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HIGHLIGHT

Brazilian recommendations of mechanical ventilation

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Sumário/Contents

New steps for the international consolidation of the Brazilian Journal of Pulmonology

Novos passos para a consolidação internacional do Jornal Brasileiro de Pneumologia

Carlos Roberto Ribeiro Carvalho, Bruno Guedes Baldi, Carlos Viana Poyares Jardim, Pedro Caruso, Rogério Souza

In 2002, the Brazilian Journal of Pulmonology (BJP) was accepted for indexing in the SciELO database. This achievement, which is the result of the determination and efforts of the then current and previous Editorial Boards, was the basis for the construction of the Journal's image and for the Journal's international exposure. Subsequently, in 2006, the BJP was accepted for indexing in PubMed, which significantly increased the Journal's visibility. In 2012, after its inclusion in the Institute for Scientific Information (ISI) Web of Knowledge database, the BJP received its first impact factor, which placed the Journal in a very prominent position among the Brazilian scientific journals. This was extremely important, especially for respiratory researchers, because it meant the existence of a vehicle, with an international circulation, for communicating research results.

However, in 2013, because of questionable criteria, Thomson Reuters, which is the international company responsible for determining journal impact factors, did not publish the impact factor of the BJP.[1,2] Although the Journal remained in the database, and therefore its publications and the citations of these publications were computed, the company chose not to publish the Journal's impact factor for that year. Considering that journal impact factors are used for journal classification by national funding agencies and for the evaluation of graduate programs, the consequences of such a penalty were huge, making it difficult to assess its extent. Unfortunately, these effects will still be felt in the coming years, unequivocally affecting subsequent impact factors.

Recently, on July 29, the annual Journal Citation Reports was published. The impact factor of the BJP for the 2013 publications was 1.268, which means that the BJP ranks sixth among the 107 Brazilian journals included in the ISI database. The return of the BJP to the annual list of the database that calculates impact factors shows, more clearly than do the ranking achieved and the impact factor itself, that the path followed by the Journal over the last few years has always been based on the dissemination of quality respiratory science. As previously mentioned, the impact factor of the BJP will still reflect the effects of that penalty over at least the next two years, because of the mechanism through which impact factors are calculated. Nevertheless, the return of the BJP to that annual list consolidates its position among the journals that are of greatest importance in disseminating knowledge in respiratory medicine worldwide.

In addition, in late July, SCImago cites per document index (which uses the Scopus database and is calculated in a similar way to the ISI impact factor) was published, and the BJP was assigned 1.45, reinforcing the Journal's position as one of the most important science journals in Brazil. This strengthens our responsibility with regard to the sustained growth of the BJP in the coming years.

To ensure this growth, structural changes have been made. We are in the final stages of changing the article submission system. As from September, we will use ScholarOne. This is the new platform that will be available to our authors, reviewers, and editors. The ScholarOne system is more modern and efficient, as well as being the model used by various journals of high international impact. The main goal of this migration is to facilitate and expedite the process of submission and review of articles, which is essential for extending the Journal's presence in the international arena, as well as being an added incentive for the participation of international authors and reviewers.

Another important achievement, which was completed on July 2014, is that the BJP content is now available through PubMed Central® (PMC), which is the U.S. National Institutes of Health/National Library of Medicine (NIH/NLM) digital archive of biomedical and life sciences journal

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literature. This tool allows free access to full-text articles from the BJP, which surely increases the visibility of articles published by researchers from other countries, contributing to their dissemination. This is only possible because all articles published in the Journal are available in English.

The major responsibilities at this point, especially for the future Editorial Board of the BJP, are to consolidate our presence in international databases, such as SciELO, PubMed, Thomson Reuters ISI Web of Knowledge, Journal Citation Reports, and SCImago, and to continuously improve the quality of the published articles. To achieve these goals, the continuous participation of all researchers, reviewers, and editors who have contributed to the growth of the BJP throughout its history is essential. We hope we can count on the collaboration of more researchers so that the BJP can not only continue to be an important journal for the dissemination of respiratory research in Brazil, but also consolidate and increase its international exposure.

Carlos Roberto Ribeiro Carvalho
Editor-in-Chief of the Brazilian Journal of Pulmonology

Bruno Guedes Baldi
Carlos Viana Poyares Jardim
Pedro Caruso
Rogério Souza
Executive Editors of the Brazilian Journal of Pulmonology

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Brazilian recommendations of mechanical ventilation 2013. Part I

Recomendações brasileiras de ventilação mecânica 2013. Parte I

The present recommendations are a joint initiative of the Mechanical Ventilation Committee of the Brazilian Intensive Care Medicine Association (*Associação de Medicina Intensiva Brasileira* - AMIB) and the Commission of Intensive Therapy of the Brazilian Thoracic Society (*Sociedade Brasileira de Pneumologia e Tisiologia* - SBPT).

Abstract

Perspectives on invasive and noninvasive ventilatory support for critically ill patients are evolving, as much evidence indicates that ventilation may have positive effects on patient survival and the quality of the care provided in intensive care units in Brazil. For those reasons, the Brazilian Association of Intensive Care Medicine (*Associação de Medicina Intensiva Brasileira* - AMIB) and the Brazilian Thoracic Society (*Sociedade Brasileira de Pneumologia e Tisiologia* - SBPT), represented by the Mechanical Ventilation Committee and the Commission of Intensive Therapy, respectively, decided to review the literature and draft recommendations for mechanical ventilation with the goal of creating a document for bedside guidance as to the best practices on mechanical ventilation available to their members. The document was based on the available evidence regarding 29 subtopics selected as the most relevant for the subject of interest. The project was developed in several stages, during which the selected topics were distributed among experts recommended by both societies with recent publications on the subject of interest and/or significant teaching and research activity in the field of mechanical ventilation in Brazil. The experts were divided into pairs that were charged with performing a thorough review of the international literature on each topic. All the experts met at the Forum on Mechanical Ventilation, which was held at the headquarters of AMIB in São Paulo on August 3 and 4, 2013, to collaboratively draft the final text corresponding to each sub-topic, which was presented to, appraised, discussed and approved in a plenary session that included all 58 participants and aimed to create the final document.

Keywords: Recommendations; Mechanical Ventilation; Respiratory Insufficiency.

Resumo

O suporte ventilatório artificial invasivo e não invasivo ao paciente crítico tem evoluído e inúmeras evidências têm surgido, podendo ter impacto na melhora da sobrevida e da qualidade do atendimento oferecido nas unidades de terapia intensiva no Brasil. Isto posto, a Associação de Medicina Intensiva Brasileira (AMIB) e a Sociedade Brasileira de Pneumologia e Tisiologia (SBPT) - representadas pelo seus Comitê de Ventilação Mecânica e Comissão de Terapia Intensiva, respectivamente, decidiram revisar a literatura e preparar recomendações sobre ventilação mecânica objetivando oferecer aos associados um documento orientador das melhores práticas da ventilação mecânica na beira do leito, baseado nas evidencias existentes, sobre os 29 subtemas selecionados como mais relevantes no assunto. O projeto envolveu etapas visando distribuir os subtemas relevantes ao assunto entre experts indicados por ambas as sociedades que tivessem publicações recentes no assunto e/ou atividades relevantes em ensino e pesquisa no Brasil na área de ventilação mecânica. Esses profissionais, divididos por subtemas em duplas, responsabilizaram-se por fazer revisão extensa da literatura mundial sobre cada subtema. Reuniram-se todos no Forum de Ventilação Mecânica na sede da AMIB em São Paulo, em 03 e 04 de agosto de 2013 para finalização conjunta do texto de cada subtema e apresentação, apreciação, discussão e aprovação em plenária pelos 58 participantes, permitindo a elaboração de um documento final.

Descritores: Recomendações; Ventilação Mecânica; Insuficiência Respiratória.

Introduction

Invasive or non-invasive mechanical ventilation (MV) must be performed in an adequate and safe manner to avoid the occurrence of ventilation-induced lung injury. Based on physiological

Completion of the drafting of the document: October 20, 2013
Conflicts of interest: With the help of the Brazilian Thoracic Society, the AMIB Division of Scientific Issues procured financial support from industrial companies and laboratories, distributed as sponsorship quotas, to cover part of the event costs (participants' air tickets, food and lodging). None of those companies participated in the drafting of the present document, nor had access to its content until it was disclosed (after its final format was approved) as brochures distributed at the Brazilian Congress of Intensive Care Medicine in Rio de Janeiro in 2013. The companies that collaborated with the present project are: Air Liquide, Covidien, GE, Intermed, Magnamed, Mindray and Philips.
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principles, evidence collected in laboratory experiments, and randomized clinical or observational studies involving actual patients that were available in the literature, current MV recommendations indicate that ventilatory support should be performed at a tidal volume (Vt) of 6mL/Kg predicted body weight, with a delta between plateau pressure and positive end-expiratory pressure (PEEP) not greater than 15cmH_2O, and end-expiratory pressure levels sufficient to avoid airway and alveolar collapse and ensure adequate gas exchange. Other recommendations include positioning the patient to guarantee adequate and harmless ventilation (such as prone positioning in cases of severe acute respiratory distress syndrome - ARDS) and the use of advanced support techniques (such as extracorporeal carbon dioxide (CO_2) removal) in cases of refractory ARDS. The development of increasingly more sophisticated ventilators allow for fine adjustment of sensitivity and include several trigger mechanisms, different inspiratory flow speeds, acceleration, mechanisms for ending inspiratory time, and monitoring options, which enable adjustment of the patient-ventilator synchrony and MV as a function of the patient's disease. In this regard, the possibility of providing differential ventilatory support for restrictive and obstructive conditions stands out.

For that reason, joint analysis of the available evidence on ventilatory support by Brazilian experts who deal with mechanical ventilation like anesthesiologists, intensivists, pneumonologists, physical therapists, nurses, nutritionists and speech therapists was necessary. Such evidence, taken together with experience gathered by the various specialties, may provide guidance to health care professionals in Brazilian intensive care units (ICU) on how to provide safe and effective respiratory support for patients with respiratory failure, based on the best evidence available, in order to avoid the occurrence of ventilator-associated lung injury.

Therefore, the aim of the present study was to review the available literature on 29 subtopics related to ventilatory support for individuals with respiratory failure, and following presentation, discussion, and approval at a plenary session including all 58 participating specialists, to present the results in the form of recommendations and suggestions.

Methods

Literature available from MEDLINE (2003-2013) and the Cochrane Central Register of Controlled Trials (CENTRAL) was reviewed by specialists with a higher education (intensivists, anesthetists, pulmonary specialists, physical therapists, and nurses) who were distributed in pairs for review of each of the 29 selected subtopics related to non-invasive and invasive ventilatory support for patients with respiratory failure.

After reviewing the articles available in the literature, each pair answered the questions formulated by the organizing commission (composed by Carmen Silvia Valente Barbas, President of the Committee of Respiratory Failure and Mechanical Ventilation of AMIB, Alexandre Marini Isola, National Coordinator of the Course of MV in ICU – VENUTI, and Augusto Manoel de Carvalho Farias, Coordinator of the Department of Intensive Care of the SBPT) according to criteria previously suggested by other authors.[1-4] Thus, the term recommendation was used when the level of evidence was high, i.e., derived from randomized studies conducted with more than 100 participants, meta-analyses, all-or-nothing effect, or patient safety. The term suggestion was used when the available evidence was weak, i.e., based on observational or case-control studies, case series, or on the experience of specialists to provide guidance for efficient and safe ventilatory support in Brazil. We therefore hoped that these evidence-based recommendations would help to avoid potential deleterious effects associated with inadequate ventilatory support in our patients.

The 58 participating specialists were requested to answer the proposed questions during an eight-hour session conducted at the Brazilian Intensive Care Medicine Association (*Associação de Medicina Intensiva Brasileira* - AMIB) on August 3, 2013. The answers were formulated based on the evidence available in the literature and on the experience of the specialists and were then presented at a plenary session that included all 58 participating specialists, which was held on August 4, 2013 at AMIB headquarters. During that session, the answers were discussed, modified when needed, voted on, and approved in accordance with the suggestions and observations of the specialists who attended the meeting.

The reports made by all the pairs of specialists were gathered by the project organizing commission, which revised, formatted and

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drafted the final document, following the authors' revisions. The document was then printed in the form of a bedside manual of recommendations to be distributed to ICUs all across Brazil, and it was also sent for publication in the Brazilian Journal of Intensive Care (*Revista Brasileira de Terapia Intensiva* - RBTI) and the Brazilian Journal of Pneumology (*Jornal Brasileiro de Pneumologia*).

Indications for noninvasive and invasive ventilatory support

Comment - Mechanical ventilation (MV) totally or partially replaces spontaneous ventilation and is indicated in acute respiratory failure (ARF) or acute exacerbations of chronic respiratory failure. MV promotes improvement of the gas exchange and reduction in the work of breathing. It can be performed in a noninvasive manner by means of an external interface, which usually consists of a face mask, or in an invasive manner through an endotracheal or a tracheostomy tube. Noninvasive ventilation (NIV) consists of the application of inspiratory pressure to ventilate the patient through a nasal/facial interface (inspiratory positive airway pressure (IPAP) and/or pressure support ventilation (PSV)) or of positive expiratory pressure to keep the airway and alveoli open and thus improve oxygenation (expiratory positive airway pressure (EPAP or PEEP)). The continuous positive airway pressure (CPAP) mode consists of the exclusive application of continuous end-expiratory pressure to the airway through a nasal/facial interface, while the patient's ventilation is fully spontaneous.

Noninvasive positive pressure mechanical ventilation: when to start

Recommendation - In the absence of contraindications (Chart 1), patients unable to maintain spontaneous ventilation (minute ventilation >4Lpm, $PaCO_2$<50mmHg, and pH>7.25) should start bi-level NIV, with a sufficient inspiratory pressure to maintain adequate ventilation; the goal is to avoid progression to muscle fatigue and/or respiratory arrest.[5]

Suggestion - NIV may be used in patients with reduced consciousness levels due to hypercapnia in chronic obstructive pulmonary disease (COPD). The level of consciousness should clearly improve one or two hours after beginning NIV.[5,6]

Recommendation - Patients who deteriorate or do not improve should be immediately intubated due to risk of loss of lower airway protection and respiratory arrest.[5]

Noninvasive positive pressure mechanical ventilation: when to discontinue

Recommendation - Use of NIV should be monitored at bedside by a health care professional within thirty minutes to two hours. For NIV to be considered successful, the following criteria should be met: reduction of the respiratory rate (f), increase in the tidal volume (Vt), improvement of the level of consciousness, reduction or cessation of the use of accessory muscles, increase in the partial pressure of oxygen (PaO_2) and/or the peripheral oxygen saturation (SpO_2), and reduction of $PaCO_2$ without significant abdominal distension. When NIV is unsuccessful, orotracheal intubation (OTI) with initiation of invasive ventilation should immediately be performed. Successful NIV is expected in 75% of hypercapnia cases and approximately 50% of hypoxia cases.[5]

Noninvasive mechanical ventilation in asthma exacerbations

Suggestion - NIV may be used together with pharmacological treatment to improve airflow obstruction and reduce respiratory effort in individuals with moderate and severe asthma attacks.[5,7]

Chart 1 - Contraindications to noninvasive ventilation

Absolute
Need for emergency intubation
Cardiac or respiratory arrest
Relative
Inability to cooperate, protect the airways, or abundant secretions
Reduced level of consciousness (excepting hypercapnic acidosis in COPD)
Non-respiratory organ failure (encephalopathy, malignant arrhythmia, severe gastrointestinal bleeding with hemodynamic instability)
Face or neurological surgery
Face trauma or deformity
High risk of aspiration
Upper airway obstruction
Recent esophageal anastomosis (avoid pressurization above 15cmH_2O)

COPD - chronic obstructive pulmonary disease.

Noninvasive mechanical ventilation in acute exacerbations of chronic obstructive pulmonary disease

Recommendation - NIV should be used in COPD exacerbations to reduce the need for intubation (relative risk - RR: 0.41 [95% confidence interval - 95%CI: 0.33-0.53]), reduce hospital length of stay and reduce mortality rates (RR: 0.52 [95%CI: 0.35-0.76).[5,6]

Acute cardiogenic pulmonary edema

Recommendation - NIV (bilevel positive airway pressure (BIPAP) with EPAP at 5 to 10 and IPAP at up to 15cmH_2O) or CPAP at 5 to 10cmH_2O must be used in individuals with acute cardiogenic pulmonary edema to reduce the need for endotracheal intubation (RR: 0.53 [95%CI: 0.34-0.83]), as well as the in-hospital mortality rate (RR: 0.6 [95%CI: 0.45-0.84]).[5,8,9]

Noninvasive mechanical ventilation in acute respiratory distress syndrome

Suggestion - NIV may be used in ARDS, especially in cases of mild ARDS; the desired therapeutic goals should be achieved within thirty minutes to two hours. Avoid delaying intubation in unsuccessful cases.[5,10]

Recommendation - NIV should be avoided in severe ARDS due to the high rate of respiratory failure and need for OTI, especially when PaO_2/FIO_2<140 and the Simplified Acute Physiology Score (SAPS) II >35.[5,10]

Noninvasive mechanical ventilation in severe community-acquired pneumonia

Suggestion - NIV may be used in severe cases of community-acquired pneumonia (CAP) with hypoxemic respiratory failure, particularly in individuals with concomitant COPD; the desired therapeutic effect should be achieved within thirty minutes to two hours. Avoid delaying intubation in unsuccessful cases.[5,11]

Post-extubation

Recommendation - NIV should be used to shorten the duration of invasive ventilation (NIV weaning-facilitating action), reduce mortality, reduce the rate of ventilator-associated pneumonia (VAP), and shorten the ICU and hospital stay of individuals with COPD and hypercapnia.[5,12,13]

Recommendation - NIV should be started immediately in high-risk patients (Chart 2) to avoid ARF and reintubation (prophylactic action).[5,12-15]

Recommendation - Avoid the use of NIV following the onset of a new respiratory failure event after extubation (curative action).[5,12-16]

Noninvasive ventilation in the postoperative period

Recommendation - NIV is indicated for the treatment of ARF that occurs in the immediate postoperative period following elective abdominal and thoracic surgery, and is associated with improvements in gas exchange, reductions in atelectasis, decreased work of breathing, and reduction in the need for OTI; furthermore, NIV may possibly reduce the mortality rate. In such cases, NIV must be used cautiously, with a full understanding of the limitations of and contraindications for its use.[5,16-19]

Suggestion - In esophageal surgery, NIV may be used to avoid ARF by maintaining lower inspiratory pressures (EPAP< 8 and IPAP < 15). This same suggestion applies to thoracic, abdominal, cardiac, or bariatric surgery.[5,17-19]

Bronchoscopy

Suggestion - NIV may be used during and after bronchoscopy to reduce the risk of complications in individuals with severe refractory hypoxemia, postoperative respiratory failure, or severe COPD.[5] Special care must be provided to

Chart 2 - Patients considered to be at risk of extubation failure and who could benefit from noninvasive ventilation immediately after extubation (prophylactic use)

Hypercapnia
Congestive heart failure
Ineffective cough or secretions retained in the airways
More than one failure in the spontaneous respiration test
More than one comorbidity
Upper airway obstruction
Age > 65 years old
Increase of severity of illness, as indicated by APACHE >12 on the day of extubation
Duration of mechanical ventilation >72 hours
Patients with neuromuscular diseases
Obese patients

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individuals subjected to transbronchial biopsy, which includes maintenance of the airway pressures at <20cmH_2O and performance of chest radiographs in cases of clinical decompensation and approximately six hours after the procedure (in order to rule out pneumothorax).

Masks and ventilators for providing noninvasive ventilation

Ventilators available in Brazil: characteristics, advantages and disadvantages

Suggestion - NIV may be performed using portable ventilators specifically designed for this purpose and that have leak compensation. The device should be coupled to a nasal/facial interface with a single-limb circuit and a built-in exhalation port. NIV may also be performed using microprocessor-controlled ventilators with software for this specific purpose, which should be coupled to the nasal/facial interface by means of an elbow connector and the ventilator's dual-limb circuit (Chart 1 - electronic supplementary material). The CPAP mode may be generated using of flow generators[20,21] (Chart 3).

Carbon dioxide rebreathing

Suggestion - Avoid or minimize CO_2 rebreathing when single-limb circuit ventilators are used. The risk of CO_2 rebreathing is lower with systems where the exhalation ports are built into the mask compared to ones where the exhalation ports are in the ventilator circuit. Other factors that might contribute to CO_2 rebreathing are use of low PEEP and reduced pressure support; special attention is needed in such cases.[22]

Oxygen supplementation

Suggestion - In the case of ventilators with a gas blender, the device allow adjustments in the oxygen (O_2) supplementation. When portable NIV devices without a gas blender are used, oxygen should be given straight to the mask beyond the exhalation port using an external O_2 source. The supplemental FiO_2 depends on the O_2 flow, position of the O_2 connector in the circuit, degree of leak in the ventilator circuit, the type of interface used, and the level of IPAP and EPAP supplied.[23-26]

Monitoring during noninvasive ventilation

Recommendation - Monitor Vt, f and SpO_2 during the use of NIV. Use a graphical monitoring system when available. Asynchrony, air leaks, auto-PEEP, efficacy of effort, and the leak compensation mechanism should be continuously monitored.[26,27]

Indications for the choice of interface in common clinical situations

Recommendation - Choose an appropriate interface, i.e., the one that adjusts best to the patient's face to achieve the greatest clinical efficiency.

Recommendation - Use interfaces without nasal compression when the estimated duration of NIV is >24 to 48 hours.

Recommendation - Use interfaces with a PEEP valve when CPAP with flow generator is used.

Recommendation - When NIV is performed with an ICU (conventional microprocessor-controlled) ventilator, use a mask connected to a dual-limb circuit. When NIV-specific ventilators are used, use a mask for single-limb circuits[20,23-25] (Chart 4).

Adaptation to and tolerance of interfaces

Nasal masks

Suggestion - Nasal masks may be used in cases of mild ARF for patients with claustrophobia or maladaptation to the facial mask.

Suggestion - Several interfaces can be combined when patients need continuous ventilatory support to avoid the occurrence of ischemia due to reduction of blood flow that is caused by the pressure of the mask on the patient's face (Chart 5).[25]

Oral-nasal (facial) masks

Recommendation - Use face masks in cases of mild to moderate ARF to achieve fast improvement of physiological parameters (gas exchange and work of breathing). Monitor the patient's tolerance and the occurrence of side effects, such as ulcers at support points and gastric distension.

Full-face mask and Helmet

Recommendation - Use these interfaces in the most severe cases of hypoxemic respiratory

Chart 3 - Types of modes of ventilation for noninvasive support

Modes	Description	Indication*
CPAP	Constant airway pressure Spontaneous ventilation	Recommendation: in cardiogenic APE, PO of abdominal surgery, and mild/moderate sleep apnea
BIPAP (BILEVEL)	Two pressure levels (IPAP and EPAP) Flow cycled	Recommendation: in acute hypercapnia, for respiratory muscle rest; in cardiogenic APE; and in immunosuppressed individuals with infection

CPAP - continuous positive airway pressure; BIPAP - bilevel positive airway pressure; APE - acute pulmonary edema; PO - postoperative period; IPAP - inspiratory positive airway pressure; EPAP - expiratory positive airway pressure. *except when contraindicated.

failure because they allow for greater airway pressurization. As those devices cover the patient's entire face, the pressure they exert on the skin is more widely distributed, and thus pressure points on the nose are minimized, consequently reducing the risk of skin injury (Chart 5).

Suggestion - Helmet-like masks can be used, when available, in less severe cases of respiratory failure. This type of mask is hermetically sealed around the patient's neck by an air cushion that is inflated by the ventilator itself, and the points of contact are on the neck, shoulders and axillary region. However, as the dead space is large, the use of helmet-like masks in individuals with ventilatory disorders is limited; such patients may need requires correction by means of higher levels of pressure support. Internal noise is another cause of discomfort that should be taken into consideration. This type of interface may induce trigger asynchrony due to delayed release of the inspiratory flow, with a consequent increase in the work of breathing[28-30] (Chart 5).

Intubation and tracheostomy

Techniques for elective, semi-elective and emergency intubation

Recommendation - Use direct laryngoscopy with visualization of the larynx as the fastest and most reliable method for insertion of the orotracheal tube in elective or emergency cases. Three unsuccessful attempts at intubation by an experienced physician are considered to characterize a difficult airway, in which case the corresponding specific guidelines should be followed.[31,32]

Chart 4 - Differences between noninvasive ventilation using portable ventilators specific for noninvasive ventilation and intensive care unit microprocessor-controlled ventilators with a non-invasive ventilation module

Circuit	ICU ventilators Dual-limb, with demand valve	NIV specific Single-limb
Exhalation	Exhalation valve	Exhalation through port or exhalation valve in the mask or circuit
Air leak	Compensated when PCV (time-cycled) or NIV-specific module is used	Automatic compensation
O_2 supplementation	Regulated by the ventilator blender	Regulated by the ventilator blender or O_2 supplementation through the mask and/or circuit
PEEP	In the ventilator exhalation valve	Ventilator exhalation valve and/or adjustable valve in mask
Type of interface	Interfaces for dual-limb circuit	Allows for use of masks with built-in exhalation valve or in the ventilator circuit

ICU - intensive care unit; PCV - pressure-controlled ventilation; NIV - noninvasive ventilation; O_2 – oxygen; PEEP - positive end-expiratory pressure.

Elective intubation

Suggestion - Elective tracheal intubation is an intubation that is performed when there are no signs of imminent failure of airway protection, ventilation, and/or oxygenation. Under such conditions, the method of tracheal intubation that is most suited to each individual patient should be selected. Use direct laryngoscopy with OTI as the first-choice method.[31,32]

Suggestion - Adequately prepare the patient for tracheal intubation, including pre-oxygenation, monitoring, and appropriate

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Chart 5 - Advantages and disadvantages of the various types of interfaces

Interface	Advantages	Disadvantages	Suggested ventilators and adjustments
Nasal	Less risk of aspiration	Mouth air leak	Continuous-flow single-limb circuit devices
	Facilitates expectoration	Depressurization through the mouth	
	Less claustrophobia	Nose irritation	
	Allows talking	Limited use in patients with nasal obstruction	
	Allows eating	Mouth dryness	
	Easy handling		
	Less dead space		
Facial	Less mouth air leak	Higher risk of pressure ulcer on the nose or support points	Continuous-flow or demand-flow devices
	More appropriate for acute conditions because it allows for greater flow rates and pressure levels	Greater claustrophobia	Single- or dual-limb circuit
		Greater risk of aspiration	When dual-limb circuit devices are used, leak automatic compensation in the circuit is necessary
		Hinders eating	
		Hinders communication	
		Risk of asphyxia in case of ventilator malfunction	
		Risk of bronchial aspiration	
Total-face	More comfortable for prolonged use	Greater dead space	Continuous-flow devices
	Easy to adjust	Should not be used in association with aerosol therapy	Single-limb circuit
	Less risk of face skin injury	Monitor for vomiting (attention to aspiration)	Use preferentially with NIV-specific ventilators or conventional ventilators with NIV module
	Minimum air leak		
Helmet	More comfortable for prolonged use	Greater risk of CO_2 rebreathing	Continuous-flow or demand-flow devices
	No risk of face or skin injury	Favors patient-ventilator asynchrony	
		Risk of asphyxia in case of ventilator malfunction	Dual- or single-limb circuit with PEEP valve in the helmet
		Should not be used in association with aerosol therapy	
		High internal noise and greater feeling of pressure in the ears	
		Need of higher pressures to compensate for the dead space	
		Skin injury can occur in the axillae	

NIV - noninvasive ventilation; CO_2 – carbon dioxide; PEEP - positive end-expiratory pressure.

positioning during the procedure in order to achieve optimal laryngoscopy.[32,33]

Suggestion - A curved-blade laryngoscope of the appropriate size is preferred. A straight-blade laryngoscope may be used to achieve appropriate larynx exposure in cases where intubation is difficult.[31,32,34]

Emergency intubation

Suggestion - Use the rapid sequence intubation technique to avoid the risk of gastric aspiration. Insert the orotracheal tube as soon as possible after loss of consciousness occurs.[32,35,36]

Suggestion - Use hypnotics (propofol, etomidate, ketamine or thiopental), opioids (fentanyl, alfentanil or remifentanil) and neuromuscular blocking drugs (rocuronium or succinylcholine). The Sellick maneuver (cricoid pressure) can be performed during the procedure to minimize the risk of gastric aspiration.[32,35-37]

Techniques and indications for tracheostomy: advantages and disadvantages

Timing of tracheostomy: recommendations based on the cause of respiratory failure

Spinal cord injury

Suggestion - Perform tracheostomy early (within seven days). High cervical spinal cord injury (C5 or above) is an independent predictor of the need for prolonged MV. Patients with injuries at lower levels should be assessed on an individual basis.[32,38]

Traumatic brain injury

Suggestion - Perform tracheostomy early (within seven days) in the most severe cases (Glasgow Coma Scale <8), as patients with traumatic brain injury usually require prolonged ventilatory support. The evidence regarding reductions in the VAP rate is contradictory, and there is no evidence that early tracheostomy reduces mortality, airway injury, or the length of hospital stay.[32,38,39]

Patients with trauma not affecting the central nervous system

Suggestion - Early tracheostomy is indicated when prolonged ventilatory support is anticipated.[32,38-40]

Patients admitted to the intensive care unit for clinical causes

Recommendation - Wait 14 days to perform a tracheostomy, as early use of this procedure does not reduce the 30-day mortality rate, length of stay in the ICU, or the need for sedation.[32,41-44]

Tracheostomy techniques

Recommendation - Perform percutaneous or conventional tracheostomy, depending on the available resources and the staff's experience. Percutaneous tracheostomy can be performed at the bedside by ICU staff. Although it is more expensive and demands that a bronchoscopy be performed to increase its safety, the associated rates of surgical wound infection are lower. Conventional tracheostomy must be performed in an operating room by specialized staff, except for the case of ICUs that are equipped with a room for surgical procedures. Both techniques have similar rates of major complications, such as bleeding, subcutaneous emphysema, pneumothorax and death.[32,45-47]

Initial adjustment of invasive ventilation and conventional ventilation modes

Ventilation adjustment

Recommendation - Use the FIO_2 needed to maintain SpO_2 at 93 - 97%.[48,49]

Recommendation - Use a Vt of 6mL/kg/predicted body weight. Reassess as a function of changes in the patient's clinical condition.[48-52]

Recommendation - Use the assist-control mode (AC) as either volume-cycled (VCV) or time-cycled pressure-limited, known as pressure controlled ventilation mode (PCV), and reassess within the first few hours based on the patient's clinical condition.[48-51]

Recommendation - Adjust the initial f = 12 to 16 breaths per minute, with an inspiratory flow rate or inspiratory time required to maintain the inspiration to expiration ratio (I:E) initially at 1:2 or 1:3. In patients with obstructive disease, the initial f can be lower (<12 breaths per minute), and in patients with restrictive disease it may be higher (e.g., >20 breaths per minute, if required by the patient's clinical condition). Reassess as soon as the first arterial blood gas results are available.[48,51-54]

Recommendation - Establish the type of ventilator triggering. The more widely available types of ventilator triggering are the time-triggered (ventilator-controlled mode) and the patient-triggered (flow or pressure triggered, also known as pneumatically triggered) modes. The ventilator's sensitivity should be adjusted to the most sensitive level to avoid auto-triggering. The ventilator can also be triggered by neural stimuli (neurally adjusted ventilatory assist-NAVA).[48,51-54]

Recommendation - Initially use a PEEP of 3 to 5cmH_2O, except in cases of diseases such as ARDS, where the PEEP value should be assessed according to the specific guidelines described in the each topic of the present recommendations.[48,49,55-57]

Recommendation - Use passive heaters and humidifiers in individuals undergoing MV. When available, active humidification and heating should be performed in patients with thick secretions, and optimal humidification should be maintained to avoid obstruction of the orotracheal tube.[58]

Recommendation - Set the alarms on an individual basis, using specificity and sensitivity parameters appropriate for the patient's clinical

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condition. Also, an apnea backup and the specific parameters for apnea should be adjusted if they are available in the device.

Recommendation - After the initial parameters are defined, check the Vt, pressure and flow curves to establish whether their values correspond to the expected parameters or if immediate readjustment is needed. Check pulse oximetry, which should be continuously monitored. Initially, set the maximum airway pressure at 40 cmH_2O to avoid barotrauma, and adjust as soon as possible based on the patient's clinical condition.[48,51-54]

Recommendation - Arterial blood gases must be assessed after 30 minutes of steady ventilation to check whether the ventilation and gas exchange goals were met. If they were not, perform necessary adjustments of the mode and cycling parameters.[48-51]

Recommendation - Assess the eventual hemodynamic repercussions of MV. Investigate the presence of hypovolemia, auto-PEEP and/or pneumothorax in patients with hypotension that is associated with positive pressure ventilation.

Recommendation - Maintain the most appropriate level of muscle work. In patients with high inspiratory flow demands, use opioids to reduce the ventilatory drive and provide appropriate comfort for the patient. Induce muscle rest for 24 to 48 hours in patients with respiratory muscle fatigue or hemodynamic instability.

Recommendation - In patients who do not need muscle rest, start an assist mode of ventilation as soon as possible, with appropriate adjustment of the ventilator's sensitivity. Avoid ventilator-induced diaphragmatic dysfunction, which usually occurs after 18 hours of controlled ventilation.

Suggestion - In older adults, patients who require prolonged use of controlled modes of ventilation, malnourished patients, patients using corticosteroids or neuromuscular blocking agents, and individuals with hypothyroidism, pay special attention to the assessment of respiratory muscle function.

Conventional modes of ventilation[59]

Suggestion - Use the volume assist-control mode (VCV) when the aim is to maintain a more stable minute volume (Vt x f). This mode of ventilation can be timed (controlled), and pressure- and flow-triggered (assisted) and is cycled off when the preset inspired Vt is achieved. The airway pressure is variable and depends on the patient's ventilatory mechanics (special attention should be paid to monitoring the peak and plateau pressures when this mode is used, and it should be ensured that the maximum airway pressure alarm is properly set). This mode is also used to measure the peak and plateau pressures for calculating the compliance and resistance of the respiratory system under a constant square-wave inspiratory flow pattern (see this specific topic in the present recommendations).

Suggestion - Use the PCV assist-control mode when respiratory mechanics are impaired (low compliance and/or high resistance), as it allows for better control of the airway and alveolar pressures. This mode characteristically limits pressure throughout all the inspiratory phase and is time-cycled. The inspiratory time is set in seconds by the caregiver. The flow is free and decelerating waveform. In this mode, the Vt is variable and depends on the administered delta pressure and the patient's ventilatory mechanics (special attention should be paid to monitoring the expired Vt and adjusting the maximum and minimum minute volume alarms). The inspiratory flow speed (ramp, rise time or slope) can be increased or reduced. The rise time can be faster in patients with obstructive disease to obtain a better Vt. Special attention should be paid to the possible occurrence of peak flow overshoot. In patients with restrictive disease, a slower rise time should be used.

Suggestion - PSV is considered the preferential mode during assisted/spontaneous ventilation. It should be started as soon as possible, based on the patient's clinical condition. This is an exclusively patient-triggered mode, and can be flow- or pressure-triggered. Characteristically, pressure is limited throughout all the inspiratory phase and is cycled off when the inspiratory flow falls, typically to 25% of the peak inspiratory flow. This cycling criterion (% of the peak inspiratory flow) can be set between 5% and 80% in some of the most modern ventilators, which allows a reduction of the inspiratory time in patients with obstructive disease (% of the cycling off >25%) and an increase in the inspiratory time in patients with restrictive disease (% of the cycling off <25%). The rise time can be faster in patients with obstructive disease, thus decreasing inspiratory time and obtaining a better Vt. Special attention should be paid to the occurrence of

peak flow overshoot. In patients with restrictive disease, use a slower rise time, which may be accompanied by a Vt gain.

Suggestion - Use pressure-cycled ventilators just if they are the only ventilators available. The ventilator can be time and pressure triggered. Characteristically, it provides a fixed flow rate until the airway pressure reaches the value predetermined by the caregiver (cycling). As a result, the Vt is unknown, and consequently the use of an external ventilometer (Wright's ventilometer) is recommended; alternatively, arterial blood gases can be assessed after 20 minutes of steady ventilation to check whether the $PaCO_2$ is compatible with the patient's clinical condition (35 to 45 mmHg in most cases). This device usually does not have a built-in O_2 blender or alarms. The multi-disciplinary staff must pay special attention to monitoring both ventilation and oxygenation.

Recommendation - Avoid the use of Synchronized Intermittent Mandatory Ventilation (SIMV) because it has been shown to be associated with a delay in MV weaning. Currently, the use of SIMV is restricted to patients in whom minimal minute volume is necessary at the beginning of MV weaning process (e.g., individuals with neuropathy, or upon immediate awakening from general anesthesia). As soon as the ventilatory drive stabilizes, SIMV should be shifted to PSV. A brief description of SIMV mode follows. Controlled cycles can be volume-cycled (V-SIMV) or pressure-limited (P-SIMV). Spontaneous cycles should be associated with PSV. SIMV is characterized by the fact that it allows for controlled, assisted and spontaneous cycles to occur within the same time window (TW), which is determined by the f of the controlled mode. Controlled cycles only occur when a patient assisted trigger did not occur in the immediately preceding TW. Otherwise, the ventilator waits for the next patient-trigger, i.e., an assisted cycle. Spontaneous cycles supported by PSV can occur in the remainder of the TW.

Asynchrony and new modes of mechanical ventilation

Patient-ventilator asynchrony

Comment - Patient-ventilator asynchrony is a lack of coordination between the patient's inspiratory effort and ventilatory needs and the support provided by the ventilator.[60] Asynchrony is a frequent event, occurring in 10% to 80% of all ventilator cycles, and is associated with prolonged of MV and ICU stays.[61]

Recommendation - The presence of asynchrony should be actively assessed during the assessment of patients subjected to MV, and it should be corrected.

Trigger asynchrony

Ineffective triggering

Comment - Ineffective triggering occurs when the patient's inspiratory effort is not enough to trigger the ventilator.[62] The reason might be a maladjustment in ventilator sensitivity or patient-related factors such as respiratory muscle weakness, central respiratory depression, dynamic hyperinflation (auto-PEEP), or longer mechanical inspiratory time relative to the neurally stimulated inspiratory time.[62,63]

Identification - Clinical examination of the patient's chest and abdomen can reveal that the inspiratory effort is not accompanied by a ventilator cycle.[64,65] Figure 1A shows how to identify this asynchrony in ventilator curves.[64,65]

Recommendation - To correct trigger asynchrony, the ventilator's sensitivity should be adjusted to the most sensitive level possible, while avoiding auto-triggering; in addition, pressure triggering can be shifted to flow triggering (which is usually more sensitive).

Suggestion - In the presence of auto-PEEP, extrinsic PEEP may be titrated up to 70 to 85% of the auto-PEEP; the effects of this adjustment on asynchrony must be checked.[62] During PSV, one might attempt to reduce the pressure that is administered or to increase the percentage of the cycling criterion.[63] When pressure-controlled ventilation (PCV) is used, one might attempt to reduce the inspiratory time, or in cases where VCV is used, to increase the inspiratory flow rate or reduce the pause time.[62,63]

Double triggering

Comment - Two consecutive cycles are triggered by a single patient inspiratory effort. The ventilator's mechanical inspiratory time is shorter than the patient's neural inspiratory time.[3]

Identification - Clinically two consecutive cycles without an interval between them can be observed; this pattern that may be repeated

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quite often. Figure 1B shows how to identify this asynchrony in the ventilator curves.[64-66]

Suggestion – In VCV, the inspiratory flow rate and/or the Vt should be increased, while still complying with the safety thresholds. Alternatively, VCV could be shifted to PCV or PSV, in which the inspiratory flow rate varies as a function of the patient's inspiratory effort. When double triggering occurs under PCV, the inspiratory time and/or delta of pression value could be increased. In PSV, one might try to increase the pressure level or reduce the percentage of the cycling criterion.[62,63]

Auto-triggering

Comment – The ventilator is triggered in the absence of a patient's inspiratory effort. This can be caused by overly high ventilatory sensitivity, leaks in the system, flow alterations due to presence of condensates in the circuit, detection of the heartbeat, or wide variations in chest pressure that are due to stroke volume (Figure 1C).[60,62]

Identification – The observed respiratory frequency is higher than the adjusted one, and the cycles are not preceded by indicators of patient inspiratory effort.[64-67]

Recommendation – Once the presence of leak or condensate in the circuit is corrected or ruled out, gradually reduce the ventilator's sensitivity to a level sufficient for auto-triggering to stop.[62,64-66]

Flow asynchrony

Insufficient inspiratory flow

Comment – In insufficient inspiratory flow, the flow offered is lower than patient ventilatory demands. This typically occurs when the flow is set by the operator and cannot be increased by the patient's inspiratory effort, as in VCV. Nevertheless, this phenomenon might also occur in PCV and PSV, when the adjusted pressure is insufficient to ensure an appropriate balance between the patient's ventilatory demands and mechanics.[67,68]

Identification - The patient exhibits discomfort and uses the accessory respiratory muscles. Figure 2 shows how to identify this asynchrony in the ventilator curves.[67,68]

Recommendation – Correct the causes of the increased ventilatory demands, such as fever, pain, anxiety, or acidosis. In VCV, increase the inspiratory flow rate and check for signs of patient comfort, as well as the shape of the pressure - time curve; shift to PCV or PSV, in which the flow is not fixed;[68] adjust the speed necessary to achieve the maximum airway pressure (rise time - speed of flow rise, or increasing the controlled pressure value).[69]

Excessive inspiratory flow

Comment – Excessive inspiratory flow can occur in VCV when the flow is set above the level desired by the patient, or in PCV or PSV when high pressures or a faster rise time are set.

Identification - In VCV, the pressure – time curve peak is achieved too early.[68,69] In PCV or PSV, the airway pressure becomes higher than the adjusted level, a phenomenon known as overshoot.[69]

Recommendation – In VCV, reduce the flow rate; in PCV and PSV, the rise time should be reduced until the overshoot disappears.[68]

Cycling asynchrony

Premature cycling

Comment – In premature cycling, the ventilator interrupts the inspiratory flow before the patient desired; in other words, the ventilator's mechanical inspiratory time is shorter than the patient's neurally controlled inspiratory time.[70] In VCV and PCV, the inspiratory time is adjusted by the operator. In PSV, premature cycling occurs when a low pressure level and/or a high percentage of the cycling criterion are adjusted.[70] Figure 3 shows how to identify this asynchrony in the ventilator curves. In some cases, the patient's inspiratory effort may suffice to trigger a new cycle (double cycling).[64,66,70]

Recommendation – In VCV, the inspiratory flow rate may be reduced and/or Vt may be increased in compliance with the safety thresholds. Alternatively, one might shift to PCV or PSV, where the inspiratory flow rate varies as a function of the patient's inspiratory effort. When premature cycling occurs in PCV, the inspiratory time and/or the delta of inspiratory pressure value may be increased. In PSV, one could try to increase the pressure level or reduce the percentage of the cycling criterion.[62,63,70]

Delayed cycling

Comment - In delayed cycling, the ventilator's mechanical inspiratory time is longer than the time desired by the patient; in other words, the ventilator cycling time is longer than the patient's neurally controlled inspiratory time. In VCV, this can occur when the inspiratory time is extended by setting a high Vt or a low inspiratory flow rate or if inadequate use is made of the inspiratory pause. In PCV, delayed cycling occurs when the inspiratory time is set beyond the time desired by the patient. In PSV, particularly in the case of obstructive diseases such as COPD, the increase in the resistance and compliance of the respiratory system gradually slows down the inspiratory flow rate, thus increasing the inspiratory time.[70] Figure 3 shows how to identify this asynchrony in the ventilator curves.[64,66]

Recommendation - In modes of ventilation in which the operator adjusts the inspiratory time, the latter should be reduced. In PSV, the percentage of the cycling criterion might be increased (e.g., from 25% to 40% or even higher).[70]

Suggestion - Patient-ventilator asynchrony should be treated by adjusting the ventilation parameters or shifting to other modes of ventilation (experts' opinion).

Advanced modes of mechanical ventilation

Comment - The choice of the mode of ventilation should be based on the severity of the patient's condition.[71] In patients with respiratory failure and asynchrony, a shift to another mode of ventilation may be an option. The number and complexity of modes of ventilation exhibited a significant rise in recent years. Despite their increasing availability, the clinical impact of these newer modes of ventilation has not yet been thoroughly investigated.[71]

Suggestion - Use advanced modes of ventilation in specific clinical situations, provided that the operator is thoroughly acquainted with the parameters of each mode and that the patient's clinical condition can benefit from the resources specific to each mode.

Pressure-regulated volume-control mode

Comment - This is a time-cycled pressure-limited ventilation mode. The ventilator readjusts

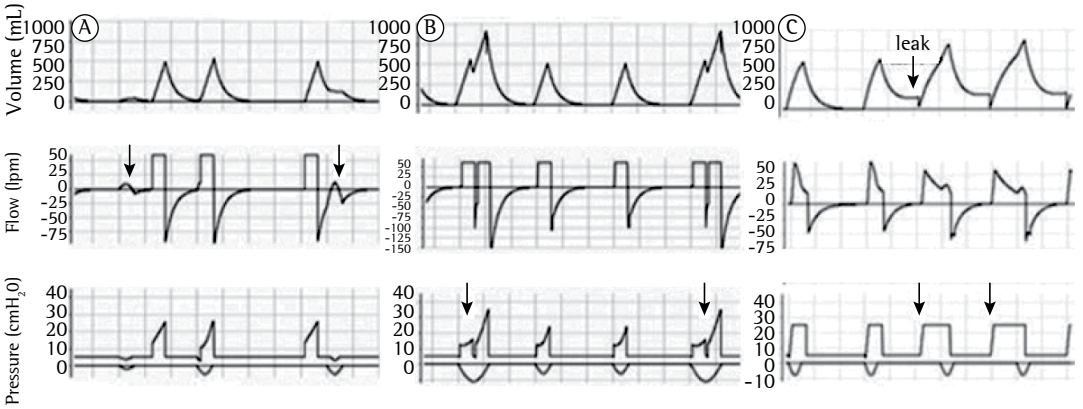

Figure 1 - Trigger asynchronies identified in volume-, flow- and pressure-time curves, indicated by arrows. Negative deflections in the pressure-time curves represent the patient's inspiratory effort (muscle pressure), which are only visible when the esophageal pressure is monitored. Panel A) Lost efforts. The first arrow indicates a weak stimulus, which is unable to trigger the ventilator, thus resulting in a small positive flow wave and minimal tidal volume. The second arrow points to effort during expiration, which failed to trigger the ventilator and merely sufficed for the flow to return to baseline and become slightly positive. Panel B) Double-triggering. Example in volume-controlled ventilation. The patient's inspiratory efforts persist at the time of cycling-off, thus triggering another cycle. The corresponding volumes are added together (stacking), and the airway pressure increases, causing the high-pressure alarm to go off. Panel C) Auto-triggering. In the support pressure mode, some cycles are triggered without a patient inspiratory effort, which can be facilitated by leaks; this is observed in the volume-time curve, which does not return to baseline (the inspired volume is greater than the expired volume). Figures obtained at Xlung.net, a virtual mechanical ventilation simulator. Available at: http//:www.xlung.net.

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the pressure limit at each cycle based on the Vt obtained in the previous one, until reaching a target Vt that has been preset by the operator.[72]

Suggestion - Indicate when limited-pressure Vt control is desired, aiming to automatically adjust the inspiratory pressure if the respiratory mechanics change.

Recommendation - Caution is required in adjusting the target Vt, as undesirable increases of the inspiratory pressure may result.

Airway pressure release ventilation and bilevel positive airway pressure ventilation

Comment - Airway pressure release ventilation (APRV) is pressure-limited and time-cycled, and is considered to be a spontaneous mode of ventilation. The operator adjusts the pressure high (PEEPhigh) and low (PEEPlow), the PEEPhigh to PEEPlow ratio, and the frequency of alternation between both PEEP levels; the time of PEEPhigh must be longer than the time of PEEPlow. The BIPAP mode also uses two PEEP levels, but the time of PEEPlow is longer than that of PEEPhigh. The patient can breathe spontaneously at both pressure levels.[73,74] Support pressure may also be applied, as its value is added to the PEEPlow value, and the final airway pressure (Paw) is the result of the sum of PSV + PEEPlow. When the PEEPhigh value is lower than PSV + PEEPlow value, during the PEEPhigh period the ventilator only complements the PSV value to reach the same level of Paw as in PEEPlow + PSV.

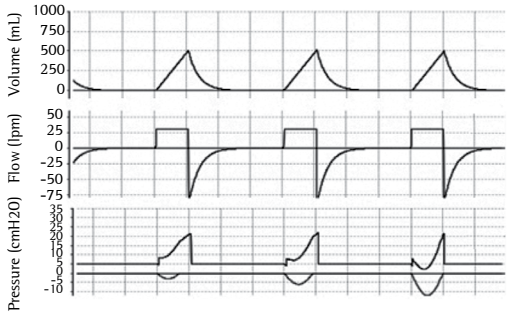

Figure 2 - Flow asynchrony. In volume-controlled mode, the flow rate was adjusted below the patient's demand; the patient thus maintained muscle effort throughout inspiration, and the curve consequently became concave and upward. The asynchrony exhibits increasing intensity from the first to the third cycle, as represented in the figure. The negative deflections in the pressure-time curve represent the patient's inspiratory effort (muscle pressure) and are only visible when esophageal pressure is monitored. Figures obtained at Xlung.net, a virtual mechanical ventilation simulator. Available at: http//:www.xlung.net.

Suggestion - Use APRV when maintenance of spontaneous ventilation and alveolar recruitment is necessary; APRV may improve gas exchange and reduce dead space and asynchrony.

Recommendation - Caution is required when regulating the alternation between the two pressure levels because in this mode, the minute volume results from the sum of the obtained Vt, when the pressures are alternated, plus the Vt generated from PSV cicles.

Proportional assist ventilation

Comment - Proportional assist ventilation (PAV) is a spontaneous ventilation mode that follows the equation of motion to generate inspiration pressure (Pvent) in proportion to the patient's inspiratory effort (Pmus). Therefore, when the Pmus decreases, Pvent also decreases, and vice-versa.[71,75-79] Some studies found better patient-ventilator synchrony when PAV, or its latest version, PAV plus (PAV+), is used compared to PSV. The PAV+ software estimates the work of breathing (WOB) of both patient and mechanical ventilator using the equation of motion, and calculates compliance and resistance through the application of 300-ms inspiratory micro-pauses every 4 to 10 ventilation cycles.

Indication - PAV is indicated for patients with respiratory drive and significant asynchrony under spontaneous modes of ventilation, PSV in particular. It is also indicated when one wants to determine the patient's WOB and mechanical measurements during assisted ventilation, e.g., for obtaining real-time intrinsic PEEP estimates.[75-79]

Recommendation - Before starting the PAV+ mode, the operator should set the type and diameter of the tracheal prosthesis, the type of humidifier, maximum Vt and maximum allowed airway pressure (limits) in the ventilator.

Recommendation - Set the percentage of initial support at 50% to achieve a patient WOB of 0.3 – 0.7 J/L with adequate Vt and f. Pvent increases proportionally with the patient's Pmus. The support percentage should not exceed 90%. If a greater percentage is needed, conventional assisted-controlled ventilation modes are recommended. Gradually reduce the support percentage in parallel with improvement of the

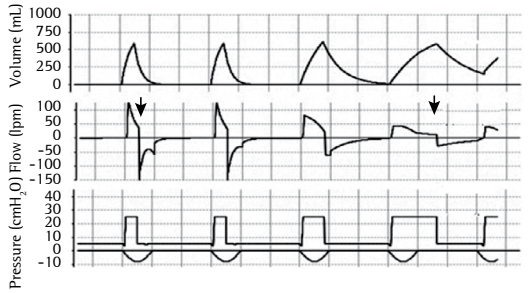

Figure 3 - Cycling asynchronies during pressure support ventilation. In the first cycle, the cutoff point of 25% of the peak inspiratory flow (percentage of the cycling criterion) was reached rapidly; the ventilator's inspiratory time was therefore shorter than the time desired by the patient. This is shown in the expiratory segment of the flow curve, which tends to return to the baseline as a result of the patient's inspiratory effort, which is still present. The last cycle represents the opposite situation, i.e., delayed cycling. The flow reduction occurs very slowly, which is typical of airway obstruction; the cycling threshold is therefore reached with some delay. Sometimes, the cycle is interrupted by a contraction of the respiratory muscles, which causes an increase above the support pressure adjusted at the end of inspiration (not shown in this figure). Figures obtained at Xlung.net, a virtual mechanical ventilation simulator. Available at: http//:www.xlung.net

patient's clinical condition, to as low as 30%. When the (abovementioned) parameters are maintained, consider to extubate the patient.

Suggestion - PAV is an alternative to PSV in patients with significant asynchrony; it has the potential to improve the patient-ventilator interaction.

Recommendation - PAV should be avoided in patients without respiratory drive, as well as in MV with leaks that impair the measurements of resistance and compliance.

Automatic tube compensation

Comment - Automatic tube compensation (ATC) is a spontaneous mode of ventilation that aims at reducing the resistive work imposed by the presence of an artificial airway - i.e., an orotracheal or tracheostomy tube. Some studies showed reductions in the work of breathing and better patient comfort with ATC compared to PSV.[80-82]

Suggestion - Use ATC plus or minus PSV to automatically compensate for the increase in the resistive work associated with the presence of a tracheal prosthesis (in PSV, the compensation should be calculated by the caregiver as a function of the prosthesis diameter; the smaller the diameter, the higher the PSV value should be, e.g., PSV=5cmH_2O for 9-mm tubes, and PSV=9cmH_2O for 6-mm tubes).

Recommendation - ATC is contraindicated for patients without respiratory drive, and care should be taken in patients who have excess secretions that interfere with inspiratory flow; the airway pressure alarms should be properly set.

Neurally adjusted ventilatory assist

Comment - Neurally adjusted ventilatory assist (NAVA) is a mode of ventilation that captures the electrical activity of the diaphragm and uses it as a criterion for triggering and cycling-off of the ventilator, thus providing inspiratory support in proportion to the electrical activity of the diaphragm. Use of NAVA requires placement of an esophageal-gastric catheter, with sensors positioned on the distal third of the esophagus to detect the electrical activity of the diaphragm.[5,6] In clinical studies, use of NAVA was associated with improved patient-ventilator synchrony when compared to PSV.

Indications - NAVA is indicated for patients with respiratory drive and significant asynchrony on spontaneous ventilation, and particularly in the case of loss of effort with PSV, as in patients with auto-PEEP (intrinsic PEEP).[77-79,83]

Recommendation - Special care is required in patients with oronasal or esophageal disorders that might hinder the passage or proper positioning of the NAVA catheter. The NAVA catheter should be properly placed and fixed, and its position should be checked on a regular basis. Once the probe is fixed, measure the electrical activity of the diaphragm (Edi), and adjust the NAVA gain as a function of the Vt, f and airway pressure (Edi versus NAVA gain). The ventilator is triggered by 0.5-μV variations in the Edi. From that point onwards, the ventilator delivers free flow as a function of the Edi reading. The maximum airway pressure results from adding [maximum Edi - minimum Edi] multiplied by the NAVA gain to the extrinsic PEEP value. Cycling-off occurs when Edi falls to 70% of the maximum Edi peak detected.[77-79,83]

Recommendation - NAVA gain is adjusted as a function of the patient's clinical condition, and should be assessed on an individual basis.

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Suggestion - NAVA may be an alternative to PSV for patients with significant asynchrony; it may improve the patient-ventilator interaction, especially in cases where there is loss of respiratory effort.

Adaptive support ventilation

Comment - Adaptive support ventilation (ASV) employs an algorithm to select the Vt and f combination necessary to reach the minute volume set by the caregiver by means of spontaneous and controlled cycles, with the lowest possible airway pressure. The version known as Intellivent-ASV employs an end-tidal CO_2 ($ETCO_2$) and a SpO_2 sensor to adjust the PEEP and FIO_2 automatically by means of a table.[83]

Indications - ASV is indicated for patients with severe respiratory failure when reductions of the work of breathing and stimulation of spontaneous respiration are desired.

Suggestion - Use ASV to ensure minute volume with appropriate lung protection in patients with unstable ventilatory drive, asynchrony or discomfort. Monitor for possible occurrence of leaks or excess secretions, which may impair the appropriate functioning of the ventilator.

Ventilators for invasive ventilation

Choice of mechanical ventilator

The following questions should be answered when choosing mechanical ventilators: in which patient population they will be used (adults, children, or newborn infants)? How often are patients with severe ventilation problems admitted (e.g., ARDS, severe obstructive disease, pulmonary fistula, etc.)? What information do ventilators provide to contribute to decision-making about ventilatory support in that particular ICU? How will patients be weaned from MV? What mode of ventilation will be used? Which clinical and mechanical measurements contribute to decision-making? How often and in which situations will NIV be used?

Suggestion - Assess the particular characteristics of various ventilators as a function of the resources available to and the needs of your service:

Ventilators with basic resources. These include one or more basic modes of ventilation without curves. As a rule, they are used for transportation of patients under MV.

Ventilators with basic resources and curves. These include the basic modes of ventilation (VCV, PCV, SIMV and PSV) and the basic ventilation curves (volume, flow and pressure).

Ventilators with curves and advanced ventilation resources. In addition to the basic modes of ventilation and curves, these also include advanced ventilation modes, such as dual-control modes (e.g., PRVC), differential modes for spontaneous ventilation (such as PAV+ and NAVA), and advanced monitoring methods (e.g., measuring the work of breathing, airway occlusion pressure [P 0.1], maximum inspiratory pressure [Plmax], volumetric capnometry, and indirect calorimetry).

Recommendation - In the hospital setting, any ventilator should include at least the following features: (1) control of the expired tidal volume (eVt); (2) basic monitoring tools (at least inspiratory pressure); and (3) a gas blender coupled to the ventilator to avoid the use of O_2 supplementation through the artificial airway.

Recommendation - In addition to the requirements mentioned above, ventilators that are to be used in the ICU should also include the following: (1) curve monitoring (at least the pressure-time curve), (2) alarms (at least for the maximum and minimum airway pressure, for detection of apnea and disconnection from the ventilator).

Comment - The electronic supplementary material includes a list of the mechanical ventilators for adults available in Brazil (in August 2013) with a description of some of their features (Tables 2, 3, 4 and 5 in the supplementary material). This list does not include ventilators that are exclusively used in the following situations: (1) for NIV, (2) in children and newborn infants, (3) at home or for sleep apnea, and (4) in anesthesia.

Monitoring the patient under ventilatory support

Monitoring of gas exchange

How to perform bedside monitoring of the ventilatory mechanics

Recommendation - The ventilatory mechanics should be routinely monitored in all patients who

are subjected to invasive mechanical ventilatory support, including the following parameters: eVt, peak pressure (maximum inspiratory pressure), plateau or inspiratory pause pressure (under controlled ventilation), extrinsic PEEP, auto-PEEP or intrinsic PEEP .[84-88]

Suggestion - Calculate the resistance of airways (Raw) and static compliance (Cst), and monitor the flow-time, pressure-time, and volume-time curves in selected cases.[84-88]

Comment - In clinical practice, the alveolar pressure can be estimated by means of an inspiratory pause lasting at least two seconds. The pressure at the end of the pause is known as plateau or pause pressure. For measurements to calculate the Raw, the inspiratory flow rate must have a "square" wave pattern and be converted to liters/second.

Recommendation - The following are mandatory requirements for accurate measurement of the pause pressure: absence of respiratory muscle effort, pause duration of two to three seconds, and absence of leaks.[84-87]

Recommendation - Avoid alveolar pressure values >28 to 30cmH_2O, which are indicative of low static lung compliance. In such case, the possible cause should be investigated (alteration of the lung parenchyma and/or the thoracic cage). In the former case, reduce the Vt and/or the driving pressure (also called distending pressure); in the latter, also other causes might be present, to wit, reduction of the chest wall compliance and/or intra-abdominal hypertension. In the latter case, the intra-abdominal pressure should be monitored and decompression should be started when needed.[84-88] Figure 4 shows how to calculate Raw and Cst.

Comment - Auto-PEEP, also called intrinsic PEEP (PEEPi), occurs when the end-expiratory pressure is higher than the airway pressure due to incomplete lung emptying.

Recommendation - Auto-PEEP is identified on the flow-time curve when the expiratory flow does not return to zero at the end of expiration.[84-87]

Recommendation - Auto-PEEP or PEEPi should be measured during controlled ventilation; for this purpose, a pause is introduced at the end of expiration (expiratory pause), with full attention to the same warnings as in the measurement of the inspiratory pause.[84-87]

Recommendation - In cases of ARDS, the distending pressure should be monitored; also known as driving pressure, this value is calculated by subtracting PEEP from the plateau pressure (Pplat). The distending pressure should always be ≤15cmH_2O in cases of moderate or severe ARDS, when higher PEEP is necessary, resulting in an increase of Pplat to 30 - 40cmH_2O (see topic: MV in ARDS in the present recommendations).[89-91]

Monitoring of gas exchange in mechanical ventilation

Arterial blood gas measurement

Recommendation - In order to ground clinical reasoning and therapeutic practice, arterial blood gas samples should be collected as soon as possible, preferably from the radial or the femoral artery, in all cases of ARF. Arterial blood gas assessments permit diagnostic assessment of the acid-base status and lung gas exchange through direct measurement of the pH, $PaCO_2$, and PaO_2, and calculation of the oxygen saturation (SaO_2), bicarbonate (HCO_3) and base excess (BE). When intoxication causing methemoglobinemia and carboxyhemoglobinemia is suspected, SaO_2 should be directly measured using co-oximetry.[92,93]

Recommendation - Collect samples for arterial blood gas measurement in all patients subjected to ventilatory support 20 minutes after the initial adjustment of the ventilator parameters, and then every day for the duration of the acute phase of the clinical problem. Samples should also be collected whenever the patient's clinical condition changes.[92,93]

Recommendation - Avoid collecting samples for arterial blood gas measurement from areas irrigated by the artery to be punctured that are at risk of ischemia, and from infected sites. In patients with coagulopathy or thrombocytopenia, samples should only be collected when the test is fully necessary.[92,93]

Care in the performance of the blood gas measurement

Suggestion - Use standard kits or 5-mL syringes with a minimum amount of lithium or sodium heparin, and a fine needle (23 to 25G), preferentially with a safety mechanism.[92,93]

Recommendation - This procedure is invasive, and thus it must be performed under aseptic conditions. Whenever possible, the procedure

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should be explained to the patient and performed only with his or her consent.[92,93]

Recommendation - The puncture site should be compressed for at least five minutes, or longer in cases of coagulopathy or use of anticoagulants.[92,93]

Recommendation - The sample should be analyzed as soon as possible. When analysis is performed outside the unit, the sample should be transported in a refrigerated container.[92,93]

Care in the interpretation of arterial blood gas measurements

Recommendation - Record the following parameters at the time of sample collection: FIO_2, Vt, f, PEEP, SpO_2, and $ETCO_2$ (when capnography is performed).

Recommendation - The PaO_2/FIO_2 ratio should be calculated in all cases to assess the efficiency of oxygenation and the patient's clinical progression.[92,93]

Suggestion - Record whether the patient is in the prone position, the mode of ventilation at the time of sample collection, and if alveolar recruitment maneuvers and PEEP titration were performed before sample collection.

Comment - The arterial blood gas measurement merely reflects a especific moment of the patient's condition. Pulse oximetry and capnography are more adequate methods for continuous monitoring.

Pulse oximetry

Recommendation - Continuous monitoring by means of pulse oximetry should be performed in all patients who are receiving O_2 supplementation, NIV, or invasive ventilatory support, as well as in patients with ARF.

Capnography

Recommendation - Perform capnography in patients with neurologic diseases who are receiving ventilatory support, to confirm the position of the ventilatory prosthesis, and whenever the CO_2 level is above 50mmHg.

Suggestion - Capnography can be used for monitoring in patients with a ventilation-perfusion imbalance to detect acute alterations in status, as well as for monitoring of specific therapies (e.g., thrombolytic therapy in pulmonary thromboembolism).

Regional monitoring

Monitoring by means of electrical impedance tomography

Comment - Electrical impedance tomography (EIT) is a noninvasive technique based on the measurement of electrical current that passes between electrodes placed around the thorax to identify areas that are more and less resistant to the passage of the current. EIT is used for monitoring ventilation, and more recently, for bedside continuous monitoring of lung perfusion.[94-98]

Suggestion - Use EIT for detection of lung ventilation disorders, such as pneumothorax, as well as for evaluating changes in ventilation when placing the patient in specific decubitus position, to check the position of the endotracheal tube, to assess pulmonary recruitment and collapse, and to assess the regional distribution of ventilation. In the future, EIT may be used for monitoring of lung perfusion.[94-98]

Computed tomography

Recommendation - Use computed tomography (CT) as a diagnostic method in cases of respiratory failure of unknown etiology; CT angiography should be used when pulmonary embolism is suspected.

Suggestion - In centers where CT is available, this method may be used to monitor alveolar recruitment and decremental PEEP titration in cases of moderate or severe ARDS, paying special attention to the care required in patient's transportation, and taking the total radiation dose into consideration.[99,100]

Chest ultrasound

Recommendation - In centers where it is available, staff should be trained to use chest ultrasound for early detection of pneumothorax and pleural effusion, and as an aid in performing therapeutic procedures.

Suggestion - Chest ultrasound can be used to estimate alveolar re-aeration in patients treated for VAP, to assess pulmonary edema, to detect post-extubation atelectasis, and to estimate PEEP-induced pulmonary recruitment.[101-103]

Sedation and analgesia during mechanical ventilation

When are sedatives and analgesics indicated and how should they be administered?

Suggestion - Use sedation and analgesia in patients treated with MV in order to control anxiety, agitation and pain. Appropriate sedation helps the patient better tolerate the ventilator, diagnostic and therapeutic procedures.[104,105]

Recommendation - The sedation level should be mild to moderate to allow for early mobilization.[106]

Recommendation - Titrate propofol and midazolam for low, moderate and deep sedation. Dexmedetomidine should not be used to induce deep sedation. The recommended opioids are fentanyl, morphine, and remifentanil.[107]

Suggestion - Avoid using ketamine as the main sedative in patients undergoing MV. Ketamine may be useful in situations in which its opioid-sparing effect is required.[107,108]

Suggestion - Have a thorough knowledge of the main drugs used for analgesia and sedation in patients under ventilatory support:

Measurement of the airway resistence (Raw) and static lung compliance (Cst) under VCV, controlled mode, square waveform flow	
Raw = (Ppeak - Pplat)/Flow Raw - 40-30/1 Raw = 10 cmH_2O/L/s	Cst = eVt/(Plat - PEEP) Cst = 500/30-5 Cst = 20 ml/cmH_2O

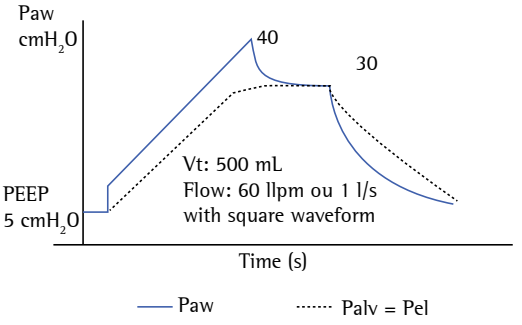

Figure 4 - Inspiratory pause maneuver and estimation of the airway resistance and pause (or plateau) pressure. VCV - volume-controlled ventilation. Paw - airway pressure; PEEP - positive end-expiratory pressure; Vt - tidal volume; Pel - elastic pressure; Palv - alveolar pressure.

Propofol - Its main action is as a gamma-aminobutyric acid (GABA) agonist. It has sedative, hypnotic, anxiolytic and anticonvulsant effects and promotes amnesia. It does not have an analgesic effect. It causes dose-dependent respiratory depression and hypotension secondary to systemic vasodilation, especially when administered by bolus. Prolonged infusion might make awakening unpredictable and cause propofol infusion syndrome (PRIS), which has an incidence of <1%. The mortality of PRIS is high, and the syndrome is characterized by worsening of metabolic acidosis, hypertriglyceridemia, arrhythmia, and hypotension with an increased need for vasopressors. The recommended initial dose is 5mcg/kg/minute over five minutes, followed by continuous infusion at 5 to 50mcg/kg/minute.[107]

Midazolam - This is a GABA agonist that promotes anxiolysis, amnesia and hypnosis. It has anticonvulsant effects. It does not have an analgesic effect. The use of this benzodiazepine for hypnosis seems to be associated with a higher incidence of delirium. Compared to propofol, midazolam may increase the length under MV. Abstinence syndrome can occur after prolonged infusions, i.e., longer than seven days. The recommended initial dose is 0.01 to 0.05mg/kg, and the maintenance dose is 0.02 to 0.1mg/kg/h in continuous infusion.[109]

Dexmedetomidine - This is an alpha-2-adrenergic agonist with central action. It has sedative effects and helps to reduce the need for analgesics/opioids. It does not have an anticonvulsant effect. It is not associated with significant respiratory depression. It is not appropriate for inducing deep sedation. The prevalence of delirium is lower in patients treated with dexmedetomidine compared to benzodiazepines. In patients admitted to the ICU, the recommended loading dose is not used, and the drug is started as a continuous infusion. After the start of infusion, its action begins in 15 minutes, and the maximum effect is reached within one hour. The recommended dose is up to 1.4mcg/kg/hour.[107]

Fentanyl - This has rapid onset of action and high potency. It does not release histamine. It tends to accumulate in parallel with the duration of continuous infusion and in patients with liver dysfunction; in some patients, chest-wall rigidity can occur. The recommended initial dose

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is 50 to 100mcg. For continuous infusion, the recommended rate is 0.7 to 10mcg/kg/h.[108,110]

Morphine - This is the opioid that is most widely used for pain exacerbations. For bedside titration, 1 to 2mg are administered every 10 minutes until adequate analgesia is achieved or side effects appear. For continuous infusion, the recommended dose is 2 to 30mg/h. It tends to accumulate in case of liver or kidney dysfunction. It releases histamine.[110]

Remifentanil - This is an opioid with analgesic potency similar to that of fentanyl. It is metabolized by plasma esterases, and its pharmacological profile does not favor accumulation, even after prolonged infusion. It does not exhibit a residual analgesic effect. The recommended loading dose is 1.5mcg/kg in about three to five minutes, and the recommended maintenance dose is 0.5 to 15mcg/kg/hour.[110]

When should neuromuscular blocking agents be used?

Recommendation - Use cisatracurium during the first 48 hours in cases of ARDS with PaO_2/FiO_2<120 to maintain controlled MV. Induction of neuromuscular blockade requires deep sedation and appropriate monitoring of the level of consciousness. The suggested dose is 37.5mg/hour.[111]

How should sedated patients under mechanical ventilation be monitored?

Recommendation - Monitor the level of sedation using the Sedation and Agitation Scale (SAS) or the Richmond Agitation and Sedation Scale (RASS). Both are tools for clinical use that have been validated for use in the ICU and must be applied systematically, by trained staff.[112,113]

Suggestion - In order to assess the degree of sedation in patients who require neuromuscular blockade or when the use of scales is not possible, use brain activity monitoring methods such as continuous electroencephalography (EEG) or the biespectral index system (BIS).

How to discontinue sedation

Recommendation - Perform daily interruption of sedation in patients receiving MV as soon as the patient's clinical condition allows it. Patients who are awake or might be easily awakened and cooperative with the current sedation strategy do not require discontinuance or interruption of sedation.[110,114]

Recommendation - Pain and delirium should be routinely and frequently assessed and treated, as they commonly cause agitation upon awakening. Maintenance of sedation can contribute to increased MV duration and difficult weaning from MV.[110]

Mechanical ventilation in asthma

Comment - Severe asthma attacks pose a risk to the patient's life. Morbidity and mortality due to asthma has decreased in the past decade as a function of the use of ventilatory strategies that aim to reduce alveolar hyperinflation.[115]

Indications for mechanical ventilation

Recommendation - Indications for invasive MV in asthma include the following: cardiac arrest; respiratory arrest; reduced level of consciousness, Glasgow Coma Scale <12; hypoxemia (PaO_2<60 mmHg; SpO_2<90%) that is uncorrected by use of face-mask oxygen supplementation (FiO_2 40-50%); severe arrhythmia; or progressive fatigue (progressive hypercapnia).

Suggestion - Suggested indications for invasive MV in asthma include myocardial ischemia and lactic acidosis after treatment with bronchodilators.[116,117]

Intubation of patients with an asthma attack

Recommendation - Perform rapid sequence intubation.

Suggestion - Place the patient in a 20-30°-degree, head-up position (which reduces the risk of passive regurgitation and aspiration).

Recommendation - Perform pre-oxygenation with O_2 mask or BIPAP; use a bag-valve mask gently (eight respiratory cycles).

Suggestion - Perform premedication with intravenous (IV) lidocaine at 1.5mg/kg, three minutes before intubation (this reduces the sympathetic reflex and the occurrence of nausea and vomiting) and fentanyl 3mcg/kg (this reduces the sympathetic reflex but may cause respiratory depression).

Suggestion - Do not perform the Sellick maneuver.

Recommendation - For inducing intubation, select ketamine 1 to 2mg/kg IV, propofol 2 to 2.5mg/kg IV, or etomidate 0.2 to 0.3mg/kg IV.

Recommendation - To induce muscle relaxation, use rocuronium 0.9mg/kg or succinylcholine 1 to 1.5mg/kg IV (fasciculation may increase the risk of regurgitation and aspiration).[118,119]

Suggestion - Another option for inducing muscle relaxation is vecuronium 0.3mg/kg (disadvantage: onset of action is 60-90 seconds).

Suggestion - Use a tube with the largest possible diameter (>8 mm of internal diameter when possible).

Ventilator settings

Suggestion - ventilatory settings are as follows: Mode: PCV or VCV; Vt: 6mL/kg predicted body weight; maximum inspiratory pressure: <50cmH_2O; plateau pressure: <35cmH_2O; auto-PEEP: <15cmH_2O; f: 8 to 12 breaths/minute; flow rate: as needed to maintain an expiratory time sufficient to end expiration; 60 to 100L/minute (VCV); free (PCV); FiO_2: as necessary to maintain SpO_2>92%; PaO_2>60mmHg; PEEP: low (3 to 5cmH_2O); in selected cases (however, with appropriate monitoring, higher values of PEEP can be used, due to its mechanical effect of opening the small airways).[116,117]

Patient monitoring and reduction of hyperinflation

Recommendation - Patients with asthma who are receiving MV should be periodically monitored for alveolar hyperinflation (plateau pressure and intrinsic PEEP) and calculation of the airway resistance. The peak pressure is not a representative measure of alveolar hyperinflation.[120,121]

Recommendation - Use a Vt of 5 to 6mL/kg predicted body weight. In cases with hyperinflation refractory to conventional treatment, consider volumes <5mL/kg and lower f (10 to 12 breaths per minute) to avoid alveolar hyperinflation. This strategy may cause hypercapnia; therefore, the $PaCO_2$ should be monitored and maintained <80mmHg, and the pH should be maintained >7.20 (permissive hypercapnia).

Suggestion - Use PEEP as a strategy to reduce alveolar hyperinflation. In such cases, use PCV with a distending pressure of ≤15cmH_2O. When PEEP increases, the expiratory volume may increase, which indicates a reduction in alveolar hyperinflation, or deflation.

Recommendation - Monitor ventilatory mechanics in patients who have hemodynamic instability in order to detect the presence of auto-PEEP, and readjust the parameters as needed to improve hemodynamic status.[122]

Recommendation - Perform chest radiographs in patients with hemodynamic instability, due to the risk of pneumothorax.[120,121]

Recommendation - Weaning from ventilation must be started as soon as bronchospasm and alveolar hyperinflation are controlled.[1,8]

Suggestion - Patients with asthma can be extubated under mild sedation.

Suggestion - In cases where ventilator weaning is difficult, investigate the presence of respiratory muscle weakness due to polyneuropathy associated with the use of corticoids and curare.

Analgesia and sedation[123-126]

Suggestion - Avoiding the use of morphine is suggested, as morphine may increase histamine release. Do not use meperidine, because it also may increase histamine release. The following agents can be used: fentanyl 1 to 3mcg/kg/hour; alfentanil 0.5 to 1mcg/kg/minute; sufentanil 0.5mcg/kg/hour; ketamine 0.25 to 0.5mg/kg/hour (bronchodilator); propofol 0.3 to 4mg/kg/hour (bronchodilator); or midazolam 0.04 to 0.06mg/kg/hour (3 to 5mg/hour).

Muscle relaxation[126-128]

Recommendation - Muscle relaxation may be performed as needed to allow intubation during the initial stage of MV. Long-lasting use should be avoided due to an associated risk of myopathy and neuropathy (a risk that is increased by the concomitant use of corticoids).

Recommendation - Rocuronium is the drug of choice, with a dose of 1mg/kg, an onset of action 45 seconds, and duration of action of 45 minutes. Sugammadex can be used as an antidote, if needed.

Suggestion - The suggested muscle relaxants for use during MV are vecuronium (0.15mg/kg, onset of action 75-90 seconds, and duration of action 30 minutes) or succinylcholine at a dose of 1 to 1.2mg/kg (up to 1.5mg/kg) for intubation at induction. Succinylcholine is contraindicated in patients with a history of malignant hyperthermia,

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neuromuscular disease, muscular dystrophy, hyperkalemia, or rhabdomyolysis, as well as for use up to 72 hours after burns or up to 72 hours after stroke.

Suggestion - Do not use pancuronium; although the risk of histamine release is low, it is higher than that of vecuronium or rocuronium.

Recommendation - Do not use atracurium or cisatracurium due to the high risk of histamine release.

Additional treatment - use of anesthetics, heliox and extracorporeal membrane oxygenation

Suggestion - Use halogen-based anesthetics (e.g., isoflurane) administered through an anesthesia ventilator for possible control of bronchospasm that is refractory to usual treatment; this therapy should not be used for longer than 12 hours. Special attention should be paid to monitoring for liver injury during their use.[122]

Suggestion - Heliox may reduce airway resistance and facilitate the delivery of bronchodilators to the lungs; its use can be attempted in refractory cases and in services where the appropriate equipment for the use of heliox is available.[129]

Suggestion - Extracorporeal membrane oxygenation (ECMO) can be considered for severe cases that do not respond to the abovementioned treatments.[130]

Mechanical ventilation in chronic obstructive pulmonary disease

Indications for invasive mechanical ventilation

Recommendation - Consider invasive MV when NIV is contraindicated or fails (25% of cases). Optimize pharmacological treatment.

Suggestion - For OTI, use tubes with the largest possible diameter, ideally > 8 mm, to reduce airway resistance and facilitate the removal of secretions.[131-133]

Aims of mechanical ventilation

Recommendation - To promote respiratory muscle rest and improvement of acute gas exchange disorders, reduce lung hyperinflation, and optimize patient-ventilator synchrony.[131,134,135]

Initial mode of ventilation

Suggestion - Any mode of ventilation (volume- or pressure-controlled) can be used in the initial treatment of COPD exacerbations, provided monitoring is adequate and the staff is thoroughly acquainted with the selected mode.[131-136]

Fraction of inspired oxygen

Suggestion - Adjust FiO_2 based on the arterial blood gas measurement and pulse oximetry so as to use the lowest FIO_2 level that can maintain SaO_2 at 92 to 95% and PaO_2 at 65 to 80mmHg.[131]

Tidal volume

Recommendation - Use a low Vt, specifically 6mL/kg predicted body weight.[131,132-136] In PCV and PSV, monitor for excess Vt that can occur when pressure levels are low.

Ventilation frequency and minute volume

Recommendation - Initially set f at 8 to 12 breaths per minute. Minute volume should be adjusted to achieve a normal pH, rather than a normal $PaCO_2$.[131,132-136]

Inspiratory flow and inspiration-to-expiration ratio

Recommendation - In the controlled-volume mode, use a decelerating inspiratory flow rate of 40 to 60L/min, and adjust the I:E rate to <1:3, thus allowing for an expiratory time long enough to promote pulmonary deflation and reduce air trapping. In the pressure-controlled mode, set the lowest distending pressure value that allows for an inspiratory time sufficient to reduce the ventilator inspiratory flow to zero (lung filling time). The I:E ratio should be kept at <1:3 to achieve a sufficient expiratory time with minimal auto-PEEP.[131,132-136]

Use of PEEP in controlled ventilation

Suggestion - Apply external PEEP to counterbalance auto-PEEP caused by the expiratory flow limitation, or as an attempt to induce lung deflation, provided that respiratory mechanics are adequately monitored. For this purpose, the plateau pressure value should be used in the VCV

and PCV modes.[131,137,138] In VCV, external PEEP-induced deflation is determined by the maintenance or fall of the plateau pressure. However, when the plateau pressure increases, external PEEP can cause additional lung hyperinflation and thus should be reduced or discontinued. In PCV, the expired Vt should be monitored in parallel with the increase in PEEP. When the expired Vt decreases, hyperinflation becomes worse, and external PEEP should be reduced or discontinued. On the contrary, when the expired Vt increases, external PEEP induces lung deflation and can be maintained.[131,137,138]

Use of PEEP in assisted/spontaneous ventilation

Suggestion - In the case of pressure-triggered ventilation, patients with auto-PEEP may find it difficult to start an assisted cycle, thus resulting in asynchrony. In such cases, flow-triggering should be used and/or external PEEP should be applied at approximately 85% of auto-PEEP to help the patient reach the ventilator trigger threshold.[134,139,140]

Monitoring of mechanical ventilation

Recommendation - In COPD exacerbations, the respiratory mechanics and lung hyperinflation should be monitored. The main parameters to monitor are: plateau pressure, peak pressure, auto-PEEP, airway resistance, as well as the flow-time, volume-time, and pressure-time curves. In cases with severe bronchospasm, a peak pressure as high as 45cmH_2O may be well tolerated, provided the plateau pressure is <30cmH_2O.[131,132]

Discontinuation of mechanical ventilation

Suggestion - Patients with COPD usually have greater difficulty in achieving an appropriate patient-ventilator interaction. Therefore, it is suggested to use modes of ventilation that afford greater comfort to the patient and facilitate monitoring. In this regard, PSV is quite useful when it is properly set. Special attention should be paid to high support pressure values, as they can hinder cycling and worsen the patient-ventilation interaction, resulting in increased auto-PEEP. PAV+ and NAVA are promising approaches for improving the patient-ventilator interaction, but more evidence is needed before these modes are routinely recommended.[141-143]

Suggestion - The deceleration of the inspiratory flow rate is lower in patients with COPD, and the inspiratory time may be increased in PSV with the usual expiratory sensitivity (25%). In ventilators that allow adjustment of PSV cycling (percentage of the cycling criterion, expiratory sensitivity or cycling-off criteria), adjust the expiratory cycling sensitivity to a higher level (40% to 60%), aiming to reduce the inspiratory time, Vt and the odds of asynchrony.[144,145]

Suggestion - To reduce the inspiratory time, with a consequent increase in the expiratory time, adjust the inspiratory flow rise time to a higher level, taking proper care to avoid inspiratory flow overshoot and monitor the patient's comfort.[144,145]

Recommendation - After 24 to 48 hours of muscle rest, use NIV for early discontinuation of invasive MV in patients with COPD exacerbations provided the staff is duly trained and the criteria described in the specific corresponding topic in the present recommendations are followed.[146]

Administration of inhaled bronchodilators

Suggestion - Administer bronchodilators per the inhalation route using nebulizers or a metered-dose spray coupled to a spacer. Advantages of the metered-dose spray include ease of manipulation, a reproducible dose, and a lower risk of contamination.[147] When beta-2-adrenergic agonists are administered using a metered-dose spray, the suggested dose is four puffs (first at 20-minute intervals for to three doses, and every two to four hours as maintenance treatment).[148]

Mechanical ventilation in community-acquired pneumonia

Comment - The following recommendations apply to patients with CAP and healthcare-associated pneumonia (HCAP) and concern invasive mechanical ventilation and NIV. In the case of pneumonia associated with ARDS, see the specific topic in the present recommendations.

Noninvasive mechanical ventilation

Suggestion - Use NIV cautiously in individuals with severe pneumonia. Use of NIV should be monitored at the bedside by a healthcare

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professional within thirty minutes to two hours. For NIV to be considered successful, the following criteria should be met: reduction of f, increase of Vt, improvement of the level of consciousness, reduction or cessation of the use of the respiratory accessory muscles, increase of PaO_2 and/or SpO_2, and reduction of $PaCO_2$ without significant abdominal distension. In unsuccessful cases, OTI and invasive MV should be performed immediately, as delay in intubation reduces the patient's' survival. A better response to NIV is achieved under the following three circumstances: patients with systolic or diastolic left-sided cardiac failure; COPD with CO_2 retention and acidosis; and immunosuppressed individuals with bilateral pneumonia. Success is expected in 75% of patients with hypercapnia and 50% of patients with hypoxia.[149,150]

Mode of ventilation

Suggestion - The choice of the mode of ventilation[151-154] should be based on three criteria: the multi-professional staff's knowledge of and skills in using the selected mode; ventilator availability; and the clinical indication, which is mainly based on the presence of respiratory stimulus, hemodynamic instability and the severity of the lung injury.

Positive end-expiratory pressure

Suggestion - In the absence of ARDS, use PEEP values of 5 to 10cmH_2O. The PEEP value should be adjusted in combination with the FiO_2 to keep SpO_2 at 90 to 95%, in order to minimize the risk of cognitive impairment. MV with very low or no PEEP is associated with greater bacterial translocation.[151-155] For patients with ARDS, see the specific topic in the present recommendations.

Adjusting the fraction of inspired oxygen

Suggestion - The FiO_2 value should be adjusted in combination with PEEP in order to keep SpO_2 at 90 to 95%, thus minimizing the risk of cognitive impairment.[151-155]

Tidal volume

Suggestion - A Vt of >6mL/kg ideal body weight increases bacterial translocation and ventilator-associated lung injury. Therefore, patients should be ventilated with a Vt of ≤6mL/kg predicted body weight.[151-155]

Decubitus

Suggestion - Patients with unilateral pneumonia and severe hypoxemia can be placed in the lateral decubitus position. However, close surveillance is needed in such cases given the unpredictability of the results, as there is a higher risk of worse oxygenation and contamination of the contralateral lung.[156]

Rescue treatment

Suggestion - Patients with unilateral pneumonia and hypoxemia that is refractory to conventional treatment may be candidates for independent MV. This treatment, however, should be performed only at centers with wide experience in independent lung ventilation and an available bronchoscopy service.[157]

Ventilator-associated pneumonia

Suggestion - Patients with VAP should be ventilated using a protective ventilation strategy (Vt=6mL/kg predicted body weight), f to maintain $PaCO_2$ at 35 to 45mmHg, and PEEP sufficient to ensure appropriate gas exchange, with either the VCV or PCV modes). Shift to assisted or spontaneous modes as soon as possible to achieve earlier discontinuation of MV.

Suggestion - Patients with unilateral pneumonia and severe hypoxemia can be placed in the lateral decubitus position. However, close surveillance is needed in such cases given the unpredictability of the results, as there is risk of worse oxygenation and contamination of the contralateral lung. New proposals for positioning to reduce the aspiration of secretion above the cuff and prevent VAP are currently being investigated, such as the Trendelenburg lateral decubitus position.[158]

Recommendation - Use the following general strategies to reduce VAP: wash/disinfect the hands with 70% alcohol; perform microbiological surveillance; monitor and remove invasive devices as soon as possible; and apply programs for the rational use of antibiotics.

Recommendation - The ventilator circuits should be replaced when they become dirty or damaged, since there is no need for planned

replacement. Replacement of humidifiers must be performed every seven days or as needed.

Recommendation - Perform aspiration of subglottic secretions when the patient requires MV for more than 72 hours; ventilation can be intermittent or controlled by a device that is specifically designed for this purpose.[159]

Suggestion - When available, use tubes with cuffs that are specifically designed to avoid microaspiration in patients who are estimated to require MV for at least 24 hours.

Recommendation - Set, monitor, and maintain the endotracheal tube cuff pressure at a level of at least 25cmH_2O.[160]

Recommendation - The patient should be placed in the 30-45°-degree, head-up position.

Recommendation - Perform oral hygiene with 2% chlorhexidine on a daily basis.[161]

Suggestion - Perform daily interruption of sedation.

Suggestion - Perform selective decontamination of the digestive tract.[162,163]

Recommendation - Use silver-coated endotracheal tubes whenever intubation is estimated to last more than 24 hours.[164]

Mechanical ventilation in patients with sepsis

Comment - ARDS is a common complication in patients with severe sepsis, although it is underdiagnosed in most cases. Observational studies showed that a diagnosis of ARDS is registered in the clinical records of only 30% to 50% of individuals who were shown to have diffuse alveolar injury on autopsy.[165-167] For this reason, special attention to the possible presence of ARDS in patients with sepsis has paramount importance. Some interventions have proven efficacy in patients with ARDS, such as ventilation with Vt between 4 to 6mL/kg of predicted body weight. However, these interventions still need to be more widely disseminated, applied, and audited in clinical practice.[168-170] Lack of appropriate diagnosis is a possible reason for the low rates of institutional adherence to appropriate treatment.[166]

Suggestion - Apply a routine system for identifying ARDS in patients with sepsis, particularly in patients with severe sepsis and septic shock. A decrease in the PaO_2/FiO_2 ratio and the presence of bilateral infiltrates on the chest radiograph as diagnostic criteria may be possible diagnostic criteria for ARDS; clinicians should also monitor patients for signs that may represent early manifestations of ARDS[171,172] (increased f, decrease of SpO2 and need for O_2 supplementation) as early alerts.

Observation - The diagnosis and management of ARDS are described in sections "Mechanical ventilation in ARDS" and "Ventilation in the prone position and extracorporeal circulation" of the present recommendations.

Recommendation - Use a Vt of approximately 6mL/kg predicted body weight in patients who are undergoing MV and have sepsis but not ARDS. A systematic review that included randomized and observational studies on patients who underwent surgery or were admitted to the ICU suggests that ventilation at a low Vt is associated with reduced mortality, as well as a reduced incidence of ARDS and pneumonia, compared to ventilation at a high Vt.[52]

Observation - Recommendations for patients undergoing MV with pneumonia and sepsis but without ARDS, are included in the section "Mechanical ventilation in pneumonia".

Mechanical ventilation in acute respiratory distress syndrome: diagnosis, recommendations and special care

Comment - Starting in 2012, ARDS was classified (Berlin Definition) into three categories (mild, moderate and severe)[173] (Chart 6).

How to ventilate patients with ARDS

Modes of ventilation

Recommendation - Initially (first 48 to 72 hours), controlled modes of ventilation (VCV or PCV) are recommended for all patients with ARDS (i.e., mild, moderate and severe cases). In PCV, the airway pressure is equal to the plateau or alveolar pressure when the respiratory flow falls to zero.

Tidal volume[55,174,175]

Recommendation - In patients with mild ARDS who require assisted ventilation, Vt should be set at 6mL/kg (predicted body weight).

Recommendation - In patients with moderate or severe cases of ARDS who require assisted or

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controlled ventilation, Vt should be set at 3 to 6mL/kg (predicted body weight).[176]

Recommendation - Use the following formulas to calculate the predicted body weight:[175] males: 50 + 0.91 x (height in cm - 152.4); females: 45.5 + 0.91 x (height in cm - 152.4).

Fraction of inspired oxygen

Recommendation - Use the lowest possible FiO_2 that suffices to ensure SpO_2>92% in all three ARDS categories.

Plateau pressure

Recommendation - Try to maintain the plateau pressure (Pplat) ≤ 30cmH_2O.[175,177]

Recommendation - Try to keep the difference between Pplat and PEEP (known as distending pressure or driving pressure) ≤15cmH_2O in all three ARDS categories.[91]

Suggestion - When high PEEP (usually >15cmH_2O) is used in patients with moderate or severe ARDS, a Pplat of up to 40cmH_2O may be tolerable, provided that the driving pressure is always maintained at ≤15cmH_2O.[91]

Respiratory rate

Recommendation - Begin with f = 20 breaths per minute, and increase to up to 35 breaths per minute as needed in order to achieve the desired $PaCO_2$ (<80mmHg), provided that auto-PEEP is not induced. In patients with moderate or severe ARDS who are subjected to permissive hypercapnia with a Vt of ≤6mL/kg predicted body weight, f may be as high as 45 breaths per minute, provided that this f does not cause auto-PEEP.[175]

PEEP adjustment

Comment - There are several strategies for adjusting PEEP in ARDS, many of which are in equipoise (i.e., the degree of evidence does not allow for a definitive conclusion on the superiority of any of them). The techniques where there is wider experience and that have proved to be safer in clinical studies are described in this topic.

Recommendation - Avoid using PEEP <5cmH_2O in patients with ARDS.[55,175]

Recommendation - Avoid using PEEP below the values described in the table "LOW PEEP versus FIO_2" (Table 1).[175]

Suggestion - Use the table "LOW PEEP versus FIO_2" (Table 1) only in cases of mild ARDS.[175]

Comment - There are two options for adjusting high PEEP, corresponding to the ALVEOLI[178] and LOVS[179] studies that are described in Table 2; these studies demonstrated very similar practical results. The LOVS table tends to subject the patient to longer periods of high PEEP.

Suggestion - Use Table 2 in cases of moderate or severe ARDS as an alternative to the decremental PEEP technique, which is described below.

Suggestion - The Express study suggests using Pplat of a maximum of 30 cmH_2O and maximum PEEP with a Vt of 6 mL/kg predicted body weight in cases of moderate or severe ARDS.[180]

Suggestion - Avoid using Table 2 in cases of mild ARDS.[181]

Decremental PEEP titration according to respiratory system compliance

Recommendation - The decremental PEEP technique is described in the following section. After a maximum recruitment strategy (MRS) is performed, the elastic compliance of the respiratory system is measured at decremental PEEP values starting at 23 to 26cmH_2O to a minimum of approximately 8 to 12 cmH_2O. PEEP is typically decreased in steps of 2 to 3cmH_2O every four minutes. Once the PEEP level that induces the best compliance is identified, or two or more reduction steps with equivalent compliance are observed, a PEEP value 2 to 3cmH_2O above that level is selected. Before the PEEP value thus found to be adequate is finally selected, a MRS is performed again. PEEP may then be directly set at 2 to 3cmH_2O above the value identified by decremental titration.[182-184]

Suggestion - Consider MRS in cases of moderate or severe ARDS.[182-184]

Decremental PEEP titration by other methods

Suggestion - Perform decremental PEEP titration using EIT in centers where it is available: following MRS, select the PEEP value that is associated with a collapse increase of less than 5%, as estimated by EIT.[95]

Suggestion - Perform decremental PEEP titration using conventional CT. Following MRS, select the PEEP value that is associated with a collapse increase of less than 5%, as estimated by CT. If this method is used, all issues related to patient care, transportation, and safety should be taken into consideration, only staff who have been specifically trained for this procedure should be involved, and low radiation doses should be used.[91]

Suggestion - Based on oxygenation after MRS, select the PEEP value that is associated with a <10% reduction in the PaO_2/FIO_2 ratio.

Estimation of the lower inflection point using the random volumes technique

Recommendation - With the patient sedated and without an active ventilatory drive, set PEEP to zero and vary the Vt in 50mL aliquots to a maximum Vt of 1,000mL or a Pplat of 40cmH_2O, and record the Pplat value after three ventilations. Record the measurements in a Vt versus Pplat table and plot an x-y graph (Vt on the y-axis and Pplat on the x-axis). A sigmoid curve is expected. Identify the curve trends and the lower inflection point (meeting point of the trend lines in the first curvature, projecting the value on the x-axis) and set PEEP 2.0cm above the lower inflection point.[55]

Estimation of the point of best compliance (compliance-PEEP technique)

Recommendation - With the patient sedated and without active ventilatory drive, set Vt at 6mL/kg predicted body weight, and vary PEEP in 2 to 3cmH_2O aliquots; record Pplat after three ventilations. Record the measurements in a PEEP versus static compliance table (for how to calculate the static compliance of the respiratory system (Cst), see section "Monitoring the patient under ventilatory support" in the present recommendations) to find the PEEP value that provides the best Cst. Set PEEP 2.0 cm H_2O above that value. If the best Cst value corresponds to two PEEP levels, the ideal PEEP should be considered to be the highest one.

Neuromuscular blocking agents

Recommendation - In cases of ARDS with pO_2/FiO_2<120mmHg under deep sedation, use cisatracurium during the first 48 hours of ventilatory support.[111]

Prone positioning

Recommendation - Use prone positioning in patients with ARDS and a PaO2/FiO2 ratio of <150 for at least 16 hours per session. (More details are provided in the specific topic of the present recommendations).[185]

Recommendation - Discontinue prone positioning as soon as a PaO2/FiO2 ratio >150mmHg is attained with a PEEP of ≤10cmH_2O in the supine position.[17]

Suggestion - In patients with moderate or severe ARDS, use prone positioning for patients with right ventricular dysfunction and controlled hypoxemia, as well as in cases where it is difficult to maintain lung protection within the safety threshold (distending pressure ≤15cmH_2O and pH >7.15).[185,186]

Maximum alveolar recruitment maneuvers or maximum recruitment strategy

Suggestion - In patients with moderate or severe ARDS, perform MRS as a part of the lung protective strategy to reduce the driving pressure following adjustment of decremental PEEP.[91,187]

Recommendation - MRS should be performed in PCV mode with a distending pressure of 15cmH_2O. Start with PEEP=10cmH_2O and increase

Chart 6 - The Berlin classification of acute respiratory distress syndrome[173]

Criterion	Mild	Moderate	Severe
Timing	Acute onset within one week of a known clinical insult or new or worsening respiratory symptoms		
Hypoxemia (PaO_2/FIO_2)	201-300 with PEEP/CPAP ≥5	101-200 with PEEP ≥5	≤100 with PEEP ≥5
Origin of edema	Respiratory failure not fully explained by cardiac failure or fluid overload		
Chest imaging	Bilateral opacities	Bilateral opacities	Bilateral opacities

PaO_2/FIO_2 - relationship between oxygen partial pressure and fraction of inspired oxygen; PEEP - positive end-expiratory pressure; CPAP - continuous positive airway pressure.

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PEEP by 5cmH_2O every two minutes until it reaches 25cmH_2O; thereafter, PEEP should be increased sequentially by 10cmH_2O until it reaches 35cmH_2O, or 45cmH_2O at most. Next, reduce PEEP to 25cmH_2O and start decremental PEEP titration (as described in the section above).[91,187]

Recommendation - Place a central venous access device and perform continuous invasive blood pressure monitoring.[91,187]

Recommendation - In patients with refractory hypoxemia that does not respond to prone positioning, perform MRS followed by readjustment of PEEP by means of the decremental technique; initiate rescue therapy in eligible patients, with full adherence to the monitoring and safety norms included in the present recommendations.[91,187]

High-frequency ventilation

Recommendation - Avoid the use of high-frequency ventilation as adjuvant therapy.[188]

Nitric oxide

Suggestion - Use inhaled nitric oxide (NO) in patients who have severe ARDS with acute pulmonary hypertension and right ventricular failure; monitor the response and titrate the dose as parts per million (ppm).[187]

Extracorporeal membrane oxygenation (venovenous)

Recommendation - In patients with refractory hypoxemia, which is defined as a P/F ratio <80mmHg with an FiO_2>80% after at least three hours of adjuvant and rescue maneuvers for severe ARDS, use veno-venous ECMO when this technology is available. More details are given in the corresponding topic in the present recommendations.[187]

Ventilation in the prone position and extracorporeal circulation

Ventilation in the prone position: when should it be performed?

Recommendation - When it is indicated, ventilation in the prone position should be performed during the first 48 hours of MV.[185,189-191]

Indications

Recommendation - Avoid routine ventilation in the prone position in mild ARDS.[185,189-191]

Suggestion - Use ventilation in the prone position in the following situations: after PEEP titration in patients with moderate ARDS,[185,189-191] when there is moderate-to-severe acute right ventricular failure (acute cor pulmonale); when protective ventilation cannot be maintained; or when a distending pressure >15cmH_2O, a f >35 breaths per minute, and a pH of <7.2 are needed.

Recommendation - Prone positioning should be started early (within 48 hours of the diagnosis of ARDS) in cases of ARDS with a PaO2/FiO2 ratio <150.[185,189-191]

How long should prone positioning be maintained?

Recommendation - Maintain prone positioning for a period of 16 to 20 hours, with continuation

Table 1 - PEEP versus FiO_2 to identify optimal PEEP in cases of mild ARDS

FiO_2	0.3	0.4	0.4	0.5	0.5	0.6	0.7	0.7	0.7	0.8	0.9	0.9	0.9	1.0
PEEP	5	5	8	8	10	10	10	12	14	14	14	16	18	18↔24

Adapted from: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. N Engl J Med. 2000;342(18):1301-8. FiO_2 - fraction of inspired oxygen; PEEP - positive end-expiratory pressure.

Table 2 - Adjustment of PEEP at high values to find the optimal PEEP in cases of moderate or severe ARDS

Study ALVEOLI table										
FiO_2	0.3	0.3	0.4	0.4	0.5	0.5	0.5↔0.8	0.8	0.9	1.0
PEEP	12	14	14	16	16	18	20	22	22	22↔24
Study LOVS table										
FiO_2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0		
PEEP	5↔10	10↔18	18↔20	20	20	20↔22	22	22↔24		

Source: based on studies ALVEOLI[178] and LOVS[179]. FiO_2 - fraction of inspired oxygen; PEEP - positive end-expiratory pressure.

of all appropriate protective measures and monitoring.[185,189-191]

What are the contraindications to the use of prone positioning?

Recommendation - Prone positioning is contraindicated in the following conditions:[185,189-191] intracranial hypertension, pelvic fractures, spine fractures, intra-abdominal hypertension (relative contraindication), laparotomy, pregnancy (relative contraindication), flail chest, severe hemodynamic instability, and inexperienced staff.

Techniques and special care for prone positioning

Recommendation - The following techniques and care procedures should be observed:[185,189-191] raise FiO_2 to 100% while shifting the patient's position; if PCV is used, pay attention to possible reductions in the exhaled volume; sedation and analgesia should be optimized; place a central venous access device and perform continuous invasive blood pressure monitoring; place pillows under the patient, distributed to reduce the pressure on the main anatomical points of support; place cushions below the pelvic and shoulder girdle to reduce abdominal compression; place protective (hydrocolloid) dressings on the patient's forehead, face, knees and shoulders; consider placing an absorbent diaper on the patient's face, and change it whenever it gets too wet; perform electrocardiogram monitoring with the electrodes placed on the patient's back; move the patient, especially his or her head, taking care with anatomical points of support of the face, at least every two hours; change the arm position above and below the interscapular line every two hours at least; maintain enteral nutrition with lower volumes; and check that the patient's eyes are closed. Prone positioning can be maintained for as long as needed, provided that signs of skin or other organ pain attributable to prone positioning do not appear. The response to the shift to prone position should be monitored based on the SpO_2, if desaturation <90% remains 10 minutes after body rotation, shift to the supine position. The patient should also be shifted to the supine position in case of cardiac arrest, severe hemodynamic aggravation, malignant arrhythmia, or suspected displacement of the ventilatory prosthesis. Repositioning should be performed by three to five persons, and specific training should be provided to the staff, using videos such as those available at the following websites (PROSEVA - three people - http://www.youtube.com/watch?v=E_6jT9R7WJs and William Harvey Hospital - five people - http://www.youtube.com/watch? v=Hd5o4ldp3c0). An arterial blood gas sample should be collected after one hour of prone positioning. A patient should be considered a responder to prone positioning when the pO_2/FiO_2 ratio increases by 20 or the PaO_2 increases by 10mmHg.

Extracorporeal gas exchange

Comment - Extracorporeal lung assist may in removing CO_2 or performing extracorporeal membrane oxygenation.[179,180,192,193]

What are the indications of extracorporeal gas exchange?

Recommendation - The mandatory criteria for initiation of extracorporeal gas exchange include the following: tracheal intubation and MV; patient ≥18 years old; acute lung disease; reversible lung injury (in some centers in which this technology is available, it is suggested for patients with irreversible lung disease awaiting transplantation); ARDS with PEEP≥10cmH_2O; and an experienced center. At least one of the following complementary criteria must also be met: hypoxic patients should have a PaO_2/FiO_2≤80, with an FiO_2 of ≥0.8 for at least three hours, despite the performance of rescue maneuvers; hypercapnic patients should have a pH of ≤7.20 with a f = 35 breaths per minute, a Vt of 4 to 6mL/kg predicted body weight, and a mandatory driving pressure of ≤15cmH_2O.

What are the contraindications for extracorporeal gas exchange?

Recommendation - Extracorporeal gas exchange is contraindicated in the following situations: dying patients; patients with a body mass index > 40 - 45kg/m^2; coma (non-sedated patients) after cardiac arrest; patients with irreversible chronic lung disease; lack of accessible and safe vascular access with an appropriate caliber catheter; a life-limiting chronic illness without the perspective of cure; and heparin-induced thrombocytopenia (HIT).[179,180,192,193]

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What devices are used for extracorporeal gas exchange?

Suggestion - Arteriovenous (A-V) interventional lung assist (ILA) is suggested for CO_2 removal in patients without hemodynamic instability.

Suggestion - Circulatory assistance using ECMO is suggested for oxygenation and CO_2 removal.

Extracorporeal membrane oxygenation - techniques and special care

Recommendation - The following techniques and procedures should be used for ECMO. A polymethylpentene membrane should be used for either CO_2 removal or ECMO. Staff must have extensive experience with the technique (knowledge of the ECMO system and patient physiology, as well as of the most common complications and how to treat them). Improvisation is not acceptable. The venous and percutaneous routes are the first choice for access; cannulas >18 Fr are preferred; if arterial access is needed, and the artery diameter is not >4mm larger than the cannula diameter, seriously consider the use of a distal perfusion cannula before the proximal cannula is placed. Provide safe anticoagulation and monitor the activated partial thromboplastin time (aPTT) and the platelet count every six hours. Initial ventilation should be ultra-protective, using the following settings: controlled ventilation at FiO_2<0.6; PEEP=10cmH_2O, distending pressure of 10cmH_2O and/or Vt <4mL/kg; and f = 10 breaths per minute.[179,180,192,193]

Recommendation - In venovenous ECMO, maintain ECMO FiO_2=1 and the lowest possible blood flow rate that is sufficient to maintain the arterial saturation > 90%; maintain the membrane ventilation flow in order to keep the pH at 7.35 - 7.40.

Recommendation - When PSV is used, attempt to achieve the lowest possible work of breathing while preserving patient-ventilation synchrony by using protective ventilation parameters (distending pressure <15cmH_2O).

Recommendation - In patients with blood flow rate >5,000 - 6,000mL/minute and SaO_2<85%, consider the following options: increase the ventilator FiO_2; control agitation; check and correct for recirculation; control the systemic temperature; increase PEEP; induce deep sedation and use neuromuscular blocking agents; perform alveolar recruitment; and consider other options, such as beta-blockers, nitric oxide, prone positioning, and permissive hypoxemia.

Interventional lung assistance - techniques and special care

Recommendation - The following techniques and procedures should be used for ILA. An echo Doppler should be performed to establish the diameter of the femoral artery and vein, and an ultrasound-guided technique should be used for insertion of the cateters. Cardiac output and perfusion pressure in the system must be ensured (maintain mean arterial pressure >70mmHg), and system flow should be monitored continuously using ultrasound. O_2 titration should begin at 1L/min and is not to exceed 10L/min. The arterial pH should be monitored in parallel with CO_2 removal, especially in patients with intracranial hypertension. Protective ventilation should be maintained as was described above for ECMO. Safe anticoagulation should be provided, with monitoring of the aPTT, the fibrinogen level, and the platelet count at least every six hours. Consider removal of cateters under direct visualization (surgical intervention) to reduce vascular complications.[179,180,192,193]

Adjuvant techniques

Nitric oxide

Comment - The aim of NO use is adjustment of the ventilation/perfusion ratio through vasodilation of the pulmonary artery territory in ventilated areas.

Recommendation - Do not use routinely.

Suggestion - NO may be used when there is acute cor pulmonale, or severe and refractory hypoxemia.

Recommendation - The following techniques and procedures should be employed when NO is used. The NO cylinder should be coupled to its own closed system, with a monitor for inhaled NO and NO_2. The initial dose should be 5ppm, and NO_2 should be maintained at <10ppm. Invasive hemodynamic monitoring by means of a thermodilution catheter is preferred. Patients should be monitored for changes in kidney function and methemoglobinemia; do not use NO in patients with methemoglobin reductase

deficiency. The patient should not be cared for by pregnant health care providers.[194]

Heliox

Comment - The aim of using heliox is to reduce airway resistance and the work of breathing.

Suggestion - Heliox may be used in conditions that are associated with lower airway obstruction to facilitate the maintenance of invasive or noninvasive ventilatory support.[195,196]

Recommendation - The following techniques and procedures should be employed. Required material should be available (including a ventilator that is prepared for the use of heliox, a heliox regulator, a gas oximeter, and two heliox cylinders, since one needs to be kept as a backup, with an helium/O_2 concentration not lower than 60/40). Heliox should be discontinued in cases of severe hypoxemia, and intubation should not be delayed, as stated in the recommendations for NIV failure.[195]

Continuous tracheal gas insufflation

Comment - The aim of continuous tracheal gas insufflation (TGI) is to remove CO_2 from the gas in the anatomic dead space, thus reducing hypercapnia to a $PaCO_2$ of <80mmHg. This resource can be used when Pplat is >30cmH_2O,Vt is low, and $PaCO_2$ is >80mmHg.

Suggestion - TGI may be indicated in patients whose f, Cst, and airway pressures are at the respiratory system protection and safety thresholds, but who have a $PaCO_2$ of >80mmHg and/or a pH of <7.2.

Recommendation - The following techniques and procedures are recommended: capnography with $ETCO_2$ measurement should be used, bearing in mind that the efficacy of TGI is greater in patients with high $ETCO_2$ levels that are close to the $PaCO_2$ level. A bronchoscopy connector should be used for the tracheal cannula, and a fine probe (6 Fr) should be inserted through the connector. The catheter tip should be placed 2 to 3cm above the carina and below the distal end of the ventilatory prosthesis (measured in a tracheal tube outside the trachea). A TGI flow that is sufficient for the expired CO_2 plateau line (now descending) to come close or contact the zero line should be used. Flow rates >10L/min should be avoided, and TGI should be used in conjunction with PSV, bearing in mind that the volumes measured by the ventilator are inaccurate and that plateau pressure cannot be accurately measured when TGI is used.[197]

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Risk factors for death in patients with severe asthma*

Fatores de risco de morte em pacientes portadores de asma grave

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Abstract

Objective: To identify risk factors for death among patients with severe asthma. **Methods:** This was a nested case-control study. Among the patients with severe asthma treated between December of 2002 and December of 2010 at the Central Referral Outpatient Clinic of the Bahia State Asthma Control Program, in the city of Salvador, Brazil, we selected all those who died, as well as selecting other patients with severe asthma to be used as controls (at a ratio of 1:4). Data were collected from the medical charts of the patients, home visit reports, and death certificates. **Results:** We selected 58 cases of deaths and 232 control cases. Most of the deaths were attributed to respiratory causes and occurred within a health care facility. Advanced age, unemployment, rhinitis, symptoms of gastroesophageal reflux disease, long-standing asthma, and persistent airflow obstruction were common features in both groups. Multivariate analysis showed that male gender, FEV_1 pre-bronchodilator < 60% of predicted, and the lack of control of asthma symptoms were significantly and independently associated with mortality in this sample of patients with severe asthma. **Conclusions:** In this cohort of outpatients with severe asthma, the deaths occurred predominantly due to respiratory causes and within a health care facility. Lack of asthma control and male gender were risk factors for mortality.

Keywords: Asthma/mortality; Asthma/therapy; Risk factors.

Resumo

Objetivo: Identificar os fatores de risco para morte em pacientes com asma grave. **Métodos**: Estudo caso-controle aninhado a uma coorte de pacientes acompanhados no Ambulatório Central de Referência do Programa para o Controle da Asma na Bahia, em Salvador (BA). No período entre dezembro de 2002 e dezembro de 2010, foram selecionados todos os pacientes com asma grave que foram a óbito e pacientes asmáticos graves vivos como controles na relação 1:4. As informações foram coletadas nos prontuários do serviço e complementadas por meio de visitas domiciliares e atestados de óbitos. **Resultados:** Foram selecionados 58 óbitos e 232 controles. Os óbitos, na sua maioria, foram atribuídos a causas respiratórias e ocorreram dentro de uma unidade de saúde. Idade avançada, inatividade laboral, presença de rinite, sintomas de doença do refluxo gastroesofágico, tempo prolongado de doença e obstrução ao fluxo aéreo persistente foram aspectos comuns em ambos os grupos. A análise multivariada mostrou que o gênero masculino, VEF_1 pré-broncodilatador < 60% do previsto e a ausência de controle dos sintomas da asma foram fatores de risco significativamente e independentemente associados à mortalidade nessa amostra de asmáticos graves. **Conclusões:** Nesta coorte ambulatorial de pacientes com asma grave, os óbitos ocorreram predominantemente por causas respiratórias em unidades de saúde. A falta de controle da asma e o gênero masculino foram os fatores de risco para óbito.

Descritores: Asma/mortalidade; Asma/terapia; Fatores de risco.

Introduction

Asthma is a common chronic respiratory disease that has a substantial impact on morbidity and mortality worldwide. It is estimated that 10% of all individuals with asthma have the severe

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form of the disease, which has negative economic and social effects, resulting in a disproportional burden in terms of the utilization of health care services, as well as impaired quality of life and immeasurable human suffering due to recurrent episodes of asphyxiation.[1,2]

Asthma mortality rates have not increased in parallel with increases in the prevalence of the disease.[1] Studies have shown that countries in which the number of deaths from asthma has decreased or remained stable are those that have adopted certain strategies aimed at controlling the disease: focusing on early diagnosis[3,4]; providing asthma treatment at primary health care facilities[4-6]; expanding/simplifying access to health care services[5,7]; developing educational programs and activities aimed at asthma control[3,4]; and providing appropriate training for health care professionals.[3]

A better understanding of the risk factors for mortality in asthma will allow the development of measures that are more effective in preventing deaths from the disease.[8] The known risk factors for death from asthma as are follows: greater asthma severity[7,9]; a lack of continuity in medical visits[8,10]; adverse socioeconomic or psychosocial conditions[7,9,11]; and poor practices in the approach to treating the disease (lack of access to effective therapies, non-adherence to treatment, and inadequate management of the symptoms).

The objective of this study was to identify factors associated with mortality among asthma outpatients followed for nearly 10 years via an asthma control program in Brazil.[12]

Methods

Study design, sample, and site

This was a nested case-control study. The study sample comprised 58 cases of death from asthma and 232 cases of severe asthma not resulting in death. All of the patients evaluated had been treated at the at the Central Referral Outpatient Clinic of the *Programa para o Controle da Asma na Bahia* (ProAR, Bahia State Asthma Control Program), in the city of Salvador, Brazil, between December of 2002 and December of 2010. The study was approved by the local research ethics committee.

The main goal of the ProAR is to coordinate activities related to the prevention of and treatment of patients with severe asthma, within the context of the Brazilian *Sistema Único de Saúde* (SUS, Unified Health Care System). Patients enrolled in the program have access, on a quarterly basis, to free medication and consultations with a multidisciplinary health care team, as well as to asthma training and education sessions. These interventions are aimed at achieving and maintaining good asthma control.[13]

At ProAR enrollment, a pulmonologist made the diagnosis of asthma, as well as classifying the severity of each case of asthma, on the basis of the symptoms identified and the measurement of PEF. The diagnosis of asthma and the classification of its severity were in accordance with the criteria established in the Global Initiative for Asthma.[14] The level of asthma control was determined with the Portuguese-language version of the six-item Asthma Control Questionnaire, validated for use in Brazil,[15] which evaluates asthma symptoms and rescue bronchodilator use in the last seven days. The cut-off score found to be the most accurate in identifying uncontrolled asthma is 1.5, patients with an average score $\geq$ 1.5 being less likely to have achieved good asthma control.[15]

Identification of cases

We evaluated all records of patients enrolled in the ProAR and clinically diagnosed with severe asthma, including only those who had been followed by a multidisciplinary team and had used an inhaled corticosteroid on a regular basis for at least three months. From among those patients, we identified those who evolved to death during the study period.

We identified deaths from asthma by reviewing the ProAR patient charts for the study period. Deaths were recorded when reported by family members or when revealed by an active search for a patient who had failed to appear for scheduled visits for six months or more.

Within the population studied, there were 62 deaths. For 8 of those deaths, the patient charts were incomplete and it was necessary to conduct home visits in attempts to obtain copies of the death certificates. In 4 cases, family members or neighbors confirmed the deaths. In the 4 remaining cases, the researchers were advised to avoid attempting to visit the residences, because

they were located in neighborhoods that are considered to be high-crime areas.

Copies of the death certificates were filed with the respective patient medical charts. When no death certificate was available, we created a provisory document, containing the pertinent information (date, time, place, and underlying cause of death), and delivered it to the Health Information Board of the Bahia State Department of Health, with a copy to the Health Information Council of the Salvador Municipal Health Department.

Identification of the controls

We selected additional patients with severe asthma to serve as controls. The controls were chosen at random from among all of the ProAR patients with severe asthma who did not evolve to death during the study period. We made the selection using a database of all existing ProAR patient charts, in the program Microsoft Excel 2010.

Data collection

The study sample was stratified by age bracket: 10-30 years; 31-50 years; and > 50 years. Each randomly selected control was also assigned to the appropriate age bracket. We selected 4 controls for every death, and controls were paired with deaths by the year of the last medical visit.

Data were collected from home visit reports, death certificates, and the medical charts on file at the health care facility. The medical charts comprised structured printouts and were organized as follows: the follow-up report of the clinical history of the patient since the previous medical visit; reports of consultations with the nursing staff, medical staff, psychologists, and social workers; records related to the enrollment of the patient in the program; copies of all examination and test results; and records of the medications dispensed by the pharmacy. The charts were systematically updated at every routine, quarterly visit.

We analyzed the following: sociodemographic data (age, gender, employment status, level of education, and place of birth); clinical data (time since enrollment in the program, number of hospital admissions, number of emergency room visits, duration of daily pulse therapy with an oral corticosteroid [≤ or > 3 days], number of asthma exacerbations, duration of the disease, and the level of asthma symptom control at the most recent ProAR evaluation); family history of asthma; smoking status; results of tests (pulmonary function tests and allergy tests); medications dispensed by the pharmacy and patient adherence to the pharmacological treatment regimen; and data related to the death (date, time, and place, as well as the underlying and contributing causes).

Statistical analysis

The data were analyzed with the Statistical Package for the Social Sciences, version 17.0 (SPSS Inc., Chicago, IL, USA). Categorical variables are presented as absolute frequencies and proportions, whereas continuous variables are presented as means and standard deviations or as medians with interquartile ranges.

The Kolmogorov-Smirnov test was used in order to assess whether the data were normally distributed. We then performed a bivariate analysis, using Pearson's chi-square or Fisher's exact test for categorical variables and the Shapiro-Wilk test or Mann-Whitney U test for continuous variables. Factors showing a significant association ($p < 0.05$) were selected for inclusion in a multiple logistic regression model.

Results

Of the 58 deaths evaluated, 25 (43.1%) occurred during the day (between 6:00 and 18:00). Among the causes of death listed on the death certificates, there was a predominance of respiratory disorders, which were listed in 35 cases (60.3%), "unspecified respiratory failure" and "asthma attack" accounting for 12 (34.3%) and 6 (17.1%), respectively. Cardiovascular events and disorders of the digestive tract were also listed as causes of death in considerable proportions (Table 1).

In the sample as a whole, the majority of the patients were unemployed and had been born in the interior (rural part) of the state of Bahia (Table 2). In our analysis of the clinical characteristics of the sample (Table 3), we observed that the patients in the study group (those who died) had been followed at the ProAR Central Referral Outpatient Clinic for a shorter time than had the control patients. In addition, the proportion of cases of controlled asthma and the rate of adherence to the standard treatment were lower in the study group.

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Table 1 - Characteristics of the patients who evolved to death among those with severe asthma treated at the Central Referral Outpatient Clinic of the Bahia State Asthma Control Program, in Salvador, Brazil, between 2002 and 2010.[a]

Characteristic	n = 58
Cause of death	
Respiratory disease	35 (60.3)
Cardiovascular disease	8 (13.8)
Disease of the digestive tract	4 (6.9)
Other	9 (15.5)
No data	2 (3.5)
Place of death	
Hospital	37 (63.8)
Emergency room	4 (6.9)
Outpatient clinic	4 (6.9)
Unspecified treatment center	5 (8.7)
Home	2 (3.4)
Public space	2 (3.4)
No data	4 (6.9)

[a]Values expressed as n (%).

Table 2 - Sociodemographic characteristics of 58 patients who evolved to death and 232 who did not among those with severe asthma treated at the Central Referral Outpatient Clinic of the Bahia State Asthma Control Program, in Salvador, Brazil, between 2002 and 2010.[a]

Characteristic	Cases of death	Control cases
Age[b]	62.2 ± 16.4	57.3 ± 14.0
Gender		
Male	33 (56.9)	47 (20.3)
Female	25 (43.1)	185 (79.7)
Level of education		
None	10 (17.2)	35 (15.1)
Elementary school	17 (29.3)	111 (47.8)
High school	10 (17.2)	52 (22.4)
College	1 (1.8)	11 (4.7)
No data	20 (34.5)	23 (10.0)
Employment status		
Unemployed	40 (69.0)	153 (65.9)
Employed	14 (24.1)	67 (28.9)
No data	4 (6.9)	12 (5.2)
Place of birth		
State capital	22 (37.9)	91 (39.2)
Other	32 (55.2)	122 (52.6)
No data	4 (6.9)	19 (8.2)

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

Pulmonary function parameters are described in Table 4. As can be seen, in the final evaluation (i.e., the last evaluation conducted before death occurred), the patients in the study group showed lower values of FEV_1 and less reversibility after administration of a short-acting bronchodilator than did those in the control group.

Table 3 - Clinical characteristics of 58 patients who evolved to death and 232 who did not among those with severe asthma treated at the Central Referral Outpatient Clinic of the Bahia State Asthma Control Program, in Salvador, Brazil, between 2002 and 2010.[a]

Clinical characteristic	Cases of death	Control cases
Length of ProAR follow-up, years[b]	2 ± 2	6 ± 2
Asthma controlled[c]	9 (15.5)	126 (54.3)
Regular use of maintenance medication[c]	35 (60.3)	205 (88.4)
Exacerbation[c]	14 (24.1)	19 (8.2)
Number of emergency room visits[c,d]	3.0 (2.0-10.0)	2.0 (1.0-5.0)
Number of hospital admissions[c]	6 (10.3)	24 (10.3)
Number of pulses of oral corticosteroid[c,d]	2.0 (1.0-4.0)	1.0 (1.0-2.0)
Missed work/school[c]	1 (1.7)	13 (5.6)
Never-smoker[e]	22 (37.9)	145 (62.5)
Family history of asthma[e]	28 (48.3)	138 (59.5)
Duration of asthma, years[d,e]	30 (10-50)	24 (10-40)
Positive allergy test result[c]	17 (29.3)	109 (47.0)
Use of a single inhaled corticosteroid[c]	23 (39.7)	100 (43.1)
Use of a long-acting bronchodilator combined with an inhaled corticosteroid[c]	51 (87.9)	225 (97.0)
Use of a short-acting bronchodilator[c]	39 (67.2)	166 (71.6)

ProAR: *Programa para o Controle da Asma na Bahia* (Bahia State Asthma Control Program). [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean ± SD. [c]Within the year preceding the death. [d]Values expressed as median (interquartile range). [e]Information obtained at enrollment in the ProAR.

In the bivariate analysis (Table 5), we observed significant differences between the study group and the control group. We identified a correlation between age and mortality, the majority of the deaths occurring in individuals over 50 years of age. In addition, 33 (56.9%) of the deaths occurred in males. As previously mentioned, the proportion of cases of controlled asthma and the rate of adherence to the standard treatment were lower in the study group than in the control group.

The variables that were significantly associated with mortality were included in the multivariate analysis (Table 5). Failure to achieve good control of asthma remained a risk factor for mortality, not only in the analysis of mortality from respiratory causes but also in that of all-cause mortality.

Discussion

In the present study, most of the deaths among patients with severe asthma were attributed to asphyxiation, asthma attack and respiratory failure being the causes of death most often listed on the death certificates. The majority of the deaths occurred at health care facilities, hospitals predominating. Being male was found to increase the risk of death, as was non-adherence to asthma treatment and failure to achieve good asthma control.

The principal objective of asthma treatment is to achieve symptom control and to reduce the risk of future complications of the disease.[14] Asthma control can be achieved through continuous use of the appropriate medication. Failure to control the symptoms of asthma can result in exacerbations and hospitalization, as well as, presumably, being associated with fatal outcomes.[2]

Most deaths from asthma are avoidable, because they represent adverse events resulting from poor asthma control, which is in turn related to factors that can be controlled[16]: failure to recognize the severity of a asthma attack and prescribe the appropriate treatment; the lack of a written asthma action plan; inappropriate emergency treatment; delayed hospital admission; and impeded access to health care services, essential medications, and treatment by health care professionals.

A lack of asthma control could be a risk factor for mortality among individuals with severe asthma. In addition to the severity of the disease *per se*, the level of asthma control can be negatively affected by patient denial or underestimation of the seriousness of the disease, the failure to

Table 4 - Lung function of 58 patients who evolved to death and 232 who did not among those with severe asthma treated at the Central Referral Outpatient Clinic of the Bahia State Asthma Control Program, in Salvador, Brazil, between 2002 and 2010.[a]

Lung function parameter	Cases of death	Control cases
Pre-BD FEV_1 > 60% of predicted	7 (12.1)	114 (49.1)
Post-BD FVC, % of predicted[b]	69.65 ± 24.03	84.39 ± 17.35
Pre-BD FEV_1, % of predicted[b]	43.36 ± 17.33	60.22 ± 19.66
Post-BD FEV_1, % of predicted[b]	48.02 ± 19.53	67.62 ± 19.82

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

Table 5 - Bivariate and multivariate analyses of risk factors for mortality among individuals with severe asthma.

Factor		Bivariate analysis			Multivariate analysis		
All-cause mortality (n = 58)	Mortality from respiratory causes (n = 35)	OR	95% CI	p	OR	95% CI	p
> 50 years of age		1.025	1.003-1.048	0.025	1.001	0.994-1.009	0.781
Male gender		5.196	2.824-9.564	< 0.001	5.392	2.373-12.254	< 0.001
Irregular use of maintenance medication in the last year		2.547	1.117-5.808	0.026	0.963	0.303-3.058	0.963
Uncontrolled asthma in the last year		5.338	2.443-11.665	< 0.001	2.796	1.135-6.890	0.025
FEV_1 > 60% of predicted		0.953	0.934-0.972	< 0.001	0.176	0.057-0.539	0.002
	Male gender	3.850	1.782-8.314	0.001	4.550	1.499-13.814	0.007
	Uncontrolled asthma in the last year	8.089	2.850-22.955	< 0.001	3.448	1.035-11.487	0.044
	Asthma exacerbation in the last year	0.162	0.061-0.427	< 0.001	0.316	0.089 -1.115	0.073
	FEV_1 > 60% of predicted	0.949	0.925-0.974	< 0.001	0.322	0.081-1.279	0.107

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use or the incorrect use of asthma medications, comorbidities, and poor patient perception of bronchial obstruction.[17,18]

The ProAR is designed to provide treatment, education, and investigation by a multidisciplinary team trained in the management of severe asthma, within the context of the SUS.[13] One of the goals of the program is to furnish asthma medication, on a regular basis and at no charge, to patients with severe persistent asthma, in order to help such patients achieve and maintain good control of the disease. Patients followed at the ProAR Central Referral Outpatient Clinic receive guidance regarding and supervision of their use of inhaled medication.[13]

Our bivariate analysis revealed that, in relation to all-cause mortality, failure to use maintenance medication doubled the risk of death. However, that association did not remain significant in the multivariate analysis.

An earlier study identified certain factors as being predictive of poor adherence to treatment among patients enrolled in the ProAR,[19] including adverse events, great distances between the patient residence/workplace and the health care facility, transportation difficulties, and short dosage intervals for prescriptions involving multiple doses. Factors related to a lack of asthma control include non-adherence to treatment, a precipitous reduction in the dose of inhaled corticosteroid, carelessness in maintaining environmental controls, and comorbidities.[19] In another ProAR study,[20] correct inhaler technique was found to be associated with asthma symptom control. The authors suggested that the inhaler techniques employed by asthma patients should be evaluated, on a regular basis, by a multidisciplinary team.

A short duration of follow-up is another factor that might be associated with difficulty in achieving good asthma control. In many cases, prolonged treatment is needed in order to achieve such control. In the present study, the rate of good asthma control in the last year was only 15.5% among the patients who died. Although some of those patients had access to a well-trained multidisciplinary team of specialists and were treated with high doses of inhaled corticosteroids, combined with long-acting β_2 agonists or other asthma medications, the evolution was unfavorable, resulting in death.

Of the deaths evaluated in the present study, the majority (63.8%) occurred in hospitals, which is in agreement with the findings of other such studies conducted in Brazil.[21] We found that 44.6% of the deaths were attributed to asphyxiation. Asthma attacks and respiratory failure, the principal causes of death in the present study, could be related to a failure to recognize the severity of the asthma exacerbation, to a failure to follow the asthma action plan prescribed, or to delays in the initiation of treatment.

A portion of asthma-related deaths result from severe, fulminant exacerbations. The reasons why asthma patients who die tend to do so in hospitals have yet to be clarified, although characteristics of the airway obstruction itself, infections, and other comorbidities could play a role. In emergency rooms, unfavorable outcomes are associated with delays in treatment, difficulty in recognizing the warning signs of asthma exacerbation, and a lack of simplified protocols for the management of such exacerbations.[16] Poor patient perception of the degree of bronchial obstruction is another, subjacent cause of fatal exacerbations. Asthma patients with a limited perception of their disease are at a greater risk of underestimating it and therefore receiving inadequate treatment.[17,22]

Asthma continues to be neglected or underestimated by governments, health care professionals, and patients. Efficient public policies and equitable access to asthma treatment could reduce the morbidity and mortality associated with the disease. In Brazil, no nationwide asthma control plan has yet been implemented. Some isolated initiatives have been quite successful, one example being the ProAR, the goal of which is to coordinate activities related to the prevention of and treatment of patients with severe asthma, conducted within the context of the SUS in the state of Bahia. Via the ProAR, asthma patients receive the necessary medication and are followed by a multidisciplinary health care team, as well as being exposed to asthma training and education, the ultimate objective being to achieve and maintain good asthma control.[13]

In our sample, being male was a risk factor for mortality. The protective effect of being female might be explained certain differences between men and women: women seek treatment more often than do men, who tend to seek treatment only when their symptoms are severe; the rate of adherence to treatment for chronic diseases

is higher in women; and the severity of such diseases tends to be greater in men.[21-25]

Asthma is a chronic inflammatory disease that can progress to partial bronchial remodeling, together with the destruction of the airways and parenchyma, leading to a progressive decline in lung function, the degree of which depends on the duration of asthma and the age of the patient.[26] The risk of asthma-related mortality increases in parallel with advancing age.[6,9] In our bivariate analysis, we identified a borderline association between advanced age and all-cause mortality, although that association did not remain significant in the multivariate analysis. Most of the deaths evaluated in the present study occurred in individuals over 50 years of age with long-standing asthma and lung function impairment greater than that observed in their younger counterparts.

Indicators of airflow limitation are also powerful predictors of mortality in patients with asthma.[27,28] Objective measures of lung function, such as PEF and FEV_1, are useful predictors of hospital admission in such patients.[29] In the present study, the patients who eventually died had presented worse lung function than had those who did not. A decline in FEV_1 has been shown to be associated with death from asthma.[7,30] Among the patients with severe asthma evaluated here, the risk of death was lower in those with better FEV_1 values. Among those who died, shorter ProAR follow-up periods might have limited any potential gains in lung function.

Our study has certain limitations. The results cannot necessarily be generalized to patients with moderate or mild asthma. Other limitations include the retrospective study design and the general unreliability of data collected from death certificates. However, the data related to the deaths evaluated here were obtained from official documents that are used in order to track most of the health indicators in Brazil. The relevance of the present study lies in the information its provides regarding characteristics of the deaths that occurred within a sample of patients with severe asthma. In addition, the retrospective nature of our study is counterbalanced by the fact that the case-control study was nested within a specific cohort, which increases the quantity and quality of the data available for analysis. To our knowledge, this was the first controlled study to evaluate the risk factors for asthma-related mortality by systematic collection of data related to multiple clinical and functional variables. We are also unaware of any previous controlled studies showing a statistically significant association between uncontrolled asthma and mortality.

Knowledge of the risk factors for asthma-related mortality is crucial to the planning and provision of individualized treatment of the disease and, consequently, to reducing the associated morbidity and mortality. Most asthma-related deaths could be avoided through early diagnosis and timely treatment of the disease, as well as improved training of health care teams, educational interventions to instruct patients in asthma self-management, and asthma-control programs.

The statistical power of our sample is limited by the relatively low number of deaths occurring within the period studied. Although we attempted to investigate all relevant deaths and employed four control cases for every case of death, the statistical power was still insufficient to make precise inferences of associations between asthma-related mortality and any of the numerous variables evaluated. However, the lack of a statistical association does not exclude the possibility that a given variable is a risk factor for mortality. Despite the low statistical power, we identified certain significant associations, which should therefore be given even more weight.

In conclusion, most of the deaths evaluated in the present study were attributed to respiratory disorders and occurred in a hospital. Uncontrolled asthma, FEV_1 pre-bronchodilator < 60% of predicted, and male gender were found to be significantly and independently associated with mortality among patients with severe asthma.

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Evaluation of von Willebrand factor in COPD patients*

Avaliação do fator de von Willebrand em pacientes com DPOC

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Abstract

Objective: To compare the absolute serum von Willebrand factor (vWF) levels and relative serum vWF activity in patients with clinically stable COPD, smokers without airway obstruction, and healthy never-smokers. **Methods:** The study included 57 subjects, in three groups: COPD (n = 36); smoker (n = 12); and control (n = 9). During the selection phase, all participants underwent chest X-rays, spirometry, and blood testing. Absolute serum vWF levels and relative serum vWF activity were obtained by turbidimetry and ELISA, respectively. The modified Medical Research Council scale (cut-off score = 2) was used in order to classify COPD patients as symptomatic or mildly symptomatic/asymptomatic. **Results:** Absolute vWF levels were significantly lower in the control group than in the smoker and COPD groups: 989 ± 436 pg/mL vs. 2,220 ± 746 pg/mL ($p < 0.001$) and 1,865 ± 592 pg/mL ($p < 0.01$). Relative serum vWF activity was significantly higher in the COPD group than in the smoker group (136.7 ± 46.0% vs. 92.8 ± 34.0%; $p < 0.05$), as well as being significantly higher in the symptomatic COPD subgroup than in the mildly symptomatic/asymptomatic COPD subgroup (154 ± 48% vs. 119 ± 8%; $p < 0.05$). In all three groups, there was a negative correlation between FEV_1 (% of predicted) and relative serum vWF activity ($r^2 = -0.13$; $p = 0.009$). **Conclusions:** Our results suggest that increases in vWF levels and activity contribute to the persistence of systemic inflammation, as well as increasing cardiovascular risk, in COPD patients.

Keywords: von Willebrand factor; Pulmonary disease, chronic obstructive; Endothelial cells.

Resumo

Objetivo: Comparar os níveis séricos absolutos e a atividade sérica em percentual do fator de von Willebrand (FvW) em pacientes com DPOC clinicamente estáveis, tabagistas sem obstrução das vias aéreas e em indivíduos saudáveis que nunca fumaram. **Métodos:** Foram incluídos no estudo 57 indivíduos, em três grupos: DPOC (n = 36), tabagista (n = 12) e controle (n = 9). Todos os participantes realizaram radiografia do tórax, espirometria e exame de sangue durante a fase de seleção. Os níveis séricos absolutos e a atividade sérica em percentual do FvW foram obtidos por turbidimetria e ELISA, respectivamente. A escala *Medical Research Council* modificada foi utilizada para classificar pacientes como sintomáticos ou assintomáticos/pouco sintomáticos no grupo DPOC (ponto de corte = 2). **Resultados:** Os níveis absolutos do FvW no grupo controle foram significativamente menores que os nos grupos tabagista e DPOC: 989 ± 436 pg/mL vs. 2.220 ± 746 pg/mL ($p < 0,001$) e 1.865 ± 592 pg/mL ($p < 0,01$). Os valores em percentual de atividade do FvW no grupo DPOC foram significativamente maiores que no grupo tabagista (136,7 ± 46,0% vs. 92,8 ± 34,0%; $p < 0,05$), assim como foram significativamente maiores no subgrupo DPOC sintomático que no subgrupo DPOC assintomático/pouco sintomático (154 ± 48% vs. 119 ± 8%; $p < 0,05$). Houve uma correlação negativa entre o VEF_1 (% do previsto) e os níveis em percentual de atividade do FvW nos três grupos ($r^2 = -0,13$; $p = 0,009$). **Conclusões:** Nossos resultados sugerem que aumentos nos níveis de FvW e de sua atividade contribuem para a manutenção da inflamação sistêmica e o aumento do risco cardiovascular em pacientes com DPOC.

Descritores: Fator de von Willebrand; Doença pulmonar obstrutiva crônica; Células endoteliais.

Introduction

Worldwide, COPD is a public health problem, affecting more than 10% of the population over the age of 50 years.[1,2] The prevalence of this disease has increased particularly in developing countries.[3] It is estimated that, in 2020, COPD will be the third leading cause of death worldwide. This obstructive disease is usually associated with smoking,[3] and COPD patients are at a higher risk of cardiovascular changes than is the general population.[4,5]

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Recently, the presence of a systemic inflammation process has been found to be associated with some complications in COPD patients, chief among which are cachexia, anorexia, osteoporosis, and atherosclerosis.[2,6] However, it has yet to be clearly established whether comorbidities are a consequence of lung disease or whether COPD can be considered a systemic disease. Inflammation is believed to also occur at the endothelial level, contributing to the formation of atherosclerotic plaques.[7] This vascular event could partially explain the higher prevalence of cardiovascular diseases in smokers who develop airway obstruction.[7] Some inflammatory and endothelial markers, such as C-reactive protein and fibrinogen, are increased in COPD patients.[7,8] Von Willebrand factor (vWF) is a marker of endothelial damage and participates in the process of atherosclerosis.[9] Increased serum vWF levels have been reported in COPD patients during exacerbations.[10] The objective of the present study was to assess the behavior of vWF levels in stable COPD patients who had not experienced a recent exacerbation, as well as attempting to correlate this endothelial marker with respiratory disease severity.

Methods

The present study was approved by the local research ethics committee, and all participants gave written informed consent before undergoing any study procedures. In addition, this project was in compliance with current ethics regulations in Brazil.

Patients were selected from among those under follow-up at the outpatient clinic of the Department of Pulmonology and Tuberculosis of the Rio de Janeiro State University, located in the city of Rio de Janeiro, Brazil, and professionals working at that clinic were invited to participate as volunteers. Between February of 2011 and July of 2012, a total of 57 subjects were recruited in three groups: COPD; smoker; and control. The inclusion criteria for the group of COPD patients were having a smoking history of at least 20 pack-years and having a post-bronchodilator FEV_1/FVC ratio < 0.7. Smokers should also have a long smoking history (at least 20 pack-years), but they should have normal spirometry results at selection. Healthy volunteers should have no history of lung disease, should be never-smokers, and should have normal spirometry results. The exclusion criteria for the three groups were as follows: having a history of asthma, atopy, or atherosclerotic cardiovascular disease; having had respiratory infection in the last three weeks; having recently been diagnosed with or being under treatment for tuberculosis; having congestive heart failure, HIV infection, diseases that are systemic and inflammatory in origin, severe dyslipidemia (serum triglyceride levels > 300 mg/dL or total cholesterol levels > 280 mg/dL), and diabetes mellitus (diagnosed in accordance with the American Diabetes Association criteria)[11]; having used systemic anti-inflammatory agents or antiplatelet drugs regularly in the last year; and having abnormal laboratory test results at selection. Patients with COPD should be using their usual medications and should not have experienced exacerbations of their disease for at least three months. During the selection phase, ancillary tests included spirometry, chest X-rays, and blood testing. Spirometry was performed with a Vitatrace spirometer (Pró Médico Ltda., Rio de Janeiro, Brazil), in accordance with the American Thoracic Society standards,[12] and all subjects underwent bronchodilator testing with albuterol (400 μg). The reference equations of Pereira et al. were used.[13] Blood testing included blood workup, coagulation profile, and determination of serum glucose, urea, creatinine, uric acid, triglyceride, total cholesterol, and HDL/LDL cholesterol levels. For the selected subjects only, a blood sample was stored at −80°C and sent for analysis of absolute vWF levels (turbidimetry) and relative serum vWF activity (ELISA). Chest X-rays were performed on the same day as spirometry and blood sample collection. The X-rays were examined by a radiologist and were used in patient selection, because healthy volunteers and smokers should not have radiographic changes. Patients with COPD often had small scarring suggestive of a history of tuberculosis or signs of hyperinflation. Patients with other X-ray findings, especially when associated with clinical changes suggesting active disease, were excluded from the study.

All 57 recruited subjects met the inclusion criteria and met none of the exclusion criteria. Of those, 36 had a diagnosis of COPD, 12 were smokers without airflow obstruction, and 9 were healthy volunteers.

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Classification of COPD was based on the Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy document.[14] Therefore, symptoms and number of exacerbations of the disease in the previous year were identified and, together with post-bronchodilator measurement of FEV_1 (% of predicted), were used to assign patients to categories A, B, C, or D. Symptoms were quantified with the modified Medical Research Council (mMRC) scale, whose scores are used to determine the presence or absence of symptoms (mMRC score ≥ 2 and mMRC score < 2, respectively).[14] On this basis, 13, 5, 7, and 11 of the 36 COPD patients were classified as belonging to subgroups A, B, C, and D, respectively. According to the spirometric classification, without considering symptoms or the presence of exacerbations, 11 patients had mild COPD, 13 had moderate COPD, and 12 had severe COPD.

Statistical analysis was performed with the GraphPad Prism software, version 6 (GraphPad Software Inc., San Diego, CA, USA). ANOVA and Dunn's post hoc test were used to compare groups, and the Mann-Whitney test was used to compare independent groups. Nonparametric Spearman's test was used to compare two variables. The level of significance was set at $p < 0.05$.

Results

Of the 57 subjects recruited, 31 were male. Age was significantly higher in the COPD group than in the other two groups, whereas it was similar in the control and smoker groups. Spirometric data for the groups are shown in Table 1. Comorbidities were found in all three groups; however, they were more common in the COPD group (Table 1).

Serum vWF levels were measured by two different methods. The first determined absolute serum vWF levels. The control group had significantly lower absolute vWF levels than did the smoker and COPD groups: 989 ± 436 pg/mL vs. 2,220 ± 746 pg/mL ($p < 0.001$) and 1,865 ± 592 pg/mL ($p < 0.01$), respectively (Figure 1). The second method used determined relative serum vWF activity. The COPD group had significantly higher values than did the smoker group (136.7 ± 46.0% vs. 92.8 ± 34.0%; $p < 0.05$; Figure 2A).

In order to assess the relationship between serum vWF levels and COPD severity, we subdivided the COPD group into four categories, i.e., GOLD groups A, B, C, and D.[14] However, neither absolute serum levels nor relative serum activity showed correlations with this classification. Likewise, we found no correlation of absolute serum vWF levels or relative serum vWF activity with the spirometric classification of COPD. The ANOVA did not allow us to distinguish among the four subgroups of patients on the basis of absolute vWF levels or relative serum vWF activity ($p > 0.05$). The 18 patients classified as GOLD group C or D were using inhaled corticosteroids, because this is the treatment approach used at

Table 1 - Demographic and spirometric data of the study participants.[a]

Variables	Groups		
	Control	Smoker	COPD
	(n = 9)	(n = 12)	(n = 36)
Age, years	47.22 ± 1.41	50.30 ± 4.94	62.75 ± 9.98
Male/Female, n/n	4/5	3/9	24/12
FVC, L	3.37 ± 1.20	3.38 ± 0.61	2.90 ± 0.95
FVC, % of predicted	100.88 ± 12.17	103.30 ± 12.10	86.08 ± 20.23
FEV_1, L	2.98 ± 0.72	2.78 ± 0.54	1.59 ± 0.69
FEV_1,% of predicted	99.31 ± 11.02	104 ± 9.87	59.84 ± 21.30
FEV_1/FVC, %	79.67 ± 5.19	83.90 ± 9.68	53.07 ± 10.54
Comorbidities[b]			
SAH	1	3	9
Hypothyroidism		2	1
Dyslipidemia		1	
Glaucoma			1
Bipolar disorder			1
Calcinosis			1

SAH: systemic arterial hypertension. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as n of patients.

out facility. No correlation was found between inhaled corticosteroid use and absolute serum vWF levels or relative serum vWF activity.

In a second analysis, COPD patients were subdivided into two groups on the basis of their level of dyspnea as measured by the mMRC scale. Patients with an mMRC score ≥ 2 were considered symptomatic. In this analysis, there was no significant difference in absolute vWF levels between the symptomatic and mildly symptomatic/asymptomatic groups. However, relative serum vWF activity was significantly higher in the symptomatic group than in the mildly symptomatic/asymptomatic group (154.0 ± 48.0% vs. 118.9 ± 38.0%; p < 0.05; Figure 2B)

Subsequently, COPD patients were further subdivided into two groups on the basis of the presence or absence of exacerbations (presence being defined as ≥ 2 exacerbations in the last year and absence being defined as < 2 exacerbation in the last year). There were no significant differences in absolute serum vWF levels or relative serum vWF activity between the two subgroups.

In the control, smoker, and COPD groups, there was a significant negative correlation between FEV_1 (% of predicted) and relative serum vWF activity ($r^2 = -0.13$; p = 0.009; Figure 3), whereas there was no correlation between FEV_1 (% of predicted) and absolute vWF levels (p = 0.077).

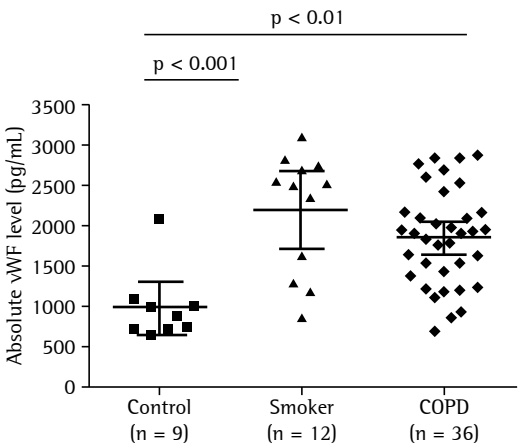

Figure 1 - Absolute serum von Willebrand factor (vWF) levels in the groups studied.

Discussion

The fourth leading cause of death worldwide, COPD affects approximately 16% of the population in the city of São Paulo, Brazil.[15] One study demonstrated that COPD is underdiagnosed in this city, because 83% of the subjects with airway obstruction did not have a clinical diagnosis of COPD.[16] This scenario remains virtually unchanged, as shown in a 9-year follow-up study, which found that 70% of the respondents had obstruction as diagnosed by spirometry.[16] In addition to destroying the alveolar septa, COPD seems to have a systemic inflammatory effect.[17] It is possible that this inflammation also affects the endothelial system,[7] the impairment of which could partially explain the high prevalence of vascular disease in COPD patients. Some studies have attempted to relate increased levels of some endothelial markers, such C-reactive protein and fibrinogen, to COPD.[7,8] One study reported increased vWF levels in COPD patients during exacerbations.[10] However, the role of this marker in COPD during the stable phase of the disease has yet

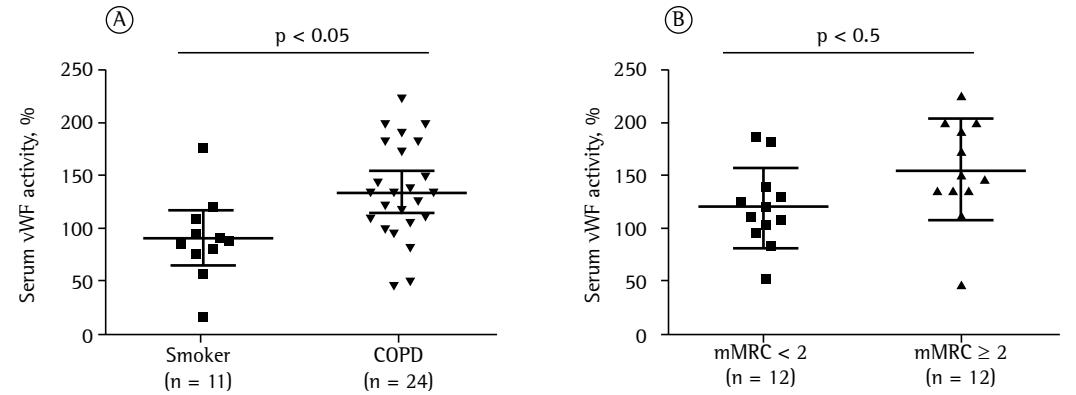

Figure 2 - Relative serum von Willebrand factor (vWF) activity. In A, comparison between the smoker and COPD groups. In B, comparison between the symptomatic COPD and mildly symptomatic/asymptomatic COPD subgroups as defined by the modified Medical Research Council (mMRC) scale scores.

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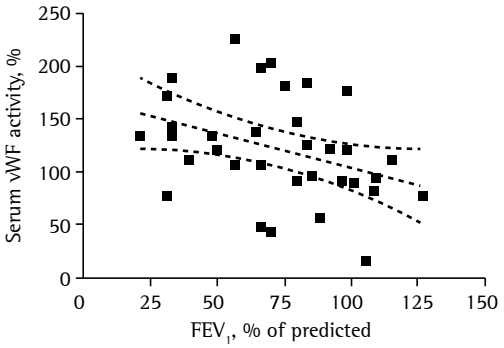

Figure 3 - Relationship between relative serum von Willebrand factor (vWF) activity and FEV_1 (% of predicted; $r^2 = -0.13$; $p = 0.0099$).

to be established. The vWF can be evaluated in two different ways: by measurement of its absolute serum levels and by measurement of its relative serum activity. The first is a quantitative evaluation, whereas the second leads us to a qualitative analysis.

In the present study, the authors found that absolute serum vWF levels were higher in smokers (with and without airflow obstruction) than in controls ($p < 0.01$). The relationship between smoking and increased vWF levels has been demonstrated in recent years, there seeming to be a significant increase of up to 76% in vWF levels after 120 minutes of tobacco use, as well as an average decrease from 144% to 123% in vWF levels in patients who quit smoking.[8] One study demonstrated that vWF activity is increased in smokers.[18] One group of authors reported that vWF levels are higher in COPD patients than in healthy subjects; however, smokers without obstruction were not included in that analysis.[19] Another study demonstrated that serum vWF levels increase in COPD patients during exacerbations.[20] In the present study, the presence of an exacerbation was considered an exclusion criterion, because our objective was to analyze vWF levels during the stable phase of COPD. Therefore, it was impossible to determine any association with that variable. The increase in relative vWF activity in COPD patients, when compared with the smoker group, suggests that vWF may play a role in the inflammatory pathophysiology of COPD and could be related to atherosclerosis and cardiovascular disease.[7]

To our knowledge, the present study is the first to attempt to correlate vWF levels with COPD severity as defined by the GOLD classification.[14] However, no statistically significant difference was found in serum vWF levels among the four COPD severity groups, nor were there any differences among the groups when the spirometric classification of COPD was considered. This suggests that, although vWF levels are high in stable COPD patients, they do not correlate with disease severity. This finding is consistent with literature reports that relate vWF levels to other inflammatory diseases, such as diabetes mellitus and rheumatoid arthritis.[10,21] It seems that vWF is a nonspecific marker of inflammation, and therefore it is not useful to grade the severity of chronic inflammatory diseases.

When we used the mMRC scale to determine the presence or absence of symptoms, we found that relative serum vWF activity was significantly higher in symptomatic patients, i.e., those with an mMRC score ≥ 2 ($p < 0.05$). This possibly indicates that the degree of inflammation is higher in symptomatic patients than in mildly symptomatic or asymptomatic patients. Following this line of reasoning, it was expected that patients with frequent exacerbations would have higher vWF levels, which was not observed in the present sample. Thus, further studies are needed to elucidate this issue.

Although there was a significant negative correlation between FEV_1 (% of predicted) and relative serum vWF activity in all three groups (control, smoker, and COPD), the correlation was not very robust (Figure 3). In addition, one study found no correlations between vWF levels and decline in FEV_1.[22] Therefore, studies involving a larger number of patients are needed to clarify this issue.

The present study has some limitations, chief among which is the fact that the control and smoker groups were not matched for age with the COPD group, which is something very difficult to achieve in studies that compare patients with and without bronchial obstruction. However, healthy volunteers (controls) and smokers were similar in age. Nevertheless, vWF levels were significantly higher in the smoker group. Another important fact is that participants were not screened for blood group (ABO blood typing system), and blood group has a small influence on vWF levels. A third limitation was the lack of evaluation of

other inflammatory parameters, such as C-reactive protein and fibrinogen. This evaluation would allow us to analyze them in comparison with related data in the literature and with serum vWF levels. In contrast, an attempt was made to exclude a large number of factors that could be related to systemic inflammation and endothelial injury. Thus, as reported in Methods, patients or volunteers with a history of cardiovascular disease or other chronic or infectious diseases, as well as those who were using medications, were excluded from the study, and this considerably limited the recruitment of participants.

Patients with COPD are at a higher risk of endothelial injury and consequent cardiovascular disease. In our study, absolute serum vWF levels were higher in smokers with and without bronchial obstruction than in controls, and relative serum vWF activity was higher in COPD patients than in smokers. It is possible that vWF participates in the systemic inflammatory process in COPD patients and thereby contributes to increasing cardiovascular risk.

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Clinical application of CT and CT-guided percutaneous transthoracic needle biopsy in patients with indeterminate pulmonary nodules*

Aplicação clínica da TC e biópsia transtorácica percutânea guiada por TC em pacientes com nódulos pulmonares indeterminados

Luciana Vargas Cardoso, Arthur Soares Souza Júnior

Abstract

Objective: To investigate the clinical application of CT and CT-guided percutaneous transthoracic needle biopsy (CT-PTNB) in patients with indeterminate pulmonary nodules (IPNs). **Methods:** We retrospectively studied 113 patients with PNs undergoing CT and CT-PTNB. Variables such as gender, age at diagnosis, smoking status, CT findings, and CT-PTNB techniques were analyzed. Data analysis was performed with the Student's t-test for independent samples the chi-square test, and normal approximation test for comparison of two proportions. **Results:** Of the 113 patients studied, 68 (60.2%) were male and 78 (69%) were smokers. The diameter of malignant lesions ranged from 2.6 cm to 10.0 cm. Most of the IPNs (85%) were located in the peripheral region. The biopsied IPNs were found to be malignant in 88 patients (77.8%) and benign in 25 (22.2%). Adenocarcinoma was the most common malignant tumor, affecting older patients. The IPN diameter was significantly greater in patients with malignant PNs than in those with benign IPNs ($p < 0.001$). Having regular contour correlated significantly with an IPN being benign ($p = 0.022$), whereas spiculated IPNs and bosselated IPNs were more often malignant (in 50.7% and 28.7%, respectively). Homogeneous attenuation and necrosis were more common in patients with malignant lesions (51.9% and 26.9%, respectively) **Conclusions:** In our sample, CT and CT-PTNB were useful in distinguishing between malignant and benign IPNs. Advanced age and smoking were significantly associated with malignancy. Certain CT findings related to IPNs (larger diameter, spiculated borders, homogeneous attenuation, and necrosis) were associated with malignancy.

Keywords: Solitary pulmonary nodule; Tomography; Image-guided biopsy.

Resumo

Objetivo: Investigar a aplicação clínica da TC e da biópsia transtorácica percutânea guiada por TC (BTP-TC) em pacientes com nódulos pulmonares indeterminados (NPIs). **Métodos:** Foram estudados retrospectivamente 113 pacientes portadores de NPIs submetidos a TC e BTP-TC. Foram analisadas variáveis como sexo, idade ao diagnóstico, tabagismo, achados tomográficos e técnicas de BTP-TC. A análise dos dados foi efetuada por meio do teste t de Student para amostras independentes, teste do qui-quadrado e teste de comparação de duas proporções por aproximação normal. **Resultados:** Dos 113 pacientes estudados, 68 (60,2%) eram do sexo masculino e 78 (69%) eram tabagistas. O diâmetro das lesões malignas variou de 2,6 a 10,0 cm. A maioria dos NPIs estava localizada na região periférica (85%). O resultado da biópsia foi maligno em 88 pacientes (77,8%) e benigno em 25 (22,2%). O adenocarcinoma foi o tumor maligno mais frequente, acometendo pacientes com idade mais avançada. O diâmetro dos NPIs foi significativamente maior nos pacientes com malignidade ($p < 0,001$). Houve uma associação significativa entre NPIs com contorno regular e lesões benignas ($p = 0,022$), enquanto os de tipo espiculado e bocelado foram mais frequentes em pacientes com lesões malignas (50,7% e 28,7%, respectivamente). Atenuação homogênea e necrose foram mais frequentes em pacientes com lesões malignas (51,9% e 26,9%, respectivamente). **Conclusões:** A TC e a BTP-TC foram úteis no diagnóstico diferencial entre lesões malignas e benignas nos pacientes com NPIs nesta amostra. Idade mais avançada e tabagismo associaram-se significativamente com malignidade. Houve associações de achados tomográficos (diâmetro maior, contorno espiculado, atenuação homogênea e necrose) com NPIs malignos.

Descritores: Nódulo pulmonar solitário; Tomografia; Biópsia guiada por imagem.

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*Study carried out at the São José do Rio Preto *Hospital de Base*, São José do Rio Preto School of Medicine, São José do Rio Preto, Brazil.

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Introduction

Some of the greatest challenges in the fields of thoracic surgery and radiology are related to the evaluation and management of pulmonary nodules.[1] A pulmonary nodule is defined as a well-demarcated, round focal opacity visible on chest X-rays or CT scans and surrounded by normal lung tissue, being up to 3 cm in diameter; pulmonary nodules larger than 3 cm are designated masses.[2]

It is extremely important to investigate pulmonary nodules because they constitute the most common manifestation of lung cancer, being a common finding on chest CT scans.[3] In the USA, approximately 150,000 pulmonary nodules are detected each year.[3,4] Of all pulmonary nodules seen on imaging, 60-70% are benign and 30-40% are malignant.[4]

A pulmonary nodule requires careful patient evaluation, including clinical history taking, physical examination, evaluation of risk factors for malignancy, and diagnostic imaging.[3,5] Diagnostic imaging methods for distinguishing between benign and malignant pulmonary nodules include X-rays, CT, magnetic resonance imaging, positron emission tomography/CT, and CT-guided percutaneous transthoracic needle biopsy (CT-PTNB).

Helical CT is critical in distinguishing between benign and malignant nodules, providing data on size, margins, and the presence of internal calcification. In addition, helical CT images can show nodular enhancement after intravenous contrast administration. Furthermore, helical CT allows greater accuracy in obtaining biopsy specimens.[6,7] Size, location, margins, contents, contrast enhancement, and doubling time are some of the nodule features that can be seen on CT scans of patients with pulmonary nodules, principally on those of those who are male, are over 50 years of age, are smokers, and have a family history of cancer or pulmonary fibrosis.

CT-PTNB has been widely used in the investigation of pulmonary nodules and masses. Samples can be collected by fine-needle aspiration biopsy (FNAB) or thick-needle aspiration biopsy, the latter being known as core biopsy.[8] Core biopsy has greatly contributed to a specific and early diagnosis of malignancy in patients with pulmonary nodules, reducing morbidity and mortality rates.[8]

The differential diagnosis of pulmonary nodules includes various diseases and tumors. Benign nodules include hamartomas, granulomas, and intrapulmonary lymph nodes.[4] Infectious granulomas account for 90% of all benign nodules and are most commonly caused by tuberculosis, histoplasmosis, and coccidioidomycosis.[4] The most common malignant tumors include adenocarcinoma and epidermoid carcinoma.[4]

Several CT criteria have been used in order to distinguish between benign and malignant nodules. Poorly demarcated nodules, absence of calcification (central, laminated, diffuse, or "popcorn" calcification) or fat in the lesion, doubling time ranging from one month to one year approximately, and nodular enhancement greater than 15 HU after intravenous contrast administration in patients past the fourth decade of life are suggestive of malignancy.[7,9,10] Small, well-demarcated nodules with concentric or "popcorn" calcification in young patients are suggestive of benign lesions.[10] The absence of lesion growth for at least two years is also suggestive of benignity.[11]

The present study is warranted because we found no studies examining the clinical application of CT and CT-PTNB in patients with pulmonary nodules in Brazil. From a clinical standpoint, early detection and CT-PTNB of malignant lesions can, in some cases, avoid invasive procedures, such as bronchoscopic biopsy, video-assisted thoracoscopic surgery, and even unnecessary surgery. They can also avoid the progression of lung cancer to advanced stages, enhancing patient quality and quantity of life.[3,12]

The objective of the present study was to investigate the clinical application of CT and CT-PTNB in patients with indeterminate pulmonary nodules, demographic characteristics, CT features, and CT-PTNB findings, as well as their correlation with the histopathological diagnosis, being taken into consideration.

Methods

Of a total of 132 patients with pulmonary nodules and masses studied between June of 2006 and May of 2007, 113 (85.6%) were retrospectively investigated (regardless of gender, age, or race), having undergone helical CT and CT-PTNB. The procedures were performed in the Department of Radiology of the São José do Rio Preto School of Medicine São José do

Rio Preto *Hospital de Base*, located in the city of São José do Rio Preto, Brazil. The study was approved by the local research ethics committee (Protocol no. 3682/2006).

We excluded 19 patients whose histopathological reports showed unsatisfactory or inconclusive results because of insufficient material.

The following data were collected from patient charts: gender; age at diagnosis; smoking status; CT findings, such as diameter (≤ 3 cm for nodules and > 3 cm for masses),[2] location (central or peripheral), lesion margins (regular, irregular, spiculated, or bosselated), and intralesional changes (homogeneous attenuation, necrosis, cavitation, calcification, and air bronchogram); CT-PTNB technique used (FNAB, core biopsy, or both); and complications.

The CT findings were independently evaluated by two radiologists who were blinded to the histopathological findings.

All CT examinations were performed with a Tomoscan® SR 4000 CT scanner (Phillips Medical Systems, Eindhoven, the Netherlands). Ten-millimeter CT scans of the chest were taken from the lung apices to the bases during inhalation, a high-resolution filter being used for image reconstruction. Subsequently, helical CT scans were taken before and after intravenous injection of a nonionic contrast medium, the following parameters being used: slice thickness, 10 mm; pitch (ratio between table movement per rotation and slice thickness), 2 cm; 120 kVp; and 150 mA.

Patients undergoing CT-PTNB were evaluated for general health, level of consciousness, pulmonary functional reserve, and coagulation parameters. All patients were informed of the complications of CT-PTNB and were instructed to hold their breath during the examination. The procedure was performed without intravenous contrast, during single breath-hold maneuvers performed during inhalation, with patients in the supine or prone position in order to allow direct access to the lesion.

The CT-PTNB protocol used in the radiology department of the institution is as follows: slice thickness, 5-10 mm; pitch, 2 cm; 120 kVp; and 150 mA. The goals are to locate the lesion, determine the site at which the needle should be introduced, and measure needle distance and angle. Sterilization of the puncture site was achieved with povidone-iodine, a sterile field being created with surgical drapes. Patients then received 10 mL of local anesthetic (2% lidocaine). A small incision was made with a scalpel (no. 14 blade), the needle being introduced into subcutaneous tissue through the incision. CT scans were taken in order to locate the tip of the needle, which was attached to a Bard Magnum® automatic pistol (Manan Medical Products, Northbrook, IL, USA).

CT-PTNB was performed by FNAB, core biopsy, or both. Needles ranging from 18 G to 20 G were used for core biopsy, and needles ranging from 22 G to 25 G were used for FNAB. After having undergone biopsy, patients were monitored for 2-3 h, CT scans being taken in order to detect complications.

For data analysis, descriptive and inferential statistics were used. For comparison of means, we used the Student's t-test for independent samples (distribution of benign and malignant lesions by age and pulmonary nodule diameter), the chi-square test (distribution of benign and malignant lesions by gender, lesion location, smoking status, and CT-PTNB technique), and the normal approximation test for comparison of two proportions (distribution of benign and malignant lesions by lesion margins and intralesional changes).[13] The level of significance was set at $p < 0.05$. All analyses were performed with Minitab software, version 15 (Minitab Inc., State College, PA, USA).[14]

Results

Of the 113 patients studied, 68 (60.2%) were male and 45 (39.8%) were female. The mean age was 59.3 ± 12.6 years, and the median age was 61 years (range, 12-82 years). Of the 113 patients studied, 78 (69%) were smokers and 35 (31%) were nonsmokers. Of the 78 smokers, 48 (61.5%) were male and 30 (38.5%) were female.

The diameter of benign lung lesions ranged from 1.8 cm to 6.5 cm, and that of malignant lung lesions ranged from 2.6 cm to 10.0 cm. The difference between benign and malignant nodules/masses was statistically significant ($p = 0.003$), malignant nodules and masses having predominated (23.0% and 54.8%, respectively). Most (85%) of the pulmonary nodules were located in the peripheral region, and 15% were located in the central region. There was a predominance of malignant tumors in the upper lobes, in 67 patients (76%). Of the 185 nodules found in the 113 patients studied, spiculated nodules

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were the most common (49.7%), followed by bosselated nodules (26.5%), irregular nodules (12.4%), and regular nodules (11.4%; Figure 1). The CT scans showed a total of 151 intralesional changes, the most common being homogeneous attenuation (42.4%), followed by necrosis (21.2%), cavitation (17.2%), calcification (11.2%), and air bronchogram (8.0%; Figure 2).

FNAB was performed in 71 patients, core biopsy was performed in 81, and a combination of the two was performed in 39. Pneumothorax was the only complication of CT-PTNB, in 37 patients (32.7%). Histopathology revealed that the pulmonary nodules were malignant in 88 (77.8%) of the 113 patients and benign in 25 (22.2%).

Adenocarcinoma was the most common malignant tumor (48.9%), affecting older patients (mean age, 65.6 ± 9.1 years). Malignant lesions ranged from 2.4 cm to 10.0 cm in diameter, whereas benign lesions ranged from 1.8 cm to 6.5 cm in diameter.

Patients with malignant lesions were found to be older than those with benign lesions, the difference being significant ($p = 0.034$); there was also a significant difference between benign and malignant lesions in terms of their size ($p < 0.001$), malignant lesions being larger in diameter (Table 1).

As can be seen in Table 2, neither age nor nodule location were significantly associated with the histopathological diagnosis ($p = 0.067$ and $p = 0.264$, respectively). The presence of regular margins was significantly associated with a pulmonary nodule being benign ($p = 0.022$). Spiculated pulmonary nodules and bosselated pulmonary nodules were more often malignant (in 50.7% and 28.7%, respectively). All intralesional changes were significantly associated with the histopathological diagnosis. Homogeneous attenuation and necrosis were more common in patients with malignant lesions (51.9% and 26.9%, respectively), whereas cavitation, calcification, and air bronchogram were more common in those with benign lesions (29.8%, 23.4%, and 17.0%, respectively). In the calculations related to the tests for comparison of proportions (Table 2), the CT findings of lesion margins and

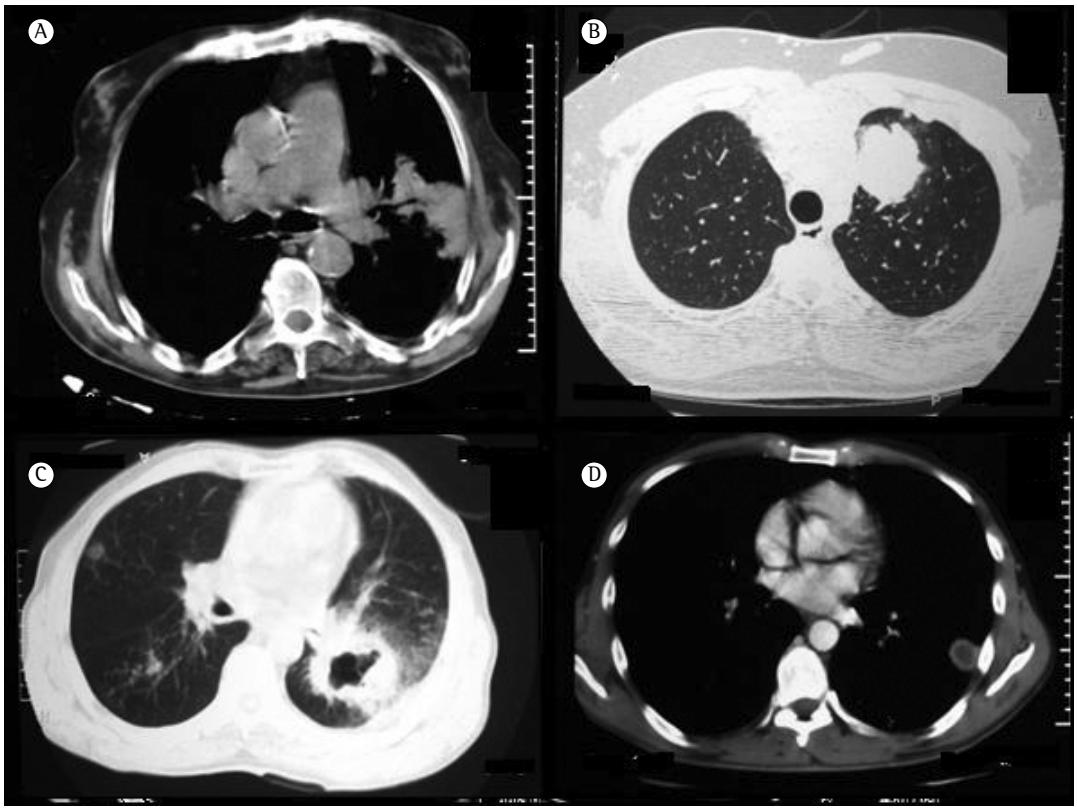

Figure 1 - Helical CT scans showing an irregular lung mass (in A; male patient, 77 years old), a bosselated lung mass (in B; male patient, 30 years old), a spiculated lung mass (in C; male patient, 64 years old), and a regular lung mass (in D; male patient, 36 years old).

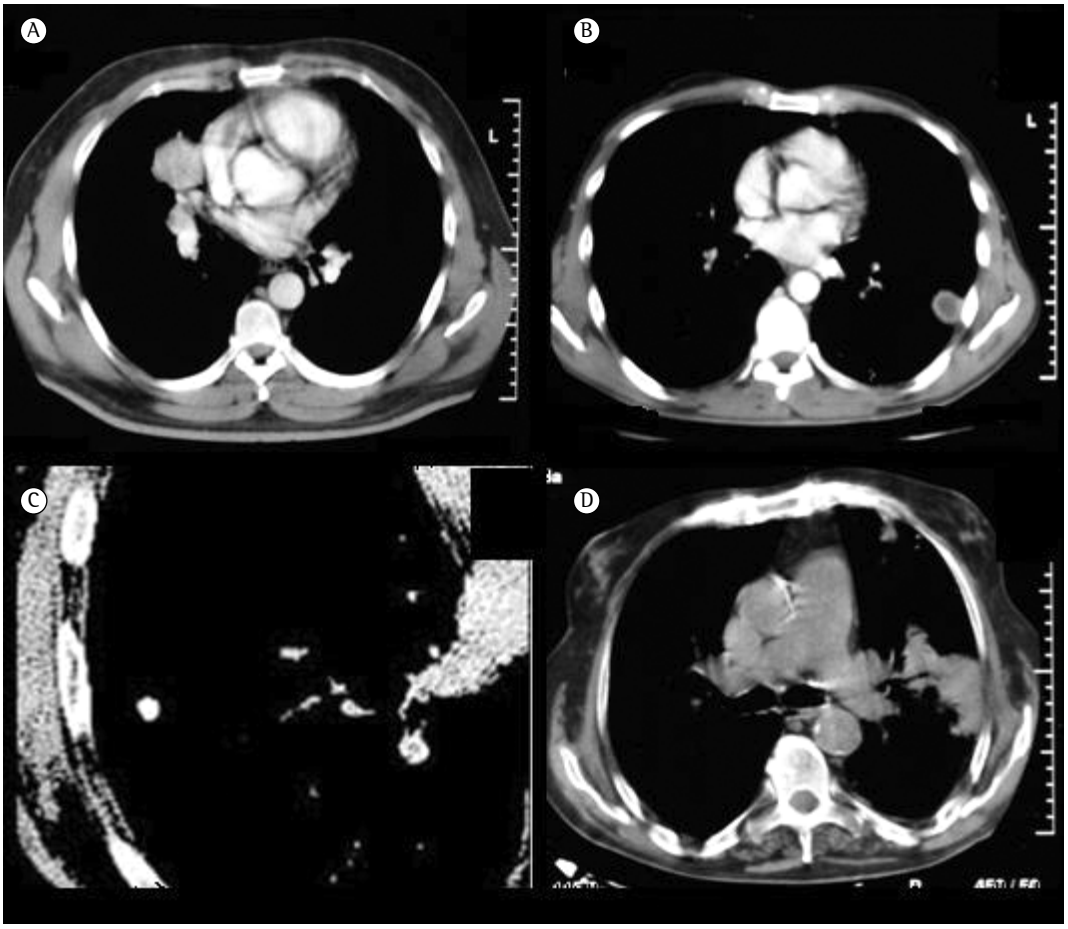

Figure 2 - Helical CT scans showing intralesional changes, including homogeneous attenuation (in A; male patient, 49 years old), necrosis (in B; male patient, 36 years old), calcification (in C; male patient, 56 years old), and air bronchogram (in D; male patient, 77 years old).

Table 1 - Distribution of malignant and benign lesions in the study sample (N = 113), by patient age and pulmonary nodule diameter.

Variable	Diagnosis	n	Mean ± SD	Median (range)	p *
Age	Malignant	88	60.7 ± 12.1	63 (30-80)	0.034
	Benign	25	54.4 ± 12.9	60 (12-82)	
Diameter	Malignant	88	5.3 ± 1.9	5.0 (2.4-10.0)	< 0.001
	Benign	25	3.7 ± 1.3	4.0 (1.8-6.5)	

*Student's t-test for independent samples.

intralesional changes were analyzed on the basis of the assumption that a given patient might present with different types of lesion margins or intralesional changes.

There was a significant association between the presence of malignant lesions and smoking (p = 0.002). Most of the patients in the study sample were smokers (n = 78). Of those, 76.1% had malignant lesions.

There was no significant association between the histopathological diagnosis and the CT-PTNB technique employed (p = 0.778). The proportions of lesions that were diagnosed as malignant by core biopsy, FNAB, or a combination of the two were similar, i.e., 29.2%, 23.0%, and 25.6%, respectively.

Table 3 shows the percentage distribution of malignant lesions by gender and CT findings. Malignant lesions were more common in male patients (55.7%). Adenocarcinoma was the most common malignant lesion in males and females (48.9%). Regarding location, peripheral lesions

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Table 2 - Distribution of malignant and benign lesions in the study sample (N = 113), by gender and CT findings.[a]

Parameter		Diagnosis		Total	p
		Malignant	Benign		
Gender	Female	39 (44.3)	06 (24.0)	45 (39.8)	0.067*
	Male	49 (55.7)	19 (76.0)	68 (60.2)	
	Total	88 (100.0)	25 (100.0)	113 (100.0)	
Location	Central	15 (17.0)	02 (8.0)	17 (15.0)	0.264*
	Peripheral	73 (83.0)	23 (92.0)	96 (85.0)	
	Total	88 (100.0)	25 (100.0)	113 (100.0)	
Lesion margins	Regular	12 (8.0)	16 (45.7)	28 (15.1)	0.022**
	Spiculated	76 (50.7)	09 (25.7)	85 (45.9)	0.597**
	Bosselated	43 (28.7)	06 (17.1)	49 (26.5)	0.118**
	Irregular	19 (12.6)	4 (11.4)	23 (12.4)	0.837**
	Total	150 (100.0)	35 (100.0)	185 (100.0)	
Intralesional changes	Homogeneous attenuation	54 (51.9)	10 (21.3)	64 (42.4)	0.001**
	Necrosis	28 (26.9)	04 (8.5)	32 (21.2)	0.007**
	Cavitation	12 (11.5)	14 (29.8)	26 (17.2)	0.004**
	Calcification	06 (5.7)	11 (23.4)	17 (11.2)	0.003**
	Air bronchogram	04 (4.0)	08 (17.0)	12 (8.0)	0.015**
	Total	104 (100.0)	47 (100.0)	151 (100.0)	

[a]Values expressed as n (%). *Chi-square test. **Normal approximation test for comparison of two proportions.

predominated (82.9%). Adenocarcinoma was the most common tumor in the peripheral region (56.2%). Regarding lesion margins, approximately half of all lesions were spiculated (50.7%). In patients with adenocarcinoma, the most common lesions were those with irregular margins (57.9%), those with spiculated margins (51.3%), and those with bosselated margins (44.2%). Homogeneous attenuation was the most common intralesional change (51.9%), followed by necrosis (26.9%). Homogeneous attenuation was most commonly found in patients with adenocarcinoma and in those with epidermoid carcinoma (38.9% and 24.1%, respectively). Cavitation was most common in cases of epidermoid carcinoma (66.7%).

Benign lesions were more common in male patients (76%), tuberculosis being the most common in males and females (72%). There was a predominance of peripheral lesions (92%). Lesions with regular margins predominated (45.7%). Cavitation was the most common intralesional change (29.8%), followed by calcification (23.4%). Air bronchogram and cavitation were most common in tuberculosis patients (87.5% and 85.8%, respectively).

Discussion

The present study evaluated the clinical application of CT and CT-PTNB in 113 patients with pulmonary nodules. The results of our study showed that CT and CT-PTNB were useful in distinguishing between malignant and benign lesions in patients with pulmonary nodules. Advanced age and smoking were significantly associated with malignancy. In patients with malignant pulmonary nodules, CT findings included larger diameter, spiculated margins, homogeneous attenuation, and necrosis. Adenocarcinoma was the most common malignant tumor, affecting mainly older patients.

The mean age of the patients in the present study was 59.3 years, being similar to that found in the literature.[15,16] In the present study, 23.0% of the patients with nodules and 54.8% of those with masses were found to have malignant lesions, the mean age of those patients ranging from 37.9 years (Hodgkin's lymphoma) to 65.6 years (epidermoid carcinoma).

In patients under 40 years of age, the incidence of lung cancer is lower than 5%.[15,16] This is due to the fact that advanced age increases the risk of lung cancer, which rarely occurs in individuals under 30 years of age.[15,17] Lung cancer is currently a public health problem and is the leading cause of cancer death in males and females, the worldwide incidence of lung cancer increasing by 0.5% per year.[17,18] In Brazil, lung cancer is the second leading cause of death

Table 3 - Distribution of malignant lesions in the study sample (N = 113), by gender and CT findings.[a]

Parameter	Malignant lesion							
	ADC	EPC	HL	SCC	NHL	MT	Other	Total
Gender								
Female	15 (38.5)	11 (28.2)	5 (12.8)	0 (0.0)	2 (5.1)	1 (2.6)	5 (12.8)	39 (44.3)
Male	28 (57.1)	5 (10.2)	3 (6.1)	6 (12.2)	2 (4.1)	3 (6.1)	2 (4.1)	49 (55.7)
Total								88 (100)
Location								
Central	2 (13.3)	0 (0.0)	8 (53.3)	2 (13.3)	1 (6.7)	0 (0.0)	2 (13.3)	15 (17.0)
Peripheral	41 (56.2)	16 (21.9)	0 (0.0)	4 (5.6)	3 (4.1)	4 (5.6)	5 (6.8)	73 (83.0)
Total								88 (100)
Lesion margins								
Regular	4 (33.3)	4 (33.3)	1 (8.3)	0 (0.0)	0 (0.0)	1 (8.3)	2 (16.7)	12 (8.0)
Spiculated	39 (51.3)	12 (15.8)	7 (9.2)	6 (7.9)	4 (5.3)	3 (3.9)	5 (6.6)	76 (50.7)
Bosselated	19 (44.2)	6 (14.0)	7 (16.3)	3 (7.0)	1 (2.3)	3 (7.0)	4 (9.3)	43 (28.7)
Irregular	11 (57.9)	3 (15.8)	0 (0.0)	3 (15.8)	0 (0.0)	1 (5.3)	1 (5.3)	19 (12.6)
Total								150 (100)
Intralesional changes								
Homogeneous attenuation	21 (38.9)	13 (24.1)	7 (13.0)	3 (5.6)	1 (1.9)	4 (7.4)	5 (9.3)	54 (51.9)
Necrosis	17 (60.7)	3 (10.7)	1 (3.6)	2 (7.1)	3 (10.7)	0 (0.0)	2 (7.1)	28 (26.9)
Cavitation	1 (8.3)	8 (66.7)	0 (0.0)	2 (16.7)	0 (0.0)	0 (0.0)	1 (8.3)	12 (11.5)
Calcification	1 (16.7)	1(16.7)	3 (50.0)	0 (0.0)	0(0.0)	0 (0.0)	1 (16.7)	6 (5.7)
Air bronchogram	3 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	4 (4.0)
Total								104 (100)

ADC: adenocarcinoma; EPC: epidermoid carcinoma; SCC: small cell carcinoma; HL: Hodgkin's lymphoma; NHL: non-Hodgkin's lymphoma; and MT: metastasis. [a]Values expressed as n (%).

in males and females.[19] In the present study, 60.2% of all males and 39.8% of all females had lung cancer. This result is similar to those found in the literature.[19-21]

Regarding smoking, the proportion of malignant pulmonary nodules was higher in smokers than in nonsmokers (76.1% vs. 23.9%), malignant pulmonary nodules being more common in males (61.5%). These findings are consistent with the literature; however, the number of cases of malignancy in females is increasing because of smoking, lung cancer in females accounting for approximately half of all cases of lung cancer.[15,17,18] Smoking is the main risk factor for lung cancer, accounting for 80-90% of all cases.[15,17,21]

In the present study, CT scans revealed malignant lesions larger than 3 cm in diameter (lung masses) in 69% of the sample as a whole, a finding that suggests that most of the patients had advanced disease. This is probably due to the delayed onset of lung cancer symptoms and the difficulty in screening the population at risk.[22] This result is consistent with the literature; the probability of malignancy is higher in individuals with lung masses (> 3 cm).[12,15,16] Nevertheless, the results of the Early Lung Cancer Action Project[16] showed that 8% of all nodules smaller than 1 cm in diameter were malignant. In the present study, malignant lesions ≤ 3 cm in diameter were detected in 23% of the patients.

In the present study, approximately half of all malignant lesions were spiculated. In patients with pulmonary lesions, the presence of spicules is a predictor of malignancy in 90% of cases.[9,10] In the present study, 28.7% of the lesions had irregular margins and 12.6% were bosselated. Although irregular margins and bosselated margins are suggestive of malignancy, they can also be found in benign lesions[9,10,23]; 25.7% of all benign lesions in the present study were found to have irregular margins, whereas 17.1% were found to have bosselated margins.

Although homogeneous attenuation was the most common intralesional change in the patients with malignant nodules (being found in 51.9%), it cannot be used in order to distinguish between benign and malignant lesions, because other changes, such as necrosis, cavitation, and air bronchogram, are also indicative of malignancy,[12] whereas calcification is the most common intralesional change in patients with benign

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lesions.[4,6,9,10] In the present study, calcification was found in only 5% of all malignant lesions.

We analyzed the histopathological reports and found that most (77.9%) of the pulmonary nodules were malignant, adenocarcinoma and epidermoid carcinoma being the most common tumors (38.0% and 14.1%, respectively). Adenocarcinoma is the most common tumor (in 30-50% of cases), followed by epidermoid carcinoma (in 30% of cases).[15,18,24] Although the proportion of patients with epidermoid carcinoma in the present study was almost half that reported in the literature, this finding is related to intralesional changes, such as necrosis and cavitation, which were more common in those with that type of tumor, a finding that is consistent with the literature.[9]

More than 50% of all adenocarcinomas found in the present study were located in the peripheral region, a finding that is similar to that of another study.[9] However, all epidermoid carcinomas in the present study were located in the peripheral region, and this is in disagreement with the results of a study showing that the central region is the most affected.[25]

Of all benign lesions found in the present study, those caused by tuberculosis were found to be the most common, a finding that is consistent with the literature showing that infectious granulomas are the most common cause of benign pulmonary nodules.[6]

Because of the characteristics of lung cancer progression, including late clinical symptoms associated with an absence of effective screening programs for the general population, lung cancer has become a serious clinical problem, helical CT being essential for detecting, characterizing, and biopsying such tumors. Lung cancer screening campaigns involving the use of multidetector CT and low radiation doses were found to reduce the risk of delayed diagnosis or lung cancer death in at-risk patients.[12,26,27] However, lung cancer screening is not part of public health programs.[26,27]

In the present study, CT-PTNB contributed to the diagnosis of pulmonary nodules, avoiding unnecessary surgery or assisting in the treatment of malignant lung tumors. Therefore, according to a group of authors,[28] pulmonary nodules require a multidisciplinary approach involving pulmonologists, thoracic surgeons, and radiologists.

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Comparison between two thoracotomy closure techniques: postoperative pain and pulmonary function*

Comparação entre duas técnicas de fechamento de toracotomia: dor pós-operatória e função pulmonar

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Abstract

Objective: To compare two thoracotomy closure techniques (pericostal and transcostal suture) in terms of postoperative pain and pulmonary function. **Methods:** This was a prospective, randomized, double-blind study carried out in the Department of Thoracic Surgery of the Luzia de Pinho Melo *Hospital das Clínicas* and at the University of Mogi das Cruzes, both located in the city of Mogi das Cruzes, Brazil. We included 30 patients (18-75 years of age) undergoing posterolateral or anterolateral thoracotomy. The patients were randomized into two groups by the type of thoracotomy closure: pericostal suture (PS; n = 16) and transcostal suture (TS; n = 14). Pain intensity during the immediate and late postoperative periods was assessed by a visual analogic scale and the McGill Pain Questionnaire. Spirometry variables (FEV_1, FVC, FEV_1/FVC ratio, and PEF) were determined in the preoperative period and on postoperative days 21 and 60. **Results:** Pain intensity was significantly greater in the PS group than in the TS group. Between the preoperative and postoperative periods, there were decreases in the spirometry variables studied. Those decreases were significant in the PS group but not in the TS group. **Conclusions:** The patients in the TS group experienced less immediate and late post-thoracotomy pain than did those in the PS group, as well as showing smaller reductions in the spirometry parameters. Therefore, transcostal suture is recommended over pericostal suture as the thoracotomy closure technique of choice.

Keywords: Thoracic surgery; Suture techniques; Acute pain.

Resumo

Objetivo: Comparar duas técnicas de fechamento de toracotomias (sutura pericostal e transcostal) em relação à dor pós-operatória e função pulmonar. **Métodos:** Estudo prospectivo, randomizado e duplo-cego realizado no Serviço de Cirurgia Torácica do Hospital das Clínicas Luzia de Pinho Melo e na Universidade de Mogi das Cruzes, na cidade de Mogi das Cruzes, Brasil. Foram incluídos no estudo 30 pacientes submetidos a toracotomias posterolaterais ou anterolaterais, com idade entre 18 e 75 anos. Os pacientes foram randomizados em dois grupos em função do tipo de fechamento da toracotomia: sutura pericostal (SP; n = 16) e sutura transcostal (ST; n = 14). A intensidade da dor no pós-operatório imediato e tardio foi avaliada por uma escala visual analógica e questionário de dor McGill. Foram avaliadas variáveis espirométricas (VEF_1, CVF, relação VEF_1/CVF e PFE) no pré-operatório e nos 21º e 60º dias pós-operatórios. **Resultados:** A intensidade da dor foi significativamente maior no grupo SP que no grupo ST. No grupo SP, houve reduções significativas nas variáveis espirométricas estudadas entre o período pré-operatório e pós-operatório. Essas reduções não foram significativas no grupo ST. **Conclusões:** Os pacientes no grupo ST apresentaram menor intensidade de dor pós-toracotomia, tanto imediata como tardia, e menor redução nos parâmetros espirométricos que os no grupo SP. Dessa forma, a técnica de fechamento de toracotomia por sutura transcostal é recomendada por apresentar vantagens sobre a técnica pericostal tradicional.

Descritores: Cirurgia torácica; Técnicas de sutura; Dor aguda.

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Introduction

Conventional thoracic surgery can cause several complications, because access to the pleural cavity requires sectioning of the intercostal muscles, opening of the parietal pleura, and spreading of the ribs. In this procedure, the costal periosteum and the intercostal neurovascular bundle can suffer injuries of varying degrees, resulting from the mechanical effects of retractors or the thermal effects of electrocautery.[1-4]

Most patients undergoing thoracotomy complain of pain, which is responsible for shallow breathing, with a consequent decrease in lung volumes and capacities, as well as secretion retention and atelectasis.[5-8] To prevent the acute pain and respiratory changes that accompany thoracic interventions, new approaches have been used, such as minimally invasive thoracotomy. The advent of video-assisted surgery two decades ago enabled the use of smaller access ports to the thoracic cavity and resection via small thoracotomy. This reduced the incidence of postoperative pain and the changes in pulmonary function.[2,9] However, conventional techniques for thoracic surgery cannot always be replaced by minimally invasive techniques, and, in such cases, acute and/or chronic pain may be present. There are still many resection cases requiring major posterolateral or anterolateral thoracotomy, especially in patients with tumors and in those with chronic infectious diseases. These major surgical procedures require some precautions, especially during thoracotomy closure, because, in practice, intercostal space closure is commonly performed with sutures around the ribs, designated pericostal sutures (PSs).

Thoracotomy closure with PSs may cause injury due to compression of the neurovascular bundle, which courses on the lower edge of the rib, as a result of its anatomical position. The structure most vulnerable to trauma is the cutaneous branch of the intercostal nerve, because of its location on the costal margin. Its trauma due to compression or crushing during the procedure of costal approximation implies pain and cutaneous paresthesia for some days or months postoperatively.[8]

In an attempt to minimize pain, some thoracic surgeons are currently replacing PS with transcostal suture (TS), which consists in passing the approximation suture through holes drilled directly into the ribs. This technique has shown positive and promising results regarding decreased pain in the postoperative period.[10-12]

The objective of the present study was to compare two thoracotomy closure techniques, i.e., PS and TS, in terms of postoperative pain and pulmonary function.

Methods

This was a prospective, randomized, double-blind study carried out between August of 2011 and September of 2012. The study project was approved by the Research Ethics Committee of the University of Mogi das Cruzes on November 18, 2010 (Protocol no. 150/2010, CAAE 0144.0.0237.000-10).

We included all patients (18-75 years of age) undergoing posterolateral or anterolateral thoracotomy through intracavitary access. The exclusion criteria were as follows: having bone metastasis; having a history of pain caused by other comorbidities; and being dependent on drugs, opioid analgesics, or any other substance that affects one's sensitivity to pain.

The patients were randomized into two groups by the type of thoracotomy closure: PS group and TS group. To that end, we used web-based randomization.

In the PS group, thoracotomy closure was performed by passing the suture around the fifth rib, close to its upper border, and around the sixth rib, away from its lower border, and drawing them together (Figure 1).

In the patients in the TS group, closure was performed as follows. The position for the suture drill holes was marked on the periosteum using an electrocautery knife. Subsequently, holes were drilled into the fifth and sixth ribs using a 7-mm diameter drill, which was rotated by a dental motor (LB100; Beltec, Araraquara, Brasil; Figures 2A and 2B). Four equidistant holes were drilled into each rib. The sutures were passed through the drill holes, and transcostal closure was performed (Figure 2B).

All closures were performed using coated synthetic absorbable polyglactin 910 suture (VICRYL®, Ethicon Endo-Surgery, Inc. Cincinnati, OH, USA), size 1, and a circular needle (40 mm).

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The study variables were postoperative pain and pulmonary function as assessed by spirometry on postoperative day (POD) 21 and POD 60 for comparisons with the values obtained in the preoperative period. According to the study protocol, pain was assessed from POD 1 to POD 10, as well as in the late postoperative period (POD 21 and POD 60).

To assess pain, we used a one-dimensional visual analog scale (VAS) and the McGill Pain Questionnaire.[13] The VAS is a 0 to 10 point scale, with 0 meaning complete absence of pain and 10 meaning the greatest level of experienced pain, with which therapists ask patients about their pain intensity. The McGill Pain Questionnaire assesses pain in four distinct domains (sensory, affective, evaluative, and mixed), on the basis of words, designated descriptors, which patients select to describe their pain.[13] Patients are instructed to choose, from among 20 groups of descriptors, those that best describe their pain at the time of the assessment.[13] The first 10 descriptors are related to the sensory dimension of pain. Descriptors 11 to 15 are related to the affective dimension of pain. Descriptor 16 addresses pain in an evaluative way, whereas descriptors 17 to 20 represent a mixed class of alternative words.[13]

Spirometry was performed in accordance with the American Thoracic Society 1995 criteria and the Brazilian Thoracic Association criteria.[14] In a stable setting, the patient sat in a comfortable position and, wearing a nose clip, performed a maximal forced expiratory maneuver, from TLC to RV. Thus, FVC, FEV_1, FEV_1/FVC ratio, and PEF were measured.[14]

Individual data are expressed as mean and standard error. Statistical analysis was performed using GraphPad Instant Software (GraphPad Software, San Diego, CA, USA). Categorical variables (gender, race, clinical diagnosis, and surgical procedure) were assessed by the chi-square test. For numerical variables (spirometry), we used the Student's t-test to compare results between the PS and TS groups and one-way ANOVA to compare preoperative and postoperative results within the same group. For the analysis of pain as measured by the VAS, we used the Student's t-test, whereas, for the analysis of pain as determined by the McGill Pain Questionnaire, we used the Mann-Whitney test.[15] The level of significance set for rejection of the null hypothesis was $p < 0.05$.[15]

Results

We included 31 patients, of whom 16 and 15 were randomized to the PS and TS groups, respectively. Only 1 patient in the TS group did not return for reassessment and was excluded

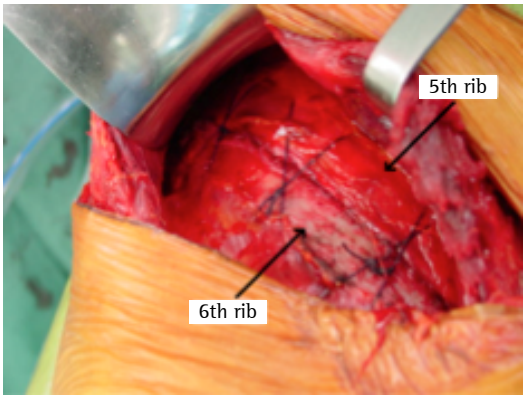

Figure 1- Closure technique with pericostal suture.

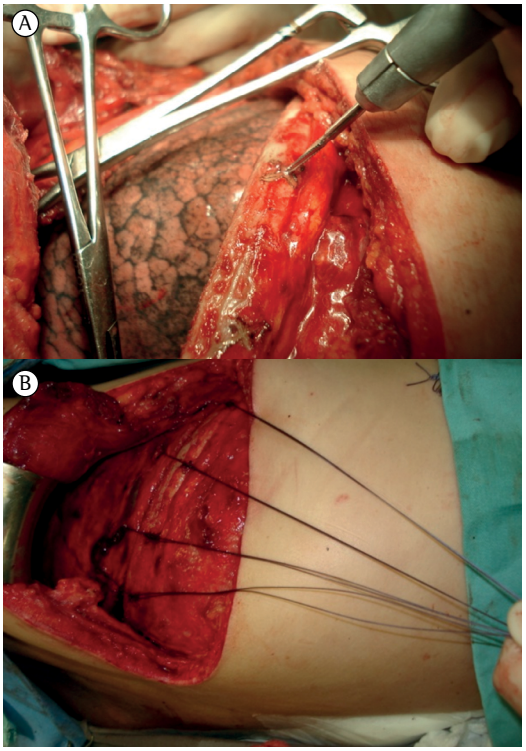

Figure 2 - In A, rib drilling. In B, approximation of the fifth and sixth ribs after the suture was passed through the drill holes.

from the study. Table 1 shows the characteristics of the sample.

The diagnosis of the patients in the PS and TS group was, respectively, as follows: adenocarcinoma, in 10 and 8 patients; epidermoid carcinoma, in 3 and 3; small cell carcinoma, in 2 and 3; and tuberculosis sequelae, in 1 and 0. Lobectomy was the most commonly performed surgical procedure (Table 1). The mean surgical time was 271.5 ± 25.7 min for the PS group and 250.3 ± 23.4 min for the TS group (p = 0.88).

Mean pain intensity (values expressed as n) was calculated for each POD. In both groups, there was a reduction in pain intensity in the follow-up period. Pain intensity was greater for the patients in the PS group than for those in the TS group from the immediate postoperative period, and this difference was statistically significant until POD 7 (p < 0.0001; Figure 3A). For the patients in the TS group, pain was minimal or absent around POD 7, whereas the patients in the PS group still reported moderate pain at that time point. In the PS group, pain was reported as minimal only on POD 60. After the McGill Pain Questionnaire was administered, we calculated and compared the mean total numbers of descriptors chosen and the mean questionnaire total scores for each assessment day. This assessment was performed from POD 1 to POD 10 and repeated on POD 21 and POD 60.

Table 1 - Sample characteristics and procedures performed in the groups studied.[a]

Variables	Groups		p
	Pericostal suture (n = 16)	Transcostal suture (n = 14)	
Age, years[b]	53.6 ± 3.4	48.9 ± 4.4	0.39
Gender			
Male	11 (68.8)	7 (50.0)	0.50
Female	5 (31.3)	7 (50.0)	
Race			
White	14 (87.5)	10 (71.4)	0.14
Afro-descendent	2 (12.5)	3 (21.4)	
Asian	0 (0.0)	1 (7.1)	
Surgery performed			
Lobectomy	9 (56.35)	8 (57.1)	0.12
Bilobectomy	4 (25.0)	3 (21.4)	
Segmentectomy	3 (18.8)	3 (21.4)	

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

When we compared the total numbers of descriptors chosen by the patients, we found that they were greater in the PS group than in the TS group. Postoperative pain intensity as assessed by this scale was found to be greater for the patients in the PS group. This difference was statistically significant between the two groups until POD 10 (p < 0.01; Figure 3B). When we compared the questionnaire total scores, we found that they were higher in the PS group than in the TS group, with this difference being statistically significant for the first 10 PODs (p < 0.001; Figure 3C).

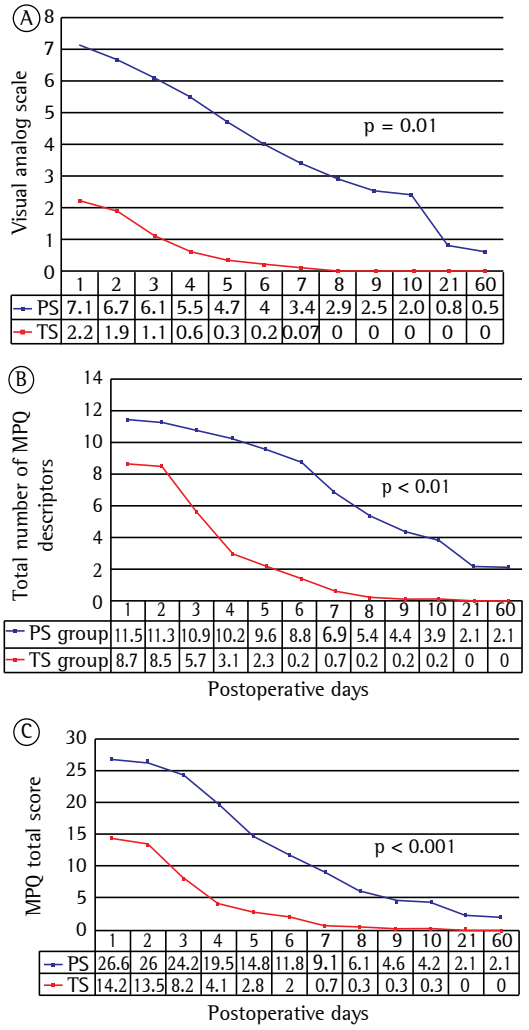

Figure 3 - Comparison of mean pain intensity on various postoperative days in the pericostal suture (PS) and transcostal suture (TS) groups. In A, visual analog scale. In B, total number of McGill Pain Questionnaire (MPQ) descriptors selected by the patients. In C, MPQ total score.

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Spirometric assessment of pulmonary function was performed at three distinct time points: in the preoperative period; on POD 21; and on POD 60. The surgical procedure led to a reduction in spirometric values in both groups, because the surgical procedure results in partial resection of the lung; however, according to the statistical analysis, the sample was homogeneous in terms of the type of surgery performed (p = 0.12). Table 2 shows the spirometric values at each time point of the study in the two groups. The FVC, FEV_1 and PEF values were significantly lower postoperatively than preoperatively in the PS group, whereas there were no significant differences in these values in the TS group.

Discussion

Confirming the interest in the subject, during the present study, a systematic review was published on thoracotomy closure techniques and their relationship with post-thoracotomy pain.[16] The authors of that review, searching the Cochrane Plus Library using the search terms "pain", "thoracotomy", and "suture", found 174 publications that linked the surgical technique employed with postoperative pain. Of those, 11 publications met the selection criteria established for that review, and, of those 11, 6 compared the thoracotomy closure technique with post-thoracotomy pain, only 4 of which were randomized studies. Those authors concluded that there is a need for further scientific evidence on certain technical aspects of thoracotomy closure techniques and their relationship with post-thoracotomy pain. In addition, they pointed out that only through the development of prospective randomized studies specifically comparing the different thoracotomy closure techniques described in the literature and assessing their relationship with post-thoracotomy pain will it be possible to make recommendations in this regard. However, the review made clear that a necessary aspect for reducing post-thoracotomy pain, and that should be common to all closure techniques, is a focus on intercostal nerve preservation.

Among the variables selected for the present study, pain was the one that caused the most difficulty in terms of assessment, because it is a continuous variable that is difficult to quantify. In addition to being a symptom, it is a subjective experience and is influenced by various factors, such as environmental, emotional, behavioral, and social factors. Therefore, we used two standard tools (a VAS and the McGill Pain Questionnaire).[13]

The largest prospective study of this subject included 280 patients undergoing posterolateral thoracotomy, divided into two groups: TS (n = 140) and PS (n = 140).[10] Pain was assessed by a numeric pain scale and the McGill Pain Questionnaire. Those instruments were administered

Table 2 - Spirometry results in the preoperative period and on postoperative days 20 and 60 in the groups studied.[a]

Variable	Time point	PS group	p	TS group	p
FVC, L	Pre	3.00 ± 0.30	0.007	2.85 ± 0.20	0.14
	POD 21	2.10 ± 0.10		2.38 ± 0.20	
	POD 60	2.26 ± 0.10		2.61 ± 0.30	
FEV_1, L	Pre	2.48 ± 0.10	0.01	2.33 ± 0.30	0.28
	POD 21	1.72 ± 0.10		1.91 ± 0.30	
	POD 60	1.89 ± 0.10		2.13 ± 0.30	
PEF, L/s	Pre	5.96 ± 0.50	0.02	5.30 ± 0.60	0.29
	POD 21	4.03 ± 0.40		4.41 ± 0.50	
	POD 60	4.80 ± 0.50		5.19 ± 0.70	
FEV_1/FVC, %	Pre	83.4 ± 2.0	0.71	79.8 ± 4.0	0.51
	POD 21	82.5 ± 2.0		83.2 ± 3.0	
	POD 60	84.1 ± 2.0		81.7 ± 3.0	

PS: pericostal suture; TS: transcostal suture; Pre: preoperative period; and POD: postoperative day. [a]Values expressed as mean ± SE.

in the second postoperative week, as well as in the first, second, and third postoperative months. The authors concluded that the patients treated with TS experienced less pain than did those undergoing PS. Although that study was not randomized, it had a consistent level of evidence to recommend the use of TS in thoracotomy closure.[10]

In an experimental study in dogs, pain was assessed in the immediate postoperative period following thoracotomy in 13 animals.[17] Seven animals underwent closure with PS close to the lower border of the lower rib, compressing the (caudal) neurovascular bundle, and 6 dogs underwent closure with TS. Pain was assessed using pain threshold scores, which were based on parameters such as HR and RR, for a period of 24 h. The study showed that the animals treated with TS experienced significantly less pain.[17] Although that experimental study used a similar methodology in terms of the surgical technique employed, which proved of great value in preventing compression of and injury to the intercostal nerve, its limitation was that it assessed pain only in the immediate postoperative period.[17]

The present study showed, through the use of the VAS and the McGill Pain Questionnaire, that the patients in the TS group experienced less pain than did those in the PS group; these results are similar to those reported in previous studies.[10,17]

In previous studies,[12,18,19] thoracotomy closure was also performed using TS; however, there was variation in the technique used to open the intercostal space during access to the pleural cavity, which means that their results are not comparable to the results of the present study or to those of another study,[10] in which technical variation in performing the thoracotomy involved harvesting of intercostal muscle flaps to protect the neurovascular bundle from the chest retractor. Therefore, the assessment of pain threshold in the postoperative period was impaired when comparing the transcostal and pericostal closure groups because there were different interventions.

The use of Finochietto retractors during chest opening is known to be responsible for much of the pain after the surgical procedure. In our study, we took this into account, which is why the same method for opening the chest wall was used in both groups, i.e., there was no variation in the technique for opening the chest wall, as previously suggested by other authors.[12,16]

The present study found that the patients in the PS group used a large number of descriptors to characterize their postoperative pain—on average, 11 descriptors on POD 1, with a mean score of 26. This has also been observed in a prospective study[6] comparing pain, as assessed by the McGill Pain Questionnaire, in 40 patients undergoing either posterolateral thoracotomy or sternotomy. The mean number of descriptors used by the patients in the group undergoing posterolateral thoracotomy was 16, with a mean score of 30, values that are very close to those found in the present study.

Regarding pulmonary function, we observed that the patients undergoing standard thoracotomy closure (PS group) showed significantly lower FVC, FEV_1, and PEF on POD 21 than in the preoperative period. These results are historically expected in the postoperative period after thoracotomy and were similar to those reported in previous studies.[20,21]

A previous study[19] investigated pulmonary function in 16 patients after major thoracotomy. Spirometry was performed on POD 14. The authors observed that FVC, FEV_1, and PEF were significantly lower postoperatively than preoperatively.[19] Patient recovery in terms of these variables was due to improvement in ventilatory capacity, reduction of the chest wall injury caused by the surgical procedure, and pain relief.

A prospective study of 33 patients undergoing thoracic surgery evaluated the impact of lung resection on pulmonary function in lung cancer patients undergoing thoracotomy.[21] Spirometry was performed in the preoperative period and in the sixth postoperative month. The FEV_1, PEF, and FVC values statistically significantly decreased relative to the values obtained in the preoperative period. Such results were expected and are related to the direct impact of surgical resection and to postoperative pain.[21]

In the present study, we expected a decrease in the spirometry variables, because the surgical procedures involve resection of lung parenchyma. However, the procedures performed in both groups were quite similar, and less postoperative pain in the TS group translated into a smaller decrease

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in FVC, FEV_1, and PEF. In the PS group, in which pain was found to be more severe, the decreases in the spirometry values were greater.

In conclusion, the patients undergoing closure of a posterolateral or anterolateral thoracotomy with TS experienced a significant decrease in immediate and late postoperative pain when compared with those undergoing closure with PS. In addition, the patients in the TS group showed smaller reductions in the spirometry parameters. Therefore, TS is recommended over PS as the thoracotomy closure technique of choice.

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Comparison between reference values for FVC, FEV_1, and FEV_1/FVC ratio in White adults in Brazil and those suggested by the Global Lung Function Initiative 2012*

Comparação entre os valores de referência para CVF, VEF_1 e relação VEF_1/CVF em brasileiros caucasianos adultos e aqueles sugeridos pela *Global Lung Function Initiative 2012*

Carlos Alberto de Castro Pereira, Andrezza Araujo Oliveira Duarte, Andrea Gimenez, Maria Raquel Soares

Abstract

Objective: To evaluate the spirometry values predicted by the 2012 Global Lung Function Initiative (GLI) equations, which are recommended for international use, in comparison with those obtained for a sample of White adults used for the establishment of reference equations for spirometry in Brazil. **Methods:** The sample comprised 270 and 373 healthy males and females, respectively. The mean differences between the values found in this sample and the predicted values calculated from the GLI equations for FVC, FEV_1, and VEF_1/FVC, as well as their lower limits, were compared by paired t-test. The predicted values by each pair of equations were compared in various combinations of age and height. **Results:** For the males in our study sample, the values obtained for all of the variables studied were significantly higher than those predicted by the GLI equations ($p < 0.01$ for all). These differences become more evident in subjects who were shorter in stature and older. For the females in our study sample, only the lower limit of the FEV_1/FVC ratio was significantly higher than that predicted by the GLI equation. **Conclusions:** The predicted values suggested by the GLI equations for White adults were significantly lower than those used as reference values for males in Brazil. For both genders, the lower limit of the FEV_1/FVC ratio is significantly lower than that predicted by the GLI equations.

Keywords: Respiratory function tests/statistics and numerical data; Respiratory function tests/diagnosis; Reference values.

Resumo

Objetivo: Comparar os valores espirométricos previstos pelas equações da *Global Lung Function Initiative* (GLI) em 2012, sugeridas como de uso internacional, com aqueles obtidos em uma amostra utilizada para derivação de valores de referência em adultos caucasianos brasileiros. **Métodos:** A amostra utilizada era composta por 270 homens e 373 mulheres saudáveis. As médias das diferenças entre os valores dessa amostra e os valores previstos calculados a partir das equações da GLI para CVF, VEF_1 e VEF_1/CVF, assim como seus limites inferiores, foram comparados por teste de t pareado. Os valores previstos pelos pares das equações foram comparados em diversas combinações de idade e estatura. **Resultados:** Nos homens da amostra, os valores obtidos para todas as variáveis estudadas foram significativamente maiores que aqueles previstos pelas equações da GLI ($p < 0,01$ para todas). Estas diferenças se tornaram mais evidentes em indivíduos com menor estatura e idade mais avançada. Nas mulheres, somente o limite inferior da relação VEF_1/CVF foi significativamente maior na amostra brasileira. **Conclusões:** Os valores previstos sugeridos pelas equações da GLI para caucasianos são significativamente menores daqueles utilizados como referência para homens brasileiros. Em ambos os sexos, o limite inferior da relação VEF_1/CVF é significativamente menor que o previsto pelas equações GLI

Descritores: Testes de função respiratória/estatística e dados numéricos; Testes de função respiratória/diagnóstico; Valores de referência.

Introduction

The interpretation of pulmonary function tests is based on comparisons between data obtained for an individual patient and (predicted) reference values derived from healthy subjects.

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Ideally, reference values should be derived from a population similar to that tested, using appropriate equipment and following standard procedures.[1]

Pulmonary function values differ substantially among different regions of the world, which has been attributed to anthropometric, environmental, social, and genetic factors, as well as to technical factors.[1-4] Attempts to compile equations by different authors were made for Europe in 1983[5] and again in 1993.[6] Those recommendations of the work group were accepted and made official by the European Respiratory Society (ERS), which supported their widespread use in Europe.

In 2005, a joint guideline of the American Thoracic Society (ATS) and the ERS recommended that the equations derived in the Third National Health and Nutrition Examination Survey (NHANES III) be adopted in the USA, but it did not endorse the use of equations for Europe, recommending that new reference values be obtained.[1] The latter recommendation was based on the fact that the derivation of reference values for spirometry in various European countries after 1993 demonstrated that the equations proposed by Quanjer et al. underestimated predicted values.[6-10] This finding was confirmed by various studies published after 2005.[11-15] Similar results were observed when reference values derived for the Brazilian population were compared with those proposed by Quanjer et al.[6,16,17]

Various limitations were identified in the derivation of the equations that were compiled by that group of authors, and it was suggested that those reference values be abandoned,[18] although studies using those reference values continue to be published.

In 2012, an even bolder proposal was suggested by Quanjer et al.: the derivation of universal equations.[19] Data on reference values derived from 72 centers in 33 countries were provided for the derivation of the equations. In Latin America, values derived in the *Projeto Latino-Americano de Investigação em Obstrução Pulmonar* (PLATINO, Latin American Project for the Investigation of Obstructive Lung Disease), which included subjects over 40 years of age, were provided.[20] We decided not to send the equations derived for adults in the Brazilian population because of the limitations observed in the previous study by Quanjer et al.[6] and because we do not believe that a universal pulmonary function equation is possible. The proponents of the universal equation acknowledge that the included data from Latin America are scarce and that that equation should not be used in the continent.

However, values for White adults were suggested, and we tested the hypothesis that those values could fit our population.

Methods

The predicted values derived from the ERS Global Lung Function Initiative (GLI) equations[19,21] for White adults were calculated for males and females by using data on gender, height, and age found in a study of reference values for the Brazilian population.[16] The patients selected completed a standard respiratory questionnaire,[22] were nonsmokers, had no respiratory symptoms, and had no cardiopulmonary disease. The Brazilian sample included 270 males (age, 25-86 years; height, 152-192 cm) and 373 females (age, 20-85 years; height, 137-182 cm).

The equations derived for males were as follows[16]:

$FVC = H \times 0.0517 - A \times 0.0207 - 3.18$
(lower limit of normality [LLN] = −0.90)

$FEV1 = H \times 0.0338 - A \times 0.0252 - 0.789$
(LLN = −0.76)

$FEV1/FVC \times 100 = 120.3 - H \times 0.175 - A \times 0.197$ (LLN = −7.6)

where H is height in cm and A is age in years.

The equations derived for females were as follows[16]:

$FVC = H \times 0.041 - A \times 0.0189 - 2.848$
(LLN = −0.64)

$FEV1 = H \times 0.0314 - A \times 0.0203 - 1.353$
(LLN = −0.61)

$FEV1/FVC \times 100 = 111.5 - H \times 0.140 - A \times 0.158$ (LLN = −8.3)

The GLI equation used to derive the parameter values is as follows:

$\log(Y) = 5a + b \times \log(H) + c \times \log(A) + AS + d \times \text{group}$

where Y is the dependent variable, H is height in cm, A is age in years, and AS is age spline.

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Group takes a value of 1 for White adults, and this value was used in the present study. The Brazilian equation for FVC and FEV_1 is linear:

$Y = a \times H - b \times A$ – constant

The mean values found in the Brazilian sample for FVC, FEV_1, and FEV_1/FVC, as well as their lower limits, were compared with the predicted values calculated from the GLI equations on the basis of the age and height of individual subjects in the Brazilian sample. Paired t-test was used for the comparisons.

Subsequently, on the basis of the Brazilian values, a linear regression analysis was performed between age (independent variable) and height (dependent variable). Regression equations were used to calculate the expected value for height at ages 25, 50, and 75 years for both genders. The values calculated from the GLI equation and those calculated from the Brazilian equation were tabulated and compared in various combinations of age and height.

All statistical procedures were performed with the IBM SPSS Statistics software, version 20.0 (IBM Corp., Armonk, NY, USA).

Results

The mean differences between the predicted values calculated from the Brazilian equations and those generated by the GLI equations, as well as their lower limits, are shown in Table 1. For males, the values found in the Brazilian sample for all of the variables studied were significantly higher than those generated by the GLI equations. For females, there were practically no differences, except for the lower limit of the FEV_1/FVC ratio, for which the values in the Brazilian sample were significantly higher than those generated by the GLI equations.

When the data tabulated for the various combinations of age and height were compared, additional data could be observed (Tables 2 and 3). For males, the differences were more evident in shorter, older subjects. In subjects aged 75 years, the differences for FVC and for its lower limit were 0.36 L and 0.38 L, respectively. For these same subjects, the difference for FEV_1 and for its lower limit was 0.29 L for both.

It is also of note that the FEV_1/FVC ratio was lower as calculated from the GLI equation, with the difference increasing with age.

Discussion

In the present study, universal reference equations for spirometry proved unable to predict spirometry values in the Brazilian population accurately.

Various reference value equations have been published in recent decades. The expected values for individuals with a certain combination of age and height can differ considerably.[1-3]

Such variations can be explained by the criteria used for selecting 'normal' populations, by the equipment used, by the measurement techniques, by the biological variability of populations, by socioeconomic and environmental factors, and by the statistical models used in the data analysis.

In 2005, the ATS and ERS published a joint guideline on pulmonary function.[1] Reference values were suggested for children and adults in the United States; however, values for other places remained to be established. As a result of this lack of recommendation, a group of authors, led by Quanjer, founded the GLI in Berlin in 2008. In April of 2010, the group received, as occurred previously,[5,6] the seal of the ERS as a task force.[19] In 2012, values derived from data sent from various places were grouped, as occurred with European data in 1993,[6] and reference values for subjects aged 3-95 years were suggested. In total, 74,187 nonsmokers from 26 countries in five continents were included in equations derived by combining various studies. The data relating to South America, which were derived from a study conducted in Latin America[20] and from a sample of children in Mexico,[23] were disregarded because of differences in height and in predicted values, as well as because of the lack of data for subjects aged 25-40 years. However, according to the published supplement, 178 cases of White adults in Brazil were included.[19]

The values for White adults were derived especially from five large studies: two conducted in the United States[24,25] and three conducted in Europe.[7,10,13] It is of note that the values derived in those studies differ, which was attributed to the different equipment used. However, various factors, such as sample selection, measurement techniques, and quality control, also influence the results obtained, which complicates the aggregation of different studies.

Comparing the values calculated from the GLI equation with the data derived from a sample used for the establishment of reference equations

Table 1 - Mean differences for the variables studied, calculated by subtracting the predicted values found in the Brazilian population[16] from those generated by the *Global Lung Function Initiative* equations[19,21], by gender.[a]

Variable	Gender					
	Male			Female		
	Δ	t	p	Δ	t	p
FVC	0.29 ± 0.62	7.81	< 0.001	−0.01 ± 0.38	−0.75	0.46
LL	0.30 ± 0.59	9.41	< 0.001	0.01 ± 0.38	0.65	0.52
FEV_1	0.28 ± 0.50	9.06	< 0.001	0.00 ± 0.33	0.36	0.72
LL	0.29 ± 0.48	10.12	< 0.001	−0.02 ± 0.33	−0.93	0.36
FEV_1/FVC	0.93 ± 4.89	3.14	0.002	0.02 ± 5.00	0.06	0.95
LL	3.27 ± 4.71	11.43	< 0.001	3.68 ± 5.23	13.55	< 0.001

LL: lower limit. [a]Values expressed as mean ± SD.

Table 2 - Predicted spirometry values for the Brazilian population[16] and those generated by the Global Lung Function Initiative (GLI) equation[19,21] for combinations of age and height in males.

Variable	Age, years	Height, cm	Pereira et al.[16]	GLI[19,21]
FVC, L	25	175	5.35	5.18
	50	170	4.58	4.48
	75	165	3.80	3.44
LL	25	175	4.45	4.19
	50	170	3.68	3.50
	75	165	2.90	2.52
FEV_1, L	25	175	4.50	4.35
	50	170	3.69	3.56
	75	165	2.90	2.61
LL	25	175	3.74	3.51
	50	170	2.93	2.78
	75	165	2.14	1.85
FEV_1/FVC	25	175	0.85	0.85
	50	170	0.81	0.80
	75	165	0.77	0.76
LL	25	175	0.77	0.73
	50	170	0.73	0.69
	75	165	0.69	0.62

LL: lower limit.

Table 3 - Comparison between predicted spirometry values for the Brazilian population[16] and those generated by the *Global Lung Function Initiative* (GLI) equation[19,21] for combinations of age and height in females.

Variable	Age, years	Height, cm	Pereira et al.[16]	GLI[19,21]
FVC, L	25	162	3.82	3.84
	50	158	3.18	3.24
	75	153	2.47	2.31
LL	25	162	3.18	3.07
	50	158	2.54	2.53
	75	153	1.83	1.63
FEV_1, L	25	162	3.23	3.30
	50	158	2.60	2.60
	75	153	1.90	1.79
LL	25	162	2.62	2.65
	50	158	1.93	2.03
	75	153	1.32	1.28
FEV_1/FVC	25	162	0.85	0.87
	50	158	0.81	0.81
	75	153	0.78	0.78
LL	25	162	0.77	0.75
	50	158	0.73	0.70
	75	158	0.68	0.64

LL: lower limit.

for spirometry in Brazil,[16] we found that, for males, the use of the GLI equation results in lower values both in terms of predicted values and of their lower limits. For females, the values are quite similar, except for the FEV_1/FVC ratio and its lower limit, for which the values in the Brazilian sample are higher than those generated by the GLI equation. These findings indicate that the use of the GLI equation will fail to diagnose reductions in FVC and, therefore, will have lower sensitivity in detecting obstructive lung disease in males. For both genders, the sensitivity for the diagnosis of obstructive lung disease will be lower with the use of the GLI equations, given that the lower limit of the FEV_1/FVC ratio as calculated from these equations is significantly lower, especially in older subjects.

The differences between the predicted values calculated from the GLI equation for the FEV_1/FVC ratio and its lower limits vary because of the regression model used; however, they are, on average, 0.11 for males and 0.12 for females,[21] which exceeds the values derived in Brazil (0.08 for males and 0.09 for females).[16]

Recent studies have compared spirometric diagnosis by the equation suggested by the GLI

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and by other equations. One study compared spirometric diagnosis by three equations in 17,572 tests (subjects aged 18-85 years) performed in laboratories in Australia and Poland.[26] The values calculated from the equations derived by the GLI were higher than those calculated from the equations derived by Quanjer et al.,[6] as expected. Differences in the lower limits resulted in a significant reduction in the diagnosis of restrictive lung disease when the GLI equation was compared with the NHANES III equation, although the latter was incorporated into the GLI equation (but comprised less than 4% of the sample). In males, restrictive lung disease was diagnosed in 22.6% by the NHANES III equation and in 17.1% by the GLI equation. In females, the proportions were 22.8% and 8.1%, respectively.

In a study conducted in Tunisia, local predicted values and those suggested by the GLI were used in 1,192 consecutive spirometries in adults aged 18-60 years.[27] Again, the proportion of cases diagnosed with restrictive lung disease by the use of the local equation (19.0%) was greater than that diagnosed by the GLI equation (8.4%).

The findings of the aforementioned studies are not surprising, given the wide range for determination of lower limits by the GLI equation, which is the result of the combination of several equations for which quality control and results were different.

In conclusion, the values suggested by the multiethnic reference equation proposed by the GLI, developed for White adults, differ significantly from the values derived for White adult males in Brazil. For females, the values derived are similar for FVC, FEV_1, and their lower limits. For both genders, the lower limit of the FEV_1/FVC ratio is significantly lower as calculated from the GLI equation.

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Anemia in hospitalized patients with pulmonary tuberculosis*

Anemia em pacientes internados com tuberculose pulmonar

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Abstract

Objective: To describe the prevalence of anemia and of its types in hospitalized patients with pulmonary tuberculosis. **Methods:** This was a descriptive, longitudinal study involving pulmonary tuberculosis inpatients at one of two tuberculosis referral hospitals in the city of Rio de Janeiro, Brazil. We evaluated body mass index (BMI), triceps skinfold thickness (TST), arm muscle area (AMA), ESR, mean corpuscular volume, and red blood cell distribution width (RDW), as well as the levels of C-reactive protein, hemoglobin, transferrin, and ferritin. **Results:** We included 166 patients, 126 (75.9%) of whom were male. The mean age was 39.0 ± 10.7 years. Not all data were available for all patients: 18.7% were HIV positive; 64.7% were alcoholic; the prevalences of anemia of chronic disease and iron deficiency anemia were, respectively, 75.9% and 2.4%; and 68.7% had low body weight (mean BMI = 18.21 kg/m^2). On the basis of TST and AMA, 126 (78.7%) of 160 patients and 138 (87.9%) of 157 patients, respectively, were considered malnourished. Anemia was found to be associated with the following: male gender (p = 0.03); low weight (p = 0.0004); low mean corpuscular volume (p = 0.03);high RDW (p = 0; 0003); high ferritin (p = 0.0005); and high ESR (p = 0.004). We also found significant differences between anemic and non-anemic patients in terms of BMI (p = 0.04), DCT (p = 0.003), and ESR (p < 0.001). **Conclusions:** In this sample, high proportions of pulmonary tuberculosis patients were classified as underweight and malnourished, and there was a high prevalence of anemia of chronic disease. In addition, anemia was associated with high ESR and malnutrition.

Keywords: Tuberculosis, pulmonary; Anemia; Malnutrition; Iron.

Resumo

Objetivo: Descrever a prevalência de anemia e de seus tipos em pacientes internados com tuberculose pulmonar. **Métodos:** Estudo descritivo e longitudinal com pacientes com tuberculose pulmonar hospitalizados em dois hospitais de referência na cidade do Rio de Janeiro (RJ). Foram avaliados o índice de massa corpórea (IMC), dobra cutânea tricipital (DCT), área muscular do braço (AMB), VHS, volume globular médio e *red blood cell distribution width* (RDW, índice de anisocitose eritrocitária), assim como os níveis de proteína C reativa, hemoglobina, transferrina e ferritina. **Resultados:** Foram incluídos 166 pacientes, sendo 126 (75,9%) do sexo masculino. A média de idade foi de 39,0 ± 10,7 anos. Alguns dados não estavam disponíveis para todos os pacientes: 18,7% eram portadores de HIV; 64,7% eram etilistas; as prevalências de anemia da doença crônica e de anemia ferropriva foram, respectivamente, de 75,9% e 2,4%; e 68,7% apresentaram baixo peso (média do IMC = 18,21 kg/m^2). Com base em DCT e AMB, respectivamente, 126/160 pacientes (78,7%) e 138/157 pacientes (87,9%) foram considerados desnutridos. A presença de anemia associou-se às seguintes variáveis: sexo masculino (p = 0,03), baixo peso (p = 0,0004), baixo volume globular médio (p = 0,03), alto RDW (p = 0,0003), alto nível de ferritina (p = 0,0005) e de VHS (p = 0,004). Houve diferenças significativas entre pacientes anêmicos e não anêmicos em relação a IMC (p = 0,04), DCT (p = 0,003) e VHS (p < 0,001). **Conclusões:** Nesta amostra, a proporção de pacientes com tuberculose pulmonar classificados com baixo peso e desnutrição foi elevada, assim como a prevalência de anemia da doença crônica. Além disso, a anemia associou-se a VHS elevada e desnutrição.

Descritores: Tuberculose pulmonar; Anemia; Desnutrição; Ferro.

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Introduction

According to the World Health Organization, one third of the world population is infected with *Mycobacterium tuberculosis*. It is estimated that approximately 8.8 million new cases of tuberculosis occur each year; Brazil ranks 18th among the 22 countries that collectively account for most such cases.[1]

In Brazil, approximately 85,000 cases of tuberculosis occur each year, approximately 5,000 deaths being associated with the disease. The incidence rate of tuberculosis in the country has been estimated at 37.2/100,000 population. Among all Brazilian states, Rio de Janeiro has the highest annual incidence rate of tuberculosis (73.27/100,000 population) and the highest mortality rate (5.0/100,000 population).[2]

According to the World Health Organization, the severity of the global tuberculosis situation is primarily due to social inequality, population aging, large migration flows, and the advent of AIDS in the 1980s.[3] In addition to AIDS, risk factors for tuberculosis include alcoholism, smoking, history of tuberculosis, diabetes mellitus, malnutrition, and low socioeconomic status.[4]

The association between tuberculosis and malnutrition consists of two interactions: the effect of tuberculosis on the nutritional status and the effect of malnutrition on the clinical manifestations of tuberculosis, as a result of immunological impairment.[3,5] Anemia has been observed in 32-94% of patients with tuberculosis [6-8]

Iron deficiency is the most common micronutrient deficiency in the world, and numerous studies have evaluated the association between serum iron levels and iron-deficiency anemia.[9,10] However, there is controversy regarding the administration of iron; some studies have shown that iron deficiency increases susceptibility to infectious processes, whereas others have shown that excess iron is more harmful to the human body than is iron deficiency, and that iron deficiency can protect against infection.[11]

Among the anemias that are characterized by altered iron metabolism, iron-deficiency anemia and anemia of chronic disease are the most common.[12]

Iron-deficiency anemia is the most common nutritional deficiency worldwide, affecting primarily individuals residing in developing countries. It occurs as a result of chronic blood loss, urinary losses, poor iron intake/absorption, and increased blood volume. In individuals with iron-deficiency anemia, a decrease in plasma iron levels occurs, limiting erythropoiesis. The risk of developing iron-deficiency anemia is highest among infants, children under 5 years of age, and women of childbearing age.[12]

Anemia of chronic disease, also known as anemia of inflammation, is a clinical syndrome characterized by the development of anemia in patients with (fungal, bacterial, or viral) infectious diseases, such as tuberculosis, inflammatory diseases, autoimmune diseases, and neoplastic diseases.[13] It is characterized by mild to moderate normocytic hypochromic anemia, and hypochromia and microcytosis can occur in 20-30% of cases. However, when microcytosis occurs, it is not as pronounced as it is in iron-deficiency anemia.[12] This type of anemia is associated with decreased serum iron levels and total iron binding capacity, as well as with increased ferritin levels.[13]

In patients with active tuberculosis, few of the studies investigating the presence of anemia have determined whether anemia is associated with iron deficiency or chronic disease or have identified variables associated with its occurrence.[6-8]

The objective of the present study was to describe the prevalence of anemia and of its types in hospitalized patients with pulmonary tuberculosis, as well as to examine the relationship between anemia and the clinical and nutritional status of anemic patients in comparison with non-anemic patients.

Methods

This was a prospective cross-sectional descriptive study, which included active pulmonary tuberculosis patients consecutively admitted to one of two tuberculosis referral hospitals in the state of Rio de Janeiro (namely *Instituto Estadual de Doenças do Tórax Ary Parreiras* and *Hospital Estadual Santa Maria*, both located in the city of Rio de Janeiro) and initiating antituberculosis treatment between March of 2007 and December of 2010. All participants gave written informed consent.

Patients under 18 years of age or over 60 years of age were excluded, as were those who had previously undergone tuberculosis treatment or who had been receiving treatment with antituberculosis drugs for more than seven days; those with diabetes mellitus receiving insulin therapy; those with renal failure on peritoneal dialysis or hemodialysis;

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those who had received blood transfusions in the 3 months preceding study entry; and those who were pregnant or lactating. For data collection, we used a standardized questionnaire and reviewed medical records. In addition, we collected blood samples and performed medical and nutritional assessment up to seven days after the initiation of pharmacological treatment. Alcohol abuse was defined as a daily intake of 30 g or more for males and of 24 g or more for females. The Cut down, Annoyed, Guilty, and Eye-opener (CAGE) questionnaire was used in order to identify alcohol abuse.[14]

Nutritional assessment included measurements of weight, height, and body mass index (BMI), in order to identify patients who were underweight,[15] as well as measurements of triceps skinfold thickness (TST) and arm muscle area (AMA), in order to identify patients who were malnourished.[15,16]

In order to classify anemia, we analyzed the following parameters: hemoglobin levels; transferrin levels; ferritin levels; and mean corpuscular volume (MCV). We used red blood cell distribution width (RDW) in order to assess the presence of anisocytosis. This classification is shown in Chart 1. In addition to the aforementioned measurements, we performed measurements of C-reactive protein (CRP) and ESR, as well as HIV testing. All tests were performed in a laboratory certified by the Brazilian Clinical Pathology Association Clinical Laboratory Accreditation Program. Iron-deficiency anemia was characterized by decreased levels of iron and ferritin and increased levels of transferrin, whereas anemia of chronic disease was characterized by decreased levels of iron and transferrin and increased levels of ferritin.[13]

Chart 1 - Parameters for the evaluation of the types of anemia studied.

Biochemical parameters	Anemia of chronic disease	Iron-deficiency anemia
Transferrin	Decreased or normal	Increased
Ferritin	Normal or increased	Decreased
MCV	Decreased or normal	Decreased
RDW	Normal or increased	Increased

MCV: mean corpuscular volume; and RDW: red blood cell distribution width.

For statistical analysis, we used descriptive statistics, including range (minimum and maximum values), mean, standard deviation, median, interquartile range, and 95% CI. We used the Kolmogorov-Smirnov test in order to test the normality of the variables and Levene's test in order to determine the equality of variances. We used the Student's t-test in order to compare means with normal distribution between the groups of patients with and without anemia. We used ANOVA in order to analyze the differences among quantitative variables and the chi-square test in order to identify associations among categorical variables. For the identification of variables associated with anemia, we used multivariate logistic regression analysis in order to assess the presence of confounding covariates. Covariates with values of $p < 0.20$ in the bivariate analysis were included in the model. Values of $p < 0.05$ were considered statistically significant. All analyses were performed with the Statistical Package for the Social Sciences, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA).

The present study was approved by the Research Ethics Committee of the Federal University of Rio de Janeiro School of Medicine Clementino Fraga Filho University Hospital on April 28, 2005 (Protocol no. 004/05).

Results

We included 166 patients, 126 (75.9%) of whom were male. The mean age was 39.0 ± 10.7 years. In our sample, 95 (62.5%) of 152 patients were non-White; 18 (18.7%) of 96 patients were HIV-positive; 97 (64.7%) of 150 patients were considered alcoholic on the basis of the CAGE questionnaire; 118 (74.7%) of 158 patients were classified as smokers or former smokers; and 47 (30.1%) of 156 patients reported illicit drug use. Of the 166 patients, 18 (10.9%) had no anemia and 148 (89.1%) had anemia. Of those, 4 (2.4%) had iron-deficiency anemia and 126 (75.9%) had anemia of chronic disease; in the remaining 18 patients, it was impossible to distinguish between the two.

We found low hemoglobin levels (mean, 10.86 ± 2.04 g/dL) in 89.2% of patients; low transferrin levels (mean, 177.28 ± 58.71 mg/dL) in 65.3%; and low MCV (mean, 82.00 ± 7.77 fL) in 39.7%. In addition, we found high ferritin levels (mean, 520.68 ± 284.26 ng/mL) in 52.7% of patients; high RDW (mean, 16.36 ± 3.47%)

in 55.4%; high CRP levels (mean, 5.84 ± 4.22 mg/dL) in 98.2%; and high ESR (mean, 60.30 ± 39.84 mm/h) in 84.3%.

On the basis of the BMI, 88 (68.7%) of 128 patients were underweight (mean, 18.21 ± 2.93 kg/m^2). On the basis of the TST, 126 (78.7%) of 160 patients were mildly, moderately, or severely malnourished (mean, 6.16 ± 3.83 mm). On the basis of the AMA, 138 (87.9%) of 157 patients were mildly, moderately, or severely malnourished (mean, 24.41 ± 9.86 cm^2).

When we compared the sociodemographic and clinical variables between the groups of patients with and without anemia, we found an association of anemia with the male gender (p = 0.03) and a trend toward an association of anemia with being a smoker or former smoker (p = 0.05; Tables 1 and 2). Table 3 shows a comparison of nutritional and laboratory variables between the groups of patients with and without anemia. Anemia was found to be associated with the following: BMI (p = 0.0004); MCV (p = 0.03); ferritin (p = 0.0005); RDW (p = 0.0003); and ESR (p = 0.004). After the multivariate analysis, ESR was the only independent variable that remained.

Table 4 shows the results of the correlation of nutritional and laboratory variables with the presence of anemia. Mean BMI and mean TST were significantly lower in the patients with anemia than in those without. However, high ESR values were significantly associated with anemia (p < 0.001). Nevertheless, there were no significant differences between the groups of patients with and without anemia regarding AMA, transferrin levels, ferritin levels, or MCV.

Discussion

In the present study, pulmonary tuberculosis was found to be more common in young adults, males, alcoholics, smokers, illicit drug users, and HIV-positive patients; this finding is similar to those reported in studies evaluating pulmonary tuberculosis inpatients at general and tuberculosis referral hospitals in Brazil.[17,18]

The prevalence of anemia in the present study (89.2%) was higher than was that in a study conducted in South Korea (32%)[6] and similar to that in studies conducted in Indonesia (63%),[7] Tanzania (96%),[8] and Malawi (88%).[19] In the present study, the proportion of patients with anemia of chronic disease was higher than was that of those with iron-deficiency anemia (75.9% vs. 2.4%), a finding that was similar to those reported in other studies[6,7] but different from those reported in another study.[8] In the bivariate analysis, anemia was found to be more common in males than in females, a finding that is inconsistent with the literature.[6-8] However, the association between anemia and the male gender was not confirmed in the multivariate analysis; likewise, we found no association between anemia and HIV infection, a finding that is in disagreement with those reported in other studies.[8,19]

On the basis of the BMI, 68.7% of patients were found to be underweight, a proportion that is higher than that reported in a study conducted in Peru (21%)[20] and similar to those reported in studies conducted in Malawi[4,21] and England.[22] This is probably due to the fact that those

Table 1 - Distribution of sociodemographic variables between the groups of patients with and without anemia.[a]

Variable	Patients with anemia (n = 148)	Patients without anemia (n = 18)	OR (95% CI)	p
Gender				
Male	116 (78.4)	10 (55.6)	2.90 (1.05-7.95)	0.03
Female	32 (21.6)	8 (44.4)		
Age, years[b]	38.6	37.6		0.71
Smoking status				
Smokers	70 (47.3)	9 (50.0)	2.70 (0.98-7.41)	0.05
Former smokers	38 (25.7)	1 (5.6)		
Never smokers	32 (21.6)	8 (44.4)		
ND	8 (5.4)	0 (0.0)		
Illicit drug use				
Yes	43 (29.1)	4 (22.2)	1.58 (0.49-5.09)	0.43
No	95 (64.2)	14 (77.8)		
ND	10 (6.8)	0 (0.0)		

ND: no data. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as mean.

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Table 2 - Distribution of clinical variables between the groups of patients with and without anemia.[a]

Variable	Patients with anemia (n = 148)	Patients without anemia (n = 18)	OR (95% CI)	p
HIV status				
Positive	17 (11.5)	1 (5.6)		
Negative	67 (45.3)	11 (61.1)	2.79 (0.33-23.1)	0.32
ND	64 (43.2)	6 (33.3)		
Alcoholism				
Yes	90 (60.8)	7 (38.9)		
No	45 (30.4)	8 (44.4)	2.28 (0.77-6.70)	0.12
ND	13 (8.8)	3 (16.7)		
Transferrin levels				
Low	86 (58.1)	10 (55.6)		
Normal	44 (29.7)	7 (38.9)	1.36 (0.48-3.83)	0.55
ND	18 (12.2)	1 (5.8)		
Ferritin levels				
High	77 (52)	1 (5.6)		
Normal	49 (33.1)	10 (55.6)	24.6 (3.2-191.2)	0.00005
Low	4 (2.7)	7 (38.9)		
ND	18 (12.2)	0 (0.0)		
MCV				
Low	63 (42.6)	3 (16.7)	3.70 (1.02-13.35)	0.03
Normal	85 (57.4)	15 (83.3)		
RDW				
Low	4 (2.7)	12 (66.7)		
Normal	52 (35.1)	6 (33.3)	0.03 (0.004-0.26)	0.0003
High	92 (62.2)	0 (0.0)		
CRP levels				
Normal	2 (1.3)	1 (0.6)		
High	145 (98.0)	17 (94.4)	0.23 (0.02-2.72)	0.21
ND	1 (0.7)	0 (0.0)		
ESR				
Normal	19 (12.8)	7 (38.9)	0.23 (0.07-0.67)	0.004
High	129 (87.2)	11 (61.1)		

ND: no data; MCV: mean corpuscular volume; RDW: red blood cell distribution width; and CRP: C-reactive protein. [a]Values expressed as n (%).

studies included high proportions of HIV-positive inpatients.

On the basis of the TST and AMA, 126 (78.7%) of 160 patients and 138 (87.9%) of 157 patients, respectively, were considered malnourished. Similar results have been reported elsewhere.[22] A 13% reduction in TST and a 20% reduction in AMA were reported in a case-control study,[22] whereas a 35% reduction in TST and a 19% reduction in AMA were reported in another study.[21]

Almost all of the patients included in our study were found to have elevated levels of CRP and ESR, a finding that is similar to those reported in the literature.[7,23,24] We believe that CRP and ESR can be useful as markers of the effect of treatment and of the resolution of inflammation, given that CRP and ESR levels decreased during antituberculosis treatment, having normalized by the end of the treatment period (data not shown).

The concentrations of most proteins are elevated in tuberculosis patients, the exception being the concentrations of transferrin and hemoglobin, which are decreased.[25] In our study, we found low concentrations of transferrin and high concentrations of ferritin, a finding that is similar to those reported by other groups of authors.[6,7,25,26]

In conditions other than inflammatory conditions, determination of ferritin levels is

Table 3 - Distribution of anthropometric variables between the groups of patients with and without anemia.[a]

Variable	Patients with anemia (n = 148)	Patients without anemia (n = 18)	OR (95% CI)	p
BMI				
Underweight	82 (55.4)	6 (33.3)	5.85 (2.00-17.07)	0.0004
Normal weight	25 (31.1)	11 (61.1)		
Overweight	3 (2.0)	1 (5.6)		
ND	17(11.5)	0 (0.0)		
Nutritional status (TST)				
Severe malnutrition	85 (57.4)	9 (50)	2.24 (0.76-6.57)	0.13
Mild/moderate malnutrition	30 (20.3)	2 (11.1)		
Normal nutritional status	28 (18.9)	6 (33.3)		
ND	5 (3.4)	1 (5.6)		
Nutritional status (AMA)				
Severe malnutrition	117 (79.1)	11 (61.1)	2.56 (0.74-8.87)	0.12
Mild/moderate malnutrition	8 (5.4)	2 (11.1)		
Normal nutritional status	15 (10.1)	4 (22.2)		
ND	8 (5.4)	1 (5.6)		

BMI: body mass index; ND: no data; TST: triceps skinfold thickness; and AMA: arm muscle area. [a]Values expressed as n (%).

Table 4 - Correlation between nutritional and laboratory variables in the groups of patients with and without anemia.

Variable	Patients with anemia		Patients without anemia		p*
	n	Mean ± SD	n	Mean ± SD	
BMI	131	18.047 ± 2.85	17	19.55 ± 3.22	0.044
TST	143	5.85 ± 3.44	17	8.7 ± 5.72	0.003
AMA	140	24.12 ± 9.95	17	26.88 ± 8.92	0.276
Transferrin	130	175.71 ± 59.64	17	189.29 ± 51.06	0.372
Ferritin	130	534.30 ± 266.98	17	416.54 ± 313.34	0.403
MCV	148	81.83 ± 7.99	18	83.42 ± 5.66	0.415
RDW	148	16.54 ± 3.50	18	14.94 ± 2.92	0.065
CRP	147	6.06 ± 4.23	18	4.04 ± 3.89	0.055
ESR	148	69.49 ± 39.56	18	25.89 ± 21.56	< 0.001

BMI: body mass index; TST: triceps skinfold thickness; AMA: arm muscle area; MCV: mean corpuscular volume; RDW: red blood cell distribution width; and CRP: C-reactive protein. *Student's t-test.

the most sensitive method for the diagnosis of iron deficiency. However, in tuberculosis patients, determination of ferritin levels should be used with caution because ferritin levels do not accurately express the amount of iron in such patients. Therefore, patients can have iron deficiency even when they have normal or increased ferritin levels.[13]

Given that microcytosis was observed in most of the patients in the present study, increased RDW might be useful to demonstrate iron deficiency,[26] although its role remains controversial.[27]

When we compared the groups of patients with and without anemia in terms of their nutritional status, we found that malnutrition was more severe in the former, who had low serum concentrations of transferrin and high serum concentrations of ferritin, as reported in one study.[7] Regarding the inflammatory state, the multivariate analysis showed that ESR was higher in the patients with anemia than in those without, the difference being significant. One group of authors[27] found that ESR increases in response to anemia, a finding that corroborates the results of the present study. However, although we excluded patients with a history of tuberculosis, those receiving insulin therapy, those on peritoneal dialysis or hemodialysis, and those who had received blood transfusions in the 3 months preceding study entry, the associations of ESR and CRP with anemia in the present study should be confirmed in studies investigating larger samples, preferably with a

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higher prevalence of iron-deficiency anemia and without the presence of comorbidities such as HIV infection, alcoholism, and smoking.

Given that it was impossible to use all of the recommended parameters for the differential diagnosis between iron-deficiency anemia and anemia of chronic disease, including transferrin receptor and bone marrow analysis,[13] the criteria used in the present study resulted in a low frequency of iron-deficiency anemia in isolation. However, we believe that some of the patients with anemia of chronic disease also had iron-deficiency anemia, as reported in one study.[8] In such cases, not all patients benefit from iron supplementation.[13] In another study,[7] after successful tuberculosis treatment, anemia was corrected without iron supplementation in most patients.

In conclusion, high proportions of pulmonary tuberculosis patients were classified as underweight and malnourished on the basis of different parameters (BMI, AMA, and TST), and there was a high prevalence of anemia of chronic disease. In addition, the degree of malnutrition was higher in the patients with anemia than in those without.

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Oxidative damage induced by cigarette smoke exposure in mice: impact on lung tissue and diaphragm muscle*,**

Dano oxidativo induzido por exposição a fumaça de cigarro em camundongos: impacto sobre o pulmão e o músculo diafragma

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Abstract

Objective: To evaluate oxidative damage (lipid oxidation, protein oxidation, thiobarbituric acid-reactive substances [TBARS], and carbonylation) and inflammation (expression of phosphorylated AMP-activated protein kinase and mammalian target of rapamycin [p-AMPK and p-mTOR, respectively]) in the lung parenchyma and diaphragm muscles of male C57BL-6 mice exposed to cigarette smoke (CS) for 7, 15, 30, 45, or 60 days. **Methods:** Thirty-six male C57BL-6 mice were divided into six groups (n = 6/group): a control group; and five groups exposed to CS for 7, 15, 30, 45, and 60 days, respectively. **Results:** Compared with control mice, CS-exposed mice presented lower body weights at 30 days. In CS-exposed mice (compared with control mice), the greatest differences (increases) in TBARS levels were observed on day 7 in diaphragm-muscle, compared with day 45 in lung tissue; the greatest differences (increases) in carbonyl levels were observed on day 7 in both tissue types; and sulfhydryl levels were lower, in both tissue types, at all time points. In lung tissue and diaphragm muscle, p-AMPK expression exhibited behavior similar to that of TBARS. Expression of p-mTOR was higher than the control value on days 7 and 15 in lung tissue, as it was on day 45 in diaphragm muscle. **Conclusion:** Our data demonstrate that CS exposure produces oxidative damage, not only in lung tissue but also (primarily) in muscle tissue, having an additional effect on respiratory muscle, as is frequently observed in smokers with COPD.

Keywords: Oxidative stress; Mice; Respiratory system; Smoking; Inflammation.

Resumo

Objetivo: Avaliar o dano oxidativo (oxidação lipídica, oxidação proteica, *thiobarbituric acid-reactive substances* [TBARS, substâncias reativas ao ácido tiobarbitúrico], e carbonilação) e inflamação (expressão de *phosphorylated AMP-activated protein kinase* e de *phosphorylated mammalian target of rapamycin* (p-AMPK e p-mTOR, respectivamente) em tecido pulmonar e músculos do diafragma em camundongos C57BL/6 machos expostos à fumaça de cigarro (FC) por 7, 15, 30, 45 ou 60 dias. **Métodos:** Trinta e seis camundongos machos da espécie C57BL/6 foram divididos em seis grupos (n = 6/grupo): grupo controle e 5 grupos expostos a FC por 7, 15, 30, 45 e 60 dias, respectivamente. **Resultados:** Comparados aos camundongos controle, os camundongos expostos à FC apresentaram menor peso corporal em 30 dias. Nos camundongos expostos à FC (comparados aos controle) as maiores diferenças (aumentos) nos níveis de TBARS foram observados no dia 7 no músculo diafragma, comparado ao dia 45 em tecido pulmonar; as maiores diferenças (aumentos) nos níveis de carbonilas foram observados no dia 7 em ambos os tipos de tecido; e os níveis de sulfidrilas foram menores, nos dois tipos de tecidos, em todos os tempos. No tecido pulmonar e no músculo diafragma, a expressão de p-AMPK exibiu um comportamento semelhante ao dos níveis de TBARS. A expressão de p-mTOR foi maior que o valor controle nos dias 7 e 15 no tecido pulmonar, assim como no dia 45 no músculo diafragma. **Conclusões:** Nossos dados demonstram que a exposição à FC produz dano oxidativo tanto no tecido pulmonar quanto (primariamente) no tecido muscular, tendo um efeito adicional no músculo respiratório, como é frequentemente observado em fumantes com DPOC.

Descritores: Estresse oxidativo; Camundongos; Sistema respiratório; Poluição por fumaça de tabaco; Inflamação

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Introduction

Cigarette smoke (CS) contains a large number of oxidants that have adverse effects on tissues through oxidative damage.[1,2] It is known that CS activates inflammatory cells, which can also increase polymorphonuclear cell production of oxidants in tissues, triggering oxidative stress, a crucial step in the pathogenesis of CS-induced tissue damage.[3-6] The combined effects of greater proteolytic damage, increased cell death, and decreased lung remodeling leads to emphysematous changes in the lungs.[7] Studies have shown that, in the blood of smokers,[8,9] as well as in various organs of animals chronically exposed to CS,[10] there are increases in lipid peroxidation, protein carbonylation, thiol oxidation, and DNA oxidization.

There is evidence that two central factors are involved in CS-induced direct injury or systemic inflammation: phosphorylated AMP-activated protein kinase and phosphorylated mammalian target of rapamycin (p-AMPK and p-mTOR, respectively). One recent study showed that p-AMPK activation inhibits or promotes inflammation, depending on the stimulus.[11] There is also increasing evidence that, in many cell types, an increase in intracellular reactive oxygen species (ROS) can activate p-AMPK.[12] A major integrator of environmental cues, mTOR controls cellular metabolism, growth, proliferation, and survival depending on mitogenic signals, as well as on the availability of nutrients and energy. It has now become clear that mTOR signaling plays a central role in regulating basic aspects of cell and organism behavior, and its dysregulation is strongly associated with progression of numerous human proliferative and metabolic diseases, including cancer, obesity, type 2 diabetes, and hamartoma syndrome.[13]

It is of great importance to elucidate the possible oxidative damage induced by CS directly in skeletal muscle, as well as the related structural abnormalities and the direct relationship between p-AMPK and p-mTOR, two factors associated with inflammation. Therefore, the aim of this animal study was to evaluate oxidative damage and inflammation in the lung parenchyma and diaphragm after 7, 15, 30, 45, and 60 days of exposure to CS.

Methods

In this study, we used 36 two-month-old male C57BL/6 mice weighing 30-35 g. The animals were used and cared for in accordance with European Communities Council Directive 86/609/EEC of 24 November, 1986. The procedures adopted in this study were approved by the Research Ethics Committee of the University of Southern Santa Catarina, in the city of Criciúma, Brazil. The mice were housed in a temperature- and humidity-controlled environment (70% humidity; 20 ± 2°C), on a 12/12-h light/dark cycle, and were given *ad libitum* access to water and chow (Nuvilab CR1; Nuvital Nutrientes Ltda., Colombo, Brazil). The animals were checked periodically in order to verify that they remained pathogen-free. For biochemical assays, the mice were randomized into six groups (n = 6/group): a control group; and five groups exposed to CS for 7, 15, 30, 45, and 60 days (designated CS-7, CS-15, CS-30, CS-45, and CS-60, respectively).

We used commercial filter cigarettes (Marlboro™ Red, 8 mg of tar and 0.6 mg of nicotine per cigarette; Philip Morris Products, Richmond, VA, USA).[14,15] Study animals were exposed to the smoke emitted from the burning of 12 cigarettes per day for 7, 15, 30, 45, and 60 days, as described previously by Menegali et al.[3] In brief, animals were placed in a covered inhalation chamber (40 cm long, 30 cm wide, and 25 cm high), positioned under an exhaust hood. A cigarette was coupled to a plastic 60-mL syringe so that each puff could be drawn in and subsequently expelled into the exposure chamber. One liter of smoke (20 puffs of 50 mL) was aspirated from each cigarette, each puff being immediately injected into the inhalation chamber. The animals were maintained in this smoke-air condition (3% smoke) for 6 min. We then removed the cover from the inhalation chamber and turned on the exhaust hood, which evacuated the smoke within 60 s. This process was immediately repeated. A total of four cigarettes were thus "smoked" in each treatment. The mice were subjected to these four-cigarette treatments three times per day (morning, noon, and afternoon), resulting in 72 min of CS exposure (12 cigarettes per day).[16] Each cigarette smoked produced 300 mg/m^3 of total particulate matter in the exposure chamber.[3] The animals were sacrificed by cervical dislocation at 24 h after the final CS exposure. Samples of lung tissue and diaphragm muscle were homogenized in buffer solution. The homogenates were centrifuged at 1000 × *g* for 10 min at 4°C,

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and the supernatants were stored at −70°C for subsequent use in the experiments.

For histological analysis, were selected all animals in each group. The right ventricle was perfused with sterile saline (0.9%) to remove blood from the lung. The right lung was fixed (by gentle infusion of 4% phosphate buffered formalin (pH 7.2) at 25 cmH_2O for 2 min through a tracheal catheter), after which it was removed and weighed. Inflated lungs were fixed for 48 h and then embedded in paraffin. Serial sagittal sections (5-μm) were obtained for histological and morphometric analyses. Macrophages and neutrophils were quantified in the alveoli. For each group, were analyzed 30 microscopic fields (10 random fields, of 26,000 mm^2 each, in 3 different sections of the right lung). The number of macrophages and neutrophils (cells/mm^2) were counted in a fluorescence microscope (BH-2; Olympus, Tokyo, Japan) equipped with a 40× objective.[3]

Oxidative damage was evaluated by quantifying sulfhydryl content, protein carbonyls, and malondialdehyde. Total thiol content was determined using the 5,50-dithiobis (2-nitrobenzoic acid)—DTNB—method (Sigma, St. Louis, MO, USA). The conditions of the DTNB test were as previously described.[17] In brief, 30 μL of a sample was mixed with 1 mL of PBS and 1 mM of EDTA (pH 7.5). The reaction was started by the addition of 30 μL of 10 mM DTNB stock solution in PBS. Control samples, which did not include DTNB or protein, were run simultaneously. After 30 min of incubation at room temperature, the absorbance was read at 412 nm and the amounts of 5-thio-2-nitrobenzoic acid (TNB) formed (equivalent to the amount of sulfhydryl groups) were measured. Protein carbonyls were determined using the 2,4-dinitrophenylhydrazine (DNPH) spectrophotometry method, as described by Levine et al.[18] In brief, samples containing either 2 N hydrochloric acid or DNPH were passed through columns containing Sephadex G-10 and rinsed with 2 N hydrochloric acid. The effluent was collected and mixed with guanidine hydrochloride, after which the absorbance determined at 360 nm in a spectrophotometer (SP 1105; Shanghai Spectrum Instruments Co., Ltd., Shanghai, China). The difference in absorbance with and without DNPH was calculated for all samples. Values are expressed as molar quantities using the extinction coefficient 22,000 [M-1]. Malondialdehyde, an important indicator of lipid peroxidation, was determined by spectrophotometry of the pink-colored product of thiobarbituric acid-reactive substances (TBARS). Total TBARS, as a proxy for lipid peroxidation (malondialdehyde levels), are expressed as mmol/mg of protein.[19]

Western blotting, the lung homogenates were prepared from the frozen lungs using a tissue lysis buffer (50 mM TRIS, pH 8.0, 5 mM EDTA, 150 mM NaCl, 1% nonionic detergent, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate) and a protease inhibitor cocktail (Sigma). The lysates were clarified by centrifugation at 13,000 g for 15 min at 4°C; 10-30 g of protein were separated by SDS-PAGE on 10% or 15% gels; and p-AMPK and p-mTOR expression (antibodies from Cell Signaling Biotechnology, Boston, MA, USA) was analyzed by immunoblot analysis. Immunoreactivity was detected by enhanced chemiluminescence (ECL; Amersham Biosciences, Buckinghamshire, UK). The band density was determined using an imaging densitometer and analyzed with the accompanying software (GS-700 and Quantity One; Bio-Rad Laboratories, Hercules, CA, USA).[20]

Data are expressed as mean ± standard error of the mean. To compare means between and among groups, we used one-way ANOVA followed by Tukey's honestly significant difference post-hoc test for multiple comparisons. The level of significance was set at $p < 0.05$. The software used for analysis of the data was the Statistical Package for the Social Sciences, version 18.0 for Windows (SPSS Inc., Chicago, IL, USA). The sample size was based on previous studies performed in our laboratory,[3] in which similar approaches were employed.

Results

Among the mice evaluated in the present study, the survival rate was 100%. In comparison with the baseline values, the body weights of the animals decreased after 30, 45, and 60 days of CS exposure (27 ± 1 vs. 23 ± 0.8 g; $p < 0.01$, 26 ± 0.5 vs. 22 ± 0.4 g; $p < 0.01$, and 25 ± 0.7 vs. 20 ± 0.3 g; $p < 0.001$, respectively). In addition, the body weights of the CS-60 group mice were significantly lower than were those of the control mice, as well as being significantly lower than were those of the CS-30 and CS-45 group mice ($p < 0.001$ for all).

In the histological analysis, lung tissue samples obtained from control mice showed thin alveolar septa and normal alveoli, whereas those obtained from mice that were exposed to CS showed destruction of the alveolar septa (starting on day 15 of exposure), alveolar enlargement, and the presence of alveolar macrophages (Figure 1A). The alveolar enlargement was significantly greater in the CS-45 group (Figure 1A). As shown in Figure 1B, the numbers of macrophages and neutrophils in the CS groups both increased significantly (in comparison with those observed for the control group) by day 7 of exposure to CS ($p < 0.01$). However, the difference in the number of neutrophils was more pronounced after 45 days of exposure ($p < 0.001$).

Figures 2, 3, and 4, respectively, show lipid peroxidation, protein carbonyls and sulfhydryl content in lung tissue samples and diaphragm muscle samples. In both tissue types, total TBARS increased after 7 days of exposure to CS, as did carbonyl levels. In the CS-7, CS-15, and CS-45 groups, there were differences between the lung tissue samples and diaphragm muscle samples,

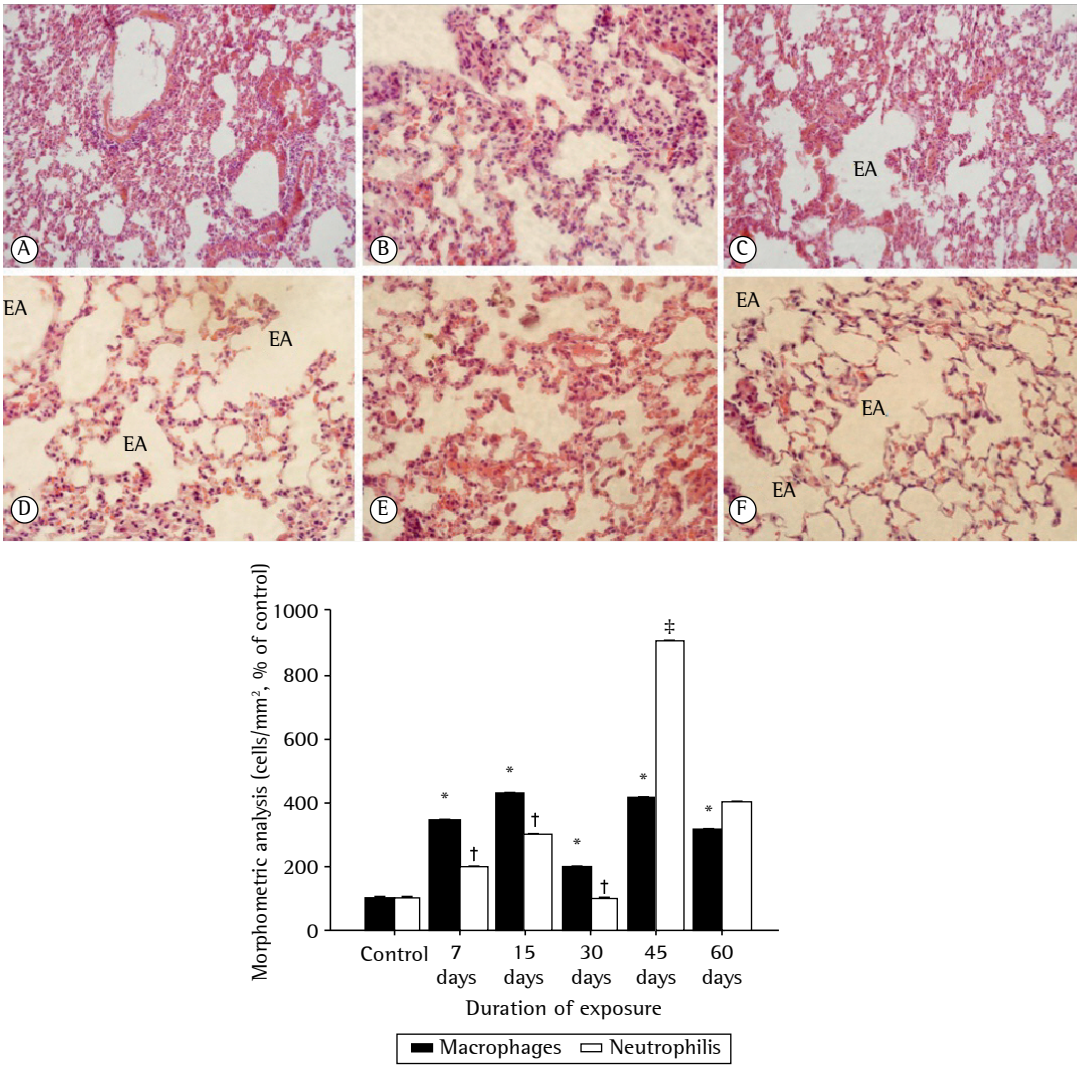

Figure 1 - In A, photomicrographs of lung tissue samples obtained from mice exposed to cigarette smoke, showing enlarged airspaces (EAs) resulting from alveolar consolidation during the development of pulmonary emphysema (magnification, ×40): a, control group; b, 7-day exposure group; c, 15-day exposure group; d, 30-day exposure group; e, 45-day exposure group; and f, 60-day exposure group. In B, Mean ± SEM of macrophages and neutrophils (cells/mm²). *$p < 0.001$ vs. control for macrophages. †$p < 0.001$ vs. control for neutrophils. ‡$p < 0.001$ vs. baseline for neutrophils.

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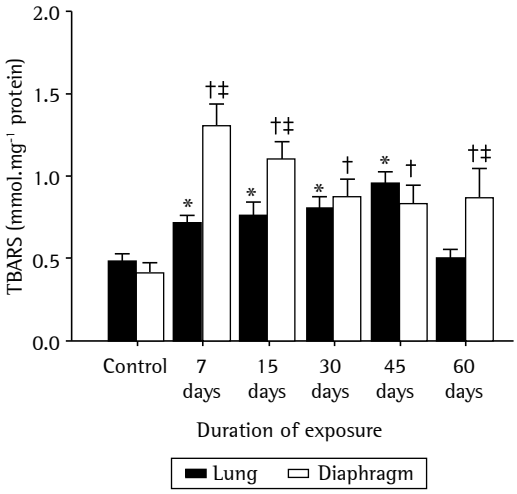

Figure 2 - Mean ± SEM of thiobarbituric acid-reactive substances (TBARS) in lung tissue and diaphragm muscle in six groups of mice: a control group; and five groups exposed to cigarette smoke for 7, 15, 30, 45, and 60 days, respectively. *p < 0.05 vs. control in lung tissue. † p < 0.05 vs. control in diaphragm muscle. ‡ p < 0.05 vs. lung tissue.

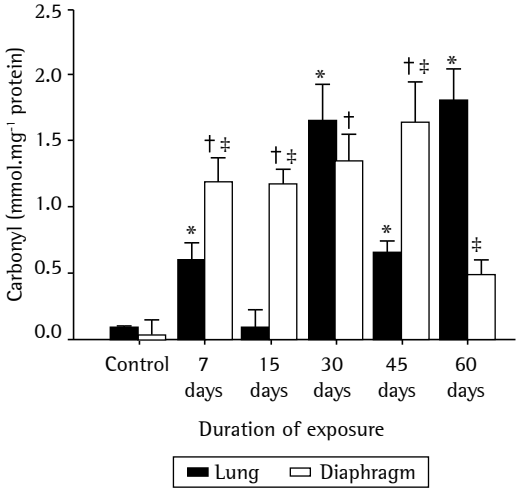

Figure 3 - Mean ± SEM of carbonyl in lung tissue and diaphragm muscle in six groups of mice: a control group; and five groups exposed to cigarette smoke for 7, 15, 30, 45, and 60 days, respectively. * p < 0.05 vs. control in lung tissue. † p < 0.05 vs. control in diaphragm muscle. ‡ p < 0.05 vs. lung tissue.

in terms of the degree to which carbonyl levels were increased. In the CS-15 group, the levels of TNB were significantly lower in lung tissue than in diaphragm muscle. However, by day 7 of CS exposure, TNB levels were lower than the control values in both tissue types.

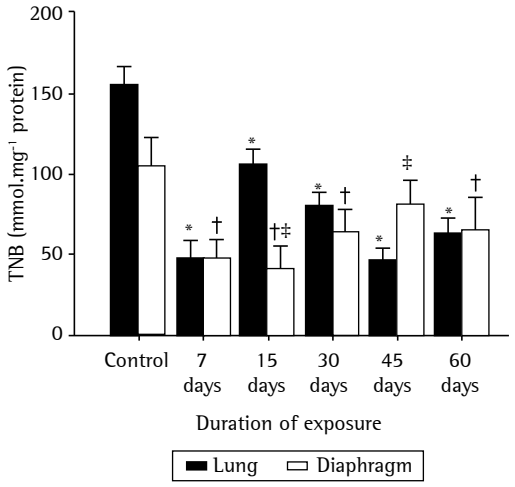

Figure 4 - Mean ± SEM of 5-thio-2-nitrobenzoic acid (TNB) in lung tissue and diaphragm muscle in six groups of mice: a control group; and five groups exposed to cigarette smoke for 7, 15, 30, 45, and 60 days, respectively. * p < 0.05 vs. control in lung tissue. † p < 0.05 vs. control in diaphragm muscle. ‡ p < 0.05 vs. lung tissue.

The lung expression of p-AMPK was higher in the CS-15 group than in the CS-7 group. Notably, in the CS-30 and CS-45 groups, p-AMPK expression was higher in diaphragm muscle than in lung tissue (Figure 5). From day 7 of CS exposure onward, the lung expression of p-mTOR was lower in all CS-exposed groups than in the control group. However, that difference was most pronounced in the CS-7 and CS-45 groups. In the diaphragm muscle samples, p-mTOR expression began to increase by day 15 of CS, peaking by day 45 (Figure 5).

Discussion

In the present study, our main objective was to characterize, at different time points, the effects induced by exposure to CS. The principal effects observed were by oxidative damage in diaphragm muscle and morphological changes in lung tissue.

The amount of neutrophils, which is associated with oxidative damage in lung tissue, was greatest on day 45 of exposure to CS. The numbers of macrophages and neutrophils are high in patients with COPD, having a direct relationship with disease severity.[21] Our data demonstrate increases in leukocytes, including macrophages and neutrophils, from day 7 to day 45 of CS exposure, which

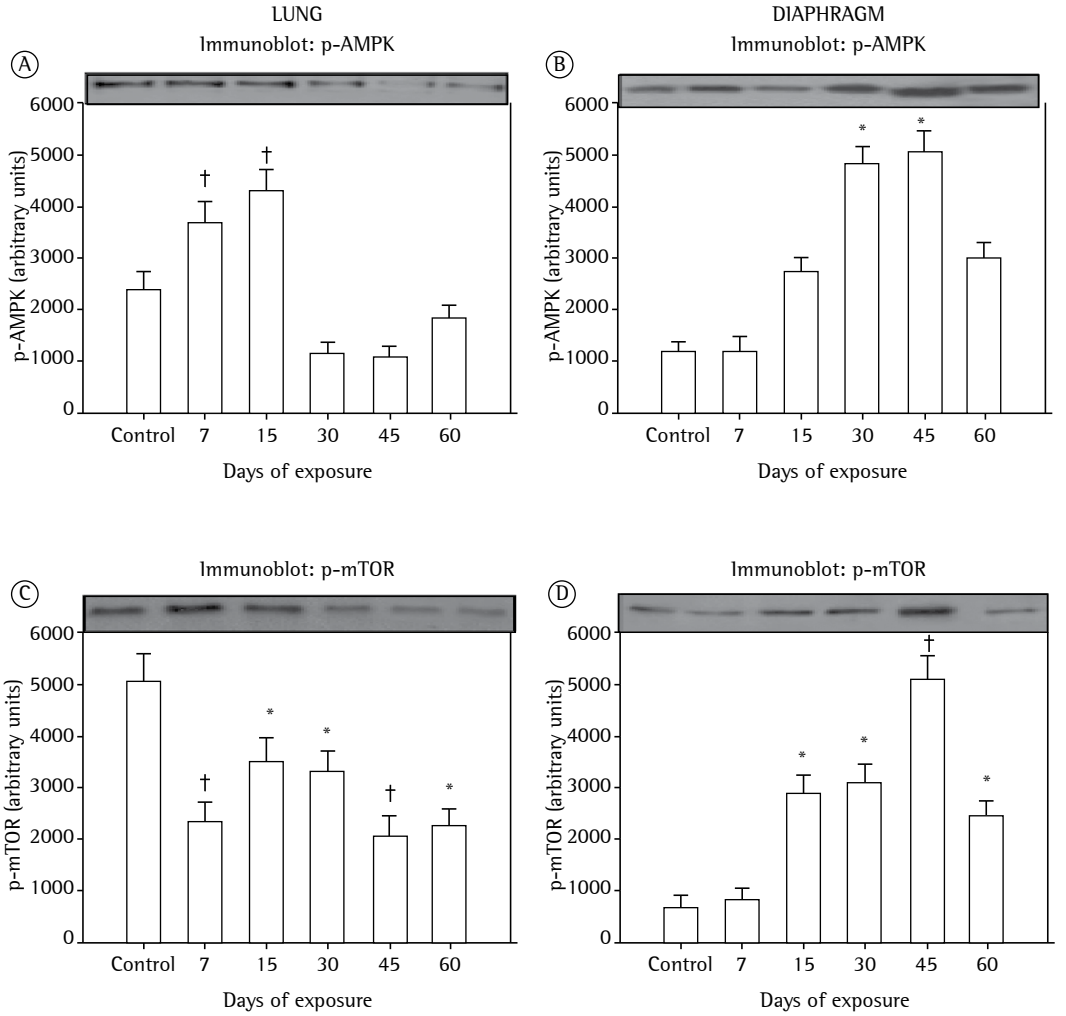

Figure 5 - In A and B, mean ± SEM for phosphorylated AMP-activated protein kinase (p-AMPK) expression in lung tissue and diaphragm muscle, respectively. In C and D, mean ± SEM for phosphorylated mammalian target of rapamycin (p-mTOR) expression in lung tissue and diaphragm muscle, respectively. Data are related to six groups of mice: a control group; and five groups exposed to cigarette smoke for 7, 15, 30, 45, and 60 days, respectively. *$p < 0.01$ vs. control. †$p < 0.001$ vs. control.

might be related to increased cell numbers and cell proliferation, resulting in immune response activation.[22] As observed, we confirmed that CS-induced pulmonary alterations appear to be the consequence of a primary inflammatory lesion characterized by the accumulation of alveolar macrophages and neutrophils in the lower respiratory tract as an immune response, which is crucial in inflammatory disease.[23] It is known that ROS play an important role in the inflammatory response to CS. Oxidative stress is characterized by higher production of ROS and decreased antioxidant levels with lipid peroxidation, thiol alterations and protein carbonylation in plasma.[24]

Pulmonary emphysema is associated with intense responses in oxidative stress, which result in a direct relationship between systemic defense activity and oxidative damage.[25,26] The oxidative damage and inflammation in lung tissue after exposure to CS have been widely studied. In addition, according to MacNee,[27] oxidative stress, as quantified by measuring plasma levels of TBARS, is associated with airflow limitation. The airflow alterations play a role in the function of respiratory muscles like the diaphragm. However, our findings demonstrate that there is an increased intensity of the inflammatory response in lung tissue starting after day 45 of exposure to CS.

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According to Park et al.,[10] exposure to CS for 30 days causes significant oxidation and depletion of the glutathione pool in the lung. Those authors also concluded that the lung is a primary target of oxidative damage by cigarette smoking in the early stages, and that CS eventually exerts its oxidative effects on all organs. In our study, it was observed that CS-induced oxidative damage caused changes not only in the lungs but also in the diaphragm. We found that exposure to CS for 30-45 days was sufficient to generate higher levels of oxidative damage in skeletal muscle (the diaphragm).

A recent study showed that the main limitation found in COPD patients might be related to the mechanism of slow cardiac output associated with airflow limitation.[28] Chiappa et al.[29] tested conditions that improve oxygen delivery and uptake as strategies in COPD patients. The authors demonstrated that one such strategy—the use of heliox (a mixture of 79% helium and 21% oxygen)—is able to ameliorate expiratory flow limitation and dynamic hyperinflation, accelerating the dynamics of peripheral muscle utilization of oxygen as a consequence of improved delivery during high-intensity exercise in patients with moderate to severe COPD. We believed that these interactions might be linked with redox balance and inflammatory responses. One recent study suggested that, in the clinical management of acute lung injury, the use of heliox has the combined therapeutic benefits of reducing mechanical and oxidative stress, thus attenuating lung inflammation.[30]

Oxidative damage generated by exposure to CS in skeletal muscle can lead to loss of muscle function, manifesting as a loss of muscle strength and a consequent higher susceptibility to fatigue.[1,31] The present investigation is the first to provide evidence of oxidative changes induced by ROS in diaphragm muscle proteins in animals chronically exposed to CS. We found that protein oxidation was significantly increased in the diaphragm after 7 days of exposure to CS. The carbonylation of the diaphragm was highest after 30-45 days of exposure, as opposed to carbonylation in the lung, which did not peak until day 60. Our data indicate that exposure to CS primarily affects the diaphragm, which can translate to a significant loss of locomotor and respiratory muscle function in pulmonary emphysema.

According to Barreiro et al.,[1] the effects of smoking-induced muscle protein oxidation appear at an earlier stage in the quadriceps muscle than in the respiratory muscles. These findings underscore the concept that CS per se is likely to be involved in direct tissue toxicity in the skeletal muscles of CS-exposed mice, regardless of lung and bronchial alterations. In addition, we observed that the same animals acutely exposed to CS exhibited a significant increase in TBARS, together with a reduction in muscle levels of sulfhydryl, immediately after exposure. Carbonylation is crucial to triggering activation of the oxidative pathway and promoting lipid peroxidation.

In this animal study of chronic CS exposure, we have shown that pulmonary function decreases in parallel with the duration of exposure, similar to what has been observed in humans.[32] In addition, chronic CS exposure has been shown to cause airflow obstruction.[33] When we analyzed the expression of p-AMPK and p-mTOR in lung tissue, we observed decreased expression of p-mTOR, a result that was expected because p-mTOR expression is associated with cell metabolism, growth, proliferation, and survival, depending on mitogenic signals, as well as on the availability of nutrients and energy.

The increased expression of p-mTOR observed in the diaphragm from day 15 to day 45 of CS exposure can be explained by the possible increase in muscle protein synthesis related to a state of physiological stress.[34] In a rat model of CS exposure, Kozma et al.[5] demonstrated that airway resistance and respiratory system resistance were higher in exposed animals than in unexposed animals. This increase in airway resistance might result in a greater diaphragmatic work, which would explain the increased diaphragm expression of p-mTOR in our CS-15, CS-30, and CS-45 groups, given that p-mTOR expression is known to be elevated in situations of muscle hypertrophy. [35] In our CS-60 group, there was a significant reduction in p-mTOR expression, which was an expected result, because myopathy is associated with reduced expression of p-mTOR.[36] Such myopathy is common in chronic lung diseases.[1] However, in our study, the expression of p-AMPK was increased only from day 30 to day 45 of CS exposure. This fact might be explained by the fact that the increased p-AMPK expression was accompanied by an increase in oxidative stress,

which is clear when we look at the increase in carbonyl by day 30 of CS exposure. Increasing evidence suggests that p-AMPK can be activated by an increase in intracellular ROS in many cell types.[12] Accordingly, whether the ROS-sensitive p-AMPK signaling pathway is involved in toxic smoke-induced lung inflammation remains to be investigated.

Perang et al.[37] were the first to report a detailed AMPK signaling pathway responsible for inducing interleukin (IL)-8 expression by toxic smoke exposure in lung epithelial cells. In this pathway, increased intracellular levels of ROS level constitute the vital trigger, because removal of intracellular ROS by N-acetyl-cysteine reduced the activation of AMPK, c-Jun N-terminal kinase, and extracellular signal-regulated kinase, as well as the induction of IL-8.[37] Previous studies have reported that toxic smoke can increase the intracellular ROS level in lung cells, although the mechanism remains unclear.[38]

In conclusion, our study shows, for the first time, that oxidative alterations in muscle proteins occur in the diaphragm as early as day 7 days of exposure to CS. In addition, this event occurred concomitantly with the parenchymal abnormalities induced by CS in the lungs, suggesting a direct toxic effect of CS on skeletal muscle proteins. However, our data also make it more obvious that pulmonary emphysema is a complex disease that has a negative impact on the whole body. Furthermore, we found that the oxidative damage caused by CS exposure occurs first in skeletal muscle and then in lung tissue.

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Lodenafil treatment in the monocrotaline model of pulmonary hypertension in rats*

Tratamento com lodenafila no modelo de hipertensão pulmonar induzida por monocrotalina em ratos

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Abstract

We assessed the effects of lodenafil on hemodynamics and inflammation in the rat model of monocrotaline-induced pulmonary hypertension (PH). Thirty male Sprague-Dawley rats were randomly divided into three groups: control; monocrotaline (experimental model); and lodenafil (experimental model followed by lodenafil treatment, p.o., 5 mg/kg daily for 28 days) Mean pulmonary artery pressure (mPAP) was obtained by right heart catheterization. We investigated right ventricular hypertrophy (RVH) and IL-1 levels in lung fragments. The number of cases of RVH was significantly higher in the monocrotaline group than in the lodenafil and control groups, as were mPAP and IL-1 levels. We conclude that lodenafil can prevent monocrotaline-induced PH, RVH, and inflammation.

Keywords: Hypertension, pulmonary; Monocrotaline; Interleukin-1.

Resumo

Avaliamos os efeitos da lodenafila na hemodinâmica e inflamação no modelo experimental de hipertensão pulmonar (HP) induzida por monocrotalina em ratos. Trinta ratos Sprague-Dawley foram randomicamente distribuídos em três grupos: controle, monocrotalina (modelo experimental) e lodenafila (modelo experimental e tratado com 5 mg/kg lodenafila v.o. por 28 dias). A pressão média de artéria pulmonar (PAPm) foi obtida por cateterismo cardíaco direito. Foram determinados a hipertrofia ventricular direita (HVD) e os níveis de IL-1 em fragmentos de pulmão. O grupo monocrotalina apresentou valores significativamente maiores de PAPm, HVD e IL-1 em comparação aos grupos controle e lodenafila. Concluímos que a lodenafila pode prevenir o desenvolvimento de HP, HVD e inflamação.

Descritores: Hipertensão pulmonar; Monocrotalina; Interleucina-1.

Pulmonary arterial hypertension (PAH) is a poor prognosis disease, which is characterized by endothelial cell proliferation, hypertrophy and proliferation of muscle cells of the media of the pulmonary arteries, reduction of the vascular lumen, and development of plexiform lesions. The reduction of the vascular lumen leads to an increase in pulmonary vascular resistance, causing right ventricle (RV) hypertrophy, cor pulmonale, and death. In addition, PAH is a public health problem, since schistosomiasis, which is one of its causes, reaches epidemic proportions in developing countries.[1,2]

The treatment of PAH is complex and costly, as well as requiring a multidisciplinary team. There are three major pathophysiological pathways, for which there are specific drugs available for treatment: the endothelin pathway; the nitric oxide pathway, and the prostaglandin pathway.[1] These pathways have been discovered using experimental models of PAH, chief among which is the monocrotaline model. Many of the drugs available for the treatment of PAH have been tested using this model.[3]

The monocrotaline model is simple, inexpensive, and feasible, being routinely used in the initial analysis of drugs with potential effects on pulmonary circulation. Monocrotaline is an alkaloid derived from the seeds of the plant *Crotalaria spectabilis*; after undergoing oxidation in the

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liver, monocrotaline produces its toxic metabolite that will cause vasculitis and medial thickening of the pulmonary arteries and arterioles.[4] Within 22 days after injection of monocrotaline, there is significant PAH.[5]

Lodenafil carbonate is a new phosphodiesterase-5 inhibitor consisting of two lodenafil molecules attached to a carbonate bridge that behaves as a pro-drug, releasing lodenafil as an active metabolite. Its safety in treating erectile dysfunction is well established in preclinical and clinical studies; however, it has never been tested in treating PAH.[6]

The objective of the present study was to assess the response to administration of lodenafil, in terms of hemodynamics and inflammation, in an experimental model of monocrotaline-induced PH.

All animals were handled humanely, in accordance with international standards for animal care.[7] The study was approved by the Research Ethics Committee of the University of São Paulo School of Medicine, located in the city of São Paulo, Brazil.

Thirty male Sprague-Dawley rats (weight, 250-300 g) were randomly divided into three groups: control group, in which the animals were given a subcutaneous injection of saline (1 mL/kg) at the study outset (D0); monocrotaline group, in which the animals were given a subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich, St. Louis, MO, USA) on D0; and lodenafil group, in which the animals were given a subcutaneous injection of monocrotaline (60 mg/kg; Sigma-Aldrich) on D0 and were given lodenafil p.o. (5 mg/kg) once daily between D0 and day 28 of the study (D28).

On D28, after deep sedation with xylazine hydrochloride (i.p., 0.3 mg/kg; Rompun®; Bayer, Leverkusen, Germany) and ketamine hydrochloride (i.p., 10 mg/kg; Ketalar®; Pfizer, New York, NY, USA), the animals were weighed. Subsequently, hemodynamic measurements were performed, being followed by euthanasia (abdominal aortic bleeding) and removal of heart and lung tissue.

The hemodynamic measurements were performed by inserting an umbilical catheter into the external jugular vein, the catheter being connected to a pressure transducer (HP 1295C; Hewlett-Packard, Palo Alto, CA, USA) coupled to a hemodynamic monitor (Monitox Dx 2020; Hewlett-Packard), in accordance with a technique described in a previous study.[8] Mean pulmonary artery pressure (mPAP) was thus measured.

The RV was dissected from the left ventricle (LV), the interventricular septum (S) having remained attached to the LV (LV+S). The ratio of RV weight to LV+S weight (i.e., RV/LV+S) was taken as the index of RV hypertrophy.[8]

To assess the degree of inflammation, IL-1 levels were determined with a capture ELISA using a commercial IL-1 kit (R&D System Inc., Minneapolis, MN, USA).[9] Peptide levels were measured in frozen lung fragments.

For the statistical analysis, ANOVA with post hoc Bonferroni correction was used to compare continuous variables among the groups. Values of $p < 0.05$ were considered significant.

Rats in the monocrotaline group developed PAH, as shown in Figures 1 and 2, as well as experiencing a significant increase in mPAP and RV hypertrophy.

Rats in the lodenafil group had significantly lower mPAP than did those in the monocrotaline group, and there was no significant difference between the former and those in the control group, i.e., lodenafil prevented the development of PAH (Figure 1). The same pattern was observed for the remodeling of the RV and for IL-1 levels (Figure 2).

To our knowledge, the present study is the first to demonstrate that lodenafil was able to prevent the development of PAH in an experimental model of monocrotaline-induced disease.

It is clear that experimental PAH models do not mimic human PAH cases closely enough. There are several factors that may be related to this limitation, among which are the speed of the onset of PAH, which occurs over years in humans but progresses rapidly in animal models, and

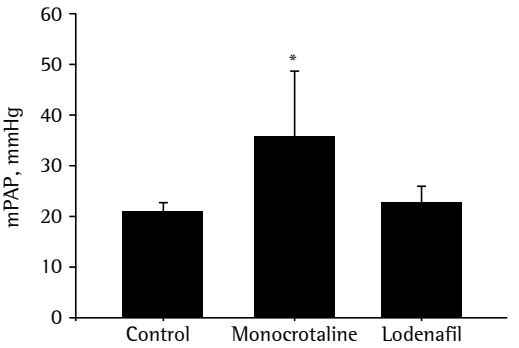

Figure 1 - Mean pulmonary artery pressure (mPAP) in the groups studied. *p = 0.001 (monocrotaline group vs. control and lodenafil groups).

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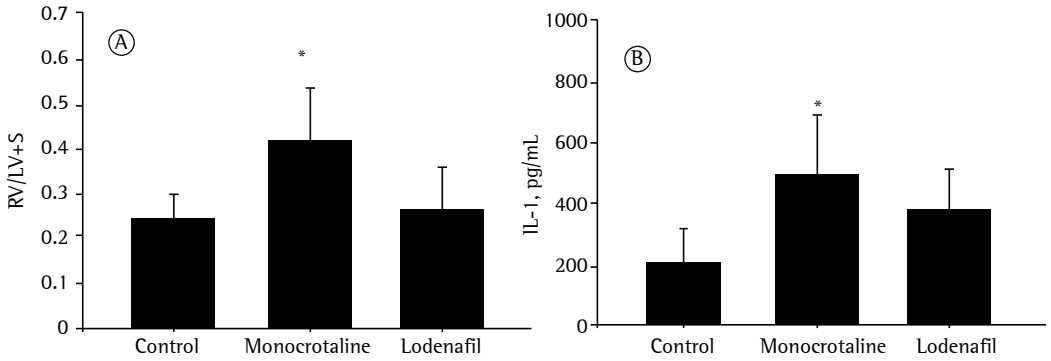

Figure 2 - Right ventricle (RV) hypertrophy and IL-1 levels in the groups studied. In A, RV hypertrophy was determined by the ratio of RV weight to left ventricle + septum weight (LV+S). In B, IL-1 levels were determined by ELISA. *p < 0.001 (monocrotaline group vs. control and lodenafil groups for both graphs).

specific characteristics of the methods used to induce PAH.[5] Specifically in the monocrotaline-induced model of disease, there is a very significant inflammatory aspect, which is not observed in the major forms of human PAH.[4] This explains why several drugs have a marked effect in various experimental models and do not produce the same results in humans, a finding that emphasizes the care that must be taken before extrapolating results derived from experimental models directly into clinical practice.

To specifically assess the inflammatory nature of the monocrotaline model, as well as the potential anti-inflammatory properties of lodenafil, we determined IL-1 levels in lung tissue. The finding of similar IL-1 levels in lodenafil-treated compared with control rats suggests that lodenafil inhibited the inflammatory cascade characteristic of the monocrotaline model. The direct mechanism of this inhibition was not the object of our study, which is a study limitation.

Survival in monocrotaline-treated rats is significantly lower, as reported in a previous study,[8] and this happens in parallel with progressive vascular involvement and progressive involvement of the RV. The same can be observed in humans with PAH, who have decreased survival because of the development of pulmonary vascular remodeling associated with progressive failure of the RV, with this being the leading cause of death in patients with PAH.[2] Our study demonstrated that lodenafil prevented the increase in mPAP, as well as the remodeling of the RV, in rats with monocrotaline-induced PAH. Although we did not investigate the role of lodenafil in reversing established PAH, our findings demonstrate the therapeutic potential of lodenafil in PAH, analogously to what has been shown for other phosphodiesterase inhibitors.[3]

In conclusion, lodenafil prevented the development of PAH and the remodeling of the RV in rats subjected to an experimental model of PAH. Our findings provide the first basis for the development of clinical studies to investigate the potential of lodenafil in the treatment of human PAH.

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Cytokine levels in pleural fluid as markers of acute rejection after lung transplantation*

Citocinas no líquido pleural após transplante pulmonar como marcadores de rejeição aguda

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Abstract

Our objective was to determine the levels of lactate dehydrogenase, IL-6, IL-8, and VEGF, as well as the total and differential cell counts, in the pleural fluid of lung transplant recipients, correlating those levels with the occurrence and severity of rejection. We analyzed pleural fluid samples collected from 18 patients at various time points (up to postoperative day 4). The levels of IL-6, IL-8, and VEGF tended to elevate in parallel with increases in the severity of rejection. Our results suggest that these levels are markers of acute graft rejection in lung transplant recipients.

Keywords: Lung transplantation; Pleural effusion; Cytokines; Graft rejection.

Resumo

Nosso objetivo foi determinar os níveis de desidrogenase lática, IL-6. IL-8 e VEGF, assim como a contagem total e diferencial de células no líquido pleural de transplantados de pulmão, correlacionando esses níveis com a ocorrência e a gravidade de rejeição após o procedimento. Foram analisadas amostras de líquido pleural coletadas de 18 pacientes em diferentes momentos (até o quarto dia pós-operatório). Os níveis de IL-6, IL-8 e VEGF apresentaram uma tendência de aumento paralelamente à gravidade de rejeição. Nossos resultados sugerem que esses níveis são indicadores de rejeição aguda do enxerto em transplantados de pulmão.

Descritores: Transplante de pulmão; Derrame pleural; Citocinas; Rejeição de enxerto.

Lung transplantation (LT) is a therapeutic option for patients with advanced lung disease. Despite advances in the treatment of lung transplant recipients, acute graft rejection remains common, affecting up to 55% of all such patients in the first postoperative year. Rejection is the major risk factor for bronchiolitis obliterans syndrome and appears to be related to a humoral response with complement activation and production of donor-specific HLA antibodies.[1] Transbronchial biopsy (TBB) is the primary method for diagnosing acute cellular rejection. Several studies have shown a correlation between elevated serum cytokine levels and postoperative complications, as well as an association of serum cytokine levels with reperfusion edema, acute rejection, and bronchiolitis obliterans syndrome.[2] The objective of the present study was to determine whether acute cellular rejection correlates with lactate dehydrogenase (LDH) levels, proinflammatory cytokine levels, and differential cell counts in the pleural fluid of lung transplant recipients. We hypothesized that elevated levels of proinflammatory cytokines in the pleural fluid of lung transplant recipients are early indicators of graft rejection.

Between August of 2006 and March of 2008, 20 lung transplant recipients were evaluated for inclusion in the present study, 18 being included. Two patients were excluded from the analysis because they died within the first 6 weeks. The overall LDH levels, overall cytokine levels, and cell counts in pleural fluid were reported elsewhere, without any reference to rejection.[3]

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Blood and pleural fluid samples were collected 6 h after surgery and daily until postoperative day 4. A TBB was performed at 2 and 6 weeks after LT in order to evaluate the severity of rejection, which was classified according to the intensity of perivascular mononuclear cell infiltration in the lung parenchyma, as follows: A0, no rejection; A1, minimal rejection; A2, mild rejection; A3, moderate rejection; and A4, severe rejection. For the final analysis, the highest degree of rejection was selected.

No TBB samples underwent blind analysis. All TBB procedures were performed by a trained pathologist as routinely done at our facility. At least 5 tissue samples were analyzed in order to ensure that the lung parenchyma was adequately represented.

In 3, 4, 8, and 3 of the 18 patients studied, the severity of rejection was classified as A0, A1, A2, and A3, respectively.

Serum levels of IL-6 and IL-8 were undetectable in all samples. Serum VEGF levels were much lower than pleural fluid VEGF levels and were not significantly different among subgroups A0, A1, A2, and A3.

In the present study, undetectable serum cytokine levels suggest that the inflammatory response in lung transplant recipients is primarily confined to the lungs and pleural cavity. Similar results have been reported elsewhere,[2,4,5] acute elevation of serum cytokine levels having been found to be more evident in patients with reperfusion edema or acute rejection.

The correlation between LDH levels and differential cell counts in pleural fluid is shown in Table 1, having been found to be higher in the subgroups of patients with graft rejection.

We found elevated levels of IL-6 and IL-8 in the pleural fluid samples collected 6 h after LT, those levels having decreased by postoperative day 4. No such variation was observed in VEGF

Table 1 - Relationship of the severity of acute rejection with the levels of lactate dehydrogenase, as well as with the total and differential cell counts, in pleural fluid samples collected at various time points.[a]

Variable	Severity of acute rejection			
	A0	A1	A2	A3
LDH, mg/dL				
6 h	1,855 ± 594	3,176 ± 530**	2,310 ± 964	4,134 ± 1,487*
24 h	1,250 ± 1,028	1,605 ± 1,285	1,779 ± 1,233	2,214 ± 958
48 h	1,181 ± 293	1,455 ± 642	966 ± 503	1,638 ± 1,201
72 h	375 ± 46	655 ± 462	576 ± 159	711 ± 203***
96 h	316 ± 33	586 ± 434	498 ± 214	621 ± 233
Total cells, cells/mL				
6 h	1,679 ± 1,612	3,812 ± 2,387	4,606 ± 3,156	10,710 ± 6,394***
24 h	595 ± 786	2,764 ± 1,250****	2,359 ± 645****	4,723 ± 2,514****,*****
48 h	645 ± 116	897 ± 626	649 ± 421	2,457 ± 2,180
72 h	246 ± 323	316 ± 168	542 ± 456	599 ± 680
96 h	167 ± 223	418 ± 288	435 ± 399	482 ± 120***
Neutrophils				
6 h	1,573 ± 1,485	3,580 ± 2,281	4,368 ± 3,074	9,884 ± 5,245******
24 h	537 ± 707	2,471 ± 942****	1,314 ± 1,255****	4,478 ± 2,553****
48 h	588 ± 147	812 ± 642	590 ± 450	2,266 ± 2,255
72 h	210 ± 274	266 ± 155	475 ± 431	374 ± 393
96 h	144 ± 192	258 ± 252	367 ± 339	235 ± 195
Lymphocytes				
6 h	90 ± 112	161 ± 56	156 ± 126	1,078 ± 1,455
24 h	52 ± 72	136 ± 140	71 ± 28	191 ± 74***
48 h	45 ± 32	65 ± 62	48 ± 50	145 ± 90***
72 h	36 ± 50	45 ± 24	36 ± 4	133 ± 166
96 h	23 ± 32	78 ± 61	41 ± 43	59 ± 62

A0: no rejection; A1: minimal rejection; A2: mild rejection; A3: moderate rejection; and LDH: lactate dehydrogenase. [a]Values expressed as mean ± SD. *p < 0.05 (A3 > A0 and A2). **p < 0.05 (A1 > A0). ***p < 0.05 (A3 > A0). ****p < 0.05 (A3, A2, and A1 > A0). *****p < 0.05 (A3 > A1 and A2). ******p < 0.05 (A3 > A0, A1, and A2).

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levels. Patients in whom graft rejection was more severe tended to have higher levels of those cytokines (Table 2). Patients in whom the severity of rejection was classified as A3 had higher levels of IL-6 and IL-8 at all time points when compared with those in whom graft rejection was less severe. In the samples collected 6 h after LT, VEGF levels were higher in the patients in whom the severity of rejection was classified as A3, A2, or A1 than in those in whom it was classified as A0.

Inflammation of the pleural space is due to the surgical trauma and the presence of a chest tube and can explain the high levels of cytokines. In our study, it is of note that, although inflammatory marker levels progressively decreased over time, proinflammatory cytokine levels remained elevated during the first 4 days, and irritation caused by the chest tube left in place for up to 96 h can explain that, although it does not explain the differences among the subgroups of patients. We speculate that the cytokine levels found in the patients classified as A0 represent the increase in cytokine levels that occurs as a result of the surgical trauma and the use of chest tubes. This level of inflammation decreases over time, as evidenced by a reduction in pleural fluid levels of IL-6 and IL-8. The cytokine levels found in the patients in whom the severity of rejection was classified as A3 were much higher than were those found in the remaining subgroups of patients, and this might be an early indicator of rejection.

Pleural fluid levels of IL-6 correlated positively with LDH levels ($r = 0.49$; $p = 0.030$) and the neutrophil count ($r = 0.90$; $p = 0.036$), and VEGF levels were strongly correlated with the neutrophil count ($r = 0.91$; $p = 0.030$) and the total leukocyte count ($r = 0.88$; $p = 0.048$). In contrast, a strong negative correlation was found between IL-8 levels and the lymphocyte count ($r = -0.97$; $p = 0.007$). In addition, a strong positive correlation was found between IL-6 levels and IL-8 levels ($r = 0.70$; $p < 0.001$), as well as between IL-6 levels and VEGF levels ($r = 0.71$; $p < 0.001$). Furthermore, there was a slight correlation between IL-8 levels and VEGF levels ($r = 0.49$; $p = 0.027$). In the present study, there was no correlation between the severity of acute rejection and the severity of primary graft dysfunction within the first 72 h after LT.

In lung transplant recipients, inflammation of the lung parenchyma leads to increased interstitial edema with increased cytokine levels. Because

Table 2 - Relationship of the severity of acute rejection with the levels of IL-6, IL-8, and VEGF in pleural fluid samples collected at various time points.[a]

Variable	Severity of acute rejection			
	A0	A1	A2	A3
IL-6, pg/mL				
6 h	14,717 (10,719-18,816)	27,368 (20,445-41,316)	38,521 (29,543-45,367)	49,854 (42,854-53,415)*
24 h	6,384 (2,304-10,464)	13,410 (10,973-17,608)	12,060 (8,886-17,824)	21,337 (17,779-48,322)**
48 h	7,642 (2,707-12,576)	11,402 (9,370-13,303)	12,211 (9,075-13,477)	15,010 (12,717-46,503)**
72 h	5,010 (2,227-7,793)	7,731 (5,301-9,687)	4,891 (3,748-6,303)	8,506 (4,871-8,605)
96 h	2,879 (2,196-3,562)	7,372 (6,292-8,461)***	4,838 (3,963-8,047)***	6,151 (4,593-7,709)***
IL-8, pg/mL				
6 h	1,318 (1,020-1,617)	1,706 (1,018-1,956)	1,696 (1,286-1,941)	2,216 (2,110-2,323)****
24 h	1,266 (996-1,536)	1,177 (767-1,608)	1,224 (799-1,706)	2,187 (2,119-2,254)****
48 h	1,091 (760-1,423)	1,455 (765-1,523)	1,037 (788-1,169)	2,036 (1,922-2,150)****
72 h	965 (579-1,351)	1,472 (377-1,633)	945 (682-1,163)	1,935 (1,775-2,096)****
96 h	981 (482-1,481)	464 (244-1,127)	690 (288-1,005)	1,859 (1,775-1,943)****
VEGF, pg/mL				
6 h	72 (34-110)	343 (290-508)***	297 (145-504)***	566 (153-879)***
24 h	123 (18-228)	188 (115-284)	279 (77-445)	382 (107-745)
48 h	121 (20-221)	123 (95-172)	283 (84-435)	123 (54-406)
72 h	142 (17-267)	143 (112-214)	294 (116-382)	123 (39-406)
96 h	144 (8-280)	134 (115-336)	280 (191-425)	280 (81-445)

A0: no rejection; A1: minimal rejection; A2: mild rejection; and A3: moderate rejection. [a]Values expressed as median (interquartile range). *$p < 0.05$ (A3 > A0 and A1). **$p < 0.05$ (A3 > A0). ***$p < 0.05$ (A3, A2, and A1 > A0). ****$p < 0.05$ (A3 > A2, A1, and A0).

lymphatic vessels are cut, the amount of interstitial fluid leaving the lungs and going through the visceral pleura increases. This can lead to high cytokine levels, which have been observed in patients with graft rejection.

One limitation of the present study was the small number of patients in each subgroup. However, it is clear that inflammatory cytokine levels were highest in the patients in whom the severity of rejection was classified as A3. The lack of a significant difference between subgroups A1 and A2 regarding cytokine levels is possibly due to the small sample size. In addition, the fact that pleural fluid collection and TBB occurred at different time points possibly influenced the results. The data presented here are old, and a larger study, based on these data, is currently under way.

The present study demonstrated that elevated levels of proinflammatory cytokines in the pleural fluid of lung transplant recipients can be markers of acute graft rejection (particularly of moderate to severe rejection). In this context, determination of cytokine levels in pleural fluid can be useful in identifying patients who will develop graft rejection. Therefore, treatment can be initiated sooner, thus reducing lung parenchymal injury.

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Overview of the biochemical and genetic processes in malignant mesothelioma*

Panorama dos processos bioquímicos e genéticos presentes no mesotelioma maligno

Leonardo Vinícius Monteiro de Assis, Mauro César Isoldi

Abstract

Malignant mesothelioma (MM) is a highly aggressive form of cancer, has a long latency period, and is resistant to chemotherapy. It is extremely fatal, with a mean survival of less than one year. The development of MM is strongly correlated with exposure to asbestos and erionite, as well as to simian virus 40. Although various countries have banned the use of asbestos, MM has proven to be difficult to control and there appears to be a trend toward an increase in its incidence in the years to come. In Brazil, MM has not been widely studied from a genetic or biochemical standpoint. In addition, there have been few epidemiological studies of the disease, and the profile of its incidence has yet to be well established in the Brazilian population. The objective of this study was to review the literature regarding the processes of malignant transformation, as well as the respective mechanisms of tumorigenesis, in MM.

Keywords: Occupational diseases; Mesothelioma; Genes, tumor suppressor; Oncogenes; Signal transduction.

Resumo

O mesotelioma maligno (MM) é um câncer extremamente agressivo, com elevado período de latência e resistente aos protocolos de quimioterapia, além de ser extremamente fatal, com taxa de sobrevivência média inferior a um ano. O desenvolvimento do MM é fortemente correlacionado com a exposição ao amianto e erionita, assim como ao vírus símio 40. Apesar de vários países terem banido o uso de amianto, o MM tem se mostrado de difícil controle e sua incidência tende a aumentar nos próximos anos. No Brasil, o MM não é amplamente estudado do ponto de vista genético e bioquímico. Além disso, poucos estudos epidemiológicos foram realizados até o momento, e o perfil de incidência do MM não está bem estabelecido na população brasileira. O objetivo deste estudo foi revisar a literatura em relação ao processo de transformação maligna e seus respectivos mecanismos de tumorigênese no MM.

Descritores: Doenças profissionais; Mesotelioma; Genes supressores de tumor; Oncogenes; Transdução de sinal.

Introduction

Malignant mesothelioma (MM) is a rapidly growing cancer that results from unregulated proliferation of the mesothelial cells lining the pleural, peritoneal, and pericardial cavities. MM is typically but not exclusively related to exposure to mineral fibers, particularly asbestos and erionite.[1] The latency period of MM, i.e., the time elapsed from exposure to the offending agent (in particular, the aforementioned mineral fibers) to diagnosis is long; however, the time elapsed from the onset of malignancy to diagnosis is indeed short, MM producing symptoms shortly after its initial growth.[2]

Histologically, MM can be classified as epithelial, biphasic, or sarcomatoid, the mean survival time being 18 months, 11 months, and 8 months, respectively.[3] Malignant pleural mesothelioma (MPM) is the most common type of MM, accounting for approximately 70% of cases.[1] As is the case with most MMs, MPM is

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commonly diagnosed at advanced stages, the survival rates for MPM being lower than 12 months.[4] Malignant peritoneal mesothelioma is less common than MPM and accounts for approximately 30% of all MMs, being extremely aggressive (mean survival rate, 6-12 months).[5,6]

In addition to the fact that MM is highly resistant to chemotherapy and radiation therapy, the benefits of surgical removal are few, and there is controversy regarding the efficacy of surgical removal alone; furthermore, not all patients can undergo surgical removal.[7] Apparently, improvements in survival rates have been achieved with a combination of surgical removal, chemotherapy, and radiation therapy; however, controversy remains regarding the efficacy and benefits of this practice.[6,8-10] It is known that cisplatin is the most active drug in the treatment of MM, the use of cisplatin in combination with pemetrexed having been approved by the US Food and Drug Administration as a standard treatment for MM.[11] However, various in vitro and in vivo studies have used other drugs, some of which have shown promising results.[12]

The first study to demonstrate that there was a relationship between asbestos and the development of MPM was conducted in South Africa in the 1960s.[13] Since then, several studies have shown strong evidence that asbestos, especially amphibole asbestos, is associated with the development of MM.[2,7] However, it is widely debated whether chrysotile asbestos is a human carcinogen.

The biochemical mechanisms responsible for the genesis of MM as a result of asbestos exposure have yet to be fully understood. In broad terms, asbestos particles become trapped in lung tissue, generating a strong inflammatory response, with the participation of TNF-α and nuclear factor kappa B (NF-κB), which generate resistance to apoptosis and accumulation of DNA damage.[14] The involvement of high mobility group protein B1, which is known to be an inflammatory marker, has recently been demonstrated. This protein increases the release of TNF-α and IL-1β, as well as increasing the activity of NF-κB.[15] In addition to eliciting this inflammatory response, asbestos can generate reactive oxygen and nitrogen species, which lead to DNA structure damage and induce genotoxicity, thus favoring the development of MM.[16,17]

In the scientific literature, there is considerable debate regarding the role of chrysotile asbestos in the genesis of MM; there are reports that chrysotile asbestos cannot cause MM in humans. Although there is no doubt about the role of amphibole asbestos in the genesis of MM, there is still much debate regarding the role of chrysotile asbestos in MM, which is why chrysotile asbestos is still used in several countries, including Brazil. Here, we will not discuss this controversial issue, the nature of which is more political than scientific. However, the dangers of chrysotile asbestos cannot be ignored, which is why chrysotile asbestos and other types of asbestos are classified as human carcinogens, their use being considered unsafe regardless of the level of exposure.[18] In addition to asbestos exposure, risk factors for the development of MM include erionite exposure, simian virus 40, and germline *BAP1* mutations.[2,19-21]

In several countries, the incidence of MM has increased significantly in recent years. Data from the USA show that the mean incidence of MM is 2,586 cases per year, with a cumulative total of 23,277 MM cases between 1999 and 2007, the incidence of MM in males being four times higher than that in females.[22] In Brazil, there have been very few epidemiological studies, all of which were based on reported cases and on data from Brazilian National Ministry of Health databases. This makes it difficult to provide a realistic picture of MM in the country.

Despite the aforementioned difficulties, one group of Brazilian researchers conducted a study[23] in which the Brazilian National Mortality Database was used in order to estimate the incidence of MM in Brazil. Between 1980 and 1995, the authors of that study used the International Classification of Diseases, 9th revision (ICD-9), codes 163.0, 163.1, 163.8, and 163.9, and all cases of pleural neoplasm were considered to be cases of mesothelioma. Between 1996 and 2003, the authors used the International Classification of Diseases, 10th revision (ICD-10), codes C45.0, C45.1, C45.2, C45.7, C45.8, C45.9, and C38.4. That study showed that the mortality rate for MM was 0.56 per 1,000,000 population in 1980, having increased by 55% in 2003.[23] In addition, the study showed that the male-to-female ratio of patients with MM was nearly 1:1,[23] which is quite different from the 5:1 ratio found in a recent study conducted in the UK.[24] However, given the methodological limitations of the aforementioned study,[23] it is possible that its findings do not reflect the true incidence of MM in Brazil. The limitations of the

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aforementioned study[23] include the low quality of the data from the Brazilian National Mortality Database, the underreporting of cases in some Brazilian states, the use of two different revisions of the ICD (i.e., ICD-9 and ICD-10), and the fact that, according to ICD-9, all pleural neoplasms are MMs. Therefore, the findings of that study are difficult to interpret, and the real incidence of MM in Brazil remains unknown. However, despite the aforementioned methodological difficulties,[23] the information provided by that study is extremely useful for estimating the incidence of MM, as well as reinforcing the idea that the appropriate authorities should monitor the incidence of MM more closely in order to provide reliable data, as is done in the USA.[22] In addition to the abovementioned epidemiological study,[23] studies have been conducted in Brazil in an attempt to improve the diagnosis of MM[25,26] and find prognostic markers of MM.[27]

Given its aggressiveness and increasing incidence worldwide, MM and its main etiologic agent (i.e., asbestos) have been the subject of international discussions aimed at banning the trade of asbestos worldwide. In Brazil, asbestos is regulated by Law no. 9,055; all forms of asbestos are prohibited, with the exception of chrysotile.[28] There have been few studies investigating MM and the profile of individuals diagnosed with MM in Brazil,[26,29-33] further studies being therefore required.

The primary objective of the present review was to provide an overview of how MM uses the cellular machinery in order to promote its growth, i.e., an overview of the genes and pathways that are activated or deactivated. This knowledge is important for the development of new drugs and therapies for this aggressive cancer. We did not seek to review the roles of asbestos and other environmental exposure factors in the development of MM. Our primary objective was to review the principal biochemical and genetic events occurring in MM and their consequences in the process of malignant transformation, in an attempt to strengthen the Portuguese-language scientific literature, which lacks a review of studies on this topic.

Genes and biochemical pathways involved in MM

An understanding of the cellular processes that favor or assist in the process of MM development is of utmost importance for the creation of therapies aimed at activating or deactivating certain biochemical pathways, the principal effect being tumor growth suppression. Research groups worldwide have been working on this, and there have been major advances, which have aided in the treatment of MM. Below, we briefly describe the genes that play a key role in the development of MM. For a more detailed analysis of the mechanics of the genes involved in MM, please refer to recently published review articles by our research group.[21,34]

It is known that each type of cancer uses a certain "group" of genes in order to grow; however, the group of genes used depends on cancer type and stage. Certain patterns of gene activation and deactivation occur in all types of cancer and are explored in the development of drugs and therapies. In the particular case of MM, the genes whose roles are well established are *p16*^{INK4a}, *p14*^{ARF}, *NF2*, and *BAP1*. Although the roles of the *TP53* and *PTEN* genes are well established in various types of cancer, their roles in MM remain controversial. Figure 1 shows an overview of the roles of the aforementioned genes in MM.

p16^{INK4a} *and p14*^{ARF}

Located on chromosome 9p21, the *p16*^{INK4a} and *p14*^{ARF} genes are important tumor growth suppressors and encode two distinct proteins, namely p16^{INK4a} and p14^{ARF}. The p16^{INK4a} protein is a cyclin-dependent kinase inhibitor and plays a role in the hyperphosphorylation of the retinoblastoma protein. This results in inactivation of the retinoblastoma protein and, consequently, failure of cell cycle arrest. In contrast, the p14^{ARF} protein inhibits the degradation of p53 through its interaction with murine double minute 2 protein (MDM2).[35] The loss of these vicinal genes has a major impact on cell cycle control, and it is therefore possible to infer the reason why these are the most frequently mutated genes in MM.

The literature shows that *p16*^{INK4a} and *p14*^{ARF} are deleted in 80-90% of cases of MM.[36,37] Approximately 70% of all cases of epithelial MM and nearly 100% of all cases of biphasic or sarcomatoid MM show changes in *p16*^{INK4a} and *p14*^{ARF}.[38] The literature shows that *p16*^{INK4a} and *p14*^{ARF}, as well as their respective proteins, play important roles in cell cycle control and that their inactivation is most frequently involved in the malignant transformation of MM.

NF2

Located on chromosome 22q12, the *NF2* gene encodes a protein designated merlin, which has a sequence of 595 amino acids and plays an important role in the upstream regulation of the cascade of the Hippo pathway, which will be explained later. In the mid-1990s, inactivation of the *NF2* gene was reported in approximately 40% of all cases of MM.[39] Subsequent studies have demonstrated the importance of *NF2* inactivation in MM.[40] Although *NF2* mutations have been found in 38% of cases of MPM, an absence of *NF2* mutations has recently been reported in non-small cell lung cancer, this being a possible approach to the differential diagnosis of the two.[41] Therefore, mutations/alterations in the *NF2* gene are important to the development of MM and currently constitute the second most common alteration in MM.

BAP1

The *BAP1* gene is a tumor suppressor gene that is located on chromosome 3p21.3 and encodes the protein BAP1, which plays an important role in the ubiquitin-proteasome pathway in histone deubiquitination, regulation of cell cycle progression, modulation of chromatin, gene transcription, and DNA repair.[42]

Germline *BAP1* mutations have recently been detected in families with a high incidence of MM, characterizing a syndrome that predisposes to MM, uveal melanoma, and, possibly, other cancers.[19,42,43] In addition to germline mutations, somatic mutations have been identified in approximately 20% of all cases of MM.[44,45] Studies[19,42,43] of the effects of germline mutations on cancer development have provided a major breakthrough, given that cancer is often associated with the effects of somatic mutations related or unrelated to external factors, including exposure to asbestos, radiation, and cigarette smoke. Therefore, it is of paramount importance to gain a better understanding of the genes involved in the development of MM, as well as of the mechanisms by which germline mutations contribute to the development of MM, because individuals with such genetic susceptibilities should avoid exposure to risk factors. To that end,

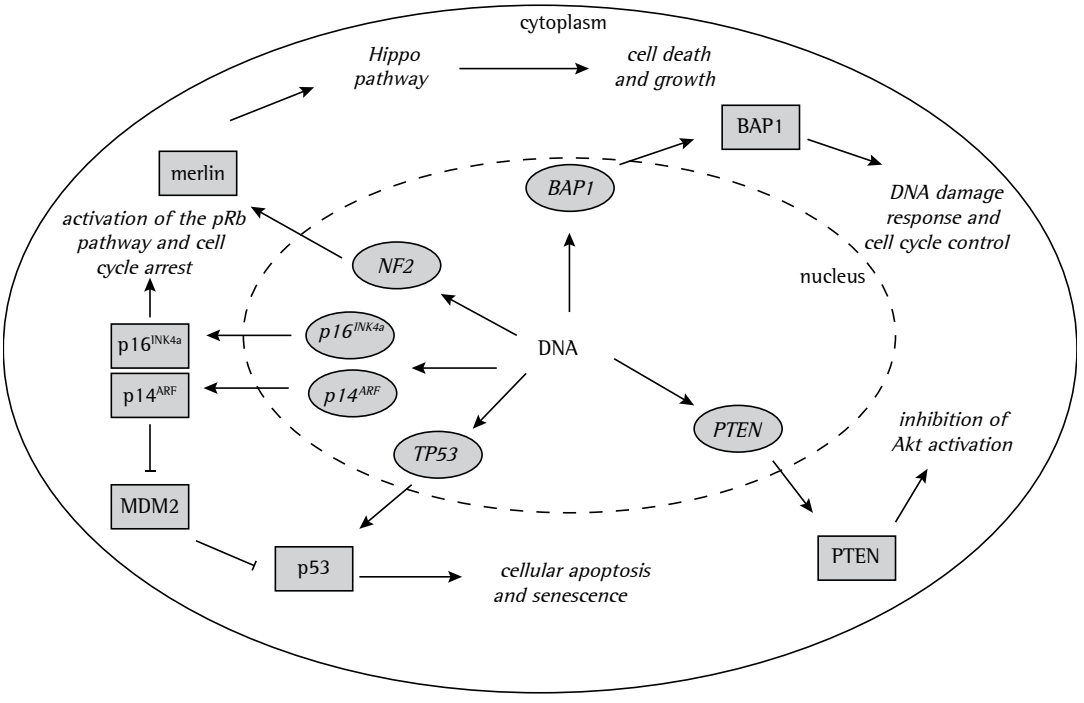

Figure 1 - Genes and proteins involved in the development of malignant mesothelioma. The $p16^{INK4a}$ protein activates the retinoblastoma protein (pRb) pathway, and the $p14^{ARF}$ protein modulates p53. The *NF2* gene encodes the merlin protein, which acts as an upstream regulator of the Hippo pathway. The *BAP1* gene encodes BRCA1 associated protein-1, which plays a role in DNA damage response and cell cycle control. The *PTEN* gene encodes the PTEN protein, which is an important negative regulator of the PI3K/Akt pathway. The p53 protein plays a key role in apoptosis control and cellular senescence.

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there is a need for techniques that can detect such mutations in the population in an inexpensive and reproducible manner, given that such screening is currently performed on a small scale and in scientific studies. In individuals suspected of having *BAP1* cancer syndrome, early diagnosis is essential to prevent the onset of diseases associated with *BAP1* mutations. Therefore, a multidisciplinary approach involving family physicians, pathologists, and geneticists is required in order to diagnose, monitor, advise, and treat individuals and families with this syndrome. There is a need for knowledge and training of health professionals (especially physicians) regarding the clinical signs of *BAP1* cancer syndrome, which can result in catastrophic harm to patients if it is not diagnosed early.[42]

TP53

Known as a DNA guardian, the p53 protein is encoded by the *TP53* gene. The p53 protein plays a role in various cellular functions that are critical for well-orchestrated cell control. In addition, *TP53* mutations are found in approximately 50% of all cancer cases, and, in most other cases, *TP53* is inactivated by mutations in other genes, by viral proteins, or both.[46,47]

From a mechanistic standpoint, several research groups have focused on gaining a better understanding of the various mechanisms of p53 cell function control. The first clues as to the role of p53 in mediating apoptosis were provided by a study published in the 1990s.[48] A new mechanism of action of p53 has recently been identified and is believed to be one of the main mechanisms used to fight the process of malignant transformation, i.e., induced cellular senescence.[49] However, the role of the p53 protein in MM has yet to be well defined; intriguingly, in most cases, MM does not neutralize p53 activity in a direct manner, i.e., through *TP53* mutations. Studies have shown that the *TP53* gene is present in its natural state, i.e., without mutations.[50,51] However, the absence of mutations in a given gene does not necessarily imply that the gene is functioning normally, given that there are various gene regulation mechanisms that can cause gene inactivation, including DNA methylation (epigenetic regulation) and RNA interference (post-transcriptional regulation).[52]

It can be speculated that other gene mutations (such as the aforementioned mutations) can lead to malignant transformation in MM, resulting in reduced selective pressure for *TP53* inactivation. In addition, it is plausible to assume that the malignant transformation of MM occurs through pathways that are independent of p53 activity. The mechanisms leading to the maintenance of wild-type *TP53* in MM have yet to be fully understood. Mutations/alterations in the *TP53* gene do not seem to be critical to the development and progression of MM.[53,54]

PTEN

Discovered in 1997 by two independent research groups,[55,56] the *PTEN* gene is a common deletion on chromosome 10. Monoallelic mutations are common in various types of cancer; however, homozygous *PTEN* mutations are frequently found in advanced cancers, such as endometrial cancer and glioblastoma.[57] Interestingly, *PTEN* is heavily regulated by various gene regulation processes, such as RNA interference, methylation, acetylation, oxidation, and ubiquitination. Therefore, analysis of gene status (i.e., mutation levels) is important but should not be used as the only predictor of gene activation and function. Analysis of protein expression levels is also required, given the potential association between protein expression and susceptibility to cancer development, as is the case with *PTEN*.[52,58]

The activity of PTEN results from the ability to antagonize the signaling pathway of the phosphatidylinositol 3-kinase (PI3K) pathway through the dephosphorylation of phosphatidylinositol-3,4,5-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate (PIP2). It is known that PIP3 is a second messenger responsible for the activation of Akt (i.e., PKB), which in turn sends signals necessary for cell growth, survival, and proliferation. In fact, various types of cancer display overexpression in this biochemical pathway. This results in uncontrolled cell growth. Loss of PTEN activity results in accumulation of PIP3 and, consequently, overactivation of Akt, PTEN being therefore commonly used in malignant processes.[59] In addition to having cytoplasmic activities, PTEN has nuclear activities that are important for cell cycle control and genomic stability.[59,60]

Interestingly, PTEN can regulate p53 levels independently of its activity as a phosphatase by maintaining p53 acetylation (Figure 2).[61] In addition, PTEN inhibits MDM2 phosphorylation, which is required for nuclear migration and,

consequently, p53 degradation. Therefore, PTEN can protect p53 from the degradation of MDM2.[62,63]

A new mechanism has recently been identified in prostate cancer, a mechanism in which complete loss of *PTEN* in combination with wild-type *TP53* surprisingly induced a strong cellular senescence response, which resulted in the inhibition of malignant cell growth. However, the combination of complete loss of *PTEN* and wild-type *TP53* was associated with a more severe form of prostate cancer. Therefore, it is plausible to speculate the reason why complete (homozygous) loss of *PTEN* is restricted to advanced cancers.[52]

The role of *PTEN* in MM remains controversial, given that the PI3K/Akt pathway is known to be overexpressed; however, whether this overexpression is due to the absence of *PTEN* or to *PTEN* inactivation and the role of *PTEN* in the development of MM are still a matter of debate.[64-67]

DNA methylation and microRNA

Recent studies have shown that DNA methylation and microRNA expression play an important role in cancer development and should be explored in the diagnosis and treatment of cancer. In MM, epigenetic analysis of the methylation profile of several genes allowed the distinction between normal and malignant tissues, a fact that is of great importance because of the difficulty in distinguishing normal and malignant tissues[68] and because epigenetic analysis of the methylation profile can be a powerful tool in the diagnosis of MM.[69]

MicroRNA studies have shown interesting results. MicroRNAs can regulate and modulate gene expression, microRNA expression being severely altered in cancer.[70] MicroRNA expression in normal tissue has been shown to be different from microRNA expression in malignant tissue, and specific microRNA expression profiles have been found in each histological type of MM.[71] Studies have proposed the use of microRNA as a diagnostic tool,[71] a prognostic marker,[72] and a treatment option for MM.[73] Therefore, the future looks promising for these two parameters and their potential benefits in the diagnosis and treatment of MM.

Biochemical pathways involved in the development of MM

Below, we briefly describe the biochemical pathways most commonly used in the malignant transformation of MM. These pathways are summarized in Figure 3. For a more detailed analysis of the mechanics of the pathways involved in MM, please refer to recently published review articles by our research group.[21,34]

Receptor tyrosine kinases

Receptor tyrosine kinases constitute a large family of receptors that regulate the cell cycle and are often activated in MM.[74] Among receptor tyrosine kinases, EGFR was detected in 44% of all cases of MPM.[75] In addition, VEGF is expressed in MM and is associated with decreased patient survival.[76] In addition, insulin-like growth factor and its receptor are also active in MM.[77]

Activation of these receptors results in the activation of biochemical cascades that lead to the transduction of abnormal cell growth signals, principally through the Ras/MAPK pathway[78,79] and the PI3K/Akt pathway[80] in MM.

PI3K/Akt/mTOR

The PI3K pathway regulates various processes that are vital to cells, including survival, metabolism, and proliferation. A major product of the PI3K pathway, PIP3 acts as a second messenger essential for the translocation of Akt to the plasma membrane, where Akt is phosphorylated. Phosphorylated Akt is responsible for sending biochemical signals responsible for cell proliferation and resistance to apoptosis.[64] The *PIK3CA* gene encodes the catalytic unit p110α, which is known for its ability to activate the PI3K pathway by converting PIP2 to PIP3.[81] It is known that Akt is phosphorylated by mammalian target of rapamycin (mTOR), and the mTOR complex plays an important role in energy balance and growth, being therefore a therapeutic target of interest in patients with MM.[82]

In MM, the PI3K/Akt/mTOR pathway is overexpressed,[64,80,83] inhibition of the activity of certain pathway components, such as PI3K and mTOR, being therefore an excellent therapeutic pathway.[84] However, the applicability of such drugs in clinical practice has proved frustrating.[85]

Recently, in an elegant study,[86] it was demonstrated that activation of colony-stimulating factor 1 receptor (CSF1R) can generate clonogenicity and resistance in untransformed mesothelial cells. It was also demonstrated that, in primary MPM cultures and MPM cell lines,

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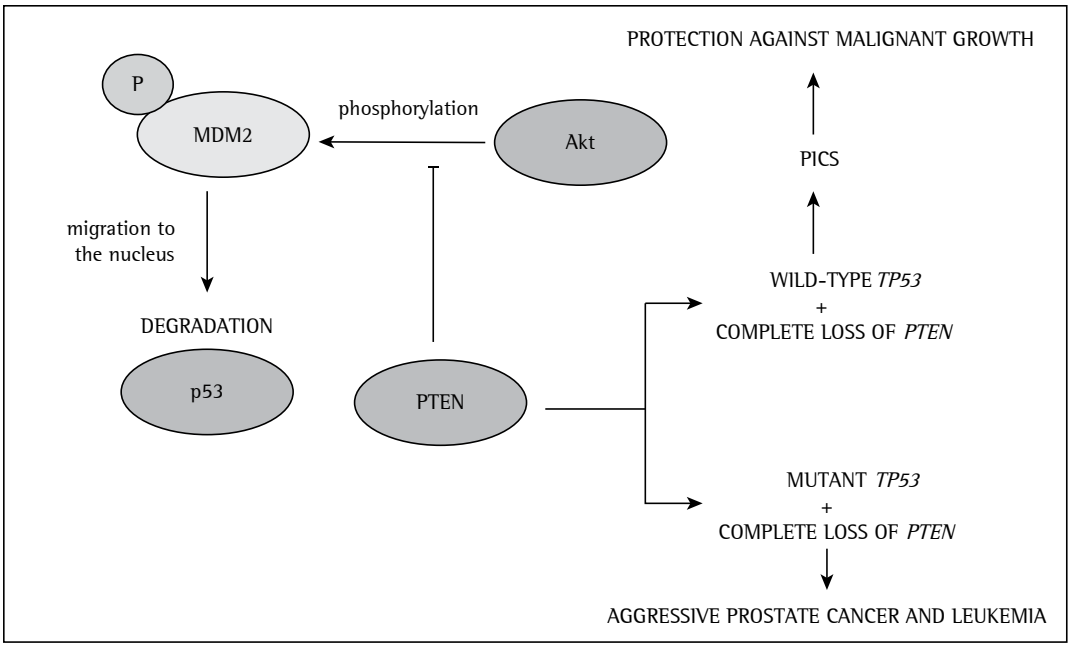

Figure 2 - The PTEN protein protects p53 from degradation by inhibiting the migration of murine double minute 2 protein (MDM2) to the nucleus. There is a cross-talk mechanism between PTEN and p53. The association between complete loss of *PTEN* and a wild-type *TP53* results in a senescence mechanism designated *PTEN* loss-induced cellular senescence (PICS), which is an important mechanism against malignant growth. The loss of *PTEN* in a cellular context with a mutant *TP53* results in prostate cancer that is more aggressive and leukemia, both mechanisms having been demonstrated in animals.

there are subpopulations of cells expressing CSF1R, which is responsible for resistance to pemetrexed via Akt and β-catenin signaling. Another interesting finding of that study was that the abovementioned subset of cells accounts for less than 10% of the total number of cells in culture, this small proportion being responsible for resistance to pemetrexed in cell lines and primary cultures; therefore, CSF1R plays an important role in the survival of cells that do not express it.[86] Given that CSF1R greatly influences cell survival and that CSF1R expression is higher in MM than in normal tissue, pharmacological inhibition of CSF1R in humans is an attractive and promising strategy to overcome the high resistance to chemotherapy observed in MM patients and expand the limited therapeutic armamentarium currently available to combat MM.[86]

Ras/MAPK

The Ras/MAPK pathway consists of several components, such as surface receptors and transcription factors, which regulate gene expression. The Ras/MAPK pathway is one of the most frequently deregulated pathways in human cancer and controls vital cellular processes, such as proliferation, growth, and senescence, as well as regulating apoptosis through its interaction with various members of the B-cell lymphoma (Bcl) family of proteins.[87] The major components of the Ras/MAPK pathway are Ras, Raf, MEK, and MAPK, which are susceptible to mutations/alterations and therefore favor the process of malignant transformation. Given the importance of the Ras/MAPK pathway, several drugs have been developed, some of which are under clinical trial.[88]

Studies have shown higher expression of MAPK in MM than in normal lung tissue,[89] as well as prolonged MAPK activation after exposure to asbestos.[78] This shows that the Ras/MAPK pathway plays a role in MM growth and that its inhibition can yield interesting results in the treatment of MM.

The Bcl family of proteins and apoptosis

Responsible for the control of apoptosis, the Bcl family of proteins is divided into two major classes, namely pro-apoptotic proteins and anti-

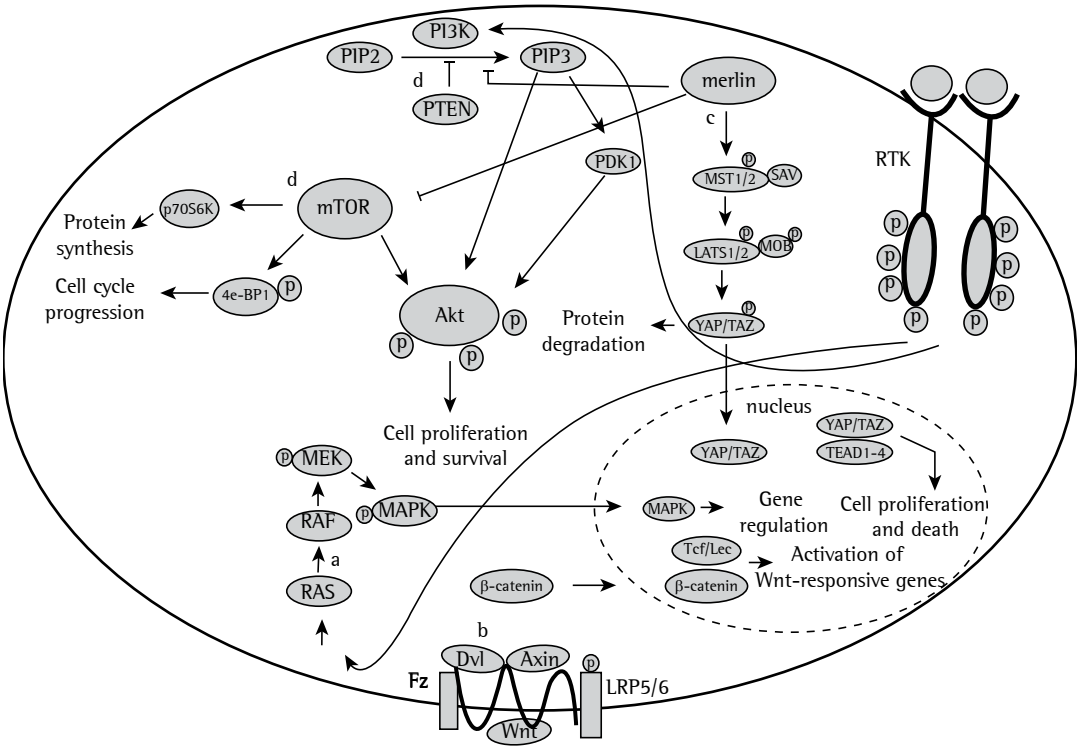

Figure 3 - Biochemical pathways most commonly altered in malignant mesothelioma. In a, receptor tyrosine kinases are frequently activated in malignant mesothelioma, thus increasing the Ras and PI3K pathways. The Ras pathway activates the Raf pathway, which phosphorylates mitogen-activated protein kinase kinase (MEK). In turn, MEK phosphorylates mitogen-activated protein kinase (MAPK), which migrates to the nucleus, thus regulating gene expression. In b, the Wnt pathway controls various cellular processes. In the presence of a Wnt ligand, a complex involving disheveled homolog (Dvl), Axin, frizzled (Fz), and low-density lipoprotein receptor-related proteins (LRP5/6), it leads to inhibition of β-catenin phosphorylation and degradation. Consequently, β-catenin migrates to the nucleus, where it interacts with the Tcf/Lef complex, thus leading to the activation of Wnt-responsive genes. In c, the merlin protein is encoded by the *NF2* gene and inhibits the PI3K pathway and mTOR, acting as an upstream regulator of the Hippo pathway. A biochemical cascade is initiated by a stimulus, macrophage stimulating 1/2 (MST1/2) phosphorylating salvador homolog 1 (SAV1), large tumor suppressor 1/2 (LATS1/2), and Mps one binder kinase 1 (MOB1). The MST1/2 and SAV1 complex phosphorylates LATS1/2; the LATS1/2 and MOB1 complex interacts directly and phosphorylates YAP/TAZ. Phosphorylated YAP/TAZ leads to protein degradation, whereas dephosphorylated YAP/TAZ enters the nucleus and binds to TEAD1-4 transcription factors in order to regulate genes involved in cell proliferation and death. In d, the PI3K/Akt/mTOR pathway is activated by the conversion of phosphatidylinositol-3,4,5-trisphosphate (PIP3) to phosphatidylinositol 4,5-bisphosphate (PIP2), PTEN acting as an antagonist of this activation. PIP3, pyruvate dehydrogenase kinase, isozyme 1 (PDK1), and mammalian target of rapamycin (mTOR) phosphorylate protein kinase B (Akt). Activated Akt participates in processes that are central to cell proliferation, survival, and motility.

apoptotic proteins.[90] Like other types of cancer, MM is resistant to apoptosis, and this hinders the destruction of malignant cells by traditional chemotherapy.[91] Various anti-apoptotic members of the Bcl family of proteins are expressed by MM,[92] and this reduces the efficacy of traditional chemotherapy. Bcl family inhibitors have been developed and have shown interesting results; however, the clinical application of such drugs remains unknown.[93]

Hippo

The Hippo pathway controls cell proliferation, growth, and death via a complex cascade of biochemical events that result in gene regulation.[94] In MM cells, the Hippo pathway was identified

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by the loss of *LATS2* and *YAP* expression, as well as by inactivation of an upstream regulator of the pathway, such as merlin, which is encoded by the *NF2* gene.[95] The Hippo pathway is used in the malignant transformation of MM. Further studies are needed in order to understand the mechanisms by which MM uses the Hippo pathway for its benefit.

Wnt

The Wnt pathway regulates important cellular processes, such as cell proliferation, polarity, and death during embryonic development and in the process of tumor progression.[96] In broad terms, activation of the Wnt pathway can be canonical (i.e., a change in the transcription process) or non-canonical (i.e., activation of non-transcriptional processes).

β-catenin is the principal Wnt pathway transcriptional effector, acting in the nucleus and forming a molecular complex that leads to the activation of specific genes.[97] The Wnt pathway has been shown to be altered in MM[98,99] and has been implicated in decreased patient survival.[100]

Relevance of altered signaling processes in cancer

An understanding of the complex and enigmatic biochemical and molecular processes that occur during malignant transformation is of paramount importance for the development of new drugs and therapies. The search for an understanding of how malignant cells can subvert the cellular machinery and all cell cycle control systems has been exhaustive, resulting in new drugs and therapies that increase the chances of survival of patients with MM.

Individualization: the future of cancer treatment

A deep understanding of how cancer uses the cellular machinery to drive its growth is of paramount importance; each type of cancer uses distinct genes and pathways, thus generating "patterns" of activation and inactivation.[101] These "patterns" can provide important clues for the development of cancer-specific drugs and therapies.

A deep understanding of the molecular processes occurring in a given patient is within the scope of personalized medicine, the objective of which is to treat each disease (e.g., cancer) individually (because of the large variability in physiological processes) in an attempt to improve treatment and prognosis. Although this approach is still in its infancy, it might be used in clinical practice in the future.[102]

In patients receiving certain drugs that are metabolized by specific enzymes, enzyme profile analysis is sometimes recommended. Genetic variations in these enzymes culminate in changes in the pharmacokinetic and pharmacodynamic profiles of drugs, resulting in increased adverse effects and treatment failure.[103]

Although a "one-size-fits-all" approach has been used in the treatment of cancer, an individualized approach is required. In order to choose the best drug or drugs for individual patients (i.e., specific drugs for cancer targets), it is essential to know the receptors and pathways expressed by a given cancer type. This individualized approach improves treatment and increases the chances of survival. Advances in knowledge and technology regarding the process of malignant transformation have resulted in the development of personalized medicine, which is based on the analysis of deregulated cellular processes in individual patients and the use of specific therapeutic tools, therefore increasing the chances of successful treatment.

Final thoughts and future directions

The primary motivation for the present study was the lack of studies reviewing the Portuguese-language literature on genetic and biochemical processes in MM. In fact, MM is not widely studied in Brazil; there are few research groups dedicated to the epidemiological study of MM in the country. In addition, there is a lack of reliable data on the profile, incidence, and prevalence of MM in the Brazilian population.

Our research group has been engaged in gaining a better understanding of the cell signaling processes that contribute to the tumorigenesis of MM. The primary objective of the present study was to provide an overview of the most common genetic and biochemical events in the malignant transformation of MM. As previously mentioned, we did not seek to provide an extensive review of the pathways involved in MM; our objective

was to provide an overview of cell signaling processes in MM. We conclude that MM is a highly aggressive cancer and has a long latency period, with very low survival rates. Worldwide, great efforts have been made to gain a better understanding of the process of tumorigenesis in MM and propose and develop alternatives for the treatment of this aggressive cancer. It should be emphasized that it was outside the scope of the present study to analyze factors associated with MM, such as exposure to asbestos, erionite, and simian virus 40.

Mutations are common in MM, affecting genes such as *p16*^{INK4a}, *p14*^{ARF}, *NF2*, and *BAP1*, whose mutations are commonly somatic. In addition, germline *BAP1* mutations have recently been identified, conferring susceptibility to the development of MM and other types of cancer. Although *TP53* and *PTEN* are known to play major roles in other types of cancer, their roles in MM require further investigation. In addition to the aforementioned genes, the PI3K/Akt/mTOR, Ras/MAPK, Bcl, Hippo, and Wnt pathways and their components are the most altered pathways in MM. Several studies have focused on the modulation of these pathways for a safe and effective reduction in the malignant growth of MM, including in vitro studies, animal studies, and clinical trials.

It is clear that further studies are needed in order to improve the understanding and characterization of the genes and pathways involved in the tumorigenesis of MM, given that few studies in Brazil have addressed this issue. In addition, there is a need for epidemiological studies aimed at analyzing the incidence of MM in the Brazilian population and establishing a realistic and reliable profile of MM in the country.

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Use of volume-targeted non-invasive bilevel positive airway pressure ventilation in a patient with amyotrophic lateral sclerosis*,**

Utilização de ventilação não invasiva com dois níveis de pressão positiva nas vias aéreas e volume alvo em paciente com esclerose lateral amiotrófica

Montserrat Diaz-Abad, John Edward Brown

Abstract

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease in which most patients die of respiratory failure. Although volume-targeted non-invasive bilevel positive airway pressure (BPAP) ventilation has been studied in patients with chronic respiratory failure of various etiologies, its use in ALS has not been reported. We present the case of a 66-year-old woman with ALS and respiratory failure treated with volume-targeted BPAP ventilation for 15 weeks. Weekly data downloads showed that disease progression was associated with increased respiratory muscle weakness, decreased spontaneous breathing, and increased use of non-invasive positive pressure ventilation, whereas tidal volume and minute ventilation remained relatively constant.

Keywords: Amyotrophic lateral sclerosis; Respiratory insufficiency; Hypoventilation; Intermittent positive-pressure ventilation; Sleep.

Resumo

A esclerose lateral amiotrófica (ELA) é uma doença neurodegenerativa progressiva. A maioria dos pacientes com ELA falece por insuficiência respiratória. Embora a ventilação não invasiva com dois níveis de pressão positiva nas vias aéreas e volume alvo tenha sido estudada em pacientes com insuficiência respiratória crônica de diferentes etiologias, sua utilização em ELA não foi relatada. Apresentamos o caso de uma mulher de 66 anos com ELA e insuficiência respiratória tratada com ventilação com dois níveis de pressão positiva e volume alvo por 15 semanas. Os dados obtidos semanalmente mostraram que a progressão da doença estava associada com aumento da fraqueza muscular respiratória, redução da respiração espontânea e maior uso de ventilação não invasiva com pressão positiva, enquanto o volume corrente e a ventilação minuto permaneceram relativamente constantes.

Descritores: Esclerose amiotrófica lateral; Insuficiência respiratória; Hipoventilação; Ventilação com pressão positiva intermitente; Sono.

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease. Most ALS patients die of respiratory failure due to progressive respiratory muscle weakness, with a median survival of less than 2 years after diagnosis.[1] Non-invasive positive pressure ventilation (NPPV) prolongs and improves the quality of life of patients with ALS.[2] The use of volume-targeted, non-invasive bilevel positive airway pressure (BPAP) ventilation, in spontaneous-timed (ST) mode with adjustment of inspiratory pressure to provide an estimated target tidal volume (V_T), has been studied in patients with chronic respiratory failure of various etiologies.[3-8] However, we are unaware of any reports of its use in a patient with ALS.

We report the case of a patient with ALS with rapidly progressive disease and hypercapnic

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respiratory failure who was treated at home with volume-targeted BPAP ST mode ventilation. Weekly monitoring of downloaded ventilator data was accompanied by routine clinical follow-up.

Case report

A 66-year-old woman without a significant past medical history and with a body mass index of 23.4 kg/m^2 presented with mild bulbar symptoms followed by right foot drop. At 11 months after symptom onset, she was diagnosed with ALS. At that time, FVC was 2.22 L (79% of predicted) and MIP was −28 cmH_2O (40% of predicted). Her ALS Functional Rating Scale (ALSFRS) score was 34 (out of 40) with a bulbar component score of 10 (out of 12), denoting mild impairment. Her Pittsburgh Sleep Quality Index (PSQI) score was 8 (out of 21), which is consistent with poor sleep quality, whereas her Epworth Sleepiness Scale (ESS) score was 4 (out of 24), indicating no evidence of excessive daytime sleepiness.

At 4 months of follow-up, marked disease progression was evident, with worsening bulbar symptoms and fatigue, as were new conversational dyspnea, orthopnea, and nonrestorative sleep. Her pulmonary function and functional status had declined—FVC, 1.58 L (57% of predicted); MIP, −25 cmH_2O (36% of predicted)—and her ALSFRS score was 28 with a bulbar component score of 8. Sleep scores were relatively unchanged (PSQI, 7; ESS, 4). An arterial blood gas could not be obtained after two attempts. Gastrostomy and NPPV were recommended. The patient requested further confirmatory testing prior to these interventions, and overnight in-laboratory polysomnography was scheduled for the following week.

Polysomnography revealed sleep hypoventilation. Three weeks later, volume-targeted BPAP ST ventilation titration (Average Volume-Assured Pressure Support; Philips-Respironics, Murrayville, PA, USA) was performed using a full face mask, per patient preference (Table 1). The patient could not tolerate the target V_T (8 mL/kg). Therefore, the final settings were V_T at 320 mL (6 mL/kg), inspiratory positive airway pressure at 8-15 cmH_2O, expiratory positive airway pressure at 6 cmH_2O (increased for flow limitation), and inspiratory time at 1.5 s, with a backup rate of 12 breaths/min. One week later, the patient returned to the clinic with continued worsening of bulbar symptoms and weakness, using a walker, and reporting dyspnea on minimal exertion. Her FVC was 1.05 L (38% of predicted), with an MIP of −19 cmH_2O (27% of predicted) and a $PaCO_2$ of 53 mmHg. Her ALSFRS score was 26, with a bulbar component score of 6, her PSQI score was 17, and her ESS score was 7. Nocturnal NPPV was started with polysomnography settings and a backup rate of 14 breaths/min.

A gastrostomy tube was inserted under radiological guidance, and the patient started home hospice, with no plans to return to the clinic. Seven weeks after starting NPPV, she was contacted to adjust settings based on symptoms and downloaded data (Table 2 and Figure 1), and the patient decided to come to the clinic for a short visit to discuss her worsening dyspnea. She had mild dyspnea at rest and required a wheelchair for mobility. Nocturnal NPPV, which was used every night,

Table 1 - Sleep study data in a patient with amyotrophic lateral sclerosis.

Parameter	Type of study[a]	
	PSG	AVAPS
Total sleep time, min	250	116
Sleep efficiency, %	55	32
Sleep latency, min	58	113
Total wake time, min (%)	203 (45)	242 (65)
Stage 1, min (%)	5 (1)	15 (4)
Stage 2, min (%)	175 (39)	99 (27)
Stage 3, min (%)	71 (16)	15 (4)
REM, min (%)	0 (0)	0 (0)
Wake after sleep onset, min	143	31
Arousal index, events/h	18	6
Spontaneous arousals, n	67	11
Periodic limb movement index, events/h	1	0
Apnea hypopnea index, events/h	0	0
Mean nocturnal SpO_2, %	95	97
Minimum SpO_2, %	93	94
Baseline $ETCO_2$, mmHg	46	47-54
Maximum $ETCO_2$, mmHg	57	57
$ETCO_2$ > 50, min	227	121
Baseline RR, breaths/min	-	24
Final $ETCO_2$, mmHg	-	35-45
Final RR, breaths/min	-	12-14

PSG: polysomnography; AVAPS: average volume-assured pressure support; REM: rapid-eye-movement sleep; and $ETCO_2$: end-tidal CO_2. [a]The patient performed both studies recumbent at approximately 45°.

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Table 2 - Weekly ventilator data downloads for an amyotrophic lateral sclerosis patient on bilevel positive airway pressure ventilation.

Variable	Week														
	1	2	3	4	5	6	7	8	9	10[a]	11	12	13	14	15
V_E, L/min	4.3	4.5	4.4	4.4	4.3	4.5	5.8	5.4	5.5	6.5	7.1	6.4	5.9	6.3	6.5
V_T, mL	271	267	266	259	265	259	295	273	252	310	354	346	336	336	338
Trigger, %	77	82	77	78	76	80	90	87	78	63	55	47	43	46	50
Daily use, h	5.0	3.9	6.1	5.7	5.4	5.3	5.6	6.0	10.1	12.9	11.8	12.4	10.3	13.7	17.6
Use ≥ 4 h/day, %	71	43	100	100	86	86	86	100	100	86	100	100	43	100	100
RR, breaths/min	19	19	20	19	20	20	22	23	25	24	22	20	20	21	22
AHI, events/h	15.2	19.4	25.8	21.6	19.1	23.2	5.2	13.4	22.3	26.8	7.7	15.1	11.4	5.5	12.7
Leak, L/min	40	40	41	38	42	38	36	39	41	38	38	36	36	35	36
IPAP, cmH_2O	11.9	11.9	12.9	12.8	12.5	12.6	10.9	11.8	13.5	15.0	13.8	13.5	14.2	14.0	14.5

V_E: minute ventilation; V_T: tidal volume; Trigger: patient-triggered (spontaneous) breaths; Daily use: device use per 24-h period; Use ≥ 4 h/day: days on which the device was used for ≥ 4 h/day; AHI: apnea-hypopnea index; Leak: total mask leak; and IPAP: inspiratory positive airway pressure. [a]Ventilator support increased between weeks 8 and 9; week 10 reflects this increase for the first complete week.

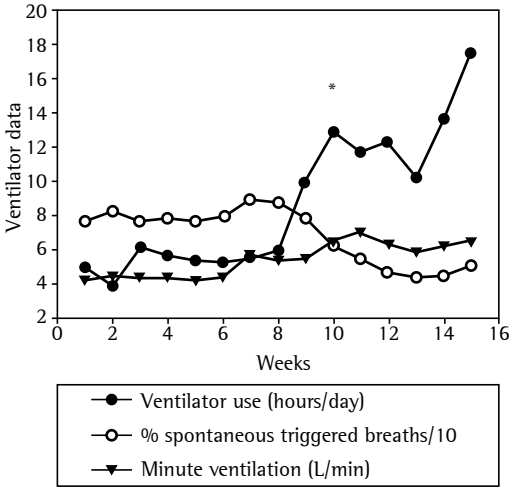

Figure 1 - Ventilator data for a 15-week period in an amyotrophic lateral sclerosis patient on bilevel positive airway pressure ventilation. Note the increased duration of daily use of ventilation with decreased ability to trigger breaths spontaneously (i.e., increased reliance on timed ventilator-delivered breaths) over time. As can be seen, minute ventilation remained relatively constant. *Ventilator support increased between weeks 8 and 9; week 10 reflects this increase for the first complete week.

helped ease breathing, allowing her to sleep better and longer. She had recently developed a mask leak due to weight loss. At that time, FVC was 1.01 L (36% of predicted), MIP was −15 cmH_2O (21% of predicted) and $PaCO_2$ was 55 mmHg. Settings were adjusted to V_T at 370 mL (7 mL/kg), inspiratory positive airway pressure at 10-17 cmH_2O, and inspiratory time at 1.2 s, with a backup rate of 18 breaths/min. Intermittent daytime NPPV use and a new mask fitting were recommended. Contact with the patient (via telephone and e-mail) was maintained, and the changes were well tolerated. At 6 weeks after her last visit, she was again contacted to adjust settings but declined to make further changes. Shortly thereafter, she died of progressive respiratory failure.

Discussion

We have presented the case of a patient with ALS treated for chronic respiratory failure with volume-targeted BPAP ST mode ventilation for 15 weeks, in whom the use of weekly monitoring of ventilator data in addition to routine care provided useful information for management of respiratory failure. Disease progression was associated with worsening respiratory muscle weakness, a decrease in spontaneous breathing, and increased use of NPPV, although V_T and minute ventilation (V_E) remained relatively constant. To our knowledge, the use of this mode of NPPV has not been reported in ALS.

Among patients with ALS, the progression of the disease is relatively rapid but varies.[9] Therefore, serial NPPV pressure adjustments may be required in order to compensate for declining respiratory muscle strength and increasing hypercapnia.[10] An NPPV mode with an inspiratory pressure range to maintain a target V_T, rather than a fixed pressure, might reduce the frequency of required adjustments over time

in some patients. This feature might also be of benefit in the short term, such as during sleep, when patients with diaphragmatic weakness are vulnerable to worsening hypoventilation, especially during rapid-eye-movement sleep. Ambogrio et al. showed that, in comparison with traditional BPAP ST mode ventilation, volume-targeted BPAP ST mode ventilation was better able to maintain V_E (by maintaining V_T) during sleep in patients with obesity hypoventilation syndrome.[4]

The built-in software of NPPV devices is proprietary, and, in the absence of independent validation, the data provided on many parameters should be considered as indicators of trends without any guarantee as to linearity of the estimations provided.[11] Despite this limitation, the available data can provide valuable information for patient management. Studies involving remote monitoring of NPPV compliance data in patients with ALS using traditional BPAP ventilation have shown that such monitoring reduces health care utilization and hospital admissions, potentially reducing overall health care costs, in comparison with routine care.[12] This monitoring modality could be particularly useful in patients with rapidly progressive or advanced ALS, who, like our patient, might be homebound. The ability to request and verify changes to the settings remotely (without a home visit) is an additional advantage.

Volume-targeted BPAP ST mode ventilation is a relatively new alternative to traditional NPPV for patients with respiratory failure, and we have reported its use for the first time in a patient with ALS. Additional studies are needed in order to compare the various NPPV modes, in terms of their effect on survival, quality of life, sleep quality, adherence, adequacy of ventilation, and health care utilization, in ALS patients.

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Accessory cardiac bronchus causing recurrent pulmonary infection

Brônquio cardíaco acessório causando infecções respiratórias de repetição

Gláucia Zanetti, Bruno Hochhegger, Marcos Duarte Guimarães, Edson Marchiori

To the Editor:

A 15-year-old female patient presented to our hospital with a history of recurrent pneumonia and complaints of productive cough and episodes of bronchospasm. Physical examination revealed crackles in the right hemithorax. Laboratory test findings were normal. A chest X-ray showed right paracardiac opacities. Axial CT (Figure 1A) demonstrated consolidations with cystic areas in the right paracardiac region. A reformatted coronal image showed an accessory cardiac bronchus (ACB; Figure 1B, arrow) arising from the medial wall of the intermediate bronchus. Three-dimensional shaded surface display coronal reformatting showed the ACB (Figure 1C, arrow) and a correspondent lobule with cystic dilatations (arrowheads). Bronchoscopy confirmed the presence of the ACB arising from the intermediary bronchus. Bronchoalveolar lavage and cultures were negative for Mycobacterium spp. and fungi. Surgery demonstrated infected cystic structures and small bronchioles and alveoli with retained secretions distally to the ACB.

Bronchial division anomalies are common, although most are encountered incidentally in asymptomatic adults. They might be isolated or associated with a variety of other congenital disorders.[1] ACB is a rare congenital anomaly of the tracheobronchial tree, characterized by an anomalous bronchus originating from the intermediate bronchus opposite to the origin of the right upper lobe bronchus or originating from the medial wall of the right main bronchus.[1-3] From its origin, it runs medially and caudally toward the heart.[2] An ACB might be a short, blind-ended structure or a long, branching bronchus that develops into a series of small bronchioles, which might end in vestigial parenchymal tissue in the bronchioles or in cystic degeneration, or it might be associated with small amounts of pulmonary parenchyma.[1,3]

Most patients with ACB are asymptomatic, and the anomaly is discovered incidentally during bronchoscopy or imaging studies conducted for unrelated reasons.[1,4] However, an ACB can become symptomatic due to recurrent infection, empyema, hemoptysis, and malignant transformation.[1,2,4,5] These symptoms are caused by the accumulation of secretions in the ACB, leading to inflammation and infection, extensive microvascularization, and hemoptysis, especially when the ACB is long or has an accessory lobe.[2,4] Thus, the short type of ACB tends to be asymptomatic, whereas the accessory-lobed and long diverticular types are more susceptible to complications.[5]

Histological examination suggested that the specimen resected from our patient was the accessory bronchus, including an accessory lobe with retained secretions. The finding of scar tissue, but no alveoli, on the peripheral accessory lobe suggested that it had been deteriorated or ruptured by constant infection, leading to bronchopneumonia and empyema.[4]

An ACB is not generally visible on chest X-ray, but it can be visualized well with other imaging modalities. Surgical resection of a long ACB or of one with an accessory lobe is advised as soon as symptoms occur.[4,5]

In conclusion, pulmonologists and radiologists should recognize normal bronchial anatomy as well as developmental bronchial anomalies because this is important to establish a correct diagnosis. Although an ACB is not pathological per se, it is occasionally associated with clinical symptoms and complications.

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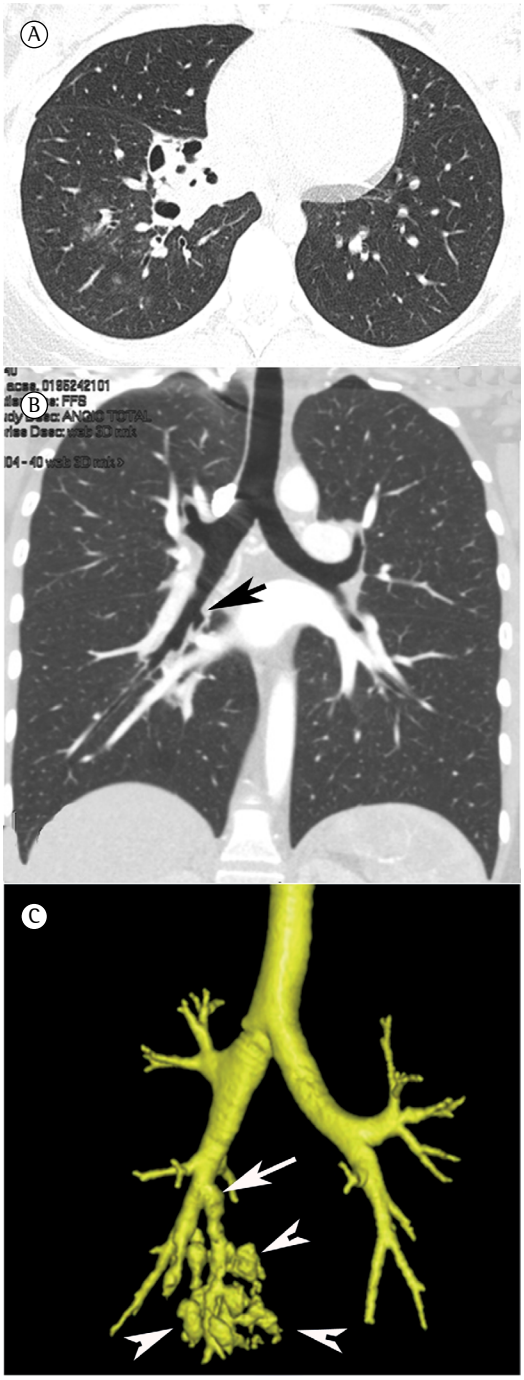

Figure 1 - In A, an axial CT image demonstrating consolidations with cystic areas in the right paracardiac region. In B, a reformatted coronal image showing an accessory cardiac bronchus (arrow) arising from the medial wall of the intermediate bronchus. In C, three-dimensional shaded surface display coronal reformatting, showing the accessory cardiac bronchus (arrow) and a correspondent lobule with cystic dilatations (arrowheads).

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Ground-glass nodules and CT-guided placement of platinum coils

Nódulos em vidro fosco e marcadores espirais de platina guiados por TC

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To the Editor:

The detection of a small growing pulmonary nodule on chest CT raises the suspicion of lung cancer, but proof of malignancy must be established by either needle biopsy or nodule resection.[1] Pulmonary nodules ≤ 10 mm with ground-glass opacity should be considered to have a high possibility of malignancy.[2] Various centers perform the excision of these small growing nodules using video-assisted thoracoscopic surgery (VATS) in order to minimize postoperative morbidity, as well as to remove as small a volume of lung tissue as possible. Small nodules are often visible with the thoracoscope if they lie within 5 mm of the visceral pleural surface; however, if they are located deeper in the lung, palpation is required in order to locate them for excision. A previous study found that, in a series of 92 consecutive patients undergoing VATS, 50 (54%) required conversion to thoracotomy.[3] The most common reason for conversion to full thoracotomy was failure to locate the nodule. Univariate and multivariate analysis of the eleven variables studied showed that if the distance from the pleural surface to the nodule edge was greater than 5 mm, the probability of failure to detect a nodule was 63%,[3] and 40% of those nodules were found to be malignant. Because of the difficulty in localizing a nodule during surgery and the increasing clinical load due to the identification of small lung nodules for lung cancer screening using CT, there has been extensive investigation for improving nodule localization techniques in order to assist the resection of small nodules during VATS. We would like to report the first use of a new technique for the intraoperative localization of such nodules in Brazil: CT-guided placement of platinum coils.

A 72-year-old woman underwent a chest CT for the evaluation of chronic cough. The CT scans demonstrated a 1-cm ground-glass nodule in the central portion of the right upper lobe (Figure 1A). The nodule was later biopsied, and the final pathological examination revealed atypical cells suspected of being adenocarcinoma *in situ* (formerly known as bronchioalveolar carcinoma). Surgical resection using VATS was planned; however, because of the ground-glass nature of the nodule and its distance from the pleural surface, preoperative wire localization was requested. Using CT guidance, the tip of the loaded Chiba needle was percutaneously placed approximately 5 mm deep into the lung nodule (Figure 1B). The guide wire was introduced up to the first mark, advancing 30 mm of the fiber-coated coil out of the Chiba needle and into the lung parenchyma, where it assumed a tightly coiled helical configuration into the nodule (Figures 1C and 1D). The patient underwent VATS, and the coil was easily localized by lung palpation through a 3-cm minithoracotomy (Figure 1E). The final diagnosis was pulmonary adenocarcinoma.

Techniques for the localization of pulmonary nodules have been classified into three types.[1,4] The first class uses intraoperative imaging (either ultrasonography or CT). Localization with intraoperative ultrasound is difficult because the lung must be completely collapsed in order to allow the visualization of small nodules.[1,4] This technique lengthens the surgical time, since the complete collapse of the lung can take 30-150 min and is often contraindicated in patients with extensive emphysema. Not only is experience with real-time CT-guided thoracoscopic resection limited, but also artifacts caused by instruments and staples degrade the CT image, and the limited space within the scanner gantry makes the procedure difficult.[1,4]

 http://dx.doi.org/10.1590/S1806-37132014000400015

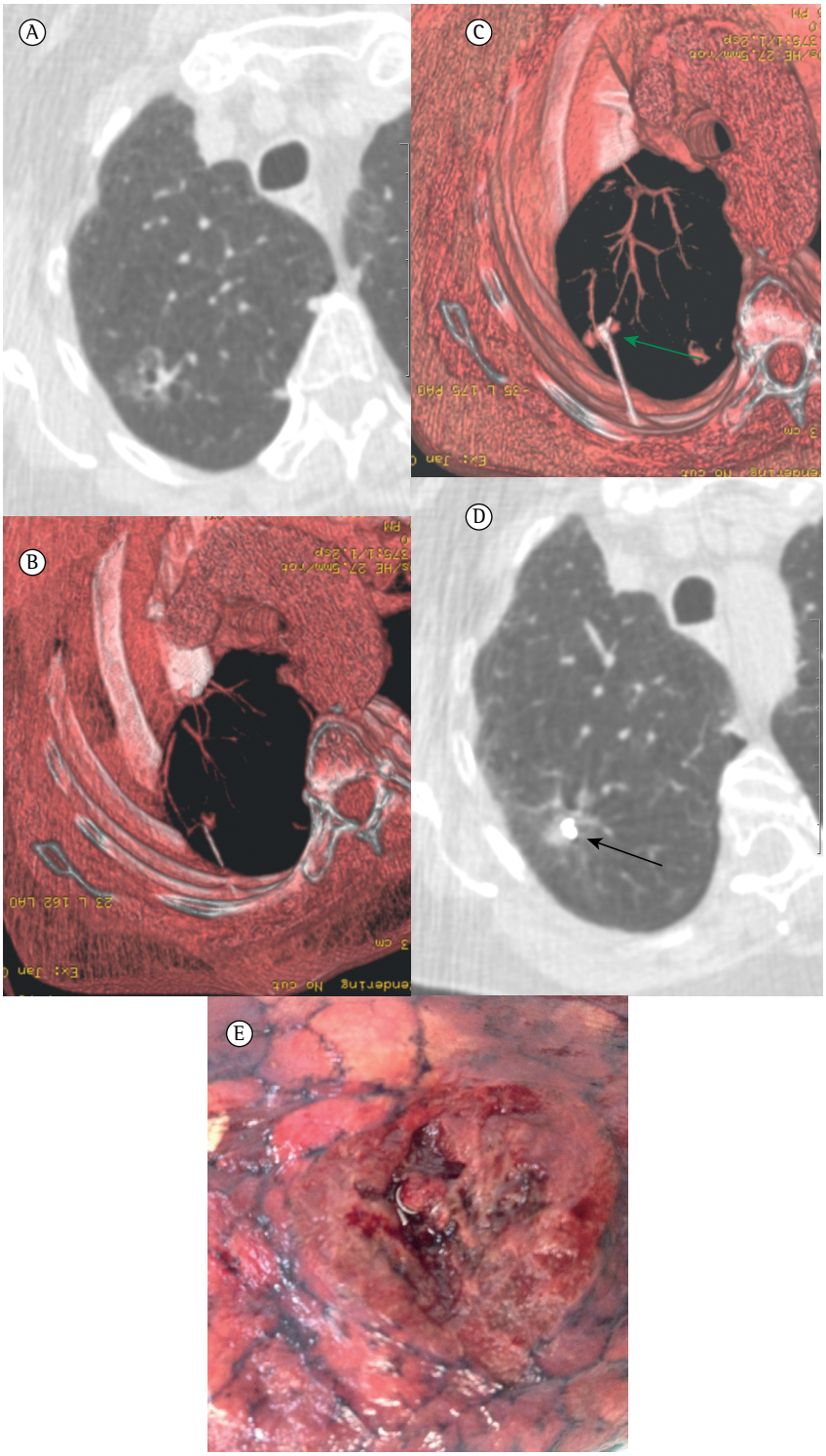

Figure 1 - In A, a CT scan demonstrating a 1-cm ground-glass nodule in the central portion of the right upper lobe. In B, volume rendering of a CT scan demonstrating the needle inside the ground-glass nodule in right upper lobe. In C, volume rendering of a CT scan demonstrating the CT-guided placement (arrow) of a platinum microcoil inside the ground-glass nodule. In D, a CT scan taken after the procedure, demonstrating the platinum coil (arrow) inside the ground-glass nodule. In E, a photograph of the surgical specimen showing the coil.

The second class of targeting techniques includes the percutaneous injection of dyes, contrast media, radionuclides, or colored adhesive agents.[1,4,5] Diffusion away from the nodule is a limitation of these techniques and imposes restrictions on the allowable time between the CT localization procedure and the thoracoscopic resection. This can cause difficulties in the operating room scheduling. In addition, certain dyes, such as methylene blue, carry a possible risk of anaphylactic reactions following their injection and are often difficult to visualize on the visceral pleural surface in patients with extensive anthracotic pigmentation of the lungs.[1,4,5] Because these materials are not water-soluble, they carry a potential risk of stroke if they gain access to the pulmonary veins.

The third class of targeting techniques uses coils or microcoils that are soft and pliable and cause little damage to lung tissue, even when dislodged. A previous study compared the use of microcoils and hook wires for the localization of nodules in freshly harvested goat lungs.[5] The authors reported that when a coil was displaced, it would uncoil, causing minimal tissue damage. In addition, the "fuzzy" fiber coating on these microcoils induces coagulation and increases the adhesion of the coil to the lung tissue. The coiled configuration and the fiber coating virtually eliminate the risk of embolization.

In conclusion, we would like to highlight this new method of nodule localization, which is a safe and effective technique and increases the success rate of nodule excision using VATS, especially for small, ground-glass nodules.

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Edital de Seleção

Brasília, 30 de maio de 2014

No período de 23 de junho a 22 de agosto estarão abertas as inscrições para candidatos a posição de Editor-Chefe do Jornal Brasileiro de Pneumologia com atuação no quadriênio janeiro de 2015 a dezembro de 2018. Os interessados ao posto deverão enviar à administração da SBPT em Brasília, suas propostas de gestão e curriculum vitae na plataforma Lattes. As propostas dos candidatos deverão abranger o campo administrativo, científico e orçamentário, e deverão ser apresentadas em relação aos quatro anos previstos para a duração do mandato. Experiência prévia em publicações científicas será importante critério para a escolha do candidato. Os candidatos deverão conhecer as normas relativas à seleção do Editor-Chefe e o funcionamento do Jornal Brasileiro de Pneumologia, explícitas em seu regulamento, o qual poderá ser obtido por meio de contato com a secretaria do Jornal em Brasília.

Prof. Dr. Jairo Sponholz Araújo
Presidente da SBPT

Prof. Dr. Carlos Roberto Ribeiro Carvalho
Editor-Chefe

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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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Organização: Ikone Eventos
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7th International Wasog Conference on Diffuse Parenchymal Lung Diseases
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06 a 10 de setembro
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- **ALCANCE DAS PEQUENAS VIAS AÉREAS,** 50% a 70% de partículas finas.[3]
- **NÃO PRECISA** ser conservado em geladeira.

Referências bibliográficas: 1. Morice AH, Peterson S, Beckman O, Kukova Z. Efficacy and safety of a new pressurised metered-dose inhaler formulation of budesonide/formoterol in children with asthma: a superiority and therapeutic equivalence study. Pulm Pharmacol Ther.2008;21(1):152-9. **2.** Noonan M, Rosenwasser LJ, Martin P, O'Brien CD, O'Dowd L. Efficacy and safety of budesonide and formoterol in one pressurised metered-dose inhaler in adults and adolescents with moderate to severe asthma: a randomised clinical trial. Drugs.2006;66(17):2235-54. **3.** Chambers F, Ludzik A. *In vitro* drug delivery performance of a new budesonide/formoterol pressurized metereddose inhaler. Journal of aerosol medicine and pulmonary drug delivery. 2009 Jun;22(2):113-20.

VANNAIR® 6/100 mcg/inalação e VANNAIR® 6/200 mcg/inalação (fumarato de formoterol di-hidratado/budesonida) VANNAIR® (fumarato de formoterol di-hidratado/budesonida) é composto por substâncias que possuem diferentes modos de ação e que apresentam efeitos aditivos em termos de redução da asma do que outros produtos isoladamente. A budesonida é um glicocorticosteróide que tem uma rápida (dentro de horas) e dose-dependente ação antiinflamatória nas vias aéreas e o formoterol é um agonista beta-2-adrenérgico seletivo de início de ação rápido (1-3 minutos) e de longa duração (pelo menos 12 horas). **Indicações: VANNAIR** está indicado no tratamento da asma nos casos em que o uso de uma associação (corticosteróide inalatório com um beta-2 agonista de ação prolongada) é apropriado. **Contra-indicações: Hipersensibilidade a budesonida, ao formoterol ou a outros componentes da fórmula. Cuidados e Advertências:** Advertências: É recomendado que a dose seja titulada quando o tratamento de longo prazo é descontinuado e este não deve ser interrompido abruptamente. Para minimizar o risco de candidíase orofaríngea, o paciente deve ser instruído a lavar a boca com água após administrar as inalações de **VANNAIR**. Uma deterioração súbita e progressiva do controle da asma é um risco potencial e o paciente deve procurar suporte médico.Pacientes que necessitaram de terapia corticosteróide de alta dose emergencial ou tratamento prolongado de altas doses recomendadas de corticosteróides inalatórios podem exibir sinais e sintomas de insuficiência adrenal quando expostos a situações de estresse grave. Administração de corticosteróide sistêmico adicional deve ser considerada durante situações de estresse ou cirurgia eletiva. **VANNAIR** deve ser administrado com cautela em pacientes com graves alterações cardiovasculares (incluindo anomalias do ritmo cardíaco), *diabetes mellitus*, hipocalemia não tratada ou tireotoxicose. Pacientes com prolongamento do intervalo QTc devem ser cuidadosamente observados (para maiores informações vide bula completa do produto). *Uso durante a gravidez e a lactação:* categoria C de risco de gravidez. Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. A administração de **VANNAIR** em mulheres lactantes deve ser apenas considerada se os benefícios esperados para a mãe superarem qualquer possível risco para a criança (para maiores informações vide bula completa do produto). Interações medicamentosas: o metabolismo da budesonida é mediado principalmente pela CYP3A4, uma subfamília do citocromo P450. Portanto, inibidores desta enzima, como o cetoconazol ou suco de *grapefruit* (pomelo), podem aumentar a exposição sistêmica à budesonida. A cimetidina apresenta um leve efeito inibidor sobre o metabolismo hepático da budesonida. Fármacos como a procainamida, fenotiazina, agentes antihistamínicos (terfenadina), inibidor da monoaminooxidase (MAO) e antidepressivos tricíclicos foram relacionados com um intervalo QTc prolongado e um aumento do risco de arritmia ventricular. **Os bloqueadores beta-adrenérgicos (incluindo os colírios oftálmicos) podem atenuar ou inibir o efeito do formoterol** (para maiores informações vide bula completa do produto). **Reações adversas:** as reações adversas que foram associadas à budesonida ou ao formoterol são apresentadas a seguir. *Comum*: palpitações, candidíase na orofaringe, cefaléia, tremor, leve irritação na garganta, tosse, rouquidão. *Incomum*: taquicardia, náusea, câibras musculares, tontura, agitação, ansiedade, nervosismo eperturbações do sono. (para outras reações adversas, vide bula completa do produto). **Posologia:** a dose de **VANNAIR** deve ser individualizada conforme a gravidade da doença. Quando for obtido o controle da asma, a dose deve ser titulada para a menor dose que permita manter um controle eficaz dos sintomas. **VANNAIR® 6/100 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Crianças (6-11 anos)*: 2 inalações duas vezes ao dia. Dose máxima diária: 4 inalações. **VANNAIR® 6/200 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Instruções de Uso*: vide bula completa do produto. **Superdose:** A superdosagem de formoterol irá provavelmente provocar efeitos típicos dos agonistas beta-2-adrenérgicos: tremor, cefaléia, palpitações e taquicardia. Poderá igualmente ocorrer hipotensão, acidose metabólica, hipocalemia e hiperglicemia. Pode ser indicado um tratamento de suporte e sintomático. A administração de uma dose de 90 mcg durante três horas em pacientes com obstrução brônquica aguda e quando administrada três vezes ao dia como um total de 54 mcg/dia por 3 dias para a estabilidade asmática não suscitou quaisquer problemas de segurança. Não é esperado que uma superdosagem aguda da budesonida, mesmo em doses excessivas, constitua um problema clínico. Quando utilizado cronicamente em doses excessivas, podem ocorrer efeitos glicocorticosteróides sistêmicos (para informações de superdosagem grave vide bula completa do produto). **Apresentações: VANNAIR® 6/100 mcg/inalação:** Aerossol bucal 6/100 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO E PEDIÁTRICO. VANNAIR® 6/200 mcg/inalação:** Aerossol bucal 6/200 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO. USO POR INALAÇÃO ORAL. VENDA SOB PRESCRIÇÃO MÉDICA.** Para maiores informações, consulte a bula completa do produto. (VAN005). AstraZeneca do Brasil Ltda., Rod. Raposo Tavares, Km 26,9 - Cotia - SP - CEP 06707-000 Tel.: 0800-0145578. www.astrazeneca.com.br **Vannair®. MS – 1.1618.0234**

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