvement.

Discussion

The patients described had a family history of HP accompanied by a clinical profile that corresponded to the subacute form of HP, with an obstructive pattern and symptoms such as cough, dyspnea on exertion, weight loss, and sometimes fever, the onset of which occurred within weeks to months of exposure to an antigen.

A high index of suspicion was essential for the diagnosis, as were analytical and laboratory criteria. Among the major criteria, defined by Schuyler et al.,[7] are symptoms consistent with the disease, evidence of exposure to antigens as per clinical history, X-ray and CT findings consistent with HP, and lymphocytosis in BAL fluid. Chief among the minor criteria are crackles at the lung bases and arterial hypoxemia. In only one of the patients was there a decrease in DLCO. In none of the cases was it possible to detect precipitating antibodies to the antigen.

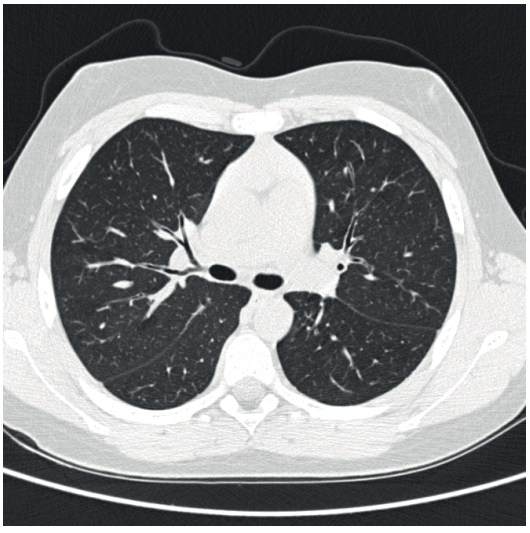

Figure 3 - HRCT scan of the chest showing pronounced ground-glass changes in both lung fields, consistent with extrinsic allergic alveolitis.

Although they are objective markers of exposure, negative results do not absolutely exclude the diagnosis.[1-4,6]

In these three cases, it was possible to detect a diffuse reticulonodular/interstitial pattern on chest X-ray, as well as a ground-glass pattern, ground-glass areas, and micronodules, characteristic of the subacute form.

The detection of lymphocytosis in the BAL fluid from the patients, as well as the finding of a CD4/CD8 ratio < 0.1% in two of them, lent support to the diagnosis of HP.

Given that the disease was not severe in any of the cases, the treatment consisted in avoiding contact with the allergen and using oral/inhaled corticosteroids, which resulted in progressive clinical improvement, as well as in improvement in radiological and ventilatory parameters.

The need for lung biopsy should be weighed in terms of its cost-benefit ratio, and it should be considered in rare cases in which there is diagnostic uncertainty or in which the clinical course or treatment response is unclear.[3]

HP is an uncommon disease in children and has nonspecific symptoms. For the diagnosis of HP, it is important that clinical evaluation be performed and allergen exposure be investigated. In all three cases described above, the patients had a family history of HP, although they were not screened for the presence of HLA. These facts lead us to believe that, although allergens are the major triggering factor for this disease, heredity is also an important cofactor. Early diagnosis of HP is crucial in order to prevent severe and irreversible complications, such as pulmonary fibrosis.

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Lymphadenitis caused by infection with an isoniazid- and rifampin-resistant strain of *Mycobacterium bovis* BCG in an infant with IFN-γ/IL-12 pathway defect*

Linfadenite por *Mycobacterium bovis* BCG resistente a isoniazida e rifampicina em lactente com defeito no eixo IFN-γ/IL-12

Lilian Martins Oliveira Diniz, Tiago Guimarães, Maria das Graças Rodrigues de Oliveira, Jorge Andrade Pinto, Silvana Spindola de Miranda

Abstract

We report a rare case in a female infant (age, 3.5 months) with primary immunodeficiency (IFN-γ/IL-12 pathway defect) who presented with suppurative lymphadenitis after *Mycobacterium bovis* BCG vaccination. The strain of *M. bovis* BCG identified was found to be resistant to isoniazid and rifampin. The patient was treated with a special pharmacological regimen involving isoniazid (in a limited, strategic manner), ethambutol, streptomycin, and IFN-γ, after which there was complete resolution of the lesions.

Keywords: BCG vaccine; Interferon-gamma; Tuberculosis, multidrug-resistant.

Resumo

Relatamos um caso raro em uma lactente com três meses e meio de idade, portadora de imunodeficiência primária (defeito no eixo IFN-γ/IL-12), que apresentou linfadenite supurativa após a vacinação por *Mycobacterium bovis* BCG, cepa essa resistente a isoniazida e rifampicina. Após o tratamento com um esquema medicamentoso especial com isoniazida (de forma estratégica e limitada), etambutol, estreptomicina e IFN-γ, houve a cura completa das lesões.

Descritores: Vacina BCG; Interferon gama; Tuberculose resistente a múltiplos medicamentos.

Introduction

BCG is an attenuated strain of *Mycobacterium bovis* that is present in the tuberculosis vaccine, which was first used in humans in 1922.[1] The vaccine produces an artificial primary infection with non-virulent bacilli in order to increase resistance to a future infection with virulent bacilli.[1]

The World Health Organization recommends vaccination with the BCG vaccine for all newborns in areas with a high prevalence of tuberculosis as a means to prevent the disease.[2] In Brazil, the use of the BCG vaccine for many years has demonstrated the effectiveness of vaccination, with minimal adverse reactions, and with severe complications occurring only rarely.[1]

During the natural course of the vaccination lesion, nonsuppurative axillary, supra-axillary, or infraclavicular lymph node swelling can be seen. However, severer lesions caused by *M. bovis* BCG strains can be found in patients with immunodeficiency, who should be treated with a combination regimen of drugs, such as isoniazid, rifampin, ethambutol, and ciprofloxacin.[1,3-5]

The treatment of disease caused by BCG can be complicated by resistance to pyrazinamide, which is inherent to all strains of *M. bovis*, as well as by intermediate resistance of some strains to isoniazid and by the emergence of acquired resistance during inappropriate therapy.[5,6]

*Study carried out at the Federal University of Minas Gerais, Belo Horizonte, Brazil.
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Financial support: This study received financial support from the *Fundação de Amparo à Pesquisa do Estado de Minas Gerais* (FAPEMIG, Foundation for the Support of Research in the state of Minas Gerais).
Submitted: 25 April 2013. Accepted, after review: 27 May 2013.

The authors report a case of an infant with primary immunodeficiency who had suppurative lymphadenitis after *M. bovis* BCG vaccination. The strain of *M. bovis* BCG identified was found to be resistant to isoniazid and rifampin.

Case report

A female infant (age, 3.5 months) was brought by her mother to the Department of Pediatric Infectious Diseases of the Federal University of Minas Gerais *Hospital das Clínicas* because of "inflammation" at the BCG vaccination site. It was reported that there was a family history of two cousins who had experienced the same adverse event after BCG vaccination and had died with suspected primary immunodeficiency in the first year of life. The physician who treated the infant noted the presence of a granulomatous lesion (not suggestive of secondary infection) at the vaccination site, as well as ipsilateral suppurative lymphadenitis (Figure 1), and the patient was started on isoniazid therapy (10 mg/kg daily for 45 days). The lymphadenitis resolved during treatment. However, after discontinuation of the drug, the lesion reappeared. The patient was prescribed isoniazid for two additional months. The lesion resolved, but, at the end of drug treatment, it returned.

The infant was referred to the Immunodeficiency Outpatient Clinic of the Federal University of Minas Gerais *Hospital das Clínicas* with suspected primary immunodeficiency. Initial immunological assessment showed that serum immunoglobulin levels and lymphocyte subpopulation levels were within the normal range. Because the initial immunological profile was normal and the clinical course was relatively benign, we hypothesized that the patient had an IFN-γ/IL-12 pathway defect. Serology for HIV was negative.

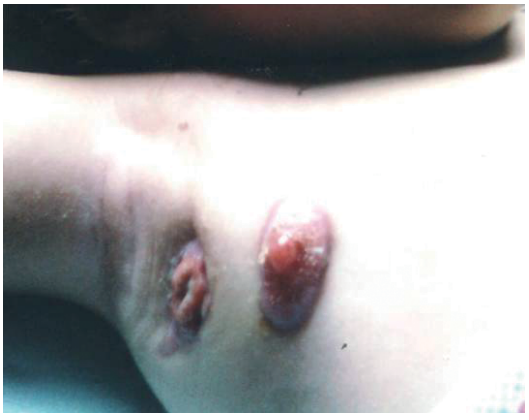

Figure 1 - Suppurative right axillary and infra-axillary lesions after BCG vaccination.

At that same time, we chose to biopsy the affected lymph node. Smear microscopy and mycobacterial culture of these clinical specimens were requested. Because of the isoniazid-induced reactivation of the lesion and the family history of primary immunodeficiency, the patient was started on a combination regimen of isoniazid (10 mg/kg daily), rifampin (10 mg/kg daily), and ethambutol (25 mg/kg daily). Ethambutol was introduced after a literature review, which showed that this drug is introduced in severer forms of infection with *M. bovis* BCG.[1,3-5] The infant was also referred to the department of ophthalmology for evaluation because of the risk of ethambutol-induced optic neuritis. In the second month of treatment, there was optic disc blurring bilaterally. At that same time, the results of smear microscopy and culture of the lymph node biopsy specimen were positive for the *M. tuberculosis* complex, and susceptibility testing showed resistance to isoniazid. In view of the partial resolution of lymphadenitis and the possibility of drug-induced optic neuritis, ethambutol was discontinued, and rifampin and isoniazid were continued despite resistance to isoniazid.

In the eighth month of treatment, the infant was hospitalized with secondary infection at the biopsy site. A chest CT performed during hospitalization showed diffuse coalescent axillary lymphadenopathy on the right, with necrotic and fistulized lymph nodes, which indicated lesion activity. A second lymph node biopsy was performed, and the specimen was sent to the Professor Hélio Fraga Referral Center, located in the city of Rio de Janeiro, Brazil, for mycobacterial and molecular analysis of the strain. The laboratory test results showed that it was the *M. bovis* BCG strain. The identification tests used were basic biochemical tests and polymerase chain reaction restriction analysis of the *hsp65* gene. Susceptibility testing was performed in BACTEC Mycobacteria Growth Indicator Tube (MGIT) 960 (Becton Dickinson, Sparks, MD, USA), the result of which showed resistance to isoniazid and also to rifampin. Sequencing of the *rpo*B gene detected the D516V mutation, and sequencing of the *kat*G gene detected the S315T mutation.

Given the results, we chose to continue treatment with isoniazid, restart ethambutol, and have the patient have ophthalmic follow-up weekly, as well as to add 30 doses of streptomycin (25 mg/kg daily) on alternate days for six months and discontinue rifampin. The ophthalmic lesion remained stable throughout the treatment period. Given the difficulty of the case, streptomycin was used because it is bactericidal and it is a first-line drug, as well as because there is a lack of knowledge about the true *in vivo* response to isoniazid.

The hypothesis of IFN-γ/IL-12 pathway defect was confirmed by molecular tests that detected a homozygous mutation in IL-12 receptor β_1, thus excluding probable severe combined immunodeficiency (SCID). Therefore, subcutaneous IFN-γ three times weekly was added to the treatment regimen.

After six months of treatment, the axillary lesion had completely resolved (Figure 2). At this writing, three years after the onset of the condition, the patient remained free of lymphadenitis, and the ocular lesion remained stable, with no impact on vision.

Discussion

Adverse reactions to the BCG vaccine vary according to the type of strain, bacterial load, administration of the vaccine, and host characteristics. Nonsuppurative reactive lymphadenitis can occur in children in the first months after administration of the vaccine and is mostly due to incorrect technique of administration. In Brazil, isoniazid has been used in the treatment of suppurative lymphadenopathy secondary to the vaccine in immunocompetent patients. This recommendation is based on the fact that the Brazilian strain of BCG is usually sensitive to this drug in in vitro tests.[7] However, in the present case, the BCG strain proved to be resistant to isoniazid and rifampin, showing probable *in vivo* response to the combination of these drugs with other drugs.

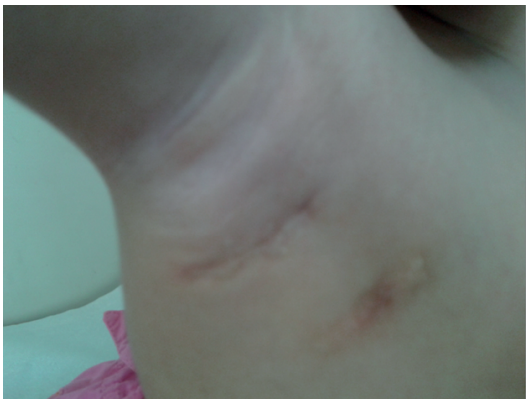

Figure 2 - Healed lesions in the right axillary and infra-axillary regions after treatment.

Nevertheless, the vaccine can cause complications that are more severe and, in most cases, occur in patients with immunosuppression. The incidence of disseminated *M. bovis* BCG disease in European countries is estimated to be two cases per million children vaccinated, and the disease occurs only occasionally in immunocompetent children.[8] In their study, Talbot et al. reported a rate of immune defects of 86% in children diagnosed with disseminated disease.[9] In the literature, there have been reports of complications in HIV-infected patients and in patients with primary immunodeficiency.[4,7] Hesseling et al. described a series of 25 patients diagnosed with severe *M. bovis* BCG infection associated with primary immunodeficiency.[10] Santos et al. described the cases of three patients with vaccine-induced infection, one of whom had IFN-γ/IL-12 pathway deficiency.[3] An IFN-γ/IL-12 pathway defect is an immunodeficiency disorder in which there is increased susceptibility to infections with microorganisms of the genera *Mycobacterium* and *Salmonella*.[11,12] It is classified as a congenital defect of phagocyte number, function, or both. Host defenses against these bacteria strongly depend on the functional integrity of mononuclear phagocytes and their interaction with T lymphocytes. T lymphocytes and natural killer cells of affected patients express a defective IL-12 receptor on their cell surfaces, leading to low production of IFN-γ, which is the major factor responsible for mycobacterial death.[13] The diagnosis of IFN-γ/IL-12 pathway defects requires a tiered approach and laboratory support.[14] In patients with severe disseminated mycobacterial infection, other immunodeficiency disorders, such as severe combined immunodeficiency, should be excluded first. In some cases, the treatment of IFN-γ/IL-12 pathway defects requires aggressive use of drugs against the mycobacterium and subcutaneous IFN-γ replacement therapy as a treatment option.[11] In the present case report, ancillary tests confirmed this deficiency, which favored the development of lesions due to *M. bovis* BCG.

A system for classification of *M. bovis* BCG disease in immunocompromised patients was developed by Talbot et al. and subsequently revised by Hesseling et al.[9,10] Infection is classified on the basis of its presentation as local, regional, distant, or disseminated disease. Regional disease is defined as that in which there is a lesion at the vaccination site and ipsilateral regional lymph node involvement, with lymphadenopathy and fistula formation and/or suppuration, as in the case described here.[9,10]

We report the course of *M. bovis* BCG infection in an infant with primary immunodeficiency who presented with a regional lesion located ipsilateral to the vaccination site. The lesion was unresponsive to the recommended treatment with isoniazid, and the strain identified was found to be resistant to two drugs (rifampin and isoniazid). Although susceptibility testing showed resistance to isoniazid, we chose to continue treatment with this drug, since testing using a critical concentration does not quantify the level of resistance (low, moderate, or high), which is determined using a minimum inhibitory concentration (MIC).[15] The level of *in vitro* resistance to isoniazid was not quantified, since an MIC was not performed, as well as not reflecting the *in vivo* reality, and the mutation found (the *kat*G S315T mutation) may be related to moderate resistance to isoniazid. Since ethambutol was withdrawn from the regimen because of suspected optic neuritis, there may have been strain selection, with acquisition of resistance to rifampin. Rifampin was discontinued, given that the mutation found in the *rpo*B region (the D516V mutation) is in most cases related to a high level of resistance.[15] There have been studies of mutations and MIC associated with clinical response for *M. tuberculosis*, but there have been no reports regarding *M. bovis* BCG.

Ethambutol-induced optic neuritis was ruled out, since the alteration in the optic disk remained stable throughout the follow-up period. The favorable outcome was possible, despite resistance to isoniazid, because of the combination of streptomycin and ethambutol, as well as because of the inclusion of the immunomodulator, resulting in resolution of the lesions.

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Solitary benign metastasizing leiomyoma: imaging features and pathological findings

Leiomioma metastático benigno solitário: aspectos de imagem e achados anatomopatológicos

Bernardo Corrêa de Almeida Teixeira, Kássia Mahfouz, Dante Luiz Escuissato, Ana Flávia Cardoso Buarque Costa, Lúcia de Noronha

To the Editor:

A 51-year-old woman presented with dyspnea on exertion, dry cough, dyslipidemia, type 2 diabetes, and liver steatosis. Twenty years prior, she had undergone hysterectomy and unilateral oophorectomy because of uterine leiomyomas.

A chest X-ray showed a solitary pulmonary nodule in the right upper lobe. The nodule was oval in shape, being approximately 24 mm × 30 mm. A CT scan of the chest confirmed that the lesion was a solitary, well circumscribed-nodule that had regular borders and was located in the posterior segment of the right upper lobe. As can be seen in Figures 1A and 1B, there was delayed contrast enhancement (50 HU before contrast injection, 55 HU within 25 s after contrast injection, and 100 HU within 5 min after contrast injection). Contrast-enhanced magnetic resonance imaging was performed, and the nodule showed slightly high signal intensity on T1-weighted images and homogeneous enhancement (Figures 1C and 1D). The lesion showed signal intensity similar to that of muscle on T2-weighted images. In-phase and out-of-phase imaging, fat-saturated imaging, and diffusion-weighted imaging provided no additional findings. A chest X-ray performed three years earlier had shown no lesions.

Because the imaging findings were inconclusive and because of the risk of malignancy, the patient underwent video-assisted thoracoscopic surgery for nodule resection. Pathological examination revealed a nodular proliferation composed of smooth muscle cells without atypia and areas of hyalinization, a finding that was consistent with leiomyoma (Figure 2A). Immunohistochemical analysis of the lesion showed that estrogen receptors and progesterone receptors were positive, and a diagnosis of benign metastasizing leiomyoma (BML) was made despite the atypical presentation, i.e., a solitary pulmonary nodule (Figures 2B and 2C).

BML is a rare neoplastic process in which leiomyomas of the uterus metastasize to distant sites, the most common of which are the lungs.[1,2] BML is usually asymptomatic, and the diagnosis is based on incidental imaging findings of multiple pulmonary nodules or, more rarely, a single nodule. The term metastasizing fibroleiomyoma of the uterus was introduced by Steiner in 1939 to described multiple nodules of proliferating smooth muscle cells in the lung of women with a history of hysterectomy.[2] Different mechanisms of spread of uterine leiomyomas have been proposed. It has been suggested that smooth muscle cells spread to the lungs after uterine extension into pelvic venous channels; that tumors gain venous access from surgical trauma during hysterectomy; and that the lesions represent metastatic foci arising from low-grade leiomyosarcomas.[3,4]

In cases of BML, pulmonary nodules can be seen 3-240 months after hysterectomy or even before the procedure. They can vary in size from millimeters to centimeters and be randomly distributed in the lung parenchyma.[3,4] A solitary nodule, as seen in our patient, is a very rare presentation of BML. In general, pulmonary nodules do not calcify and can remain unchanged or even regress spontaneously. Both CT and magnetic resonance imaging can be used in order to characterize pulmonary nodules in patients with BML; such nodules have a nonspecific appearance and usually show homogeneous contrast enhancement.[3,5] The efficacy of 18F-fluorodeoxyglucose positron emission tomography with CT (FDG-PET/CT) in detecting uterine leiomyomas is controversial. In the few reports available in the literature, FDG-PET/CT was unable to detect BML.[6] In the case reported here, FDG-PET/CT was not performed.

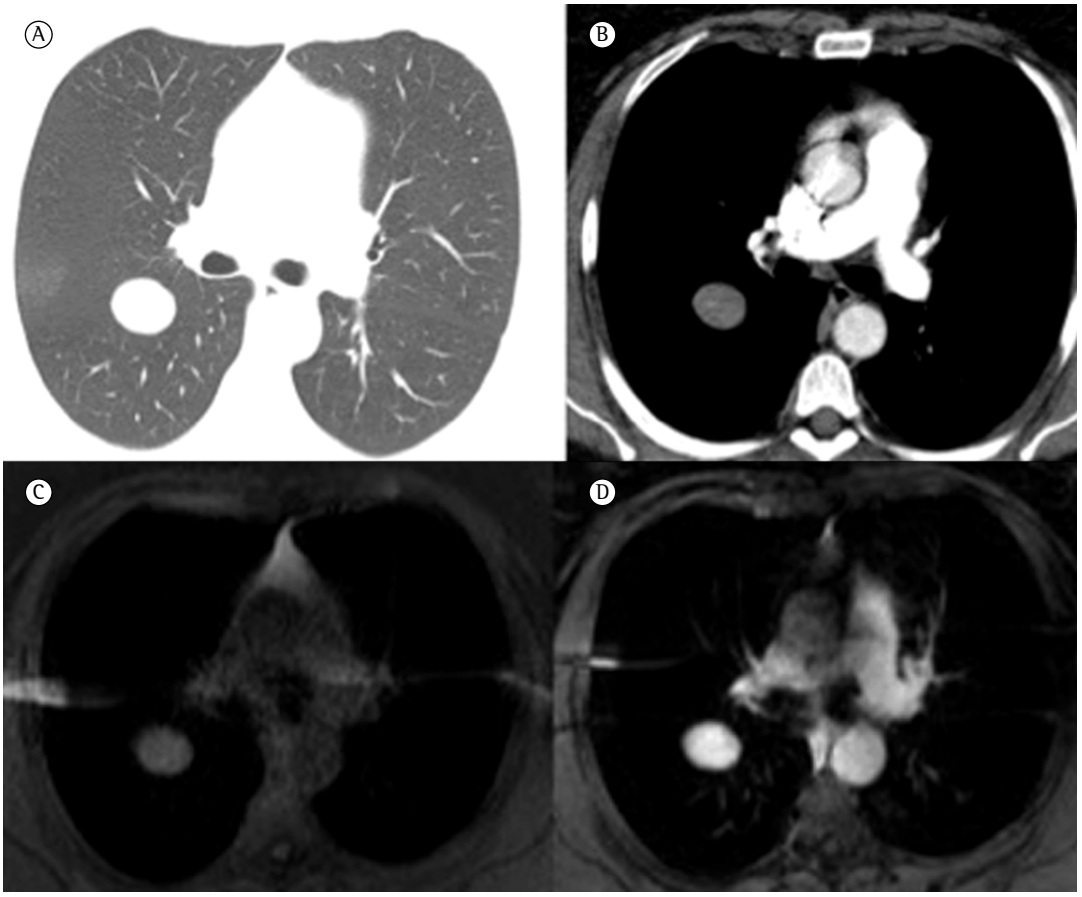

Figure 1 - CT scans and magnetic resonance imaging of the chest. In A and B, chest CT scans (lung window, in A, and mediastinal window, in B) showing an oval nodule with homogeneous density, well-defined margins, and contrast enhancement. In C and D, fat-suppressed T1-weighted images (before injection of a paramagnetic contrast agent, in C, and after injection of a paramagnetic contrast agent, in D), on which the nodule is slightly hyperintense and homogeneously enhanced.

Macroscopically, pulmonary nodules are ovoid, well circumscribed, and homogeneously white. Microscopic examination reveals proliferation of well-differentiated, benign-appearing spindle cells with eosinophilic cytoplasm, moderate degree of vascularization, insignificant nuclear atypia, mitotic activity, anaplasia, necrosis, vascular invasion, or inflammatory host tissue response. The presence of estrogen receptors and progesterone receptors in cases of BML has been well documented and constitutes evidence that BML originates from uterine smooth muscle. Extrauterine leiomyomas are uniformly estrogen receptor negative. In contrast, most BMLs are estrogen receptor positive.[4] The disease course varies and seems to depend on the estrogen status of the patient. In postmenopausal women, the disease is indolent, patient mortality being commonly due to an unrelated disease process, whereas, in premenopausal women, the progression of the disease can result in death.[1,3]

Because BML is a rare disease, with few reported cases, there is no established treatment protocol. Given that BML is a hormonally responsive tumor, the prognosis is favorable.[1,3] Treatment includes hysterectomy, bilateral oophorectomy, and long-term hormone therapy. Expectant management and pulmonary nodule resection are also therapeutic options. Menopause has been associated with lesion regression.

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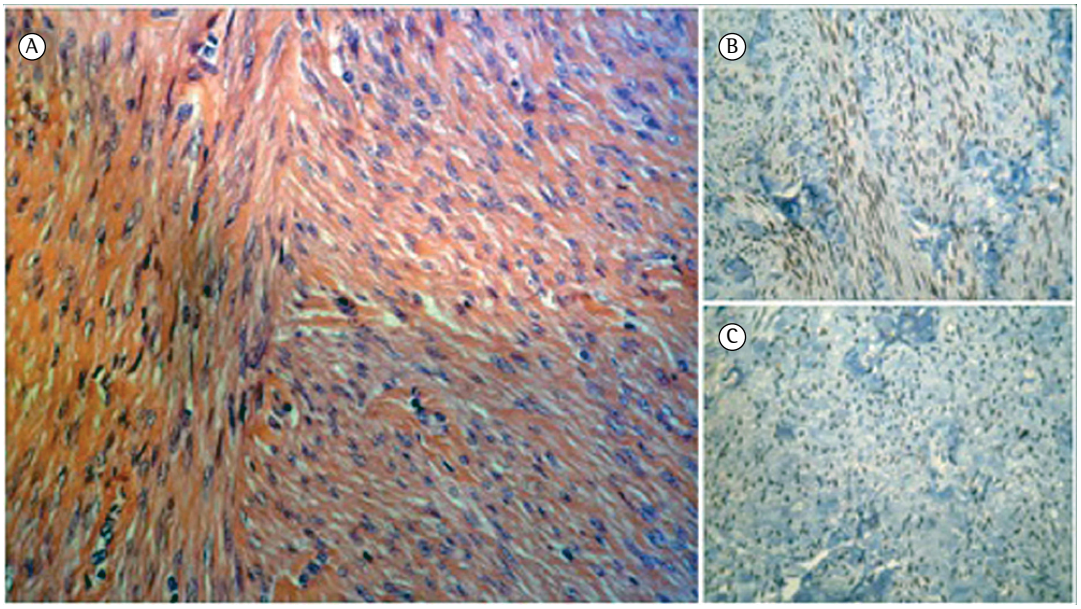

Figure 2 - Photomicrographs of the pulmonary nodule. In A, note that the nodule consisted of smooth muscle tissue arranged in multidirectional bundles, without atypia or mitosis (H&E; magnification, ×400). In B, note progesterone receptor positivity (immunohistochemistry; magnification, ×100). In C, note estrogen receptor positivity (immunohistochemistry; magnification, ×100).

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Submitted: 20 August 2013. Accepted, after review: 27 August 2013.

Low-dose CT screening for lung cancer in Brazil: a study protocol

Rastreamento de câncer de pulmão por meio de TC de baixa dosagem no Brasil: protocolo de pesquisa

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To the Editor:

Because of the lack of studies aimed at screening for lung cancer (LC) in the Brazilian population, a project that is integrated into the Program for the Support of the Institutional Development of the Brazilian National Ministry of Health Unified Health Care System and whose objective is to evaluate the efficacy of low-dose CT (LDCT) scans of the chest in screening for LC was launched. The objective of the present letter was to describe the design and methods of the *Projeto de Detecção Precoce do Câncer de Pulmão* (ProPulmão, Project for Early Detection of Lung Cancer), which was approved by the Research Ethics Committee of the *Instituto Israelita de Ensino e Pesquisa do Hospital Albert Einstein* (Protocol no. CAAE 02087012.1.0000.0071).

For the development of the project, the final sample will comprise 1,000 individuals recruited as of 2013 via public calls in vehicles of communication in the greater metropolitan area of São Paulo, as well as via partnerships with other community care services. The sample size was calculated on the basis of previous international studies addressing this issue.[1]

The inclusion criteria are as follows[2]: having no respiratory symptoms; being in the 55-74 year age bracket; being a smoker with a smoking history of at least 30 pack-years or having been a former smoker for 15 years at most; and agreeing to participate in the study by giving written informed consent. The exclusion criteria are as follows: being unable to undergo CT scans; being pregnant; having previously undergone radiation therapy to the chest; and having severe chronic disease, such as cardiovascular disease, lung disease, liver disease, kidney disease, and metabolic disease.

The primary outcome measure is early diagnosis of LC. Nevertheless, participants will undergo a multidisciplinary evaluation for smoking-related diseases and infectious diseases that are common in Brazil, such as tuberculosis.

At the initial visit, demographic and smoking history data will be collected; health-related quality of life will be assessed by the Medical Outcomes Study 36-item Short-form Health Survey[3]; the presence of anxiety or depression will be determined by the hospital anxiety and depression scale[4]; and the presence of nicotine dependence in current smokers will be determined by the Fagerström test.[5]

After the initial evaluation, individuals will be referred for LDCT screening, the scans being analyzed by two radiologists with experience in thoracic diseases. Indeterminate pulmonary nodules $\geq$ 4 mm in size will be evaluated by a medical team comprising radiologists, pulmonologists, and thoracic surgeons, who will decide on the follow-up strategy (Chart 1).

In cases of solid nodules > 8 mm in size, radiological features alone are not enough to distinguish between benign and malignant nodules. Therefore, it is important to estimate the clinical probability of malignancy. This estimation is known as pre-test probability and aids in reducing interobserver variability regarding the probability of malignancy. A multivariate logistic regression model developed at Mayo Clinic[6] on the basis of six independent predictors of malignancy—including patient age (in years), being a smoker or former smoker, having a history of extrathoracic cancer diagnosed more than 5 years prior, nodule diameter (in mm), presence of spicules, and upper lobe involvement—will be used in the study.

Chart 1 - Follow-up strategies to monitor high-risk patients for solid nodules, ground-glass opacity, and nonsolid nodules, based on the National Comprehensive Cancer Network Guidelines for Lung Cancer Screening and on the Fleischner Society guidelines.[a]

Size	Solid nodules in high-risk patients
≤ 4 mm	Follow-up LDCT scans should be taken after one year. If there are no changes, patients should undergo annual follow-up examinations.
> 4 mm and ≤ 6 mm	Follow-up LDCT scans should be taken after 6 months and after one year. If there are no changes, patients should undergo LDCT after 18 months and after 24 months.
> 6 mm and ≤ 8 mm	Follow-up LDCT scans should be taken after 3 months and after 6 months. If there are no changes, patients should undergo LDCT after 9 months, after 12 months, and after 24 months. If the nodule increases in size, biopsy or surgical resection is recommended.
> 8 mm	Calculate pre-test probability. Probability of malignancy: • Low (< 5%): serial LDCT scans • Intermediate (5-60%): PET-CT (if negative, serial LDCT scans; if positive, biopsy or surgical resection) • High (> 60%): biopsy or surgical resection
	Ground-glass opacity and nonsolid nodules
Pure ground-glass opacity ≤ 5 mm	Follow-up LDCT scans should be taken after one year. If there are no changes, patients should undergo annual follow-up examinations. If the nodule increases in size or becomes solid, patients should undergo LDCT after 3 months and after 6 months. Alternatively, the possibility of performing a biopsy or surgical resection should be considered.
Pure ground-glass opacity > 5 mm	Follow-up LDCT scans should be taken after 3 months. If there are no changes, patients should undergo annual follow-up examinations. If the nodule increases in size or if there are changes in the characteristics of the nodule, the possibility of performing a biopsy or surgical resection should be considered.
Part-solid nodule	Follow-up LDCT scans should be taken after 3 months. If there are no changes and the part-solid nodule is > 8 mm, the possibility of performing a PET-CT scan should be considered, possible follow-up strategies including LDCT scans, biopsy, and surgical resection. If the nodule increases in size or if there are changes in the characteristics of the nodule, the possibility of performing a biopsy or surgical resection should be considered.

LDCT: low-dose CT; and PET-CT: positron emission tomography-CT. [a]Adapted from the National Comprehensive Cancer Network,[7] MacMahon et al.,[8] and Patel et al.[9]

After undergoing LDCT, all patients will return for a follow-up evaluation, in which the LDCT findings will be recorded and the follow-up strategy will be proposed. At that visit, current smokers will be referred to a smoking cessation program. Although participation in the program is encouraged, enrollment is voluntary.

Abnormal CT findings will be recorded on a specific form, analyzed by the expert panel, and classified on the basis of the level of suspicion of malignancy, follow-up strategies being subsequently decided on (Figure 1).

In cases of lung cancer, the nodules seen on the follow-up LDCT scans will be compared with those seen on the initial LDCT scans; the parameters for all CT scans will be the same, therefore allowing the examination of possible changes.

Subsequent visits, occurring in the second year of follow-up, will be conducted in accordance with the flowchart shown in Figure 1, specific findings in each individual in the previous year being taken into consideration (Chart 1).

The attending physician at the outpatient clinic will give the participants the results of the LDCT examinations. In addition, the medical team will inform the participants of the suspicion or diagnosis of LC.

After diagnostic confirmation and surgical treatment (when appropriate), patients will be referred for oncological follow-up via the Brazilian

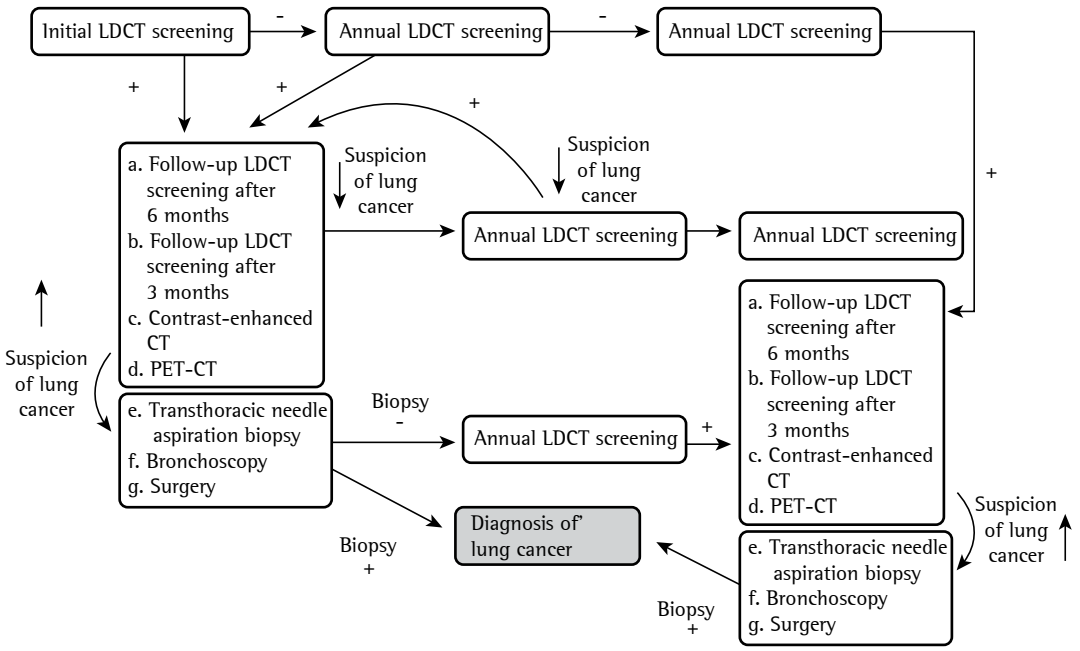

Figure 1 - Flowchart of possible follow-up strategies. LDCT: low-dose CT; and PET-CT: positron emission tomography-CT.

Unified Health Care System or the private health care system and will receive adjuvant therapy as medically indicated.

To date, there have been no studies of LDCT screening for LC in developing countries, in which the incidence of infectious diseases of the chest is higher. This raises many questions regarding the sensitivity and specificity of the method for LC screening.

The use of LDCT screening in Brazil is of fundamental importance because it will provide specific information for the validation of the method as a population screening tool for LC.

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Submitted: 9 August 2013. Accepted, after review: 19 August 2013.

Chest wall reconstruction with titanium plates after desmoid tumor resection

Reconstrução de parede torácica com placas de titânio após ressecção de tumor desmoide

Fernando Luiz Westphal, Luís Carlos de Lima, José Corrêa Lima Netto, Stephany da Cunha Seelig, Katienne Frota de Lima

To the Editor:

Chest wall reconstruction becomes necessary when there are wall defects larger than 5 cm in diameter that compromise respiratory dynamics. Its purpose is to restore wall integrity, as well as to maintain waterproofing of the pleura, an aesthetic chest contour, and respiratory dynamics. In addition, the purpose is to protect vital intrathoracic organs, thus preventing lung herniation and paradoxical breathing and preserving lung compliance.[1,2]

The indication for bony reconstruction of the chest wall is related to the size and location of the defect. Defects in the anterior, lateral, and sternal wall require reconstruction, whereas defects in the posterior wall can be covered by the posterior muscles or by the scapula and do not require the use of prostheses.[1]

There is as yet no consensus on the ideal material for use in rib reconstruction. The literature suggests the use of prostheses consisting of titanium plates (STRATOS™, Strasbourg Thoracic Osteosynthesis System; Diagnostic Medical Systems, Pérols, France) for that purpose, and, therefore, we report the case of a 25-year-old female patient who presented with a nearly one-year history of chest pain and dyspnea, as well as with a volume increase in the left costal margin.

Physical examination revealed a tumor in the lower third of the anterior chest wall, affecting the left thoracoabdominal junction. An axial CT scan of the chest showed a soft-tissue tumor that affected the region of the left anterior costal margin, extended to the abdominal region, and compressed the left hepatic lobe, the anterior pericardium, and the lung parenchyma in the left lower lobe. However, there were no signs of structural invasion. The tumor measured 12.0 × 11.0 × 7.5 cm.

The patient underwent chest wall resection, which included soft tissues and the anterior portion of the sixth, seventh, and eighth ribs, as well as the costal margin (Figure 1A). Histopathological examination of the tumor showed that it was a desmoid tumor—a rare, benign, unencapsulated neoplasm with strong infiltrative capability locally and a high rate of recurrence after surgical resection.[3] Chest wall reconstruction was performed with PHYSIOMESH™ (ETHICON®; Johnson & Johnson, Somerville, NJ, USA) and three titanium plates (STRATOS™; Figure 1B).

The ideal material for reconstruction should have the following characteristics: being adaptable; being durable; being transparent to X-ray; causing minimal inflammatory reaction; and being resistant to infection. Typically, the materials used are nylon, silicone, acrylic, Silastic® (Dow Corning Corp., Midland, MI, USA), Prolene® mesh, Vicryl® mesh (polygalactin; ETHICON®), Gore-Tex® (polytetrafluoroethylene; Gore Company, Flagstaff, AZ, USA), and Marlex mesh (polypropylene).[1,2]

Currently, Marlex mesh is the most widely used material, because it is easy to handle, permeable, highly resistant, durable, and inexpensive. In addition, it is hardly susceptible to infection. However, in contact with the lung, it causes adhesions and an intense fibrotic reaction hindering possible thoracic reoperations, as well as not providing proper support for the chest wall.[2]

The mesh used in our patient (PHYSIOMESH™) is composed of two layers: a Monocryl® film (polyglecaprone 25), which is partially absorbable and reduces adhesion to the visceral organs (in the present case, the lung, the diaphragm, and the pericardium), thus facilitating performing another surgical intervention if necessary; and a Prolene® film (polypropylene), which is consistent with the required resistance for the chest wall, thus providing comfortable healing. This mesh is placed between the lung and the titanium

prostheses, thus preventing lung herniation and protecting the lung from contact with the plates.

STRATOS™ consists of titanium bars and clips that form a vertical expandable prosthetic system. It has recently been used for fixation of rib fractures and for chest reconstruction after tumor resection.[4]

The titanium plates, once integrated into the chest wall, will form an oxide layer that is highly resistant to corrosion. They have the highest strength-to-weight ratio among all metals, i.e., titanium plates have low weight but have stiffness similar to that of the ribs. The titanium plates have the facility of integrating with the bones, which prevents detachment from the ribs over time, and are highly resistant to infections. They do not interfere with imaging or preclude magnetic resonance imaging.[5]

Previously published reports of patients who were operated on and received STRATOS™ have shown that its material does not affect the chances of local tumor recurrence. One group of authors used STRATOS™ in a male patient with an Ewing's sarcoma of proportions similar to those of the tumor in our patient, and, after a 21-month follow-up period, the patient had no tumor recurrence.[6]

Although there are no studies that define STRATOS™ as the ideal system for use in chest wall reconstruction, it is technically simple and well tolerated. In addition, other case reports and articles comparing this system with older techniques have reported better restoration of the contour of the ribs (Figure 2) and preservation of respiratory mechanics, as well as greater comfort. Maintaining chest wall symmetry prevents localized chest deformity, as well as the scoliosis seen over time in patients with a partially collapsed chest.[4,6]

We emphasize the importance of the present report, given that, to our knowledge, it is the first such report in the Brazilian literature. The existing option of a substitute for ribs increases the chances of major chest wall resections, which is an important factor in treating tumors with oncologic margins.

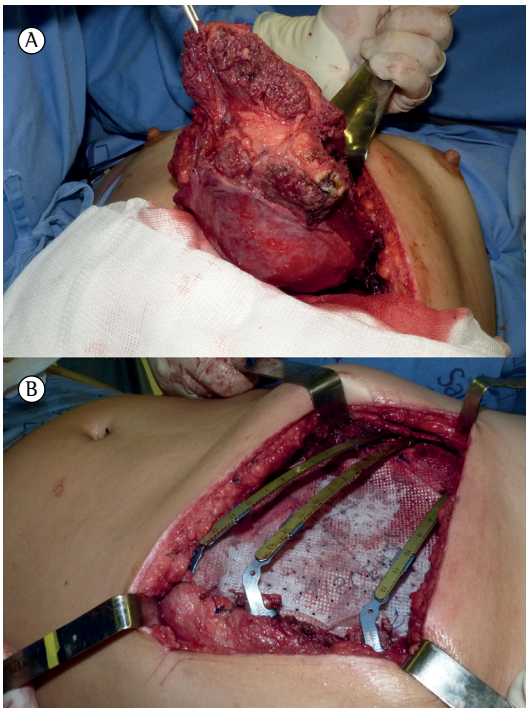

Figure 1 - Images of the desmoid tumor resection and chest wall reconstruction. In A, exposure of the tumor of the left costal margin during the surgical procedure, including ribs, muscles, the distal portion of the sternum, and the diaphragm. In B, chest wall reconstruction with a two-layered mesh and titanium bars.

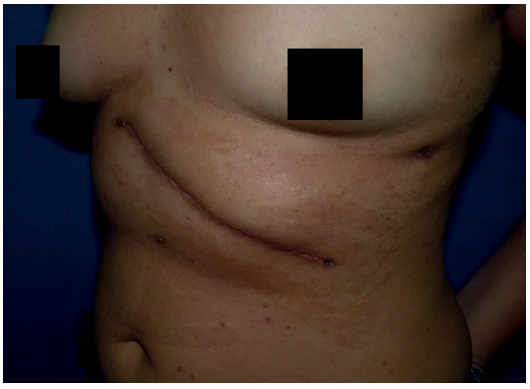

Figure 2 - Patient appearance in the sixth postoperative month. Note that the contour of the costal margin was maintained, being stable and symmetrical.

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Submitted: 10 October 2013. Accepted, after review: 15 October 2013.

Extracorporeal membrane oxygenation for postpneumonectomy ARDS

Oxigenação extracorpórea por membrana no tratamento da SARA pós-pneumonectomia

Maurício Guidi Saueressig, Patrícia Schwarz, Rosane Schlatter, Alexandre Heitor Moreschi, Orlando Carlos Belmonte Wender, Amarilio Vieira de Macedo-Neto

To the Editor:

Although ARDS is an uncommon complication of pneumonectomy, the associated mortality is high (ranging from 50% to 100%).[1] Here, we report the case of a patient with postpneumonectomy ARDS that was satisfactorily managed by extracorporeal membrane oxygenation (ECMO). A 31-year-old White female patient diagnosed with cystic fibrosis 10 years prior presented with recurrent pneumonia secondary to bronchiectasis, predominantly in the left lung (Figure 1A). In the last two years,

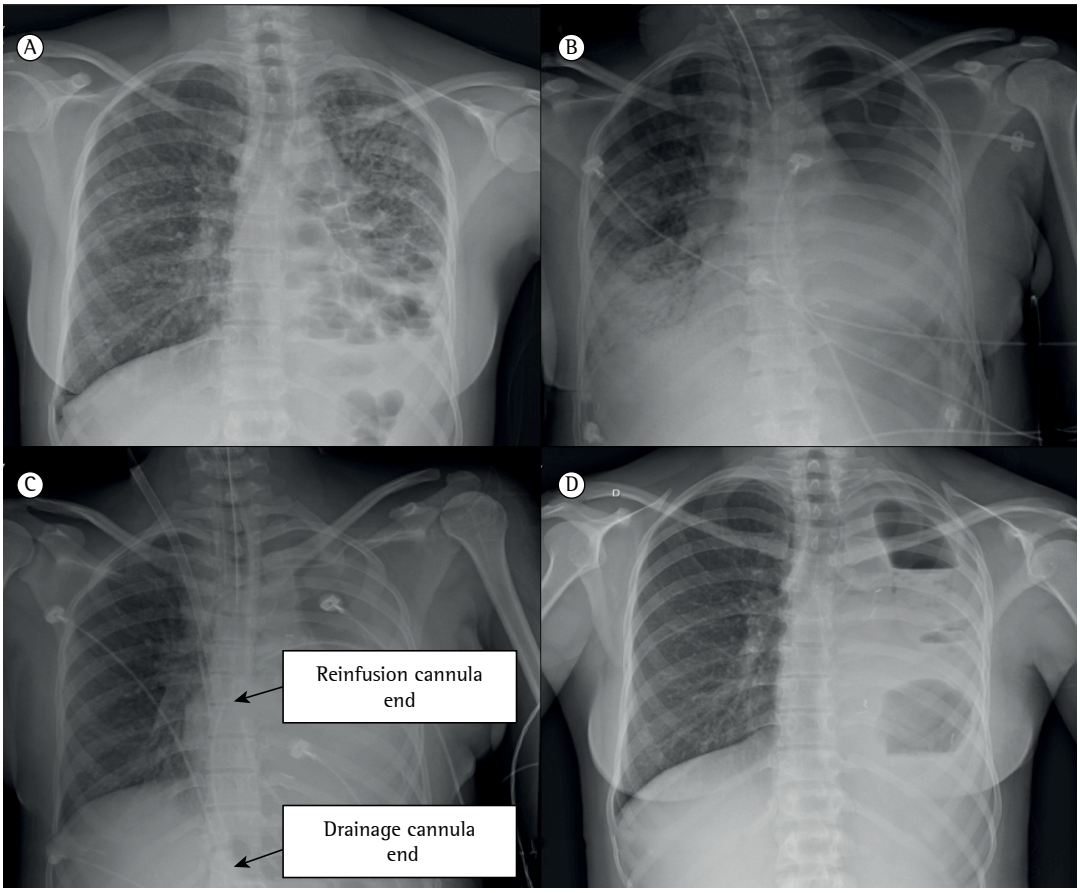

Figure 1 - Chest X-rays showing the progression of the patient. In A, chest X-ray taken before pneumonectomy, showing extensive bronchiectasis, reduced lung volume, and left pleural thickening. In B, chest X-ray taken on postoperative day 3, showing extensive areas of consolidation on the right and the postpneumonectomy pleural space on the left. In C, chest X-ray taken on the day of weaning from extracorporeal membrane oxygenation (i.e., on postoperative day 8), showing resolution of the right-sided consolidation. Note the venous cannulae and their ends in the right atrium (for reinfusion) and in the intrahepatic portion of the inferior vena cava (for drainage). In D, chest X-ray taken three months after hospital discharge, showing nearly complete closure of the pneumonectomy cavity.

Table 1 - Clinical status before initiation of and on the day of weaning from extracorporeal membrane oxygenation.

Arterial blood gas results	Before ECMO	On the day of weaning from ECMO
pH	7	7.5
PaO_2, mmHg	108	136
$PaCO_2$, mmHg	115	57
PaO_2/FiO_2	107	388
Mechanical ventilation		
PEEP, cmH_2O	8	6
FiO_2	1.00	0.35
RR, breaths/min	28	15
Tidal volume/ideal weight, mL/kg	4.4	5.8
Plateau pressure, cmH_2O	35	22
Peak inspiratory pressure, cmH_2O	45	26
Static lung compliance, mL/cmH_2O	9	20
Hemodynamics		
Norepinephrine, $\mu g \cdot kg^{-1} \cdot min^{-1}$	0.16	0.00
Mean arterial pressure, mmHg	66	90
HR, bpm	90	80
Serum test results		
Lactate, mmol/L	0.4	0.9
Base excess, mmol/L	−3.5	17.0
C-reactive protein, mg/L	231	17
Hemoglobin, g/dL	10	9
Sedation/neuromuscular blockade/scores		
Midazolam, $mg \cdot kg^{-1} \cdot h^{-1}$	0.2	0
Fentanyl, $\mu g \cdot kg^{-1} \cdot h^{-1}$	4	2
Atracurium, $mg \cdot kg^{-1} \cdot h^{-1}$	0.6	0.0
SOFA score	14	7
Murray score	3.0	1.2

ECMO: extracorporeal membrane oxygenation; PEEP: positive end-expiratory pressure; and SOFA: Sequential Organ Failure Assessment.

despite continuous use of antibiotics (500 mg of azithromycin p.o. three times a week), the patient had had seven respiratory infections, as well as purulent sputum between episodes. Therefore, a decision was made to perform a left pneumonectomy. The postoperative course was satisfactory. However, on postoperative day 2, the patient showed dyspnea, cough with purulent sputum, tachypnea, left-sided chest pain, inspiratory rales in the lower lung fields, and hypoxemia (SaO_2 < 70%). Initial management with noninvasive ventilation was ineffective, the patient being therefore placed on mechanical ventilation on postoperative day 3 (Figure 1B). After 17 h of mechanical ventilation, she still had ARDS, hypoxemia, and a pH < 7.2, despite alveolar recruitment maneuvers, attempts to reduce tidal volume, oxygen insufflation into the trachea, and neuromuscular blockade (Table 1). Therefore, a decision was made to place her on venovenous ECMO (a Revolution™ centrifugal pump and an EOS ECMO adult membrane oxygenator; Sorin, Milan, Italy). Percutaneous cannulation of the femoral and jugular veins was performed by the Seldinger technique, a 19-F arterial cannula being inserted into the right internal jugular vein for reinfusion and a 29-F venous cannula being inserted into the right femoral vein for drainage (Maquet, Rastatt, Germany). Venipuncture and cannulation of the jugular and femoral veins were performed under ultrasound guidance at the bedside. Continuous i.v. infusion of unfractionated heparin was used in order to achieve an activated clotting time of 160-200 s. Initially, ECMO blood flow was 60 $mL \cdot kg^{-1} \cdot min^{-1}$, being subsequently adjusted to maintain a PaO_2 > 50 mmHg, whereas gas flow (sweep gas) was titrated to maintain a pH ≥ 7.3. The temperature of the patient remained at 35.5-36.5°C. Lung rest was achieved by pressure-controlled ventilation at protective ventilator settings (i.e., a plateau pressure ≤ 25 cmH_2O, a positive

end-expiratory pressure of 5-15 cmH_2O, and an FiO_2 ≤ 0.4). The patient showed progressive radiological improvement (Figure 1C), as well as progressive improvement in arterial blood gas parameters and lung compliance, meeting the criteria for weaning on ECMO day 5 (Table 1). Three hours later, she was successfully extubated. The patient was discharged on postadmission day 21. There were no hemorrhagic or thromboembolic complications of ECMO. The total cost of ECMO, in Brazilian reals (R$), was 33,470.16, R$ 26,315.00 having been spent on the ECMO circuit plus medical supplies (including cannulae), R$ 5,594.93 having been spent on the ICU stay, and R$ 1,560.23 having been spent on diagnostic tests. However, the amount paid by the *Sistema Único de Saúde* (SUS, Brazilian Unified Health Care System) via the Authorized Hospital Admissions system was R$ 5,917.88.

The incidence of ARDS after left pneumonectomy is approximately 4%.[2] Possible triggers include reduced lymphatic drainage and single-lung ventilation with hyperoxia.[3] Supportive care consists of mechanical ventilation; however, in cases of refractory hypoxemia, rescue therapies include prone positioning[4] and ECMO.[5]

An invasive method, ECMO corrects severe hypoxemia and hypercapnia (pH ≤ 7.2) and reduces FiO_2 (< 0.5) and plateau pressure to safer levels, allowing the lung to rest in cases of ARDS.[6,7] Despite a PaO_2/FiO_2 ratio > 100 mmHg, early ECMO was recommended because of the presence of an FiO_2 > 0.8, a plateau pressure > 30 cmH_2O, a pH < 7.2, and a $PaCO_2$ > 100 mmHg in our patient. In addition, her Sequential Organ Failure Assessment score was 14, indicating the absence of multiorgan involvement and showing that the ECMO team at the Porto Alegre *Hospital de Clínicas*, located in the city of Porto Alegre, Brazil, abides by the policy that ARDS patients who are not at risk of imminent death should be recognized as candidates for ECMO. This approach has been advocated by other ECMO teams in Brazil.[8,9]

A novel technique, ECMO is currently not covered by the SUS; the reimbursement that our hospital received from the SUS covered less than 20% of the actual costs. Therefore, there is a need for an economic evaluation of ECMO in Brazil in order to inform the decision of whether ECMO should be included in the range of procedures covered by the SUS.

On the basis of the case reported here, we recommend early rescue therapy with venovenous ECMO for patients with postpneumonectomy ARDS accompanied by hypoxemia and respiratory acidosis refractory to mechanical ventilation, provided that the medical team has sufficient experience with the procedure, which is complex and costly.

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Submitted: 25 September 2013. Accepted, after review: 15 October 2013.

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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/

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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. 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