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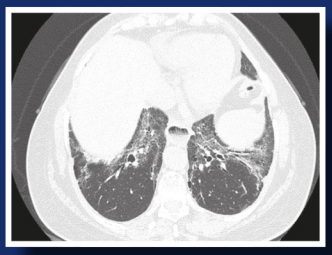
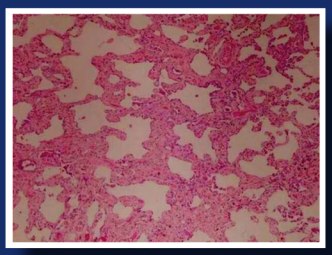
HIGHLIGHT

Lung-dominant connective tissue disease





Review Article

Editorial: Aryeh Fischer

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DAXAS® diminui as exacerbações e melhora a qualidade de vida[1,2]

Exclusivo
modo de ação anti-inflamatória[3,4]

Reduz
a taxa de exacerbação em pacientes exacerbadores frequentes e não frequentes[5]

Melhora
a função pulmonar em + 80 mL em estudo com broncodilatador de longa ação[6]

É indicado para
adultos com doença pulmonar obstrutiva crônica (DPOC) grave (VEF1 pós-broncodilatador < 50% do predito) associada com bronquite crônica que apresentam histórico de exacerbações frequentes, em complementação ao tratamento com broncodilatadores.[7]

DAXAS® ESTÁ DISPONÍVEL EM CAIXAS COM 30 COMPRIMIDOS[7]

1 comprimido ao dia
Associado ao tratamento de manutenção[7]

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INDO ALÉM DOS SINTOMAS.[1]

Referências bibliográficas: 1. Rabe KF. Update on roflumilast, a phosphodiesterase 4 inhibitor for the treatment of chronic obstructive pulmonary disease. *Br J Pharmacol*. 2011;163(1):53-67. **2.** Calverley PM et al. Roflumilast in symptomatic chronic obstructive pulmonary disease: two randomised clinical trials. *Lancet*. 2009;374(9691):685-94. **3.** Hatzelmann A et al. The preclinical pharmacology of roflumilast – A selective, oral phosphodiesterase 4 inhibitor in development for chronic obstructive pulmonary disease. *Pulm Pharmacol Ther*. 2010; 23(4):235-56. **4.** Sanz MJ, Cortijo J, Morcillo EJ. PDE4 inhibitors as new anti-inflammatory drugs: effects on cell trafficking and cell adhesion molecules expression. *Pharmacol Ther* 2005; 106(3):269-297. **5.** Wedzicha JA et al. Efficacy of roflumilast in the chronic obstructivepulmonary disease frequent exacerbator phenotype. *Chest*. 2012. doi:10.1378/chest.12-1489. **6.** Fabbri LM et al. Roflumilast in moderate-to-severe chronic obstructive pulmonary disease treated with long acting bronchodilators: two randomised clinical trials. *Lancet*. 2009;374(9691):695-703. **7.** Daxas® [Bula]. São Paulo: Nycomed Pharma.

DAXAS® roflumilaste. **USO ORAL. USO ADULTO.** Apresentações e composição: Comprimidos revestidos com 500 mcg de roflumilaste. Embalagens com 30 unidades. Indicações: para o tratamento de manutenção de pacientes com doença pulmonar obstrutiva crônica (DPOC) grave (VEF1 pós-broncodilatador < 50% do predito) associada com bronquite crônica (tosse e expectoração crônicas) que apresentam histórico de exacerbações (crises) freqüentes, em complementação ao tratamento com broncodilatadores. **Contraindicações:** Este medicamento não deve ser usado por pacientes com hipersensibilidade ao roflumilaste ou a qualquer dos componentes da formulação. **Este medicamento é contraindicado para pacientes com insuficiência hepática moderada e grave (classes 'B' e 'C' de Child-Pugh), pois não existem estudos sobre o uso do roflumilaste nestes pacientes. Precauções e advertências:** DAXAS® deve ser administrado exclusivamente pela via oral. DAXAS® **não está indicado para melhora de** broncoespasmos agudos. Cada comprimido de DAXAS® contém 199 mg de lactose. Perda de peso: nos estudos de 1 ano (M-124, M-125), uma diminuição no peso corporal ocorreu mais freqüentemente em pacientes tratados com DAXAS® comparativamente aos pacientes que receberam placebo. Depois da descontinuação do DAXAS®, a maioria dos pacientes recuperou o peso corporal após 3 meses. Na ocorrência de uma inexplicada e pronunciada perda de peso, a administração de DAXAS® deve ser descontinuada, se julgado necessário. Intolerância persistente: apesar das reações adversas como diarréia, náusea, dor abdominal e cefaléia serem transitórios e resolverem espontaneamente com a manutenção do tratamento, o tratamento com DAXAS® deve ser revisto em caso de intolerância persistente. Gravidez e Lactação: as informações disponíveis sobre o uso de DAXAS® em gestantes são limitadas, mas não indicaram eventos adversos do roflumilaste sobre a gestação ou sobre a saúde do feto/recém-nato. Não são conhecidos outros dados epidemiológicos relevantes. Estudos em animais demonstraram toxicidade reprodutiva. O risco potencial para humanos ainda não está estabelecido. DAXAS® **não deve ser administrado durante o período gestacional.** É possível que roflumilaste e/ou seus metabólitos sejam excretados no leite materno durante a amamentação; estudos em animais (ratos) em fase de amamentação detectaram pequenas quantidades do produto e dos seus derivados no leite dos animais. Categoria B de risco na gravidez – Este medicamento não deve ser utilizado por mulheres grávidas ou que estejam amamentando sem orientação médica ou do cirurgião-dentista. Pacientes idosos: os cuidados de uso de DAXAS® por pacientes idosos devem ser os mesmos para os demais pacientes; não são recomendados ajustes na dosagem da medicação. Pacientes pediátricos (crianças e adolescentes menores de 18 anos de idade): o produto não é recomendado para uso neste grupo de pacientes, pois não são disponíveis dados sobre a eficácia e a segurança da administração oral de DAXAS® nesta faixa etária. Pacientes com insuficiência hepática: não é necessário ajuste da dosagem em pacientes com insuficiência hepática leve (classe 'A' de Child-Pugh). No entanto, para pacientes com insuficiência hepática moderada ou grave (classes 'B' e 'C' de Child-Pugh), o uso deste medicamento não é recomendado pois não existem estudos sobre o seu uso nestes pacientes. Pacientes com insuficiência renal: não é necessário ajuste da dose em pacientes com insuficiência renal crônica. Pacientes fumantes com DPOC: não é necessário ajuste da dose. Efeitos na habilidade de dirigir e operar máquinas: É improvável que o uso deste medicamento tenha qualquer efeito na capacidade de dirigir veículos ou de usar máquinas. Pacientes portadores de doenças imunológicas graves, infecciosas graves, câncer (exceto carcinoma basocelular) ou tratados com imunossupressores: o tratamento com DAXAS® não deve ser iniciado ou deve ser suspenso nestes casos. Pacientes portadores de insuficiência cardíaca classes III e IV (NYHA): não existem estudos nesta população de pacientes, portanto o uso neste grupo não é recomendado. Pacientes portadores de doenças psiquiátricas: DAXAS® não está recomendado em pacientes com histórico de depressão associada com ideação ou comportamento suicida. Os pacientes devem ser orientados a comunicar seu médico caso apresentem alguma ideação suicida. **Interações medicamentosas:** Estudos clínicos de interações medicamentosas com inibidores do CYP3A4 (eritromicina e cetoconazol) não resultaram em aumentos da atividade inibitória total de PDE4 (exposição total ao roflumilaste e ao N-óxido roflumilaste). Estudos de interações medicamentosas com o inibidor do CYP1A2 fluvoxamina e com os inibidores duplos CYP3A4/1A2 enoxacina e cimetidina resultaram em aumentos na atividade inibitória total de PDE4. Desta forma, deve ser esperado um aumento de 20% a 60% na inibição total de PDE4 quando o roflumilaste for administrado concomitantemente com potentes inibidores do CYP1A2, como a fluvoxamina, enquanto não são esperadas interações com os inibidores do CYP3A4 como o cetoconazol. Não são esperadas interações medicamentosas clinicamente relevantes. A administração de rifampicina (um indutor enzimático de CYP450) resultou em uma redução na atividade inibitória total de PDE4 de cerca de 60% e o uso de indutores potentes do citocromo P450 (como fenobarbital, carbamazepina, fenitoína) pode reduzir a eficácia terapêutica do roflumilaste. Não foram observadas interações clinicamente relevantes com os seguintes fármacos: salbutamol inalado, formoterol, budesonida, montelucaste, digoxina, varfarina, sildenafil, midazolam. A co-administração com antiácido e não altera a absorção nem as características farmacológicas do produto. A co-administração com teofilina aumento em 8% a atividade inibitória sobre a fosfodiesterase 4. Quando utilizado com contraceptivo oral contendo gestodeno e etinilestradiol a atividade inibitória sobre a fosfodiesterase 4 aumentou em 17%. Não existem estudos clínicos que avaliaram o tratamento concomitante com xantinas, portanto seu uso em associação não está recomendado. **Reações adversas:** DAXAS® foi bem avaliado em estudos clínicos, e cerca de 16% experimentaram reações adversas com o roflumilaste em comparação com 5,7% com o placebo. As reações adversas relatadas com mais frequência foram diarreia (5,9%), perda de peso (3,4%), nausea (2,9%), dor abdominal (1,9%) e cefaleia (1,7%). A maior parte destas reações foram leves ou moderadas e desapareceram com a continuidade do tratamento. Os eventos adversos classificados por frequência foram: Reação comum (> 1/100 e < 1/10): Perda de peso, distúrbios do apetite, insônia, cefaléia, diarreia, náusea, dor abdominal. Reação incomum (> 1/1.000 e < 1/100): Hipersensibilidade, ansiedade, tremor, vertigem, tontura, palpitações, gastrite, vômitos, refluxo gastro-esofágico, dispepsia, rash, espasmos musculares, fraqueza muscular, mal-estar, astenia, fadiga, dor muscular, lombalgia. Reação rara (> 1/10.000 e < 1/1.000): Depressão e distúrbios do humor, ginecomastia, disgeusia, hematoquesia, obstipação intestinal, aumento de Gama – GT, aumento de transaminases, urticária, infecções respiratórias (exceto pneumonia), aumento de CPK. Em estudos clínicos, raros casos de pensamento e comportamento suicida (incluindo suicídio completo) foram reportados. Pacientes devem ser instruídos a informar o prescritor sobre qualquer ideação suicida. Posologia e modo de usar: A dose recomendada de DAXAS® é de um comprimido uma vez ao dia. Não é necessário ajuste posológico para pacientes idosos, com insuficiência renal ou com insuficiência hepática leve (classes 'A' de Child-Pugh). DAXAS® ***não deve ser administrado à*** pacientes com insuficiência hepática moderada ou grave (classe 'B'ou 'C' de Child-Pugh). Os comprimidos de DAXAS® devem ser administrados com uma quantidade de água necessária para facilitar a deglutição. Podem ser administrados antes, durante ou após as refeições. Recomenda-se que o medicamento seja administrado sempre no mesmo horário do dia, durante todo o tratamento. Este medicamento não deve ser partido ou mastigado. MS – 1.0639.0257. DX_0710_0512_VPS

CONTRAINDICAÇÃO: ALERGIA AOS COMPONENTES DA FÓRMULA E PACIENTES COM INSUFICIÊNCIA HEPÁTICA MODERADA A GRAVE. **INTERAÇÃO MEDICAMENTOSA:** A ADMINISTRAÇÃO DE INDUTORES ENZIMÁTICOS DO CITOCROMO P450, COMO RIFAMPICINA E ANTICONVULSIVANTES, PODE REDUZIR A EFICÁCIA TERAPÊUTICA DO ROFLUMILASTE. NÃO EXISTEM ESTUDOS CLÍNICOS QUE AVALIARAM O TRATAMENTO CONCOMITANTE COM METILXANTINAS, PORTANTO, SEU USO EM ASSOCIAÇÃO NÃO ESTÁ RECOMENDADO.

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Sumário / Contents

Interstitial lung disease in suggestive forms of connective tissue disease

Doença intersticial pulmonar em formas sugestivas de doença do tecido conectivo

Aryeh Fischer

Patients with the characterizable forms of connective tissue disease (CTD) are at risk for developing interstitial lung disease (ILD), and there is a growing appreciation that ILD can be the first or only clinically relevant manifestation of an underlying CTD.[1-3] Less is understood—and there is far greater controversy—about ILD associated with suggestive or "undifferentiated" forms of CTD.[2,3]

In the present issue of the *Brazilian Journal of Pulmonology*, Pereira et al. review the clinical scenario of autoantibody positivity in ILD and discuss how these serologic tests may be interpreted.[4] In addition, they provide useful insights into the evaluation of patients with ILD suspected of having occult forms of CTD. Finally, the authors argue in favor of implementing the concepts that have been put forth in a recent commentary on "lung-dominant CTD"[5]: a proposed, provisional category that describes ILD patients with an autoimmune flavor that fall short of meeting established criteria of any of the characterizable forms of CTD.

Identifying occult CTD in patients presenting with what is initially considered to be an idiopathic interstitial pneumonia (IIP) can be challenging. Sometimes patients that subsequently develop a classifiable CTD cannot be identified before the specific systemic manifestations of the CTD appear. There is no universally accepted approach to these evaluations, and current practice includes an assessment for extrathoracic features of CTD, testing of a broad array of circulating autoantibodies, and consideration of specific radiographic and histopathologic features.[2,6] Various centers have also found that a multidisciplinary evaluation—including rheumatologic consultation—can be useful.[7-9]

A number of recent studies have shown that patients with IIP often have subtle extrathoracic or other clinical features suggestive of an underlying autoimmune process and yet do not meet established criteria for any of the characterizable forms of CTD.[9-17] Sometimes these subtle symptoms or signs occur in the absence of serologic abnormalities, or a serum autoantibody known to be highly specific for a certain CTD (e.g. anti-Jo-1 with the anti-synthetase syndrome) may be present without typical systemic or extrathoracic features. Other scenarios exist whereby specific radiologic or histopathologic features are suggestive of an underlying CTD and yet the absence of extrathoracic or serologic findings precludes reliable classification as CTD-ILD.

In an area without consensus regarding terminology, the terms "undifferentiated CTD" (UCTD),[10,16] "lung-dominant CTD"[5] and "autoimmune-featured ILD"[17] have been used to describe such patients with suggestive forms of CTD-ILD. Each of these categories has a unique set of proposed criteria, represent the ideas of investigative teams from distinct ILD referral centers, and have yet to be prospectively validated.

UCTD

The first descriptions of "undifferentiated diseases" were made in the late 1960's by Sabo.[18] In 1980, LeRoy et al. proposed the concept of "undifferentiated connective tissue syndromes" to define early rheumatic disease mainly manifested by the presence of Raynaud's phenomenon and digital edema.[19] Subsequently, UCTD has been defined as symptoms and signs suggestive of a CTD (e.g., arthralgias or arthritis, Raynaud's phenomenon, leukopenia, anemia, and dry eyes or dry mouth) with antinuclear antibody positivity, but not fulfilling existing classification criteria for a specific CTD.[20] Approximately 60% of the patients with UCTD will remain "undifferentiated," and, in the minority that develops a classifiable CTD, it usually does so within the first 5 years after the UCTD diagnosis.[20] Although UCTD may evolve into any CTD, it most often evolves into systemic lupus erythematosus. An important distinguishing characteristic of UCTD is the absence of major organ involvement or damage.[20] In 2007,

a broader set of UCTD criteria were proposed and retrospectively applied to a cohort of patients with IIP evaluated at an ILD referral center.[16] Those defined as having UCTD were more likely to be female, younger, non-smokers and more likely to have radiographic and histopathologic evidence of non-specific interstitial pneumonia (NSIP). As nearly 90% of those with NSIP were defined as UCTD-ILD, the authors suggested that most patients with "idiopathic" NSIP might actually have an autoimmune disease and that idiopathic NSIP may be the lung manifestation of UCTD.[16] Corte et al. explored the clinical relevance of these broader UCTD criteria in a cohort of IIP patients from their ILD referral center.[10] In their retrospective study, CTD features were found to be quite common; 31% of NSIP cases and 13% of patients with idiopathic pulmonary fibrosis (IPF) fulfilled the traditional UCTD criteria, and an astounding 71% of NSIP cases and 36% of IPF patients fulfilled the broader, less specific UCTD set of criteria. The clinical relevance of these classification schemes was called into question, as the diagnosis of UCTD by either set of criteria had no prognostic significance.[10]

Autoimmune-featured ILD

Vij et al. described a cohort of UIP-predominant ILD patients retrospectively identified as having a suggestive form of CTD-ILD.[17] Among 200 patients evaluated in an ILD referral center, 63 were considered to have "autoimmune-featured ILD". A classification of autoimmune-featured ILD required the presence of a sign or symptom suggestive of a CTD and a serologic test reflective of an autoimmune process. The cohort that met their case definition of autoimmune-featured ILD had a demographic profile resembling that of IPF: most were older (mean age of 66 years) and male. The most common clinical symptoms were dry eyes, dry mouth, or gastroesophageal reflux disease. In that cohort, 75% of the patients with autoimmune-featured ILD had a lung injury pattern of UIP and similar overall survival to that of IPF patients and worse than in patients with classifiable forms of CTD-ILD.[17]

Lung-dominant CTD

In 2010, Fischer et al. proposed the provisional classification of "lung-dominant CTD".[5] The concept—and this classification—was meant to be applied to patients with ILD who fail to meet criteria for a characterizable CTD, yet have an "autoimmune flavor" to their disease as manifested by specific autoantibodies or histopathologic features. The presence of objective extrathoracic features is important, but their absence should not preclude a classification of lung-dominant CTD.[5] Benefits of such a classification scheme include: 1) objective and measurable criteria; 2) exclusion of non-specific symptoms (such as dry eyes, myalgia, arthralgia, or gastroesophageal reflux disease), non-specific inflammatory markers, and low-titer, less specific autoantibodies; 3) a notion that surveillance for the development of characterizable CTD is warranted; and 4) an emphasis that such a classification provides a framework by which natural history, pathobiology, treatment, and prognostic studies can be implemented.[5]

Statement of the problem

Because of the generally improved outcomes associated with CTD-ILD and because different treatment approaches are often implemented in patients with CTD-ILD, determining whether these suggestive forms of CTD-ILD represent a spectrum of CTD-ILD—rather than IIP—is important. In essence, it is important to know whether these suggestive forms of CTD-ILD have a similar natural history as the classifiable forms of CTD-ILD and whether the approach to their management should be similar to that of CTD-ILD or to that of IIP. Current strategies for identifying and classifying these patients are controversial and inadequate, there is far too little interdisciplinary dialogue in this arena, and the advancement of this field would be well served by efforts to bridge these divides. Furthermore, the lack of consensus regarding terminology and the varying sets of existing classification criteria limit the ability to conduct multicenter and multidisciplinary prospective studies needed to answer the many fundamental questions about this ILD subgroup.

A path forward

In an effort to move beyond the current impasses and address these areas of controversy, the American Thoracic Society/European Respiratory Society Task Force—"An International Working Group on Undifferentiated Forms of CTD-ILD"—has been recently formed. This Task Force is

comprised of an international, multidisciplinary panel of CTD-ILD experts, including investigators from the centers that have put forth the differing existing criteria for UCTD, lung-dominant CTD, and autoimmune-featured ILD. The primary objective of the Task Force is to develop consensus regarding the nomenclature and criteria for the classification of suggestive forms of CTD-ILD. The Task Force will also identify key areas of uncertainty that are worthy of further research in this cohort. Once there is international and multidisciplinary consensus surrounding the nomenclature and classification criteria of these suggestive forms of CTD-ILD, the requisite platform will be in place to enable the much-needed prospective, multicenter, and multidisciplinary studies to further our understanding of this subgroup of ILD.

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References

1. Cottin V. Interstitial lung disease: are we missing formes frustes of connective tissue disease? Eur Respir J. 2006;28(5):893-6. http://dx.doi.org/10.1183/09031936.00101506 PMid:17074915
2. Fischer A, du Bois R. Interstitial lung disease in connective tissue disorders. Lancet. 2012;380(9842):689-98. http://dx.doi.org/10.1016/S0140-6736(12)61079-4
3. Cottin V. Interstitial lung disease. Eur Respir Rev. 2013;22(127):26-32. http://dx.doi.org/10.1183/09059180.00006812 PMid:23457161
4. Pereira DA, Kawassaki AM, Baldi BG. Interpretation of autoantibody positivity in interstitial lung disease and lung-dominant connective tissue disease. J Bras Pneumol. 2013;39(6):728-741.
5. Fischer A, West SG, Swigris JJ, Brown KK, du Bois RM. Connective tissue disease-associated interstitial lung disease: a call for clarification. Chest. 2010;138(2):251-6. http://dx.doi.org/10.1378/chest.10-0194 PMid:20682528 PMCid:PMC3662187
6. Fischer A, du Bois RM. A Practical Approach to Connective Tissue Disease-Associated Lung Disease. In: Baughman RP, du Bois RM, editors. Diffuse Lung Disease---A practical approach. 2nd ed. New York: Springer; 2012. p. 217-37. http://dx.doi.org/10.1007/978-1-4419-9771-5_12
7. Castelino FV, Goldberg H, Dellaripa PF. The impact of rheumatological evaluation in the management of patients with interstitial lung disease. Rheumatology (Oxford). 2011;50(3):489-93. http://dx.doi.org/10.1093/rheumatology/keq233 PMid:20685802
8. Fischer A. Interstitial lung disease: a rheumatologist's perspective. J Clin Rheumatol. 2009;15(2):95-9. http://dx.doi.org/10.1097/RHU.0b013e31819b715d PMid:19265357
9. Mittoo S, Gelber AC, Christopher-Stine L, Horton MR, Lechtzin N, Danoff SK. Ascertainment of collagen vascular disease in patients presenting with interstitial lung disease. Respir Med. 2009;103(8):1152-8. http://dx.doi.org/10.1016/j.rmed.2009.02.009 PMid:19304475
10. Corte TJ, Copley SJ, Desai SR, Zappala CJ, Hansell DM, Nicholson AG, et al. Significance of connective tissue disease features in idiopathic interstitial pneumonia. Eur Respir J. 2012;39(3):661-8. http://dx.doi.org/10.1183/09031936.00174910 PMid:21920896
11. Fischer A, Meehan RT, Feghali-Bostwick CA, West SG, Brown KK. Unique characteristics of systemic sclerosis sine scleroderma-associated interstitial lung disease. Chest. 2006;130(4):976-81. http://dx.doi.org/10.1378/chest.130.4.976 PMid:17035427
12. Fischer A, Pfalzgraf FJ, Feghali-Bostwick CA, Wright TM, Curran-Everett D, West SG, et al. Anti-th/to-positivity in a cohort of patients with idiopathic pulmonary fibrosis. J Rheumatol. 2006;33(8):1600-5. PMid:16783860
13. Fischer A, Solomon JJ, du Bois RM, Deane KD, Olson AL, Fernandez-Perez ER, et al. Lung disease with anti-CCP antibodies but not rheumatoid arthritis or connective tissue disease. Respir Med. 2012;106(7):1040-7. http://dx.doi.org/10.1016/j.rmed.2012.03.006 PMid:22503074 PMCid:PMC3753791
14. Fischer A, Swigris JJ, du Bois RM, Groshong SD, Cool CD, Sahin H, et al. Minor salivary gland biopsy to detect primary Sjogren syndrome in patients with interstitial lung disease. Chest. 2009;136(4):1072-8. http://dx.doi.org/10.1378/chest.08-2839 PMid:19429722 PMCid:PMC3662204
15. Fischer A, Swigris JJ, du Bois RM, Lynch DA, Downey GP, Cosgrove GP, et al. Anti-synthetase syndrome in ANA and anti-Jo-1 negative patients presenting with idiopathic interstitial pneumonia. Respir Med. 2009;103(11):1719-24. http://dx.doi.org/10.1016/j.rmed.2009.05.001 PMid:19497723 PMCid:PMC2857337
16. Kinder BW, Collard HR, Koth L, Daikh DI, Wolters PJ, Elicker B, et al. Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease? Am J Respir Crit Care Med. 2007;176(7):691-7. http://dx.doi.org/10.1164/rccm.200702-220OC PMid:17556720 PMCid:PMC1994238
17. Vij R, Noth I, Strek ME. Autoimmune-featured interstitial lung disease: a distinct entity. Chest. 2011;140(5):1292-9. http://dx.doi.org/10.1378/chest.10-2662 PMid:21565966 PMCid:PMC3205845
18. Sabo I. The ianthanic or undifferentiated collagen disease. Hiroshima J Med Sci. 1969;18(4):259-64. PMid:5375831
19. LeRoy EC, Maricq HR, Kahaleh MB. Undifferentiated connective tissue syndromes. Arthritis Rheum. 1980;23(3):341-3. http://dx.doi.org/10.1002/art.1780230312 PMid:7362686
20. Mosca M, Tani C, Carli L, Bombardieri S. Undifferentiated CTD: a wide spectrum of autoimmune diseases. Best Pract Res Clin Rheumatol. 2012;26(1):73-7. http://dx.doi.org/10.1016/j.berh.2012.01.005 PMid:22424194

Metformin synergistically enhances antiproliferative effects of cisplatin and etoposide in NCI-H460 human lung cancer cells*

Metformina sinergicamente potencializa os efeitos antiproliferativos de cisplatina e etoposídeo em linhagem de células de câncer humano de pulmão NCI-H460

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Abstract

Objective: To test the effectiveness of combining conventional antineoplastic drugs (cisplatin and etoposide) with metformin in the treatment of non-small cell lung cancer in the NCI-H460 cell line, in order to develop new therapeutic options with high efficacy and low toxicity. **Methods:** We used the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and calculated the combination index for the drugs studied. **Results:** We found that the use of metformin as monotherapy reduced the metabolic viability of the cell line studied. Combining metformin with cisplatin or etoposide produced a synergistic effect and was more effective than was the use of cisplatin or etoposide as monotherapy. **Conclusions:** Metformin, due to its independent effects on liver kinase B1, had antiproliferative effects on the NCI-H460 cell line. When metformin was combined with cisplatin or etoposide, the cell death rate was even higher.

Keywords: Carcinoma, non-small-cell lung; Drug therapy, combination; Metformin.

Resumo

Objetivo: Testar a eficácia da combinação terapêutica de antineoplásicos convencionais (cisplatina e etoposídeo) com metformina em linhagem celular NCI-H460 de câncer de pulmão não pequenas células, a fim de desenvolver novas possibilidades terapêuticas com eficácia superior e reduzida toxicidade. **Métodos:** Foi utilizado o ensaio de brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio (MTT) e calculado o índice de combinação dos fármacos estudados. **Resultados:** Observamos que o uso de metformina em monoterapia reduziu a viabilidade celular metabólica da linhagem de células estudada. O uso de metformina em combinação com cisplatina ou etoposídeo foi sinérgico e superior à monoterapia com cisplatina ou etoposídeo. **Conclusões:** A metformina, devido às suas ações independentes *em liver kinase B1*, apresentou atividade antiproliferativa na linhagem NCI-H460 e, em combinação com cisplatina ou etoposídeo, ampliou a taxa de morte celular.

Descritores: Carcinoma pulmonar de células não pequenas; Quimioterapia combinada; Metformina.

Introduction

Lung cancer (LC) is the most common and most deadly cancer worldwide.[1] In Brazil, LC is one of the five most common cancer types, according to estimates for 2012, and it is the cancer type that took the most lives in 2010.[2] With this data, it is evident that LC is a health challenge in Brazil and worldwide. It is known that 85% of all cases of LC are classified as non-small cell LC (NSCLC), a group composed of cancer types that are different in histology, such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma, but that have similar clinical and pathological characteristics.[3,4]

Chemotherapy is used in patients staged as III or IV, in order to extend survival, control the disease, and improve quality of life.[5] In general, platinum derivatives, such as cisplatin and carboplatin, fulfill this role when combined

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with third-generation antineoplastic agents, such as etoposide.[6] Unquestionably, combination therapy is advantageous in cancer treatment, because it makes it possible to attack multiple molecular targets, with an increase in therapeutic efficacy, a reduction in dosage, and a consequent reduction in toxicity, as well as with a reduction or delay in resistance phenotype acquisition.[7]

In this scenario, there is metformin, a biguanide that is widely used as the first-line treatment for patients with type 2 diabetes mellitus.[8] However, since the 1970s, Dilman has suggested the use of antidiabetic biguanides as anti-aging and antineoplastic agents.[9]

In this context, the objective of the present study was to determine whether there was summation, synergism, or antagonism between antineoplastic agents used in the treatment of NSCLC and metformin, in the NCI-H460 cell line, in order to evaluate new therapeutic options for the treatment of NSCLC.

Methods

Initially, the NSCLC cell line NCI-H460, which is a representative of the histologic group of large cell carcinoma, was grown to subconfluence at 37°C and 5% CO_2 in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% (v/v) bovine fetal serum (Gibco/Invitrogen, Grand Island, NY, USA) and a stabilized solution of penicillin (100 IU/mL), streptomycin (100 µg/mL), and 0.5% (w/v) amphotericin B (Gibco/Invitrogen). Provided through donations from collaborators, cisplatin ((Incel, 1 mg/mL injectable solution; Laboratório Darrow, Areal, Brazil), etoposide (Posidon, 20 mg/mL injectable solution; Piére, Buenos Aires, Argentina), and metformin hydrochloride (Pharma Nostra, Rio de Janeiro, Brazil) were diluted in 1× PBS.

In the in vitro cell metabolic viability test, cells were grown at a density of 7.5×10^4 cells/well in 96-well plates, and, after 24 h, they were treated with each drug, in a dose-dependent manner, for 24 h. Subsequently, the medium was removed, 15 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) were added to 5 mg/mL in each well, and the plates were incubated for 4 h. Finally, 100 µL of analytical grade DMSO (Vetec, Rio de Janeiro, Brazil) were added in order to dissolve the formazan crystals. Therefore, the production of formazan by mitochondrial succinate dehydrogenase activity reflects the proportion of live cells undergoing oxidative stress caused by the drugs under investigation.[10] In this experiment, the absorbance values obtained from the wells of untreated cells, i.e., cells exposed only to RPMI 1640 medium, represent 100% cell viability. In vitro cytotoxicity was determined by the estimate calculation of the values for inhibitory concentration of 50% for cell proliferation (IC_{50}) on the basis of the dose-response curve for each drug, after calculation of metabolic viability, evaluated with the MTT assay (absorbance at 570 nm), by using a spectrophotometer. It is of note that the experiments to obtain the dose-response curves for each drug as monotherapy and estimate the IC_{50} values were performed in quadruplicate in three independent experiments, whereas the experiments to determine the combination index were performed in triplicate in three independent experiments. In addition, drug interaction was assessed by analysis of the combination index, the value of which was calculated by the classical isobologram equation described by Chou & Talalay, with the use of the CompuSyn software program, version 1.0 (ComboSyn, Paragon, NJ, USA).[11] In this context, combination index values greater than 1.1 indicate antagonistic interactions, values between 0.9 and 1.1 indicate additive interactions, and values lower than 0.9 indicate synergistic interactions. Regarding the statistical analysis, the mean and standard deviation of the absorbances were calculated on the basis of the results of the MTT assay, whereas the estimated IC_{50} values were calculated by the Prism software program, version 5 (GraphPad Inc., San Diego, CA, USA). All data on combination therapy are expressed as mean ± SD and were analyzed by one-way ANOVA followed by the Bonferroni test, with the use of the same software program.

Results

Initially, we performed the cell viability experiments to determine the IC_{50} concentrations for metformin, cisplatin, and etoposide in the large cell carcinoma cell line NCI-H460, which are shown in Table 1.

Subsequently, once these data were obtained, each drug was diluted to achieve concentrations in the IC_{50} range, as well as below and above this range, and in vitro experiments combining metformin with cisplatin and combining metformin with etoposide were performed to allow the

construction of dose-response plots with the software program. However, only the most relevant data will be discussed. The data from the plots were statistically analyzed and are shown in Figures 1 and 2. Finally, the effects of the combinations were used to calculate the combination index, the values of which are shown in Table 2.

The results show that combining metformin (60.58 mM) with cisplatin (0.19 mM) produced greater antiproliferative effects than did the use of metformin (60.58 and 30.29 mM) or cisplatin (0.09 mM) as monotherapy. In addition, combining metformin (60.58 and 30.29 mM) with cisplatin (0.19 mM) produced synergy. Combining metformin (30.39 mM) with etoposide (0.18 mM) was more effective in reducing the metabolic viability of the cell line tested than was the use of etoposide (0.18 and 0.09 mM) or metformin (30.29 and 15.14 mM) as monotherapy. Furthermore, combining metformin with etoposide (30.39 mM and 0.18 mM, respectively, and 15.14 mM and 0.09 mM, respectively) produced a synergistic effect.

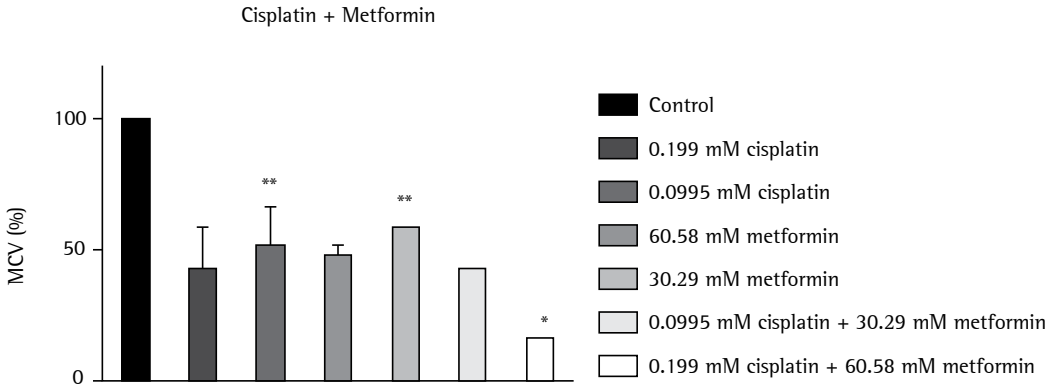
Table 1 - Inhibitory concentration of viability at 50% (IC_{50}) values in the NCI-H460 cell line with the drugs tested.

Drug	IC_{50}, mM
Cisplatin	0.19
Etoposide	0.37
Metformin	60.58

Discussion

Various epidemiological studies have reported the effects of metformin on the prevention and treatment of cancer.[12-15] There have also been studies reporting that metformin can improve treatment and thus survival in patients with NSCLC and diabetes who undergo chemotherapy.[16] Because the safety and use of metformin has been well established since it first underwent a clinical trial in 1957, its use as a potential anticancer agent is certainly an interesting strategy.[17]

Although in the present study the cytotoxic activity of metformin was found to be up to 200 times lower than that of etoposide or cisplatin, as determined by the IC_{50} values, the use of metformin as monotherapy reduced the metabolic viability of large cell carcinoma cells. However, in polytherapy regimens, metformin (60.58 and 30.29 mM) combined with cisplatin at 0.19 mM produced a synergistic effect and was more effective than was metformin combined with half the IC_{50} value for cisplatin (0.09 mM). Combining metformin (30.39 mM) with etoposide (0.18 mM) produced a synergistic effect and was more effective in reducing the metabolic viability of the NCI-H460 cell line than was the use of etoposide (0.18 and 0.09 mM) or metformin (30.29 and 15.14 mM) as monotherapy. In line with our findings, preclinical studies have reported that oral administration of metformin combined with chemotherapy can block tumor growth



(p < 0,001) vs. control

*(p < 0.05) vs. 60.58 mM metformin

**(p < 0.05) vs. 0.199 mM cisplatin + 60.58 mM metformin

Figure 1 - Evaluation of the effects of metformin and cisplatin (used as monotherapy or in combination therapy, at different concentrations) on the reduction in metabolic cell viability (MCV) in the NCI-H460 cell line.

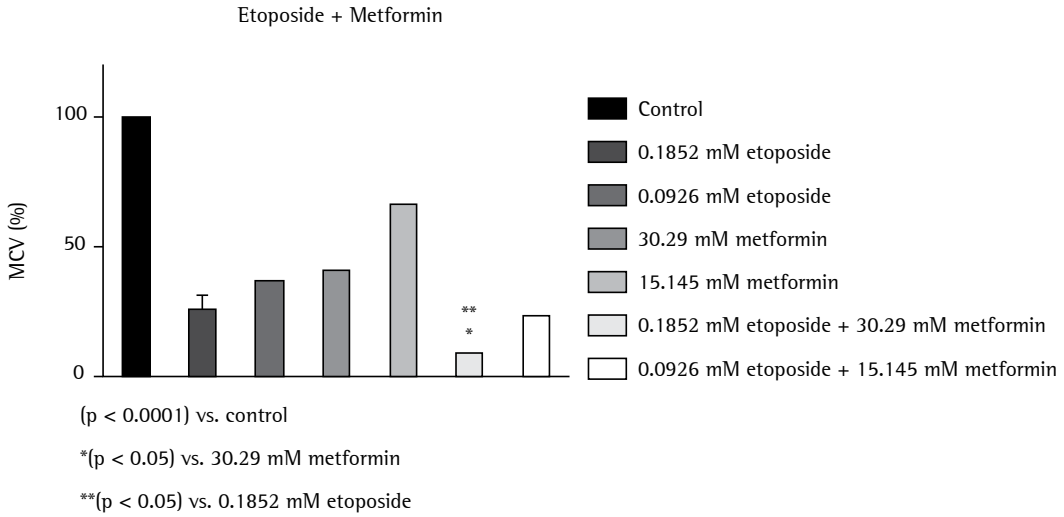


Figure 2 - Evaluation of the effects of metformin and etoposide (used as monotherapy or in combination therapy, at different concentrations) on the reduction in metabolic cell viability (MCV) in the NCI-H460 cell line.

Table 2 - Combination index for metformin plus cisplatin or etoposide in the NCI-H460 cell line.

Concentration, mM			Combination index
Metformin	Cisplatin	Etoposide	
60.58	0.19	0.00	0.70
30.29	0.19	0.00	0.90
30.29	0.00	0.18	0.81
15.14	0.00	0.09	0.90

and prevent recurrence, as well as making it possible to reduce the doxorubicin doses used in the treatment.[18]

Although the use of metformin is well established in the treatment of diabetes, the mechanism through which metformin can act in tumor suppression still needs to be further elucidated. The major mechanism of action of biguanides might be related to their capacity to inhibit mitochondrial complex I and consequently cause energy imbalance, triggering liver kinase B1 (LKB1)-dependent phosphorylation of the enzyme AMP kinase (AMPK).[19,20] Under conditions of energy stress, LKB1 is the primary regulator of AMPK, which, under conditions of reduction of intracellular ATP, modulates cell growth and metabolism.[21,22]

Allelic loss at *LKB1* occurs in various types of cancer—such as NSCLCs—especially in lung adenocarcinoma. In addition, it is believed that most mutations in *LKB1* in NSCLC lead to loss of function of the encoded protein.[23,24] The large cell carcinoma cell line used in the present study (NCI-H460) has a nonsense mutation in *LKB1*, which results in lack expression of its protein product.[24] It is therefore possible to attest that the mechanisms of action of metformin in these cells are independent of LKB1 activity. Shackelford et al. showed that phenformin, a member of the biguanide class of drugs, can be used as an anticancer agent in LKB1-deficient tumors because it induces apoptosis possibly in response to metabolic stress.[25] It has thus been suggested that a possible mechanism of action of metformin and other biguanides in LKB1-defficient tumor cells is mitochondrial complex I inhibition, which induces the production of reactive oxygen species and consequent apoptosis, because these species cannot be effectively neutralized.[26]

Another plausible reason for the antiproliferative and synergistic effects of metformin is its preferential activity against tumor stem cells, a group of cells within the tumor mass that can be related to tumor formation, maintenance of the tumor mass, recurrence, and metastasis. Because of these characteristics, drugs that selectively target these cells are extremely promising, since, in combination with conventional chemotherapy, they could make treatment more effective and prevent disease recurrence.[27] Hirsch et al. found that, in xenographic models, metformin preferentially inhibits proliferation of tumor cells expressing a stem cell phenotype.[26]

In summary, the present study has shown for the first time that metformin has antiproliferative effects in a large cell carcinoma cell line (NCI-H460) and that, when combined with cisplatin or etoposide, it acts synergistically, increasing the cell death rate. However, larger studies are still needed to elucidate further the mechanisms involved.

References

1. Globocan 2008 [homepage on the Internet]. Lyon: International Agency for Research on Cancer.[updated 2008; cited 2013 May 26]. Globocan 2008 Fast Stats. Available from: http://globocan.iarc.fr/factsheets/populations/factsheet.asp?uno=900#BOTH
2. INCA [homepage on the Internet]. Rio de Janeiro: Ministério da Saúde. [updated 2012; cited 2013 May 5]. Tipo de câncer – Pulmão. Available from: http://www2.inca.gov.br/wps/wcm/connect/tiposdecancer/site/home/pulmao/definicao
3. SEER - Surveillance, Epidemiology, and End Results Program [homepage on the Internet]. Bethesda: National Cancer Institute. [updated 2009; cited 2013 Jun 30]. SEER Cancer Statistics Review, 1975-2007. Available from: http://seer.cancer.gov/csr/1975_2007/
4. IARC - International Agency for Research on Cancer [homepage on the Internet]. Lyon: International Agency for Research on Cancer. [updated 2004; cited 2013 Jul 01]. Pathology and Genetics of Tumours of the Lung, Pleura, Thymus and Heart. [Adobe Acrobat document, 344p.]. Available from: http://www.iarc.fr/en/publications/pdfs-online/pat-gen/bb10/BB10.pdf
5. NCBI - National Center for Biotechnology Information [homepage on the Internet]. Bethesda: National Center for Biotechnology Information. [updated 2011; cited 2013 Jul 05]. The Diagnosis and Treatment of Lung Cancer (Update). [Adobe Acrobat document, 198p.]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK99021/pdf/TOC.pdf
6. Ettinger DS, Bepler G, Bueno R, Chang A, Chang JY, Chirieac LR, et al. Non-small cell lung cancer clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2006;4(6):548-82. PMid:16813724
7. Chou TC. Theoretical basis, experimental design, and computerized simulation of synergism and antagonism in drug combination studies. Pharmacol Rev. 2006;58(3):621-81. Erratum in: Pharmacol Rev. 2007;59(1):124. http://dx.doi.org/10.1124/pr.58.3.10 PMid:16968952
8. IDF – International Diabetes Federation [homepage on the Internet]. Brussels: International Diabetes Federation. [updated 2005; cited 2013 Apr 02]. Global Guideline for Type 2 Diabetes. [Adobe Acrobat document, 82p.]. Available from: http://www.idf.org/webdata/docs/IDF%20GGT2D.pdf
9. Dilman VM. Age-associated elevation of hypothalamic, threshold to feedback control, and its role in development, ageing, and disease. Lancet. 1971;1(7711):1211-9. http://dx.doi.org/10.1016/S0140-6736(71)91721-1
10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4
11. Chou TC, Talalay P. Quantitative analysis of dose-effect relationships: the combined effects of multiple drugs or enzyme inhibitors. Adv Enzyme Regul. 1984;22:27-55. http://dx.doi.org/10.1016/0065-2571(84)90007-4
12. Evans JM, Donnelly LA, Emslie-Smith AM, Alessi DR, Morris AD. Metformin and reduced risk of cancer in diabetic patients. BMJ. 2005;330(7503):1304-5. http://dx.doi.org/10.1136/bmj.38415.708634.F7 PMid:15849206 PMCid:PMC558205
13. Currie CJ, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. Diabetologia. 2009;52(9):1766-77. http://dx.doi.org/10.1007/s00125-009-1440-6 PMid:19572116
14. Jiralerspong S, Palla SL, Giordano SH, Meric-Bernstam F, Liedtke C, Barnett CM, et al. Metformin and pathologic complete responses to neoadjuvant chemotherapy in diabetic patients with breast cancer. J Clin Oncol. 2009;27(20):3297-302. http://dx.doi.org/10.1200/JCO.2009.19.6410 PMid:19487376 PMCid:PMC2736070
15. Larsson SC, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. Int J Cancer. 2007;121(4):856-62. http://dx.doi.org/10.1002/ijc.22717 PMid:17397032
16. Tan BX, Yao WX, Ge J, Peng XC, Du XB, Zhang R, et al. Prognostic influence of metformin as first-line chemotherapy for advanced nonsmall cell lung cancer in patients with type 2 diabetes. Cancer. 2011;117(22):5103-11. http://dx.doi.org/10.1002/cncr.26151 PMid:21523768
17. Sterne J. Du Nouveau dans les antidiabétiques. La NN diméthylamino guanyl guanidine (N.N.D.G.). Maroc Med 1957;36:1295-6.
18. Iliopoulos D, Hirsch HA, Struhl K. Metformin decreases the dose of chemotherapy for prolonging tumor remission in mouse xenografts involving multiple cancer cell types. Cancer Res. 2011;71(9):3196-201. http://dx.doi.org/10.1158/0008-5472.CAN-10-3471 PMid:21415163 PMCid:PMC3085572
19. El-Mir MY, Nogueira V, Fontaine E, Avéret N, Rigoulet M, Leverve X. Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I. J Biol Chem. 2000;275(1):223-8. http://dx.doi.org/10.1074/jbc.275.1.223 PMid:10617608
20. Hawley SA, Ross FA, Chevtzoff C, Green KA, Evans A, Fogarty S, et al. Use of cells expressing gamma subunit variants to identify diverse mechanisms of AMPK activation. Cell Metab. 2010;11(6):554-65. http://dx.doi.org/10.1016/j.cmet.2010.04.001 PMid:20519126 PMCid:PMC2935965
21. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251-62. http://dx.doi.org/10.1038/nrm3311 PMid:22436748
22. Inoki K, Zhu T, Guan KL. TSC2 mediates cellular energy response to control cell growth and survival. Cell. 2003;115(5):577-90. http://dx.doi.org/10.1016/S0092-8674(03)00929-2
23. Avizienyte E, Loukola A, Roth S, Hemminki A, Tarkkanen M, Salovaara R, et al. LKB1 somatic mutations in sporadic tumors. Am J Pathol. 1999;154(3):677-81. http://dx.doi.org/10.1016/S0002-9440(10)65314-X
24. Carretero J, Medina PP, Pio R, Montuenga LM, Sanchez-Cespedes M. Novel and natural knockout lung cancer cell lines for the LKB1/STK11 tumor suppressor gene. Oncogene. 2004;23(22):4037-40. http://dx.doi.org/10.1038/sj.onc.1207502 PMid:15021901

25. Shackelford DB, Abt E, Gerken L, Vasquez DS, Seki A, Leblanc M, et al. LKB1 inactivation dictates therapeutic response of non-small cell lung cancer to the metabolism drug phenformin. Cancer Cell. 2013;23(2):143-58. http://dx.doi.org/10.1016/j.ccr.2012.12.008 PMid:23352126
26. Hirsch HA, Iliopoulos D, Tsichlis PN, Struhl K. Metformin selectively targets cancer stem cells, and acts together with chemotherapy to block tumor growth and prolong remission. Cancer Res. 2009;69(19):7507-11. Erratum in: Cancer Res. 2009;69(22):8832. http://dx.doi.org/10.1158/0008-5472.CAN-09-2994 PMid:19752085 PMCid:PMC2756324
27. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. Nat Rev Cancer. 2009;9(4):265-73. http://dx.doi.org/10.1038/nrc2620 PMid:19262571

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Descriptive analysis of and overall survival after surgical treatment of lung metastases*

Análise descritiva e sobrevida global do tratamento cirúrgico das metástases pulmonares

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Abstract

Objective: To describe demographic characteristics, surgical results, postoperative complications, and overall survival rates in surgically treated patients with lung metastases. **Methods:** This was a retrospective analysis of 119 patients who underwent a total of 154 lung metastasis resections between 1997 and 2011. **Results:** Among the 119 patients, 68 (57.1%) were male and 108 (90.8%) were White. The median age was 52 years (range, 15-75 years). In this sample, 63 patients (52.9%) presented with comorbidities, the most common being systemic arterial hypertension (69.8%) and diabetes (19.0%). Primary colorectal tumors (47.9%) and musculoskeletal tumors (21.8%) were the main sites of origin of the metastases. Approximately 24% of the patients underwent more than one resection of the lesions, and 71% had adjuvant treatment prior to metastasectomy. The rate of lung metastasis recurrence was 19.3%, and the median disease-free interval was 23 months. The main surgical access used was thoracotomy (78%), and the most common approach was wedge resection with segmentectomy (51%). The rate of postoperative complications was 22%, and perioperative mortality was 1.9%. The overall survival rates at 12, 36, 60, and 120 months were 96%, 77%, 56%, and 39%, respectively. A Cox analysis confirmed that complications within the first 30 postoperative days were associated with poor prognosis (hazard ratio = 1.81; 95% CI: 1.09-3.06; p = 0.02). **Conclusions:** Surgical treatment of lung metastases is safe and effective, with good overall survival, especially in patients with fewer metastases.

Keywords: Neoplasm metastasis; Survival analysis; Thoracic surgery; Metastasectomy.

Resumo

Objetivo: Descrever características demográficas, resultados operatórios, complicações pós-operatórias e taxa de sobrevida global em pacientes com metástases pulmonares tratados cirurgicamente. **Métodos:** Análise retrospectiva de 119 pacientes submetidos a um total de 154 cirurgias de ressecção de metástase pulmonar entre 1997 e 2011. **Resultados:** Do total de 119 pacientes, 68 (57,1%) eram do sexo masculino, e 108 (90,8%) eram brancos. A mediana de idade foi de 52 anos (variação, 15-75 anos). Nessa amostra, 63 pacientes (52,9%) apresentaram comorbidades, sendo as mais frequentes hipertensão arterial sistêmica (69,8%) e diabetes (19,0%). Tumores primários colorretais (47,9%) e musculoesqueléticos (21,8%) foram os principais sítios de origem das metástases. Aproximadamente 24% dos pacientes foram submetidos a mais de uma ressecção das lesões, e 71% fizeram tratamento adjuvante prévio à metastasectomia. A taxa de recidiva de metástase pulmonar foi de 19,3%. A mediana do intervalo livre de doença foi de 23 meses. A principal via de acesso usada foi toracotomia (78%), e o tipo de ressecção mais frequente foi em cunha e segmentectomia (51%). O índice de complicações pós-operatórias foi de 22% e o de mortalidade perioperatória foi de 1,9%. As taxas de sobrevida global em 12, 36, 60 e 120 meses foram, respectivamente, de 96%, 77%, 56% e 39%. A análise de Cox confirmou que complicações nos primeiros 30 dias pós-operatórios associaram-se a pior prognóstico (*hazard ratio* = 1,81; IC95%: 1,09-3,06; p = 0,02). **Conclusões:** O tratamento cirúrgico das metástases pulmonares oriundas de diferentes sítios tumorais é efetivo e seguro, com boa sobrevida global, especialmente nos casos com um menor número de lesões pulmonares.

Descritores: Metástase neoplásica; Análise de sobrevida; Cirurgia torácica; Metastasectomia.

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Introduction

For many years, the diagnosis of lung metastasis was considered the end stage of the disease course in patients with malignant neoplasms. Initially, the treatment proposed consisted of chemotherapy and hormone therapy, and unsatisfactory responses were seen in more than 70% of cases In 1882, Weinlechner incidentally performed the first lung metastasis resection during the resection of a primary tumor, a procedure in which the metastasis was removed *en bloc* with the surgical specimen.[2] Since then, various other cases of metastasectomy have been reported, many with positive results.

In 1951, Ehrenhaft started selecting candidates for lung metastasis resection,[3] and, subsequently, it was possible to establish well-defined criteria.

Surgical treatment evolved over the 20th century because of greater knowledge about tumors that gave rise to lung metastases only. Surgical treatment of lung metastases has been given a boost in the last 15 years, as has been shown in the literature, and some of the major prognostic factors for this condition, such as disease-free interval and type of resection performed, have been identified,.[4,5] However, in comparison with major centers worldwide, there have been few studies of Brazilian patients and this treatment modality in Brazil.

Therefore, the objective of the present study was to evaluate the results of surgical treatment of lung metastases at a university hospital in the city of Campinas, Brazil.

Methods

This was a retrospective study using surgical records of the Department of Thoracic Surgery of the State University at Campinas *Hospital de Clínicas*, located in the city of Campinas, Brazil. We reviewed all records of resection of lung lesions performed in patients with a previous diagnosis of primary cancer in other organs, as well as all records of complete resection of all lesions and pathological confirmation of metastatic disease, between January 1, 1997 and December 31, 2011.

We included all patients with lung lesions who were selected for surgery on the basis of the Thomford et. al. criteria for surgical treatment of metastatic lung lesions,[6] namely: a) complete resection of all metastatic disease should be possible; b) the patient should have sufficient pulmonary function to withstand the risk of surgery and survive the postoperative period; c) the primary tumor should be (or can be) controlled; d) there should be no evidence of extrapulmonary metastases, except for colon tumors; and e) there should be no effective treatment other than surgery.

Pulmonary function was assessed on the basis of arterial blood gas analysis results and pulmonary function test results. If the pulmonary function test was not technically possible, the six-minute walk test, which has been proven to be well tolerated and safe in the assessment of patient functional capacity, was used.[7]

In addition, the records were reviewed for the postoperative presence of systemic inflammatory response syndrome (SIRS), which was defined by the presence of at least two of the following parameters, on the same day, within three days after surgery: an axillary temperature above 38°C; an RR > 24 breaths/min; an HR > 90 bpm; and a leukocyte count greater than 10,000 cells/mm^3 or less than 5,000 cells/mm^3.

Overall survival was defined as the date of first surgery to the last follow-up visit or to death (from any cause). To determine disease-free intervals, we used the dates of primary cancer treatment and the date of referral for thoracic surgery for treatment of lung metastasis.

We designed a data collection form that was used by two trained physicians to obtain the patient medical record information needed to meet the objectives of the present study. This form contained the following fields: patient initials; medical record number; gender; race; date of birth; date of surgery of the primary tumor; date of referral for thoracic surgery; symptoms at diagnosis of metastasis (dyspnea, cough, hemoptysis, and chest pain); smoking; anatomical site of the primary tumor (breast, head, neck, colorectum, kidney, musculoskeletal system, gonads, etc.); presence and type of comorbidity; history of resection for liver metastasis; neoadjuvant or adjuvant therapy to treatment of lung metastasis; number of lung resections; number and location of nodules found intraoperatively; date of resection of the metastasis; type of resection performed (segmentectomy, wedge resection, nodule resection, lobectomy, bilobectomy, and pneumonectomy); use of a stapler; extubation in the operating room; number of chest tubes and side of placement; unilateral or bilateral approach; surgical access

(thoracotomy, bilateral sequential thoracotomy, sternotomy, and video-assisted surgery); need for blood transfusion; postoperative recovery in the ICU; development of SIRS; length of hospital stay; presence and type of complication within 30 days after surgery; postoperative day of onset of the complication; current status of the patient (alive/dead); date of the last follow-up visit to our facility; and whether follow-up was conducted at another facility.

Data on postoperative complications, defined as any type of event experienced by patients within 30 days after surgery, were extracted from patient medical records. These data were grouped by major system or pathology involved, listed by frequency, and stratified as a variable in the overall survival curve.

The present study was approved by the Research Ethics Committee of the State University at Campinas School of Medical Sciences (Protocol no. 001/2013).

For categorical variables, we conducted an exploratory descriptive analysis and, subsequently, we used Pearson's, Spearman's, and Fisher's tests, whereas, for continuous variables, we used the t-test (two groups) or the Kruskal-Wallis test (more than two groups). In cases with missing data, the valid percent of variables was calculated. The Kaplan-Meier method was used for survival analyses, and the log-rank test was used for survival comparisons. Cox regression analysis (by Wald's backward stepwise method) was performed to identify the predictive variables over time. For univariate and multivariate analyses, the level of significance was set at $p < 0.10$ and $p < 0.05$, respectively. The analyses were performed with the Statistical Package for the Social Sciences, version 14.0 (SPSS Inc., Chicago, IL, USA).

Results

During the study period, 119 patients underwent a total of 154 surgical procedures. Among those patients, 68 (57.1%) were male and 108 (90.8%) were White. The median age was 52 years (range, 15-75 years). The main sites of origin of the primary tumor were the colorectum, in 47.9% of the patients; the musculoskeletal system, in 21.8%; the head and neck, in 7.5%; and the gonads, in 5.9%. Comorbidities were found in 63 patients (52.9%), and the most common were systemic arterial hypertension, in 69.8%; diabetes, in 19.0%; asthma/COPD, in 7.9%; dyslipidemia, in 7.9%; hyperthyroidism, in 6.3%, and heart disease, in 6.3%. Some patients had more than one comorbidity. A total of one, two, three, and four surgical procedures, respectively, were performed on 91 (76.5%), 24 (20.2%); 3 (2.5%), and 1 (0.8%) of the patients. Ten patients (8.4%) underwent resection for liver metastasis before the diagnosis of lung metastasis. Likewise, 85 patients (71.4%) underwent chemotherapy and/or radiotherapy before lung metastasis resection. After resection, 88 patients (75.9%) underwent adjuvant chemotherapy (Table 1). The rate of lung metastasis recurrence was 19.3%. The median disease-free interval was 23 months (range, 1-172 months).

The mean length of hospital stay was 8 days (range, 3-40 days) for the 154 surgical procedures.

The preferential location of the resected lesions was the left lower lobe (in 24.8%), followed by the left upper lobe (in 24.4%) and the right lower lobe (in 21.5%). A total of 511 lesions were described.

In 25 surgical procedures (16.3%), bilateral lesions were resected during the same surgical session, whereas, in 129 procedures (83.7%), only one of the lungs was treated. Of those 129 surgical procedures, 20 (in 10 patients) were performed on average 2 months apart in order to treat bilateral disease, which was present in 35 patients (29.4%) at diagnosis of lung metastasis.

The most common surgical access was thoracotomy, in 78.6% of the cases, followed by sternotomy, in 9.1%; bilateral sequential thoracotomy, in 8.5%; and video-assisted surgery, in 3.8%. Bilateral thoracotomy, despite consisting of two different access routes, was considered as a single surgical procedure.

The types of resection performed were wedge resection or segmentectomy, in 51.3% of the cases; nodule resection, in 29.9%; lobectomy or bilobectomy, in 15.6%; and pneumonectomy, in 3.2%.

In 107 (69.5%) of a total of 154 surgical procedures, a linear stapler was used to assist in suturing the lung parenchyma.

In 10 procedures (6.5%), the patients were not extubated in the operating room, and, in 12 (7.8%), the patients did not recover from the immediate postoperative period in the ICU environment.

Table 1 - Demographic and clinical characteristics of the 119 study patients.

Variable	n	Valid %
Gender		
Male	51	57.1
Female	68	42.9
Race		
White	108	90.8
Non-White	11	9.2
Symptoms at diagnosis of metastasis		
Yes	36	30.3
No	83	69.7
Smoking		
Yes	35	29.7
No	83	70.3
Origin of the primary tumor[a]		
Colorectum	57	47.9
Musculoskeletal system	26	21.8
Head - neck	09	7.5
Gonads	07	5.9
Kidney	04	3.4
Breast	04	3.4
Melanomas	04	3.4
Others	08	6.7
Surgical procedures		
1	91	76.5
2	24	20.2
3	03	2.5
4	01	0.8
History or resection for liver metastasis		
Yes	10	8.4
No	109	91.6
CHT/RT before resection		
Yes	85	71.4
No	34	28.6
CHT/RT after resection		
Yes	88	75.9
No	28	24.1
Comorbidities		
Yes	63	52.9
No	56	47.1

CHT/RT: chemotherapy and/or radiotherapy. [a]Eight missing cases: endometrial tumor (3 cases); ovary tumor (1 case); lung tumor (1 case); pancreas tumor (1 case); liver tumor (1 case); and stomach tumor (1 case).

After the surgical procedures, 28 patients (18.2%) developed SIRS, and 9 of those patients had some type of perioperative complication.

In the perioperative period, 34 patients (22.1%) had some type of complication, the most common being pulmonary conditions (pneumonia, respiratory failure, prolonged mechanical ventilation, retained clot, pulmonary edema, persistent air fistula) in 25 (16.2%) and extrapulmonary conditions (heart failure, arrhythmia, urinary tract/urinary tract wall infection, and thromboembolism in 12 (7.8%). In 3 cases, concomitant pulmonary and extrapulmonary complications were found.

Perioperative mortality was 3 patients (1.9%), 1 of whom died from an irreversible arrhythmia during the procedure and 2 of whom died from pulmonary complications (Table 2).

Of the 119 patients studied, 64 (53.8%) continued with follow-up treatment, whereas 22 (18.5%) lost contact with the facility for more than 1 year, and 33 (27.7%) died.

Table 3 shows the overall survival rates of the patients at 12, 36, 60, and 120 months, which were 96%, 77%, 56%, and 39%, respectively. Overall survival for the groups of patients who underwent and who did not undergo chemotherapy/radiotherapy before surgery (48% and 72%) and after surgery (51% and 72%) showed a significant difference ($p = 0.02$) in favor of those who did not undergo chemotherapy/radiotherapy, but this was not confirmed in the multivariate analysis. A Cox analysis in which patients with and without complications within the first 30 days after surgery were compared (67% vs. 24%; $p < 0.0001$) confirmed that the former had poor prognosis (hazard ratio = 1.81; 95% CI: 1.09-3.06; $p = 0.02$; Figure 1).

Survival stratification by anatomical site of the primary tumor showed that overall survival at 60 months was 68% for colorectal carcinoma, 26% for musculoskeletal carcinoma, and 56% for the other types of tumor (which are less common than the former two; Figure 2).

Survival at 60 months in the patients who had up to 6 lesions resected ranged from 60% to 76%, whereas, in those who had more than 7 nodules resected, survival at 60 months dropped to 13%. In the patients with bilateral disease at diagnosis, overall survival was 63%, and, in those with unilateral disease, overall survival was 55%. This difference was not statistically significant.

Discussion

The present study showed that the population analyzed had characteristics similar to those reported in major studies: there was a slight predominance of males; the histology of the

Table 2 - Characteristics of the 154 surgical procedures performed during the study period.

Variable	n	Valid %
Type of approach		
Unilateral	129	83.7
Bilateral	25	16.3
Preferential location of the nodules		
LLL	126	24.8
LUL	125	24.4
RLL	110	21.5
RUL	97	19.0
Surgical access		
Thoracotomy	121	78.6
Bilateral thoracotomy	13	8.5
Sternotomy	14	9.1
Video-assisted surgery	06	3.8
Most common resection performed		
Wedge resection + segmentectomy	79	51.3
Nodule resection	46	29.9
Lobectomy + bilobectomy	24	15.6
Pneumonectomy	05	3.2
Mechanical suture or linear stapler		
Yes	107	69.5
No	47	30.5
Extubation in the operating room		
Yes	144	93.5
No	10	6.5
Recovery in the ICU		
Yes	142	92.2
No	12	7.8
SIRS		
Yes	28	18.2
No	126	81.8
Complication within the first 30 days after surgery		
Yes	34	22.1
No	120	77.9

LLL: left lower lobe; LUL: left upper lobe; RLL: right lower lobe; RUL: right upper lobe; and SIRS: systemic inflammatory response syndrome.

primary tumor was predominantly colorectal adenocarcinoma; and there was approximately 30% of bilaterality of disease at diagnosis of lung metastasis.[8]

The most common surgical access in all studies is thoracotomy, and there have been few reports of treatment of the two lungs during the same surgical procedure.[9-12] In such cases, we preferentially choose sternotomy; however, depending on the location of the lesion, bilateral thoracotomy may be the best alternative. The choice to use video-assisted surgery as access for resection of metastatic lesions remains controversial in the literature, even in single lesion cases, because careful palpation of the entire lung parenchyma is impossible and imaging methods are limited in the preoperative evaluation of such lesions. With the increasing sensitivity of imaging methods, especially in cases in which the lesions are located peripherally, we believe that minimally invasive accesses will be more widely in the near future.

Table 3 - Overall survival data.

Survival time, months	Survival %	95% CI
12	96	94-98
36	77	69-85
60	56	48-64
120	39	29-49

The surgical treatment of lung metastases in major studies has followed the principle that all lesions should be completely resected and disease-free margins should be obtained and that resection of lung parenchyma should be as conservative as possible. Among the surgical techniques for excision of lesions, wedge resection and segmentectomy were the most commonly used in various studies, followed, in smaller numbers, by lobectomy and pneumonectomy.[13,14] All these data were extensively reviewed in a recent meta-analysis conducted by Pfannschmidt et al. (2007), in which 20 large studies of surgical treatment of lung metastases from colorectal cancer were compared.[15]

Currently, surgery is a significant component of the treatment of lung metastatic disease, and its practice is widely accepted worldwide, resulting in low mortality rates and increased five-year survival rates. In the study by Pfannschmidt et al.,[15] the perioperative mortality rate ranged from 0% to 2.02%, whereas, in the study conducted in Brazil by Younes et al.,[8] the rate was 0.4%. These results are in good agreement with the value of 1.9% found in the present study, showing the safety of the method.

The overall survival rate at 60 months in our sample, which was approximately 56%, was found to be similar to those reported in the meta-analysis by Pfannschmidt et al., which were as high as 62.7% also at five years.[15,16] However, since we calculated survival from treatment of the primary tumor, we observed that various

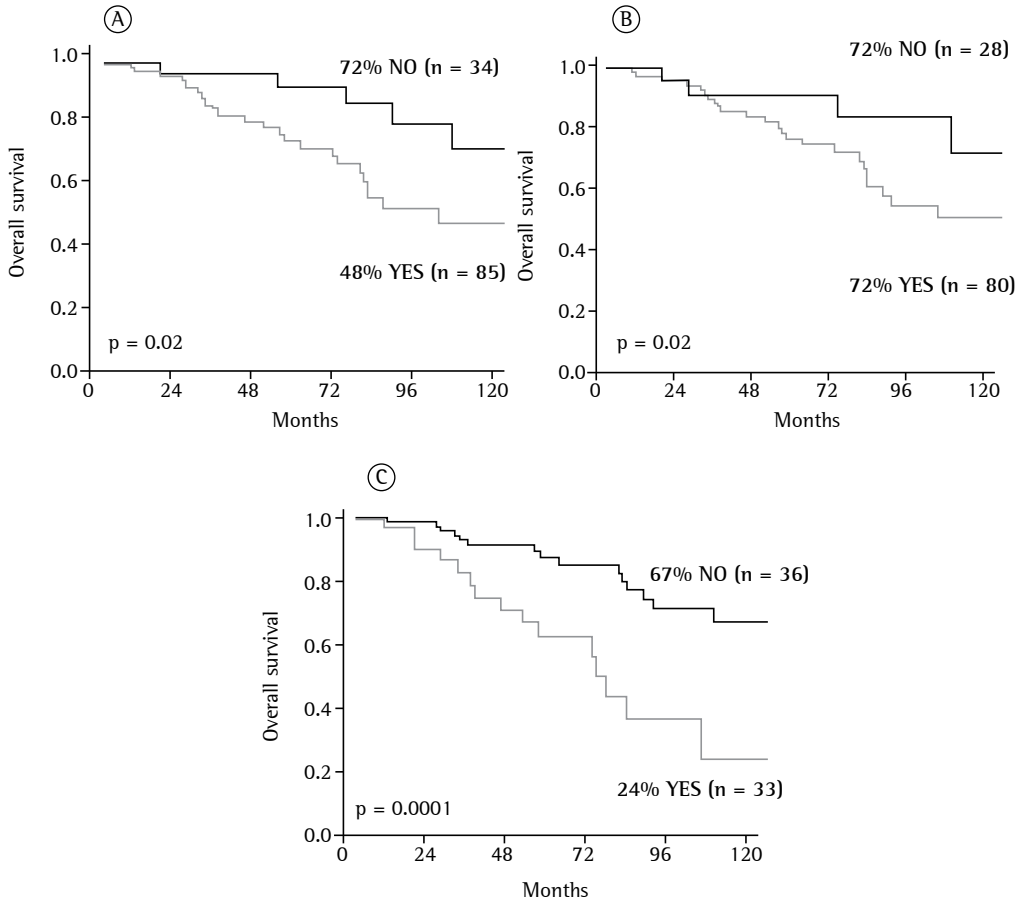

Figure 1 - In A, overall survival in the groups of patients who underwent and who did not undergo chemotherapy/radiotherapy (CHT/RT) before surgery (n = 119). In B, overall survival in the groups of patients who underwent and who did not undergo CHT/RT after surgery (n = 116). In C, overall survival in the groups of patients who had and who did not have complications within 30 postoperative days (n = 119).

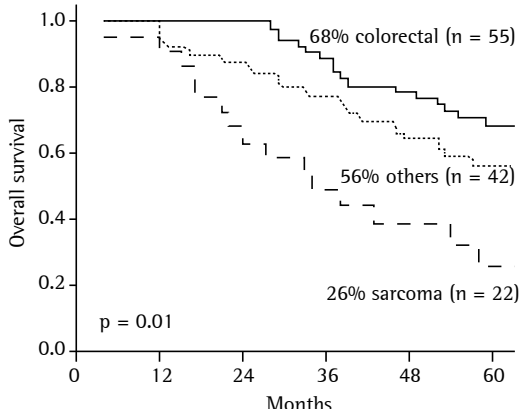

Figure 2 - Overall survival by type of primary tumor.

studies reported much lower results, given that, in them, survival was calculated from resection of the metastasis. For instance, in the study by Younes et al.,[8] survival at 60 months ranged from 10% to 40%, depending on the primary tumor site.

The prevalence of colorectal cancer as the site of origin of the lung metastases (47.9% in our study) led us to perform a separate analysis of overall survival in the patients with this type of cancer as compared with those with musculoskeletal cancer and those with cancer in other anatomical sites. We found that the patients with colorectal cancer had the best survival at 60 months (68%), whereas those with musculoskeletal cancer had the worst survival (26%). The vast majority of studies have investigated disease secondary to colorectal cancer only and reported rates of overall survival at 60 months that range from 24.0% to 62.7%.[11,16] Therefore, we believe that further studies comparing treatment of lung metastases from colorectal cancer with that of lung metastases

from cancer in other anatomical sites that are less common are needed in order to determine with accuracy the true impact and benefits of this treatment option for specific groups.

In our study, survival at 60 months in the patients who had up to 6 lesions resected ranged from 60% to 76%, whereas, in those who had more than 7 nodules resected, survival at 60 months dropped to 13%. Most studies have evaluated survival by stratifying patients based on the presence of one resected lesion or multiple resected lesions, with better results being found in patients with single lesions.[8,10,14] However, those same authors reviewed controversial results from various studies that did not find significant differences in the survival of patients with multiple nodules,[17,18] and the difference in results may lie in the heterogeneity of the biological behavior of the different histological types of tumors studied, suggesting the need for larger, specific studies of this variable.

Few studies have addressed laterality of lesions at diagnosis of metastasis and the need for more than one surgical procedure to resect the lesions. In our study, we found that 35 patients (29.4%) were diagnosed with bilateral disease and that 28 underwent more than one surgical procedure (1 of whom underwent four surgical procedures). According to the literature, patients undergoing repeat resection of new pulmonary metastatic lesions have increased survival in comparison with those treated with a single surgery (48% vs. 34%),[19] which leads us to believe in the benefits of and to continue to recommend surgical treatment of recurrent lesions as long as the patient is clinically fit to undergo the surgical procedure. With regard to the treatment of bilateral lesions, we found that overall survival was 63% in the patients with bilateral disease at diagnosis and 55% in those with unilateral disease, i.e., the difference was not statistically significant, which is in agreement with the findings of the few studies that have addressed this issue and highlights the importance of resection in bilateral disease.[8]

Another factor that stood out in the analyses of overall survival was the poorer survival of the patients who underwent chemotherapy/radiotherapy as compared with those who did not. We understand this finding to mean that the need for neoadjuvant and adjuvant treatment indicates disease in more advanced stages and weakness associated with sequential multimodal treatment. However, this finding is in disagreement with the results reported by Younes et al.,[8] who found a significant improvement in survival in the group of patients who underwent chemotherapy/radiotherapy, as well as with the finding of an absence of significant differences between these two groups in the main studies[20-22] reviewed in the meta-analysis by Pfannschmidt,[15] which leads us to agree with the opinion of those authors who claim that the different protocols for the different types of primary tumor may hinder the understanding of the true impact on the survival of patients undergoing complementary treatment.

There have been few studies addressing perioperative complications and survival in this specific group. The study by Younes et al.[8] found an overall rate of complications of 3.2%. However, there was no definition of the parameters used for classifying these events, nor was there an analysis of survival in this group only. In the present study sample, the rate of (minor and major) complications was 22%, and a Cox analysis confirmed a poor prognosis for this group of patients. Therefore, by considering even minor events (such as air leaks for more than five days) as a potential morbidity, we emphasize the importance of the fact that even these events, alone or in combination, can be predictive of a poor prognosis for the patient.

In conclusion, surgical treatment of lung metastases from different tumor sites is safe and effective, with significant overall survival, especially in patients with a smaller number of lung lesions. Survival was found to be poor in the patients with metastases from sarcomas and in those who underwent neoadjuvant or adjuvant chemotherapy/radiotherapy. Large multicenter prospective studies using better biological and molecular classification should provide a more refined understanding of and new prognostic parameters for these systemic malignant neoplasms.

References

1. Aberg T, Malmberg KA, Nilsson B, Nöu E. The effect of metastasectomy: fact or fiction? Ann Thorac Surg. 1980;30(4):378-84. http://dx.doi.org/10.1016/S0003-4975(10)61278-7
2. Weinlechner JD. Tumorenan der brustwand und derenbehand-lung (Resektion der rippen, eroffnung der brusthohle, partielleentfernun der lunge). Wiener Med Wschr. 1882;20:589-91.

3. Ehrenhaft JL. Pulmonary resections for metastatic lesions. AMA Arch Surg. 1951;63(3):326-36. http://dx.doi.org/10.1001/archsurg.1951.01250040332007 PMid:14868186
4. Fujisawa T, Yamaguchi Y, Saitoh Y, Sekine Y, Iizasa T, Mitsunaga S, et al. Factors influencing survival following pulmonary resection for metastatic colorectal carcinoma. Tohoku J Exp Med. 1996;180(2):153-60. http://dx.doi.org/10.1620/tjem.180.153 PMid:9111764
5. Dellai RCA, Chojniak R, Marques E, Younes RN. Detecção de nódulos pulmonares por tomografia computadorizada em pacientes com metástases pulmonares submetidos à cirurgia. J Pneumol. 1994;20(Suppl 3):28.
6. Thomford NR, Woolner LB, Clagett OT. The surgical treatment of metastatic tumors in the lungs. J Thorac Cardiovasc Surg. 1965;49:357-63. PMid:14265951
7. Morales-Blanhir JE, Palafox Vidal CD, Rosas Romero Mde J, García Castro MM, Londono Villegas A, Zamboni M. Six-minute walk test: a valuable tool for assessing pulmonary impairment. J Bras Pneumol. 2011;37(1):110-7. http://dx.doi.org/10.1590/S1806-37132011000100016 PMid:21390439
8. Younes RN, Haddad F, Ferreira F, Gross JL. Surgical removal of pulmonary metastasis: prospective study in 182 patients [Article in Portuguese]. Rev Assoc Med Bras. 1998;44(3):218-25. PMid:9755551
9. Moore KH, McCaughan BC. Surgical resection for pulmonary metastases from colorectal cancer. ANZ J Surg. 2001;71(3):143-6. http://dx.doi.org/10.1046/j.1440-1622.2001.02057.x
10. Pfannschmidt J, Muley T, Hoffmann H, Dienemann H. Prognostic factors and survival after complete resection of pulmonary metastases from colorectal carcinoma: experiences in 167 patients. J Thorac Cardiovasc Surg. 2003;126(3):732-9. http://dx.doi.org/10.1016/S0022-5223(03)00587-7
11. Rena O, Casadio C, Viano F, Cristofori R, Ruffini E, Filosso PL, et al. Pulmonary resection for metastases from colorectal cancer: factors influencing prognosis. Twenty-year experience. Eur J Cardiothorac Surg. 2002;21(5):906-12. http://dx.doi.org/10.1016/S1010-7940(02)00088-X
12. Saito Y, Omiya H, Kohno K, Kobayashi T, Itoi K, Teramachi M, et al. Pulmonary metastasectomy for 165 patients with colorectal carcinoma: A prognostic assessment. J Thorac Cardiovasc Surg. 2002;124(5):1007-13. http://dx.doi.org/10.1067/mtc.2002.125165 PMid:12407386
13. Toscano E. Tratamento cirúrgico das metástases nodulares do pulmão [thesis]. Rio de Janeiro: Universidade Federal Fluminense; 1991.
14. Rama N, Monteiro A, Bernardo JE, Eugénio L, Antunes MJ. Lung metastases from colorectal cancer: surgical resection and prognostic factors. Eur J Cardiothorac Surg. 2009;35(3):444-9. http://dx.doi.org/10.1016/j.ejcts.2008.10.047 PMid:19136273
15. Pfannschmidt J, Dienemann H, Hoffmann H. Surgical resection of pulmonary metastases from colorectal cancer: a systematic review of published series. Ann Thorac Surg. 2007;84(1):324-38. http://dx.doi.org/10.1016/j.athoracsur.2007.02.093 PMid:17588454
16. Ike H, Shimada H, Ohki S, Togo S, Yamaguchi S, Ichikawa Y. Results of aggressive resection of lung metastases from colorectal carcinoma detected by intensive follow-up. Dis Colon Rectum. 2002;45(4):468-73; discussion 473-5. http://dx.doi.org/10.1007/s10350-004-6222-0 PMid:12006927
17. Inoue M, Ohta M, Iuchi K, Matsumura A, Ideguchi K, Yasumitsu T, et al. Benefits of surgery for patients with pulmonary metastases from colorectal carcinoma. Ann Thorac Surg. 2004;78(1):238-44. http://dx.doi.org/10.1016/j.athoracsur.2004.02.017 PMid:15223436
18. Monteiro A, Arce N, Bernardo J, Eugénio L, Antunes MJ. Surgical resection of lung metastases from epithelial tumors. Ann Thorac Surg. 2004;77(2):431-7. http://dx.doi.org/10.1016/j.athoracsur.2003.06.012 PMid:14759411
19. Groeger AM, Kandioler MR, Mueller MR, End A, Eckersberger F, Wolner E. Survival after surgical treatment of recurrent pulmonary metastases. Eur J Cardiothorac Surg. 1997;12:703-5. http://dx.doi.org/10.1016/S1010-7940(97)00239-X
20. Mauro Rossi B, Lopes A, Paulo Kowalski L, de Oliveira Regazzini RC. Prognostic factors in 291 patients with pulmonary metastases submitted to thoracotomy. Sao Paulo Med J. 1995;113(3):910-6. http://dx.doi.org/10.1590/S1516-31801995000300005 PMid:8728726
21. Kanemitsu Y, Kato T, Hirai T, Yasui K. Preoperative probability model for predicting overall survival after resection of pulmonary metastases from colorectal cancer. Br J Surg. 2004;91(1):112-20. http://dx.doi.org/10.1002/bjs.4370 PMid:14716804
22. Lee WS, Yun SH, Chun HK, Lee WY, Yun HR, Kim J, et al. Pulmonary resection for metastases from colorectal cancer: prognostic factors and survival. Int J Colorectal Dis. 2007;22(6):699-704. http://dx.doi.org/10.1007/s00384-006-0218-2 PMid:17109105

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Levels of physical activity and predictors of mortality in COPD*

Níveis de atividade física e preditores de mortalidade na DPOC

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Abstract

Objective: To compare the Body mass index, airway Obstruction, Dyspnea, and Exercise capacity (BODE) index scores and its individual components between COPD patients with and without severe physical inactivity, as well as to correlate the number of steps/day with scores of physical activity questionnaires, age, and the BODE index (including its components). **Methods:** We included 30 patients, who were evaluated for body composition, pulmonary function (FEV_1), perception of dyspnea (modified Medical Research Council scale), and exercise capacity (six-minute walk distance [6MWD]). The patients also completed the International Physical Activity Questionnaire (IPAQ), short version, and the modified Baecke questionnaire (mBQ). The level of physical activity was assessed by the number of steps/day (as determined by pedometer), using the cut-off of 4,580 steps/day to form two groups: no severe physical inactivity (SPI–) and severe physical inactivity (SPI+). We used the Mann-Whitney test or t-test, as well as Pearson's or Spearman's correlation tests, in the statistical analysis. **Results:** In comparison with the SPI– group, the SPI+ group showed more advanced age, higher mBQ scores (leisure domain), lower 6MWD (in m and % of predicted), and lower IPAQ scores (metabolic equivalent-walk/week domain and total). The IPAQ scores showed weak correlations with steps/day (r = 0.399), age (r = –0.459), and 6MWD—in m (r = 0.446) and in % of predicted (r = 0.422). **Conclusions:** In our sample, the cut-off of 4,580 steps/day was not sensitive enough to identify differences between the groups when compared with the predictors of mortality. The IPAQ, short version score correlated with steps/day.

Keywords: Pulmonary disease, chronic obstructive/mortality; Pulmonary disease, chronic obstructive/prevention and control; Motor activity.

Resumo

Objetivo: Comparar a pontuação do índice *Body mass index*, *airway Obstruction*, *Dyspnea*, *and Exercise capacity* (BODE) e seus componentes individuais em pacientes com DPOC com grave inatividade física ou não, assim como correlacionar o número de passos diários com pontuações de questionários de atividade física, idade, índice BODE e seus componentes. **Métodos:** Foram incluídos 30 pacientes, os quais foram avaliados quanto a sua composição corporal, função pulmonar (VEF_1), percepção de dispneia (escala *modified Medical Research Council*) e capacidade de exercício distância percorrida no teste de caminhada de seis minutos (DTC6). Além disso, os participantes responderam ao *International Physical Activity Questionnaire* (IPAQ) versão curta e questionário de Baecke modificado (QBm). O nível de atividade desses pacientes foi avaliado pelo número de passos diários por pedômetro, utilizando-se o ponto de corte de 4.580 passos para a formação de dois grupos: grupo sem grave inatividade física (GIF–) e grupo com grave inatividade física (GIF+). Foram utilizados os testes de Mann-Whitney ou t não pareado, assim como os testes de correlação de Spearman ou de Pearson, na análise estatística. **Resultados:** Idade mais avançada, maiores escores no QBm (domínio lazer), menor DTC6 (em m e em % do previsto) e menores escores no IPAQ (domínios equivalentes metabólicos em caminhada e total por semana) foram encontrados no grupo GIF+ do que no grupo GIF–. Houve correlações fracas dos escores do IPAQ com o número de passos diários (r =0,399), idade (r = -0,459), DTC6 em m (r = 0,446) e em % do previsto (r = 0,422). **Conclusões:** Na amostra estudada, o ponto de corte de 4.580 passos diários não foi sensível para identificar diferenças entre os grupos estudados quando comparado com os preditores de mortalidade. O questionário IPAQ versão curta correlacionou-se com o número de passos diários.

Descritores: Doença pulmonar obstrutiva crônica/mortalidade; Doença pulmonar obstrutiva crônica/prevenção e controle; Atividade motora.

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Introduction

Currently, COPD is considered the leading cause of morbidity and the fourth leading cause of mortality worldwide.[1] Its prevalence has been increasing substantially because of the aging of the population, and it is estimated that, in 2020, COPD will be the third most common cause of death in the world.[2]

In addition to pulmonary involvement, which leads to airflow limitation that is not fully reversible and dyspnea,[3] COPD is characterized by extrapulmonary impairment, such as skeletal muscle dysfunction, which is related to decreased exercise capacity, which, together with dyspnea, leads to physical inactivity in these patients, establishing a vicious cycle or downward spiral.[4]

Because COPD is multisystemic in nature, with various factors contributing to the severity of the condition, the risk of mortality in patients with COPD began to be evaluated not only by using the Global Initiative for Chronic Obstructive Lung Disease staging system, which is based on the degree of obstruction (FEV_1),[3] but also by using the Body mass index, airway Obstruction, Dyspnea, and Exercise capacity (BODE) index. The BODE index is a multi-grading system consisting of the body mass index (BMI); FEV_1, for determination of the degree of airway obstruction; the modified Medical Research Council (mMRC) scale, for assessment of the degree of dyspnea; and the six-minute walk test (6MWT), for assessment of exercise capacity. The BODE index is considered a better predictor of mortality.[5]

However, physical inactivity in patients with COPD has now been implicated as a factor directly related to an increased risk of exacerbations and as the best predictor of early mortality in COPD.[6-8] A prospective observational study[8] involving 169 patients with COPD showed that four-year mortality was strongly associated with a physical activity level (PAL) < 1.4 in these patients. The PAL is an index calculated by dividing total daily energy expenditure (kcal/day) by resting energy expenditure.[8,9]

In a recent study, pedometers were considered the most feasible way to monitor physical activity in clinical practice.[10] In addition to being objective, they may serve as a surrogate for higher-cost options, such as accelerometers, and for questionnaires for physical activity assessment, such as the International Physical Activity Questionnaire (IPAQ),[11] short version, and the modified Baecke questionnaire,[12,13] both of which have been translated and validated for use in Brazil; however, these questionnaires are considered inaccurate, especially when administered to older populations.[14]

An association had yet to be established between a given number of steps/day and a PAL value of 1.40 (related to marked physical inactivity) in patients with COPD[8]; however, in a study published by Depew et al.,[10] this value was associated with a minimum number of 4,580 steps/day, i.e., lower numbers than that correspond to COPD patients with severe physical inactivity and, consequently, at increased risk of early mortality.

Therefore the primary objective of the present study was to compare the scores on the BODE index, which is a predictor of mortality, in patients with COPD divided into two groups: those who achieved the recommended minimal number of 4,580 steps/day and those who did not achieve this value. The secondary objective of the study was to correlate pedometer-determined steps/day with age, the BODE index (including its individual components), and scores of instruments that assess the level of physical activity in these patients.

The present study tested the hypothesis that patients who do not achieve the minimum recommended number of steps/day have higher scores on the BODE index, i.e., shorter six-minute walk distances (6MWDs), higher BMI values, a greater degree of pulmonary obstruction, and a stronger sensation of dyspnea during daily activities, reflecting a poorer prognosis.

Methods

This was a cross-sectional study involving 38 participants recruited from a convenience sample of male and female COPD patients with moderate to very severe obstruction ($FEV_1/FVC < 70\%$ and $FEV_1 < 80\%$ of predicted),[3] as diagnosed by spirometry, which had been requested by the attending pulmonologist. The patients included in the study were former smokers or nonsmokers, were oxygen-dependent or non-oxygen-dependent, and had been clinically stable in the two months prior to the study (without respiratory system infections or exacerbations). Patients with exacerbated lung disease, decompensated cardiovascular disease, rheumatic diseases, neuromuscular diseases, or orthopedic diseases that prevented them from

performing the proposed tests or affected their performance on those tests were excluded, as were those who underwent a change in medication during the study period and those who had uncontrolled arterial hypertension.

Evaluations were performed between March and September of 2012. All participants were informed of the procedures involved in the study and gave written informed consent. The study was approved by the local research ethics committee (Ruling no. 213/2012).

All participants underwent the following: anamnesis; anthropometric data collection; administration of the mMRC scale, the modified Baecke questionnaire, and the IPAQ, short version; the 6MWT; and monitoring of the number of steps/day by using a pedometer. The procedures were performed on different days, the first of which included anamnesis, anthropometric data collection, and administration of the questionnaires and the mMRC scale, as well as fitting of the pedometer for monitoring of the number of steps/day. The patients were instructed to return after three days of monitoring for pedometer data collection and for undergoing the 6MWT.

Anthropometric data (weight and height) were obtained with the use of a calibrated mechanical scale (Welmy S.A., Santa Bárbara do Oeste, Brazil) in order to calculate the BMI, which is used for the calculation of the BODE index.[5]

To assess the sensation of dyspnea, we used the validated Brazilian-Portuguese language version of the mMRC scale, which was administered as an interview. Patients were asked about the extent to which their sensation of dyspnea limits their activities of daily living and were instructed to choose only one alternative. The mMRC scale comprises five grades, characterizing the different activities that lead to the sensation of dyspnea.[15]

The 6MWT was performed along a 30-m-long by 1.5-m-wide course with markings every 2 m, as recommended by the American Thoracic Society.[16] For patients on home oxygen therapy, the test was supplemented with the same oxygen flow as that used at home. Predicted 6MWD for each patient was calculated by the Iwama et al. equation.[17]

To determine the risk of mortality, we used the BODE index, which was calculated considering the following variables: BMI; FEV_1 (% of predicted),[18] mMRC score, and 6MWD. The BODE index (total score) ranges from 0 to 10, with higher scores indicating greater disease severity. On the basis of these scores, the BODE index was calculated as a predictor of mortality, being divided into quartiles as follows: quartile 1 (0-2 points); quartile 2 (3-4 points); quartile 3 (5-6 points), and quartile 4 (7-10 points).[5]

The level of physical activity was subjectively assessed by using two questionnaires (the IPAQ, short version and the modified Baecke questionnaire), which were administered as an interview. The IPAQ, short version allows an estimate of time spent per week on different physical activities, in order to classify individuals as sedentary, irregularly active (A or B), active, or very active.[11] For the purpose of analysis, this classification was converted to continuous values expressed as metabolic equivalent minutes per week (MET-min/wk),[19] for each domain alone (walking activity, moderate physical activity, and vigorous physical activity) and in combination (sum of all physical activity). The modified Baecke questionnaire, validated by Pols et al.,[13] was administered to assess the patient's usual exercise capacity subjectively. It comprises 12 questions related to three domains: activities of daily living; sports; and leisure activities. The activities of daily living domain contain 10 questions, which are answered on a 0 to 3 scale, with 0 meaning "never does the task" and 3 meaning "always does the task". The other domains comprise open-ended questions in which patients report the time of year when they do sports and leisure activities and the amount of time they spend on these activities. For the activities of daily living domain, the final score was calculated by adding up the points assigned to each question and dividing the result by the total number of questions in that domain; for the other domains, the final score was calculated with a code that classifies the energy expenditure level of the given activity. Finally, the three domain scores were added up, and the level of physical activity of the patient was determined.[12]

The number of steps/day was quantified by a Yamax Digi-Walker SW-700 pedometer (Yamax, Tokyo, Japan), which is considered the most accurate commercially available pedometer.[20,21] It consists of a small sensor and a mechanical counter that have the purpose of recording the movements made in response to the vertical acceleration of the body. The oscillations are computed by adding up the total number of

accumulated movements and determine the total number of steps taken over the period assessed.[21] In our study, the equipment was positioned at the patients' waistline, at the level of the right anterosuperior iliac crest, being clipped to their belt or clothes. The volunteers were instructed not to change their habitual routines and to wear the pedometer continuously throughout the period of wakefulness for three consecutive days[22] in order to characterize a usual week's pattern, excluding the weekend. For the analysis, we considered the mean of the values obtained on the three days, so that a single value was regarded as the level of activity of daily living. No benchmark minimum number of steps/day was provided to the participants. In addition, the patients were instructed to keep a diary to report the activities performed every one hour.

Data were analyzed with the Statistical Package for the Social Sciences, version 17.0 (SPSS Inc., Chicago, IL, USA). Normality of the data was assessed by the Shapiro-Wilk test. The patients who achieved the minimum number of 4,580 steps/day were compared with those who did not by using the Mann-Whitney test or the unpaired t-test according to the data distribution pattern. Correlations of pedometer-determined steps/day with the scores on the IPAQ, short version and the modified Baecke questionnaire, which are subjective instruments, were tested by using Spearman's correlation coefficient. The level of statistical significance was set at $p < 0.05$. The power of the test was calculated by using the Ene software, version 2.0 (GlaxoSmithKline, Madrid, Spain). To that end, we considered the standard deviation of the variable "pedometer-determined steps/day" and a level of significance of 5%, which resulted in a power of over 80%.

Results

The study sample initially consisted of 38 patients, of whom 30 completed the study and 8 were excluded because of failure to complete all of the steps of the evaluations. Of the sample, 15 patients achieved more than 4,580 steps/day[10] and were considered to have no severe physical inactivity (SPI– group), whereas 15 did not achieve this number and were considered to have severe physical inactivity (SPI+ group). Of those 30 patients, 6 were oxygen-dependent (3 in each group).

Table 1 shows the demographic and anthropometric characteristics, the BODE index and its components, and objective and subjective characteristics of the level of physical activity of the COPD patients in the total sample and by group.

Intergroup analysis showed that the patients had similar values for BMI, degree of airway obstruction, classification of prognosis for mortality, perception of dyspnea in activities of daily living (mMRC scale), modified Baecke questionnaire scores (total score, leisure domain score, and sports domain score), and IPAQ, short version scores (MET-min/wk of moderate and vigorous physical activity). However, the patients in the SPI+ group were found to have significantly higher values for age and for the leisure domain score of the modified Baecke questionnaire, which means more time spent on activities that do not involve body displacement; in addition, these patients had lower functional exercise capacity and reported less time spent on walking activity and total physical activity in comparison with those in the SPI– group.

Pedometer-determined steps/day showed a weak but statistically significant correlation with the IPAQ, short version total score ($r = 0.399$; $p = 0.029$) in the total sample of patients (Figure 1). However, no significant correlation was found between steps/day and the modified Baecke questionnaire total score ($r = -0.129$; $p = 0.496$). In addition, steps/day showed statistically significant correlations with age ($r = -0.459$; $p = 0.011$) and 6MWD—in m ($r = 0.446$; $p = 0.013$) and in % of predicted ($r = 0.422$; $p = 0.020$). The correlations of steps/day with the BODE index and its components (BMI, FEV_1, and mMRC) were not statistically significant.

Discussion

The results of our study showed that, after the patients were divided into two groups, i.e., the SPI+ and SPI– groups, on the basis of a minimum number of 4,580 steps/day, which is considered a strong predictor of mortality by Depew et al.,[10] there was no difference between the groups regarding the classification of severity by the BODE index or the scores on the BODE index and its components "mMRC score", "BMI", and "FEV_1 (% of predicted)".

One possible factor that may have limited these aforementioned findings was the reduced

Table 1 - Demographic characteristics, anthropometric characteristics, and level of physical activity of the patients studied.[a]

Characteristic	Total (n = 30)	SPI– group (n = 15)	SPI+ group (n = 15)	p*
Age, years	68 ± 10	62 ± 8	74 ± 8	0.001
Men/women, n/n	23/7	10/5	13/2	0.671
BMI, kg/cm^2	24.6 ± 4.7	23.9 ± 4.4	24.3 ± 5.2	0.432
Steps/day	4.227 ± 2.075	5.780 ± 1.355	2.674 ± 1.384	0.000
FEV_1, % of predicted	48.0 ± 14.9	47.1 ± 16.1	49 ± 14.2	0.732
6MWD, m	380.3 ± 108.3	434.6 ± 95.8	326.2 ± 93.8	0.004
6MWD, % of predicted	70.1 ± 18.7	79.2 ± 16.4	60.8 ± 16.7	0.005
mMRC score[b]	2 (0.00-2.25)	2 (0-3)	2 (0-2)	0.589
BODE index	3.5 ± 1.9	3.3 ± 2.1	3.7 ± 1.7	0.567
Quartile[b]	2 (1-3)	2 (1-3)	2 (1-3)	0.760
Quartile 1[c]	10 (33.3)	5 (33.3)	5 (33.3)	
Quartile 2[c]	10 (33.3)	5 (33.3)	5 (33.3)	
Quartile 3[c]	9 (30.0)	4 (26.7)	5 (33.3)	
Quartile 4[c]	1 (3.3)	1 (6.7)	0 (0.0)	
mBQ[b]				
Household domain	1.1 (0.6-1.7)	1.3 (0.3-1.9)	1.0 (0.6-1.5)	0.466
Sports domain	1.4 (0.0-4.1)	2.5 (0.0-4.1)	0.2 (0.0-4.2)	0.622
Leisure domain	1.2 (1.0-2.9)	1.1 (0.6-1.4)	1.4 (1.2-3.5)	0.023
Total	5.3 (2.5-7.2)	4.8 (3.3-5.9)	5.7 (2.2-8.3)	0.604
IPAQ, short version[b]				
MET-min/wk of W	231.0 (0.0-358.8)	346.5 (82.5-528.0)	33.0 (0.0-247.5)	0.005
MET-min/wk of MPA	160 (0-510)	240 (0-600)	80 (0-360)	0.297
MET-min/wk of VPA	0 (0-0)	0 (0-0)	0 (0-0)	0.317
MET-min/wk of TPA	338.2 (153.0-979.5)	586.5 (330.0-1.150)	247.5 (0.0-657.0)	0.028

SPI–: no severe physical activity; SPI+: severe physical activity; BMI: body mass index; 6MWD: six-minute walk distance; mMRC: modified Medical Research Council; IPAQ: International Physical Activity Questionnaire; BODE: (B: body mass index, O: airflow obstruction, D: dyspnea, E: exercise capacity); mBQ; modified Baecke questionnaire; MET-min/wk of W: metabolic equivalent minutes per week of walking activity; MET-min/wk of MPA: metabolic equivalent minutes per week of moderate physical activity; MET-min/wk of VPA: metabolic equivalent minutes per week of vigorous physical activity; MET-min/wk of TPA: metabolic equivalent minutes per week of total physical activity. [a]Values expressed as mean ± dp; except where otherwise indicated. [b]Values expressed as median (interquartile range). [c]Values expressed as n (%). *Mann-Whitney test or unpaired t-test.

number of patients in the last two quartiles (10 of the total sample), as well as the fact that the groups showed no differences in the classification of severity by the BODE index. One group of authors[23] showed that level of physical activity correlates only modestly with classification of severity in COPD by the BODE index, which is more sensitive when differences in the level of daily physical activity are analyzed between patients with mild to moderate disease and patients with severe to very severe disease. Although quartile 1 and quartile 2 patients show a decrease in the level of physical activity, the decrease is subtle, whereas in quartile 3 and quartile 4 patients, this decrease is more marked.

When the two groups were compared for age, we found that there was a significant difference and correlation, age being greater in the SPI+ group than in the SPI–, which shows that, as age increases, there is a trend toward a decrease in the number of steps/day. In a descriptive meta-analysis,[24] it was found that, in groups with a mean age over 65 years, the number of steps/day is significantly lower; one possible explanation is that, as age increases, gait velocity decreases, which does not allow the pedometer to record an accurate step count. However, in our study, we believe that, since the groups showed a significant difference also in the domain "MET-min/wk of walking activity" and in 6MWD, the difference between the two groups occurred because of a decrease in walking activity among the older patients, rather than because of a possible limitation of pedometers.

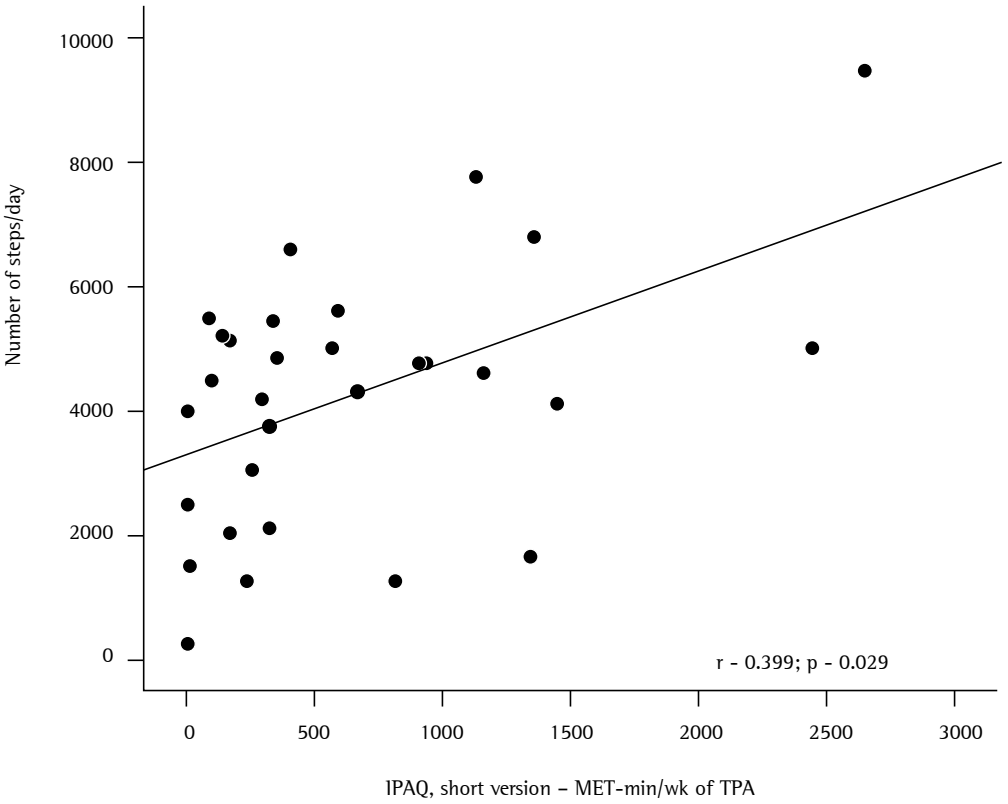

Figure 1 - Correlation between pedometer-determined steps/day and the International Physical Activity Questionnaire (IPAQ), short version score for the domain "metabolic equivalent minutes per week of total physical activity" (MET-min/wk of TPA).

Pitta et al.[25] found that daily walking time was weakly correlated with FEV_1 (r = 0.28) and BMI (r = –0.08) but strongly correlated with the 6MWT (r = 0.76). In our study, we found no significant differences or correlations between the groups when we analyzed FEV_1 (% of predicted), which shows that impairment related to airflow obstruction was not associated with a lower level of daily living activity. Oga et al.[26] stated that patient activity can be limited by an increased degree of pulmonary obstruction; however, those authors reported that FEV_1 is not beneficially influenced by physical activity. As in the study by Pitta et al.,[25] we found a relationship between pedometer-measured physical activity and 6MWD, with the group with a higher level of physical activity having greater functional capacity (as assessed by the 6MWT) than the group with a lower level of physical activity.

Regarding the differences found in the leisure domain of the modified Baecke questionnaire, the results show that the patients in the SPI+ group, who took less than 4,580 steps/day, spent more time on these activities. The fact that most of the activities reported within this domain were reading magazines, watching TV, performing manual tasks, etc, explains why the patients in the SPI+ group took less than 4,580 steps/day on average, because these activities do not involve body displacements, which are recorded by the pedometer.

Regarding the correlation of pedometer-determined steps/day with the scores on the physical activity questionnaires, which are subjective instruments, we found that only the IPAQ, short version score showed a significant but weak correlation (r = 0.399) with steps/day. A systematic review[27] reported that most validation studies found only weak correlations between IPAQ scores and objective measures of physical activity. In the present study, the modified Baecke questionnaire score showed no correlation with pedometer-determined steps/day. Unlike in our study, Mazo et al.[28] found weak to moderate concurrent validity between the modified Baecke questionnaire score and

pedometer-determined steps/day in a group of elderly women.

Limitations of our study include the fact that the sample was selected by convenience, not being representative of the general population, and that the number of patients in each BODE index quartile was not similar, there being only 1 patient in quartile 4. In addition, three days of pedometer monitoring, despite references in the literature, might have underestimated or overestimated the step counts; the number of steps might also have been underestimated by the fact that COPD patients tend to have slow gait velocity, which impacts on the detection of oscillations during gait, and this can lead to inaccurate step counts.

Therefore, the present study showed that the cut-off of 4,580 steps/day[10] was not sensitive enough to identify differences between the groups in our sample when compared with established predictors of mortality. Of the scores on the two questionnaires for physical activity assessment, the IPAQ, short version score correlated better with pedometer-measured physical activity.

References

1. National Heart, Lung, and Blood Institute. Morbidity and Mortality: Chartbook on Cardiovascular, Lung, and Blood Diseases. Bethesda: National Heart, Lung, and Blood Institute; 2009.
2. World Health Organization. World Health Statistics 2012. Geneva: World Health Organization; 2012.
3. Global Initiative for Chronic Obstructive Lung Disease [homepage on the Internet]. Bethesda: Global Initiative for Chronic Obstructive Lung Disease. [cited 2013 Jan 10]. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease – Revised 2011. [Adobe Acrobat document, 80p.]. Available from: http://www.goldcopd.org/uploads/users/files/GOLD_Report_2011_Feb21.pdf
4. Polkey MI, Moxham J. Attacking the disease spiral in chronic obstructive pulmonary disease. Clin Med. 2006;6(2):190-6. http://dx.doi.org/10.7861/clinmedicine.6-2-190
5. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(10):1005-12. http://dx.doi.org/10.1056/NEJMoa021322 PMid:14999112
6. Garcia-Aymerich J, Lange P, Benet M, Schnohr P, Antó JM. Regular physical activity reduces hospital admission and mortality in chronic obstructive pulmonary disease: a population based cohort study. Thorax. 2006;61(9):772-8. http://dx.doi.org/10.1136/thx.2006.060145 PMid:16738033 PMCid:PMC2117100
7. Yohannes AM, Baldwin RC, Connolly M. Mortality predictors in disabling chronic obstructive pulmonary disease in old age. Age Ageing. 2002;31(2):137-40. http://dx.doi.org/10.1093/ageing/31.2.137 PMid:11937477
8. Waschki B, Kirsten A, Holz O, Müller KC, Meyer T, Watz H, et al. Physical activity is the strongest predictor of all-cause mortality in patients with COPD: a prospective cohort study. Chest. 2011;140(2):331-42. http://dx.doi.org/10.1378/chest.10-2521 PMid:21273294
9. Watz H, Waschki B, Boehme C, Claussen M, Meyer T, Magnussen H. Extrapulmonary effects of chronic obstructive pulmonary disease on physical activity: a cross-sectional study. Am J Respir Crit Care Med. 2008;177(7):743-51. http://dx.doi.org/10.1164/rccm.200707-1011OC PMid:18048807
10. Depew ZS, Novotny PJ, Benzo RP. How many steps are enough to avoid severe physical inactivity in patients with chronic obstructive pulmonary disease? Respirology. 2012;17(6):1026-7. http://dx.doi.org/10.1111/j.1440-1843.2012.02207.x PMid:22672739 PMCid:PMC3409325
11. Matsudo S, Araujo T, Marsudo V, Andrade D, Andrade E, Oliveira LC, et al. Questionário Internacional de Atividade Física (IPAQ): Estudo de validade e reprodutibilidade no Brasil. Rev Bras Ativ Fis Saude. 2001;6(2):5-18.
12. Florindo AA, Latorre MR. Validação e reprodutibilidade do questionário de Baecke de avaliação da atividade física habitual em homens adultos. Rev Bras Med Esportes. 2003;9(3):121-8.
13. Pols MA, Peeters PH, Bueno-De-Mesquita HB, Ocké MC, Wentink CA, Kemper HC, et al. Validity and repeatability of a modified Baecke questionnaire on physical activity. Int J Epidemiol. 1995;24(2):381-8. http://dx.doi.org/10.1093/ije/24.2.381 PMid:7635600
14. Rabacow FM, Gomes MA, Marques P, Benedetti TR. Questionários de medidas de atividade física em idosos. Rev Bras Cineantropom Desempenho Hum. 2006;8(4):99-106.
15. Bestall JC, Paul EA, Garrod R, Garnham R, Jones PW, Wedzicha JA. Usefulness of the Medical Research Council (MRC) dyspnoea scale as a measure of disability in patients with chronic obstructive pulmonary disease. Thorax. 1999;54(7):581-6. http://dx.doi.org/10.1136/thx.54.7.581 PMid:10377201 PMCid:PMC1745516
16. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med. 2002;166(1):111-7. http://dx.doi.org/10.1164/ajrccm.166.1.at1102 PMid:12091180
17. Iwama AM, Andrade GN, Shima P, Tanni SE, Godoy I, Dourado VZ. The six-minute walk test and body weight-walk distance product in healthy Brazilian subjects. Braz J Med Biol Res. 2009;42(11):1080-5. Erratum in: Braz J Med Biol Res. 2010;43(3):324. http://dx.doi.org/10.1590/S0100-879X2009005000032 PMid:19802464
18. Knudson RJ, Slatin RC, Lebowitz MD, Burrows B. The maximal expiratory flow-volume curve. Normal standards, variability, and effects of age. Am Rev Respir Dis. 1976;113(5):587-600. PMid:1267262
19. Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc. 2003;35(8):1381-95. http://dx.doi.org/10.1249/01.MSS.0000078924.61453.FB PMid:12900694
20. Welk GJ, Differding JA, Thompson RW, Blair SN, Dziura J, Hart P. The utility of the Digi-walker step counter to assess daily physical activity patterns. Med Sci

Sports Exerc. 2000;32(9 Suppl):S481-8. http://dx.doi.org/10.1097/00005768-200009001-00007 PMid:10993418
21. Schneider PL, Crouter S, Bassett DR. Pedometer measures of free-living physical activity: comparison of 13 models. Med Sci Sports Exerc. 2004;36(2):331-5. http://dx.doi.org/10.1249/01.MSS.0000113486.60548.E9 PMid:14767259
22. Trost SG, McIver KL, Pate RR. Conducting accelerometer-based activity assessments in field-based research. Med Sci Sports Exerc. 2005;37(11 Suppl):S531-43. http://dx.doi.org/10.1249/01.mss.0000185657.86065.98 PMid:16294116
23. Mantoani LC, Hernandes NA, Guimarães MM, Vitorasso RL, Probst VS, Pitta F. Does the BODE index reflect the level of physical activity in daily life in patients with COPD? Rev Bras Fisioter. 2011;15(2):131-7. http://dx.doi.org/10.1590/S1413-35552011000200008 PMid:21789363
24. Bohannon RW. Number of pedometer-assessed steps taken per day by adults: a descriptive meta-analysis. Phys Ther. 2007;87(12):1642-50. http://dx.doi.org/10.2522/ptj.20060037 PMid:17911274
25. Pitta F, Troosters T, Spruit MA, Probst VS, Decramer M, Gosselink R. Characteristics of physical activities in daily life in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;171(9):972-7. http://dx.doi.org/10.1164/rccm.200407-855OC PMid:15665324
26. Oga T, Nishimura K, Tsukino M, Sato S, Hajiro T. Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. Am J Respir Crit Care Med. 2003;167(4):544-9. http://dx.doi.org/10.1164/rccm.200206-583OC PMid:12446268
27. Lee PH, Macfarlane DJ, Lam TH, Stewart SM. Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. Int J Behav Nutr Phys Act. 2011;8:115. http://dx.doi.org/10.1186/1479-5868-8-115 PMid:22018588 PMCid:PMC3214824
28. Mazo GZ, Mota J, Benedetti TB, Barros MV. Validade concorrente e reprodutibilidade: teste-reteste do Questionário de Baecke modificado para idosos. Rev Bras Ativ Fis Saude. 2001;6(1):5-11.

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Nocturnal hypoxemia in children and adolescents with cystic fibrosis*

Hipoxemia noturna em crianças e adolescentes com fibrose cística

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Abstract

Objective: To determine the prevalence of nocturnal hypoxemia and its association with pulmonary function, nutritional status, sleep macrostructure, and obstructive respiratory events during sleep in a population of clinically stable children and adolescents with cystic fibrosis (CF). **Methods:** This was a cross-sectional study involving 67 children and adolescents with CF between 2 and 14 years of age. All of the participants underwent polysomnography, and SpO_2 was measured by pulse oximetry. We also evaluated the Shwachman-Kulczycki (S-K) scores, spirometry findings, and nutritional status of the patients. **Results:** The study involved 67 patients. The mean age of the patients was 8 years. The S-K scores differed significantly between the patients with and without nocturnal hypoxemia, which was defined as an SpO_2 < 90% for more than 5% of the total sleep time (73.75 ± 6.29 vs. 86.38 ± 8.70; p < 0.01). Nocturnal hypoxemia correlated with the severity of lung disease, FEV_1 (r_s = −0.42; p = 0.01), FVC (r_s = −0.46; p = 0.01), microarousal index (r_s = 0.32; p = 0.01), and apnea-hypopnea index (r_s = 0.56; p = 0.01). **Conclusions:** In this sample of patients with CF and mild-to-moderate lung disease, nocturnal oxygenation correlated with the S-K score, spirometry variables, sleep macrostructure variables, and the apnea-hypopnea index.

Keywords: Cystic fibrosis; Sleep; Oximetry.

Resumo

Objetivo: Determinar a prevalência de hipoxemia noturna e sua associação com função pulmonar, estado nutricional, macroestrutura do sono e eventos respiratórios obstrutivos durante o sono em uma população de crianças e adolescentes com fibrose cística (FC) clinicamente estáveis. **Métodos:** Estudo de corte transversal envolvendo 67 crianças e adolescentes com FC e idade entre 2 e 14 anos. Todos os participantes foram submetidos a polissonografia com medição da SpO_2 por oximetria de pulso. O escore de Shwachman-Kulczycki (S-K), a espirometria e o estado nutricional dos pacientes também foram avaliados. **Resultados:** Foram incluídos 67 pacientes. A média de idade foi de 8 anos. Os resultados do escore de S-K diferiram significativamente entre os pacientes com e sem hipoxemia noturna, definida como SpO_2 < 90% por mais que 5% do tempo total de sono (73,75 ± 6,29 vs. 86,38 ± 8,70; p < 0,01). A presença de hipoxemia noturna correlacionou-se com a gravidade da doença pulmonar, VEF_1 (r_s = −0,42; p = 0,01), CVF (r_s = −0,46; p = 0,01), índice de microdespertares do sono (r_s = 0,32; p = 0,01) e índice de apneia e hipopneia (r_s = 0,56; p = 0,01). **Conclusões:** Nesta amostra de pacientes com FC e doença pulmonar leve a moderada, o nível de oxigenação noturna correlacionou-se com escore de S-K, variáveis espirométricas e da macroestrutura do sono, assim como o índice de apneia e hipopneia.

Descritores: Fibrose cística; Sono; Oximetria.

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Introduction

Cystic fibrosis (CF) is a multisystem autosomal recessive genetic disease that primarily affects the epithelia of various organs, leading to significant morbidity and mortality. It results from a mutation in the gene encoding the cystic fibrosis transmembrane conductance regulator protein, with approximately 250 kb, and located on the long arm of chromosome 7. Abnormal ion transport causes dehydration of luminal secretions, with formation of viscous mucus and subsequent stasis. This defect impairs mucociliary clearance, leading to chronic infection, airway remodeling, and, consequently, to air trapping, an imbalance in the ventilation/perfusion ratio, and increased work of breathing.[1] There is progressive airflow obstruction due to mucus plugging and inflammation within the bronchial walls, with bronchiectasis and destruction of lung parenchyma. Despite continuous improvement in the care of patients with CF, much of the morbidity and mortality result from lung disease, the end stage of which is characterized by hypercapnic respiratory failure.[2]

During sleep, there are drops in oxyhemoglobin saturation, and these occur primarily during rapid eye movement sleep; in patients with CF, these drops are associated with a decrease in intercostal muscle activity, with breathing pattern irregularity, and with hypoventilation caused by a reduction in tidal volume and minute ventilation.[3] Episodic hypoxemia can occur during sleep, as well as during exercise and during CF infectious exacerbations. There are reports of its occurrence during sleep and during exercise in clinically stable adult patients with CF, who do not exhibit daytime hypoxemia.[4] However, there has been reference to severe episodes of oxyhemoglobin desaturation during sleep in adolescents and adults with CF, and hypoxemia can be a stimulus for disruption of normal sleep patterns and quality of life in these patients.[3] Evidence in the literature suggests that hypoxemia plays a role in the pathogenesis of lung injury and in cor pulmonale in these patients, as do brief episodes of desaturation, which can increase pulmonary artery pressure.[5] Hypoxemia in these patients is potentially important because it leads to clinical complications, such as pulmonary hypertension and right heart failure; evidence suggests that hypoxemia can exacerbate lung inflammation and affect the bacterial profile of the lung in these patients.[6,7]

The definitions of hypoxemia during sleep vary among different countries and among published studies in the medical literature; some authors define it as a pulse oximetry-measured arterial oxyhemoglobin saturation (SpO_2) < 90% for more than 5% of the total sleep time (TST), whereas others define it as an SpO_2 < 90% for more than 30% of TST.[8] The lack of a clear definition of "significant hypoxemia" during sleep makes it difficult to determine its prevalence and severity. In adults, PaO_2 measurement is considered crucial; however, this measurement is not always considered to be practical or possible in children. In pediatric practice, arterial oxyhemoglobin saturation measured by pulse oximetry remains the main tool.[1]

Considering the few existing reports in the literature that address abnormalities resulting from nocturnal hypoxemia in pediatric patients with CF, our objective was to estimate the prevalence of nocturnal hypoxemia and correlate it with abnormalities in sleep macrostructure, pulmonary function data, nutritional status, and obstructive respiratory events during sleep in a sample of children with CF.

Methods

This was a prospective, cross-sectional and descriptive-analytical study. We evaluated 67 children and adolescents with CF, who were under follow-up and were consecutively recruited at a referral center for the treatment of CF, between November of 2006 and April of 2008. Patients self-reported their skin color as being white, black, light brown, medium brown, or dark brown.[9] A diagnosis of CF was confirmed by at least two abnormal sweat chloride results.

The study involved male and female patients aged 2 to 14 years who agreed to participate and whose parents or legal guardians gave written informed consent. The exclusion criteria were as follows: having undergone oral or intravenous antibiotic therapy in the month prior to the study's outset and having experienced pulmonary exacerbation in that period; having comorbidities, such as diabetes mellitus, gastroesophageal reflux disease, sleep-disordered breathing, genetic disease, primary cardiac disease, neuromuscular disease, craniofacial anomalies, psychiatric disease, and Down syndrome; being on enteral or parenteral

feeding, antidepressants, hypnotics, and home oxygen therapy; and having undergone lung transplantation.

The following instruments were used for clinical and functional assessment of the patients.

Disease severity was assessed by the Shwachman-Kulczycki (S-K) score, which is based on four major criteria—general activity, nutrition, radiological findings, and physical examination. The overall score ranges from 20 to 100, with patient's status being classified as excellent (score, 86-100), good (score, 71-85), average (56-70), poor (score, 41-55), or severe (score, ≤ 40).[10]

For assessment of nutritional status, height and weight were measured, and the values obtained were compared with data from the United States National Center for Health Statistics and converted to z scores for weight/age, height/age, and weight/height, on the basis of age and gender, by using Epi Info software program, version 3.4.1; malnutrition was defined as weight z scores below two standard deviations. In addition, we evaluated body mass index, calculated as weight in kilograms divided by height in meters squared (kg/m^2).[11]

Overnight polysomnography was performed in a standardized fashion with the use of a computerized system (BrainNet BNTÒ; LYNX Tecnologia Eletrônica, Rio de Janeiro, Brazil), which included electroencephalography, electro-oculography, leg and mentalis electromyography, measurement of airflow with an oronasal thermistor and a nasal cannula, and pulse oximetry (Onyx® II 9650 Bluetooth; Nonim Medical Inc., Plymouth, MN, USA). We estimated SpO_2 as the mean of at-rest values obtained over a five-minute period, with the patient being in a sitting position and awake, at initiation of the sleep study. A desaturation event was defined as a decrease of 4% or more in SpO_2. Mean and minimum SpO_2 were determined. Analysis of sleep stages, analysis of microarousals, and scoring of respiratory events during sleep were performed in accordance with the recommendations of the American Academy of Sleep Medicine.[12] Although controversy surrounds the definition of nocturnal hypoxemia, we chose to define it as an $SpO_2 < 90\%$ for more than 5% of TST, with a nadir of at least 85%; these values were based on previous investigations in patients with CF.[8]

Spirometry was performed with a Microlab Spirometer 3500K, version 5.X.X Carefusion (Micro Medical Ltd., Rochester, United Kingdom), in accordance with the criteria established by the American Thoracic Society[13] and the Brazilian Thoracic Association,[14] in patients ≥ 6 years of age. The parameters studied were FVC, FEV_1, and $FEF_{25-75\%}$, expressed as a percentage of predicted for gender, age, and height, in accordance with the Knudson et al. equation for children and adolescents.[15]

To build the database and perform the statistical calculations, we used the Statistical Package for the Social Sciences, version 13.0 (SPSS Inc., Chicago, IL, USA).[16] Continuous variables are expressed as median (interquartile range) or mean (standard deviation), whereas categorical variables are expressed as frequency and proportion. The chi-square test and Fisher's exact test were used for the comparison of proportions. Means were compared by the Student's t-test (for variables with normal distribution) or the Mann-Whitney test (for variables without normal distribution). Correlations of TST spent at an $SpO_2 < 90\%$ with pulmonary function variables and polysomnography variables were calculated by Spearman's linear correlation coefficient (r_s), which measures the degree of association between two numerical variables. This coefficient ranges from -1 to 1; values closer to −1 or 1 indicate a stronger association between the two variables, and values closer to zero indicate a weaker relationship. Values of $p < 0.05$ were considered statistically significant.

The present study was approved by the Research Ethics Committee of the Oswaldo Cruz Foundation (Ruling no. 119/2007). Polysomnography was performed during spontaneous sleep, and parents remained with their children during the procedure. The present study did not contravene the ethical precepts regarding research. All of the participants received verbal and written information on the nature and purpose of the study and gave written informed consent.

Results

The Referral Center for Cystic Fibrosis of the Octávio Mangabeira Specialized Hospital follows 200 adult and pediatric patients. We interviewed 85 pediatric patients with CF in order to possibly involve them in the study, and, of the 74 who were considered eligible, only 67 agreed

to participate in the study. The study sample consisted of clinically stable children, most of whom were male and self-reported their skin color as being black or brown. The demographic data of the study population are shown in Table 1.

The means of mean SpO_2 and of SpO_2 nadir, during sleep, were 94.3 ± 2.1% and 81.2 ± 5.9%, respectively. Maximum sleep time spent at an SpO_2 < 90% was 221 minutes (53% of TST), and only one patient had an SpO_2 ≤ 80% for 11 minutes (4.5% of TST). We found that 18 patients (26.9%) had an SpO_2 < 85% during sleep and that only 11 (16.4%) did not have any nocturnal desaturation events.

The patients were divided into two groups on the basis of their having or not having the outcome of interest, i.e., nocturnal hypoxemia, which was defined as an SpO_2 < 90% for more than 5% of TST, with a nadir of at least 85%. The group with nocturnal hypoxemia consisted of 4 patients (6%, Table 2). The two groups were compared regarding clinical characteristics, demographic characteristics, and polysomnography findings. Analysis of the z scores for weight/age, height/age, and weight/height revealed no statistically significant differences between the groups. However, FEV_1, FVC, and S-K score values were significantly lower in the patients with nocturnal hypoxemia than in those without nocturnal hypoxemia (Table 2).

A comparison of the two groups regarding polysomnography findings showed that the group with nocturnal hypoxemia spent a greater proportion of time in stage R sleep; however, all other sleep architecture parameters were similar between the groups. Nine patients (13.4%) had an apnea-hypopnea index (AHI) ≥ 5, and mean AHI was found to be higher in the group with nocturnal hypoxemia than in the group without nocturnal hypoxemia (12.8 ± 11.4 vs. 3.7 ± 3.1), although not statistically significantly so (Table 2).

According to Spearman's correlation coefficient, the period of time with SpO_2 < 90% for more than 5% of TST correlated significantly with FEV_1 (r_s = –0.42; p = 0.01), FVC (r_s = –0.46; p = 0.01), microarousal index (r_s = 0.32; p = 0.01), and AHI (r_s = 0.56; p = 0.01; Figure 1).

Table 1 - Demographic and clinical data of the 67 cystic fibrosis patients studied.[a]

Characteristic	Result
Male gender	38 (56.7)
Age, years[b]	8 (5-10)
Black or brown skin color	54 (80.6)
z score for weight/age[b]	–0.54 (–1.3 to 0.2)
z score for height/age[b]	–0.50 (–1.0 to 0.5)
BMI percentile[b]	34 (11-64)
Total S-K score[c]	85.6 ± 9.1
FEV_1, % of predicted[b]	78.5 (67.0-92.8)

BMI: body mass index; and S-K score: Shwachman-Kulczycki score. [a]Values expressed as n (%), except where otherwise indicated. [b]Values expressed as median (interquartile range). [c]Values expressed as mean ± SD.

Discussion

In the present study, 6% of the 67 clinically stable children with CF were shown to have hypoxemia during sleep, which was characterized as an SpO_2 < 90% for more than 5% of TST, whereas 26.9% of the children studied had significant nocturnal desaturation events (SpO_2 < 85%). These events were found to be associated with lower S-K scores, lower FVC (% of predicted), and lower FEV_1 (% of predicted), as well as with a greater proportion of time spent in stage R sleep. There was no association between these events and nutritional status. We found that the period of time with SpO_2 < 90% for more than 5% of TST correlated negatively with FVC (% of predicted) and FEV_1 (% of predicted) and positively with microarousal index and AHI.

Uyan et al.[17] evaluated children aged 8 to 12 years with CF and mild or moderate lung disease and reported higher mean values for mean and minimum SpO_2 than those observed in our study (96.1 ± 1.3% and 88.9 ± 3.9% vs. 94.3 ± 2.1% and 81.2 ± 5.9%, respectively). We studied patients aged 2 to 14 years and observed intermittent and frequent drops in SpO_2, even in younger individuals with normal or slightly abnormal pulmonary function test results. Of the 67 patients studied, 56 (83.6%) had desaturation during sleep, most of whom had more than six desaturation events during the monitoring period.

Villa et al.,[4] who studied young children with CF, showed that even those with mild lung disease had nocturnal oxyhemoglobin desaturation. Oxyhemoglobin desaturation has been reported to be more prevalent in patients with CF and severe lung disease, but there is limited information on nocturnal SpO_2 in patients with CF and milder lung disease.[8] Castro-Silva et al.[18] studied 30 patients with CF and clinically significant lung disease and compared them

Table 2 - Comparisons of demographic, clinical, functional, and polysomnography characteristics in relation to total sleep time spent at an SpO_2 < 90% in the patients studied.[a]

Characteristic	Hypoxemia during sleep[b]		p*
	Yes	No	
	(n = 4)	(n = 63)	
Age, months	99.75 ± 61.52	93.01 ± 37.14	0.74
Hospitalizations in the last year	1.25 ± 1.89	0.63 ± 1.21	0.34
Episodes of pneumonia in the last year	1.00 ± 1.89	0.75 ± 1.16	0.67
S-K score	73.75 ± 6.29	86.38 ± 8.70	< 0.01
Z score for height/age	−0.19 ± 2.16	−0.28 ± 1.27	0.89
Z score for weight/age	−1.19 ± 2.41	−0.48 ± 1.12	0.60
Z score for weight/height	−0.91 ± 1.56	−0.24 ± 0.92	0.19
Z score for BMI	−0.78 ± 1.83	−0.45 ± 1.35	0.64
FVC, % of predicted	43.00 ± 21.21	81.14 ± 17.31	< 0.01
FEV_1, % of predicted	49.00 ± 26.87	79.59 ± 17.37	0.02
$FEF_{25-75\%}$, % of predicted	44.00 ± 18.38	72.19 ± 25.81	0.14
Sleep efficiency, %	84.00 ± 6.22	80.75 ± 11.48	0.58
Latency to sleep onset, min	16.75 ± 11.70	25.98 ± 34.55	0.60
Latency to REM sleep, min	100.50 ± 51.86	171.07 ± 111.78	0.22
Microarousal index, per hour of sleep	9.25 ± 6.40	6.71 ± 2.99	0.49
TST spent in stage N1, %	6.00 ± 3.27	7.56 ± 4.00	0.45
TST spent in stage N2, %	40.75 ± 7.41	39.63 ± 11.47	0.42
TST spent in stage N3, %	20.50 ± 13.63	21.63 ± 6.53	0.88
TST spent in REM sleep, %	21.75 ± 1.71	14.68 ± 7.70	< 0.0001
AHI	12.75 ± 11.35	3.73 ± 3.09	0.21

S-K score: Shwachman-Kulczycki score; BMI: body mass index; REM: rapid eye movement; TST: total sleep time; N: non-REM sleep; and AHI: apnea-hypopnea index. [a]Values expressed as mean ± SD. [b]Hypoxemia during sleep was defined as an SpO_2 < 90% for more than 5% of TST. *Student.'s t-test

with 10 patients with CF and less severe lung disease and with 20 controls. The mean ages in the three groups were 12.8 years, 13.3 years, and 15.5 years, respectively. In the group with clinically significant lung disease, 5 patients (15%) had an SaO_2 < 90% for more than 30% of TST, and 11 (36.6%) had an SaO_2 nadir < 85%. That study found no differences in sleep macrostructure between the group with clinically significant lung disease and the group with mild lung disease. Unlike our study, which evaluated a younger population, that study observed greater nocturnal desaturation, and it is possible that these findings indicate a population with longer disease duration and greater involvement of the respiratory tract.

One group of authors[5] compared young adult CF patients with and without nocturnal desaturation and observed that those with desaturation experienced a decrease in SaO_2 and an increase in $PaCO_2$ during sleep, as well as decrease in FEV_1, relative to those without desaturation. Perin et al.[19] showed similar findings, because, in the comparison of patients with and without nocturnal desaturation, there were significantly lower values for FEV_1 (46.4 ± 13.6% vs. 66.9 ± 23.0%), FVC (35.8 ± 11.4% vs. 79.6 ± 19.1%), the S-K score (62.7 ± 9.0 vs. 73.9 ± 11.8), and daytime SpO_2 (92.2 ± 1.3% vs. 96.4 ± 1.2%), these data being similar to our findings. Those authors also observed that, among the patients with desaturation, there was a greater proportion of pulmonary hypertension. There have been reports of significant repercussions of nocturnal hypoxemia on the pulmonary circulation, including arterial remodeling and increased vascular resistance, thereby resulting in right ventricular dysfunction and cor pulmonale in these patients.[1]

The present study found negative correlations between nocturnal hypoxemia and pulmonary function values, revealed by a reduction in FVC (% of predicted) and in FEV_1 (% of predicted). The effects of the severity of lung disease on sleep have been reported in previous studies[20,21]; however, only patients with moderate or severe

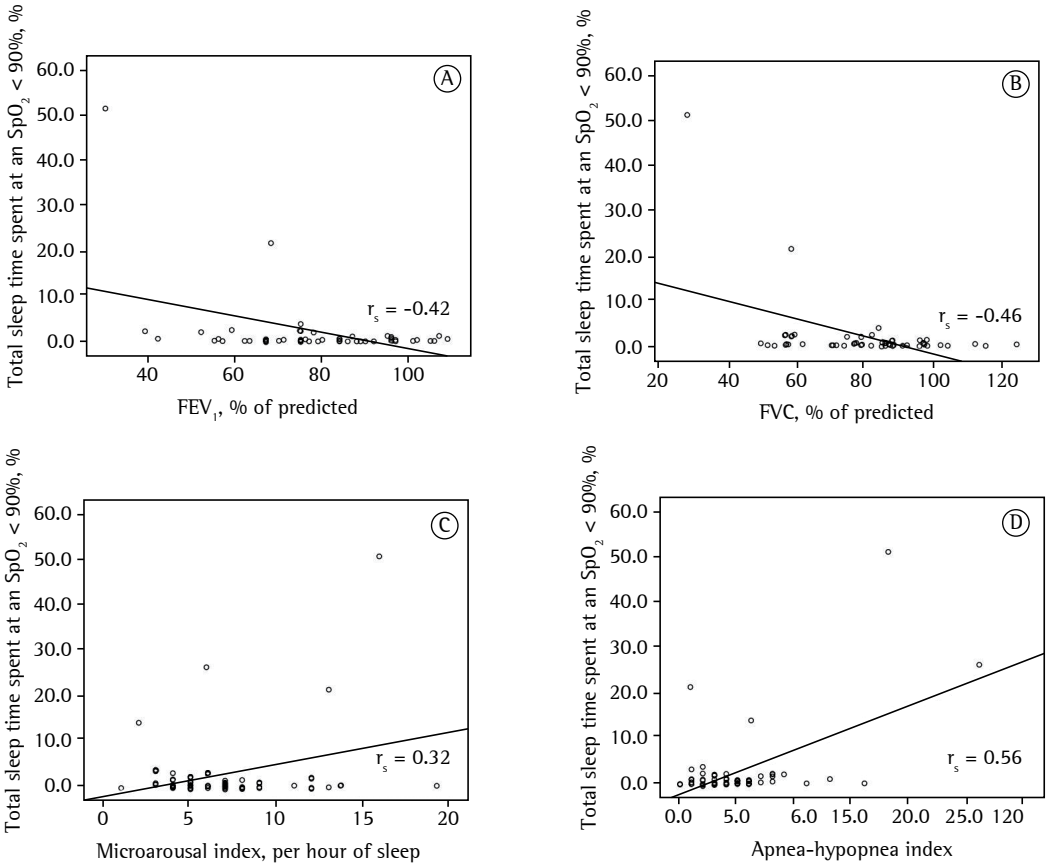

Figure 1 - Representation of the correlations between the proportion of total sleep time spent at an SpO_2 < 90% and the following: FEV_1 (% of predicted; in A); FVC (% of predicted; in B); microarousal index (per hour of sleep; in C); and apnea-hypopnea index (in D).

disease were studied. The FEV_1 variable has been proposed as a simple parameter to identify patients at risk for nocturnal desaturation.[22]

In the present study, the patients who had hypoxemia during sleep spent a greater proportion of time in stage R sleep. Hypoventilation has been reported in patients with CF during this stage of sleep,[23] and this might explain our finding; it is possible that the occurrence of a large number of apnea and hypopnea events during this stage of sleep, which are associated with hypoventilation, contributed to increased sleep fragmentation. In addition, we found a positive correlation between nocturnal hypoxemia and the number of microarousals; therefore, nocturnal desaturation would be related to lower sleep efficiency, a finding that is supported by data published in other studies.[1,5] Amin et al.[20] reported that, in their study sample, children with CF had lower sleep efficiency and more frequent awakenings than did those without CF. However, the literature offers conflicting results regarding abnormalities in sleep macrostructure in patients with CF.[24,25]

Another important finding of the present study was that 13.4% of the patients studied had obstructive sleep apnea syndrome (OSAS), a prevalence that is higher than the data reported in the literature.[17,18] In addition to being a major cause of nocturnal sleep fragmentation, OSAS might be associated with hypoxemia during sleep in patients with CF.[25] Spicuzza et al.,[26] who studied a population of children with CF and a mean age similar to that of our study population, found that 28 (70%) of 40 children had mild-to-moderate OSAS (defined as an AHI > 2). In a previous study of a similar patient population, Ramos et al.[27] showed that the patients with OSAS had signs of chronic rhinosinusitis. In the present study, we found that nasal polyposis, which is a

characteristic of chronic rhinosinusitis, was more common in patients with hypoxemia during sleep than in those without (p = 0.05), as was OSAS; however, regarding OSAS, there was no statistical significance between the groups (p = 0.21). We found a positive correlation between nocturnal hypoxemia and AHI (r_s = 0.56). It is possible that OSAS in these patients contributed to the frequent sleep disruptions; data in the literature show that sleep fragmentation can adversely affect many aspects of children's lives and of their behavioral and neurocognitive development.[28,29] However, Naqvi et al.,[30] who evaluated children and adolescents with CF, observed that the severity of lung disease was associated with the severity of sleep disruption.

The strength of our study included the evaluation of clinical stable patients in order to limit variability in nocturnal oxyhemoglobin desaturation, which occurs during CF exacerbations, and the use of laboratory polysomnography, which allowed the observation of abnormalities in sleep architecture. However, there are limitations that need to be considered. The study was cross-sectional in design, which precludes causal inferences. In addition, we identified the need for a larger sample in order to correct all potential confounding factors, as well as for a balance between the number of patients with and the number of patients without hypoxemia during sleep in order to provide power to the study.

In conclusion, our study showed that nocturnal hypoxemia is common in clinically stable children and adolescents with CF, being associated with abnormalities in pulmonary function and sleep macrostructure, as well as with OSAS. Future studies are needed to evaluate the effects of nocturnal hypoxemia on clinical outcomes; our findings draw attention to the need to include nocturnal polysomnography and cardiorespiratory monitoring in the routine evaluation of patients with CF, as well as to prescribe nocturnal home oxygen therapy.

References

1. Urquhart DS, Montgomery H, Jaffé A. Assessment of hypoxia in children with cystic fibrosis. Arch Dis Child. 2005;90(11):1138-43. http://dx.doi.org/10.1136/adc.2005.071795 PMid:16243867 PMCid:PMC1720198
2. Fauroux B. Why, when and how to propose noninvasive ventilation in cystic fibrosis? Minerva Anestesiol. 2011;77(11):1108-14. PMid:21602746
3. Ballard RD, Sutarik JM, Clover CW, Suh BY. Effects of non-REM sleep on ventilation and respiratory mechanics in adults with cystic fibrosis. Am J Respir Crit Care Med. 1996;153(1):266-71. http://dx.doi.org/10.1164/ajrccm.153.1.8542127 PMid:8542127
4. Villa MP, Pagani J, Lucidi V, Palamides S, Ronchetti R. Nocturnal oximetry in infants with cystic fibrosis. Arch Dis Child. 2001;84(1):50-4. http://dx.doi.org/10.1136/adc.84.1.50 PMid:11124784 PMCid:PMC1718605
5. Young AC, Wilson JW, Kotsimbos TC, Naughton MT. The impact of nocturnal oxygen desaturation on quality of life in cystic fibrosis. J Cyst Fibros. 2011;10(2):100-6. http://dx.doi.org/10.1016/j.jcf.2010.11.001 PMid:21195036
6. Fraser KL, Tullis DE, Sasson Z, Hyland RH, Thornley KS, Hanly PJ. Pulmonary hypertension and cardiac function in adult cystic fibrosis: role of hypoxemia. Chest. 1999;115(5):1321-8. http://dx.doi.org/10.1378/chest.115.5.1321 PMid:10334147
7. Borriello G, Werner E, Roe F, Kim AM, Ehrlich GD, Stewart PS. Oxygen limitation contributes to antibiotic tolerance of Pseudomonas aeruginosa in biofilms. Antimicrob Agents Chemother. 2004;48(7):2659-64. http://dx.doi.org/10.1128/AAC.48.7.2659-2664.2004 PMid:15215123 PMCid:PMC434183
8. Frangolias DD, Wilcox PG. Predictability of oxygen desaturation during sleep in patients with cystic fibrosis: clinical, spirometric, and exercise parameters. Chest. 2001;119(2):434-41. http://dx.doi.org/10.1378/chest.119.2.434
9. Lessa I, Magalhães L, Araújo MJ, de Almeida Filho N, Aquino E, Oliveira MM. Arterial hypertension in the adult population of Salvador (BA)--Brazil. Arq Bras Cardiol. 2006;87(6):747-56. http://dx.doi.org/10.1590/S0066-782X2006001900011 PMid:17262113
10. Shwachman H, Kulczycki LL. Long-term study of one hundred five patients with cystic fibrosis; studies made over a five- to fourteen-year period. AMA J Dis Child. 1958;96(1):6-15. http://dx.doi.org/10.1001/archpedi.1958.02060060008002
11. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. Ann Arbor: University of Michigan Press; 1990. p. 31-42.
12. Iber C, Ancoli-Israel S, Chesson A Jr, Quan S, editors. The AASM Manual for the scoring of sleep and associated events: rules, terminology and technical specifications. Westchester, IL: American Academy of Sleep Medicine; 2007 PMCid:PMC2134956
13. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36. http://dx.doi.org/10.1164/ajrccm.152.3.7663792 PMid:7663792
14. Sociedade Brasileira de Pneumologia e Tisiologia. Diretrizes para Teste de Função Pulmonar. J Pneumol. 2002;28(Suppl 3):S1-S238.
15. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and aging. Am Rev Respir Dis. 1983;127(6):725-34. PMid:6859656
16. IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Version 13.0. Armonk, NY: IBM Corp.
17. Uyan ZS, Ozdemir N, Ersu R, Akpinar I, Keskin S, Cakir E, et al. Factors that correlate with sleep oxygenation in children with cystic fibrosis. Pediatr Pulmonol. 2007;42(8):716-22. http://dx.doi.org/10.1002/ppul.20643 PMid:17595040
18. de Castro-Silva C, de Bruin VM, Cavalcante AG, Bittencourt LR, de Bruin PF. Nocturnal hypoxia and sleep disturbances

in cystic fibrosis. Pediatr Pulmonol. 2009;44(11):1143-50. http://dx.doi.org/10.1002/ppul.21122 PMid:19824056
19. Perin C, Fagondes SC, Casarotto FC, Pinotti AF, Menna Barreto SS, Dalcin Pde T. Sleep findings and predictors of sleep desaturation in adult cystic fibrosis patients. Sleep Breath. 2012;16(4):1041-8. http://dx.doi.org/10.1007/s11325-011-0599-5 PMid:21948072
20. Amin R, Bean J, Burklow K, Jeffries J. The relationship between sleep disturbance and pulmonary function in stable pediatric cystic fibrosis patients. Chest. 2005;128(3):1357-63. http://dx.doi.org/10.1378/chest.128.3.1357 PMid:16162729
21. Jankelowitz L, Reid KJ, Wolfe L, Cullina J, Zee PC, Jain M. Cystic fibrosis patients have poor sleep quality despite normal sleep latency and efficiency. Chest. 2005;127(5):1593-9. http://dx.doi.org/10.1378/chest.127.5.1593 PMid:15888833
22. Fauroux B, Pepin JL, Boelle PY, Cracowski C, Murris-Espin M, Nove-Josserand R, et al. Sleep quality and nocturnal hypoxaemia and hypercapnia in children and young adults with cystic fibrosis. Arch Dis Child. 2012;97(11):960-6. http://dx.doi.org/10.1136/archdischild-2011-300440 PMid:22984185
23. Milross MA, Piper AJ, Norman M, Willson GN, Grunstein RR, Sullivan CE, et al. Night-to-night variability in sleep in cystic fibrosis. Sleep Med. 2002;3(3):213-9. http://dx.doi.org/10.1016/S1389-9457(02)00030-8
24. Dancey DR, Tullis ED, Heslegrave R, Thornley K, Hanly PJ. Sleep quality and daytime function in adults with cystic fibrosis and severe lung disease. Eur Respir J. 2002;19(3):504-10. http://dx.doi.org/10.1183/09031936.02.00088702 PMid:11936530
25. Milross MA, Piper AJ, Dobbin CJ, Bye PT, Grunstein RR. Sleep disordered breathing in cystic fibrosis. Sleep Med Rev. 2004;8(4):295-308. http://dx.doi.org/10.1016/j.smrv.2004.03.004 PMid:15233957
26. Spicuzza L, Sciuto C, Leonardi S, La Rosa M. Early occurrence of obstructive sleep apnea in infants and children with cystic fibrosis. Arch Pediatr Adolesc Med. 2012;166(12):1165-9. http://dx.doi.org/10.1001/archpediatrics.2012.1177 PMid:23090511
27. Ramos RT, Salles C, Gregório PB, Barros AT, Santana A, Araújo-Filho JB, et al. Evaluation of the upper airway in children and adolescents with cystic fibrosis and obstructive sleep apnea syndrome. Int J Pediatr Otorhinolaryngol. 2009;73(12):1780-5. http://dx.doi.org/10.1016/j.ijporl.2009.09.037 PMid:19854521
28. Halbower AC, Degaonkar M, Barker PB, Earley CJ, Marcus CL, Smith PL, et al. Childhood obstructive sleep apnea associates with neuropsychological deficits and neuronal brain injury. PLoS Med. 2006;3(8):e301. http://dx.doi.org/10.1371/journal.pmed.0030301 PMid:16933960 PMCid:PMC1551912
29. Giordani B, Hodges EK, Guire KE, Ruzicka DL, Dillon JE, Weatherly RA, et al. Changes in neuropsychological and behavioral functioning in children with and without obstructive sleep apnea following Tonsillectomy. J Int Neuropsychol Soc. 2012;18(2):212-22. http://dx.doi.org/10.1017/S1355617711001743 PMid:22272653
30. Naqvi SK, Sotelo C, Murry L, Simakajornboon N. Sleep architecture in children and adolescents with cystic fibrosis and the association with severity of lung disease. Sleep Breath. 2008;12(1):77-83. http://dx.doi.org/10.1007/s11325-007-0123-0 PMid:17610099

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Can the single-breath helium dilution method predict lung volumes as measured by whole-body plethysmography?*

Pode o método de diluição do hélio em respiração única estimar os volumes pulmonares medidos pela pletismografia de corpo inteiro?*

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Abstract

Objective: To compare TLC and RV values obtained by the single-breath helium dilution (SBHD) method with those obtained by whole-body plethysmography (WBP) in patients with normal lung function, patients with obstructive lung disease (OLD), and patients with restrictive lung disease (RLD), varying in severity, and to devise equations to estimate the SBHD results. **Methods:** This was a retrospective cross-sectional study involving 169 individuals, of whom 93 and 49 presented with OLD and RLD, respectively, the remaining 27 having normal lung function. All patients underwent spirometry and lung volume measurement by both methods. **Results:** TLC and RV were higher by WBP than by SBHD. The discrepancy between the methods was more pronounced in the OLD group, correlating with the severity of airflow obstruction. In the OLD group, the correlation coefficient of the comparison between the two methods was 0.57 and 0.56 for TLC and RV, respectively ($p < 0.001$ for both). We used regression equations, adjusted for the groups studied, in order to predict the WBP values of TLC and RV, using the corresponding SBHD values. It was possible to create regression equations to predict differences in TLC and RV between the two methods only for the OLD group. The TLC and RV equations were, respectively, $\Delta TLC_{WBP-SBHD}$ in L = 5.264 – 0.060 × FEV_1/FVC ($r^2 = 0.33$; adjusted $r^2 = 0.32$) and $\Delta RV_{WBP-SBHD}$ in L = 4.862 – 0.055 × FEV_1/FVC ($r^2 = 0.31$; adjusted $r^2 = 0.30$). **Conclusions:** The correction of TLC and RV results obtained by SBHD can improve the accuracy of this method for assessing lung volumes in patients with OLD. However, additional studies are needed in order to validate these equations.

Keywords: Plethysmography, whole body; Total lung capacity; Residual volume.

Resumo

Objetivo: Comparar resultados de CPT e VR obtidos pelo método de diluição de hélio em respiração única (DHRU) com aqueles obtidos por pletismografia de corpo inteiro (PCI) em indivíduos com função pulmonar normal, portadores de distúrbio ventilatório obstrutivo (DVO) e portadores de distúrbio ventilatório restritivo (DVR) com diferentes níveis de gravidade e elaborar equações para estimar CPT e VR por DHRU. **Métodos:** Estudo transversal retrospectivo com 169 indivíduos, dos quais, respectivamente, 93, 49 e 27 apresentavam DVO, DVR e espirometria normal. Todos realizaram espirometria e determinação de volumes pulmonares pelos dois métodos. **Resultados:** Os valores de CPT e VR foram maiores por PCI que por DHRU. A discrepância entre os métodos foi mais acentuada no grupo com DVO e se relacionou com a gravidade da obstrução ao fluxo aéreo. No grupo com DVO, o coeficiente de correlação da comparação entre os dois métodos foi de 0,57 e 0,56 para CPT e VR, respectivamente ($p < 0,001$ para ambos). Para predizer os valores de CPT e VR por PCI utilizando os respectivos valores por DHRU foram utilizadas equações de regressão, corrigidas de acordo com os grupos estudados. Somente foi possível criar equações de regressão para predizer as diferenças de CPT e VR entre os dois métodos para pacientes com DVO. Essas equações foram, respectivamente, $\Delta CPT_{PCI-DHRU}$ em L = 5,264 – 0,060 × VEF_1/CVF ($r^2 = 0,33$; r^2 ajustado = 0,32) e $\Delta VR_{PCI-DHRU}$ em L = 4,862 – 0,055 × VEF_1/CVF ($r^2 = 0,31$; r^2 ajustado = 0,30). **Conclusões:** A correção de CPT e VR obtidos por DHRU pode melhorar a acurácia desse método para avaliar os volumes pulmonares em pacientes com DVO. Entretanto, estudos adicionais para validar essas equações são necessários.

Descritores: Pletismografia total; Capacidade pulmonar total; Volume residual.

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Introduction

Lung volume measurements are useful for characterizing the severity of respiratory diseases, evaluating the results of interventions, and determining prognosis.[1,2] Patients with restrictive lung disease (RLD) can present with reduced TLC, whereas those with chronic obstructive lung disease (OLD) can present with increased TLC (usually as a result of increased RV).[3] Various methods can be used in order to determine lung volumes, including whole-body plethysmography (WBP), gas dilution methods, and radiological techniques.[4-8] Currently, WBP and the multiple-breath helium dilution method are used in pulmonary function laboratories in order to measure TLC and RV. Any of the abovementioned methods can be used provided that the equipment and maneuvers are in accordance with the recommended technical standards.[8,9] However, WBP is considered the gold standard by some researchers.[10]

The single-breath helium dilution (SBHD) method is a simpler, cheaper, and more widely available alternative method for evaluating alveolar volume with equipment that is less complex than a plethysmograph, having the operational advantage of being performed in conjunction with DLCO determination. However, because the SBHD method depends on the air that is mobilized during a single respiratory maneuver, it can underestimate lung volumes when compared with WBP in patients with a markedly uneven distribution of ventilation. [11,12] Although determination of alveolar volume by the SBHD method for measuring DLCO is used worldwide, the value of this technique in determining lung volumes in patients with OLD or RLD has yet to be established.

In view of the abovementioned considerations, the objective of the present study was to compare lung volumes as measured by the SBHD method with lung volumes as measured by WBP in individuals with normal lung function, as well as in patients with OLD or RLD of varying degrees of severity. In addition, we sought to develop equations to estimate TLC and RV as measured by WBP (TLC_{WBP} and RV_{WBP}, respectively) on the basis of TLC and RV as measured by the SBHD method (TLC_{SBHD} and RV_{SBHD}, respectively) and adjusted for the degree of airflow obstruction.

Methods

This was a retrospective cross-sectional study involving 142 consecutive patients who underwent spirometry, static lung volume measurements by WBP, and single-breath pulmonary diffusing capacity measurements in the pulmonary physiology laboratory of a referral hospital. We selected patients with OLD caused by COPD and patients in whom spirometry results were suggestive of RLD. We excluded patients whose spirometry results showed mixed obstructive and restrictive lung disease, indeterminate lung disease, or OLD of causes other than COPD. The control group (n = 27) comprised 8 patients (6 of whom were smokers) who had normal spirometry results and who had undergone WBP and pulmonary diffusing capacity measurements in routine care, as well as 19 volunteers who were recruited from the community, who were nonsmokers, who had no respiratory complaints or diseases, who had normal chest X-rays, and who had undergone the three tests in the same period in order to determine whether the reference standards used in the laboratory were appropriate. The ethical and methodological aspects of the study project were approved by the local research ethics committee.

We collected data regarding age (years), gender, weight (kg), height (cm), body mass index (kg/m^2), and smoking history (pack-years). All pulmonary function tests were performed with a MasterScreen Body spirometer (Jäeger, Würzburg, Germany) and were in accordance with the technical recommendations in the Brazilian Thoracic Association guidelines for pulmonary function testing.[9] Patients underwent spirometry, WBP, and DLCO testing always in the same sequence and at the same time of day. All tests were performed before and after the administration of 400 µg of albuterol. We calculated TLC_{SBHD} by summing the alveolar volume as measured by DLCO testing and the anatomical dead space. We calculated RV_{SBHD} by subtracting FVC from TLC_{SBHD}. The gases and plethysmograph were calibrated daily before test initiation. The reference values for spirometry, lung volumes, and DLCO were those described elsewhere.[13-15]

In order to confirm the diagnosis of COPD by spirometry, we used an FEV_1/FVC ratio after bronchodilator use ≤ 0.70.[16] The severity of airflow obstruction was determined on the basis of FEV_1, as recommended by the American Thoracic Society.[17] Mild to moderate OLD was defined as an $FEV_1 \geq 50\%$ of the predicted value; severe OLD was defined as an FEV_1 of 35-49% of the predicted value; and very severe OLD was defined

as an FEV_1 < 35% of the predicted value. Only 5 patients presented with mild OLD. Therefore, they were evaluated in conjunction with those who presented with moderate OLD.

For the diagnosis of RLD, we used the following criteria: an FEV_1/FVC ratio after bronchodilator use > 0.80; reduced VC (an FVC < 80% of the predicted value); and reduced TLC (a TLC < 80% of the predicted value). For data analysis, we used the median in order to divide the patients with RLD into two groups according to the severity of the disease (a TLC > 72% of the predicted value indicating less severe disease and a TLC < 72% of the predicted value indicating more severe disease).

Statistical analysis was performed with the Statistical Analysis System software, version 9.1 (SAS Institute, Cary, NC, USA). The collected data were expressed as mean, SE, and 95% CI. For the evaluation of variables with one observation, one-way ANOVA was performed to determine the differences among the groups. Whenever Levene's test revealed heterogeneity of variance, Welch's ANOVA was performed. For complementation of results, Tukey's test was performed. The possible influence of the covariates gender, age, weight, height, body mass index, and smoking history was tested by analysis of covariance. For the evaluation of variables for each lung function method, mixed-model ANOVA was performed. For complementation of significant effects in relation to the method and group, the Tukey-Kramer test was performed ($p < 0.05$). The goodness of fit of the model was tested by analysis of residuals and determination of normality by the Kolmogorov-Smirnov test ($p > 0.01$), the Anderson-Darling test, and the Cramér-von Mises test ($p > 0.005$). In both models, we obtained partial or residual Pearson correlations, eliminating the effect of factors in order to determine the association between variables.

Bland-Altman plots[18] were used in order to determine the differences in TLC and RV between the two methods. The limits of agreement were calculated as ± 1.96 SDs of the differences. We developed prediction equations to estimate the differences in TLC and RV between the two methods by means of stepwise multiple linear regression, variables with a value of $p < 0.10$ being included in the models. For all analyses, we used the values obtained after bronchodilator use.

Results

In the present study, 169 individuals underwent spirometry and lung volume measurements by WBP and the SBHD method in an open system. Of the 169 individuals, 27 had normal spirometry results, 93 had OLD, and 49 had RLD. The patients with OLD were stratified as follows: patients with mild to moderate OLD (29 patients); patients with severe OLD (29 patients); and patients with very severe OLD (35 patients). The patients with RLD were stratified as follows: patients with less severe disease (25 patients); and patients with more severe disease (24 patients).

Anthropometric data, duration of smoking, smoking history, and pulmonary function test results, stratified by lung function status, are presented in Table 1.

Figure 1 shows mean TLC and RV (in absolute values and in percentage of predicted). In all groups, TLC_{WBP} and RV_{WBP} values were higher than TLC_{SBHD} and RV_{SBHD} values ($p < 0.01$), the discrepancy between the two methods being most pronounced in the OLD group and the difference increasing progressively as the severity of airflow obstruction increased. A comparison between the RV/TLC ratio obtained by WBP and the RV/TLC ratio obtained by the SBHD method provided further evidence of the discrepancy between the two methods. In the individuals with normal lung function, those ratios were 0.36 and 0.29, respectively. In the group of patients with mild to moderate OLD, those ratios were 0.53 and 0.38, respectively; in the group of patients with severe OLD, those ratios were 0.60 and 0.43, respectively; and in the group of patients with very severe OLD, those ratios were 0.66 and 0.47, respectively. In the group of patients with less severe RLD, those ratios were 0.44 and 0.32, respectively, and in the group of patients with more severe RLD, those ratios were 0.45 and 0.39, respectively.

Table 2 shows the differences in TLC and RV between WBP and the SBHD method (in absolute values and in percentage of predicted) in the groups studied. In the groups of patients with RLD and normal spirometry results, the difference in TLC between the two methods ranged from 0.61 L to 0.80 L (from 10.8% of the predicted value to 13.1% of the predicted value; $p > 0.05$). In addition, the difference in RV between the two methods ranged from 0.52 L to 0.75 L (from 30.8% of the predicted value to

Table 1 - Anthropometric characteristics, smoking history, and functional parameters in 169 individuals stratified by lung function status.

Variable	Lung function status					
	Normal	Obstructive lung disease			Restrictive lung disease	
		Mild/ moderate	Severe	Very severe	Less severe	More severe
	(n = 27)	(n = 29)	(n = 29)	(n = 35)	(n = 25)	(n = 24)
Age, years	46.40 ± 13.70[b]	64.90 ± 8.88[a]	70.10 ± 6.62[a]	65.80 ± 8.78[a]	47.30 ± 15.90[b]	54.42 ± 21.36[b]
Weight, kg	66.60 ± 16.13[a]	71.20 ± 13.21[a]	68.40 ± 12.95[a]	66.60 ± 13.94[a]	70.50 ± 9.90[a]	66.53 ± 14.71[a]
Height, cm	161.00 ± 11.28[a]	161.00 ± 9.20[a]	163.00 ± 8.63[a]	166.00 ± 9.09[a]	1.62 ± 6.69[a]	1.63 ± 8.32[a]
BMI, kg/m^2	25.50 ± 6.23[a]	27.30 ± 4.46[a]	25.70 ± 3.37[a]	24.20 ± 4.03[a]	27.10 ± 4.14[a]	25.08 ± 5.16[a]
Duration of smoking, years	5.89 ± 11.50[b]	41.86 ± 12.01[a]	44.86 ± 9.17[a]	36.40 ± 12.56[a]	10.60 ± 13.37[b]	12.17 ± 17.57[b]
Smoking history, pack-years	1.27 ± 2.10[b]	62.20 ± 37.35[a]	59.00 ± 38.69[a]	54.70 ± 35.34[a]	12.30 ± 17.86[b]	20.98 ± 43.25[b]
FVC, L	3.55 ± 1.10[a]	2.82 ± 0.81[b]	2.30 ± 0.54[c]	2.27± 0.57[c]	2.20 ± 0.42[cd]	1.89 ± 0.46[d]
FVC, % of predicted	98.32 ± 12.96[a]	81.86 ± 13.76[b]	67.70 ± 10.97[c]	60.23 ±11.58[c]	62.07 ± 7.65[c]	51.91 ± 6.81[d]
FEV_1, L	3.06 ± 0.92[a]	1.66 ± 0.51[b]	1.14 ± 0.29[c]	0.82 ± 0.19[d]	1.96 ± 0.37[b]	1.70 ± 0.43[b]
FEV_1, % of predicted	10.99 ± 12.92[a]	63.19 ± 10.95[bc]	42.41 ± 4.96[d]	27.59 ± 4.97[e]	67.34 ± 8.03[b]	58.01 ± 9.43[c]
FEV_1/FVC	85.35 ± 4.55[a]	58.80 ± 6.60[b]	49.85 ± 8.60[c]	36.90 ± 7.92[d]	89.61 ± 4.82[a]	89.73 ± 5.13[a]
DLCO, mL . min^{-1} . mmHg^{-1}	23.67 ± 6.15[a]	13.04 ± 4.32[b]	10.39 ± 4.09[c]	8.85 ± 3.47[d]	11.03 ± 4.38[cd]	9.71 ± 5.86[cd]
DLCO, % of predicted	86.42 ± 14.47[a]	56.41 ±15.31[b]	46.27 ±15.53[bc]	36.25 ± 14.65[d]	41.51 ± 13.22[cd]	37.05 ± 17.50[cd]
DLCO/AV	4.98 ± 0.61[a]	2.85 ± 0.58[b]	2.48 ± 0.89[bc]	2.10 ± 0.80[c]	3.68 ± 1.14[b]	3.23 ± 1.49[b]
DLCO/AV, % of predicted	94.04 ± 24.12[a]	66.27 ± 15.08[b]	63.91 ± 24.25[bc]	51.61 ± 21.04[c]	71.66 ± 19.86[b]	69.42 ± 27.20[b]

BMI: body mass index; and AV: alveolar volume. Values presented as mean ± SD. Matching letters indicate absence of significant difference between groups, whereas non-matching letters indicate significant difference between groups. One-way ANOVA (groups); $p < 0.05$.

43.4% of the predicted value; $p > 0.05$). In the groups of patients with mild to moderate OLD and severe OLD, the difference in TLC between the two methods ranged from 1.58 L to 2.00 L (from 30.5% of the predicted value to 38.2% of the predicted value; $p > 0.05$), and the difference in RV between the two methods ranged from 1.46 L to 2.03 L (from 80.7% of the predicted value to 99.4% of the predicted value; $p > 0.05$). The group of patients with very severe OLD differed from all of the other groups analyzed, showing the largest differences between the two methods regarding TLC (3.09 L; 50.4% of the predicted value) and RV (2.89 L; 139.5% of the predicted value).

The correlations between FEV_1/FVC and the differences in TLC and RV values between the two methods in the individuals with normal spirometry results, in the patients with OLD, and in the patients with RLD are shown in Figure 2 (panels A, B, and C, respectively). The best correlations were observed in the group of patients with OLD ($r = -0.47$ for $\Delta TLC_{WBP\text{-}SBHD}$ in % of predicted and $r = -0.54$ for $\Delta RV_{WBP\text{-}SBHD}$ in % of predicted). When the individuals with normal spirometry results and the patients with OLD were analyzed as a whole, the correlation between FEV_1 in % of predicted and $\Delta TLC_{WBP\text{-}SBHD}$ in % of predicted increased to $r = -0.61$ ($p < 0.0001$), as did the correlation between FEV_1 in % of predicted and $\Delta RV_{WBP\text{-}SBHD}$ in % of predicted ($r = -0.640$; $p < 0.0001$).

The association between TLC_{WBP} and TLC_{SBHD} was assessed by Pearson's correlation test. For the sample as a whole (n = 169), there was a moderate positive correlation between TLC_{WBP} and TLC_{SBHD} ($r = 0.71$; $p < 0.001$), as well as between RV_{WBP} and RV_{SBHD} ($r = 0.62$; $p < 0.0001$). In the group of individuals with normal spirometry results and in that of patients with RLD, the coefficients

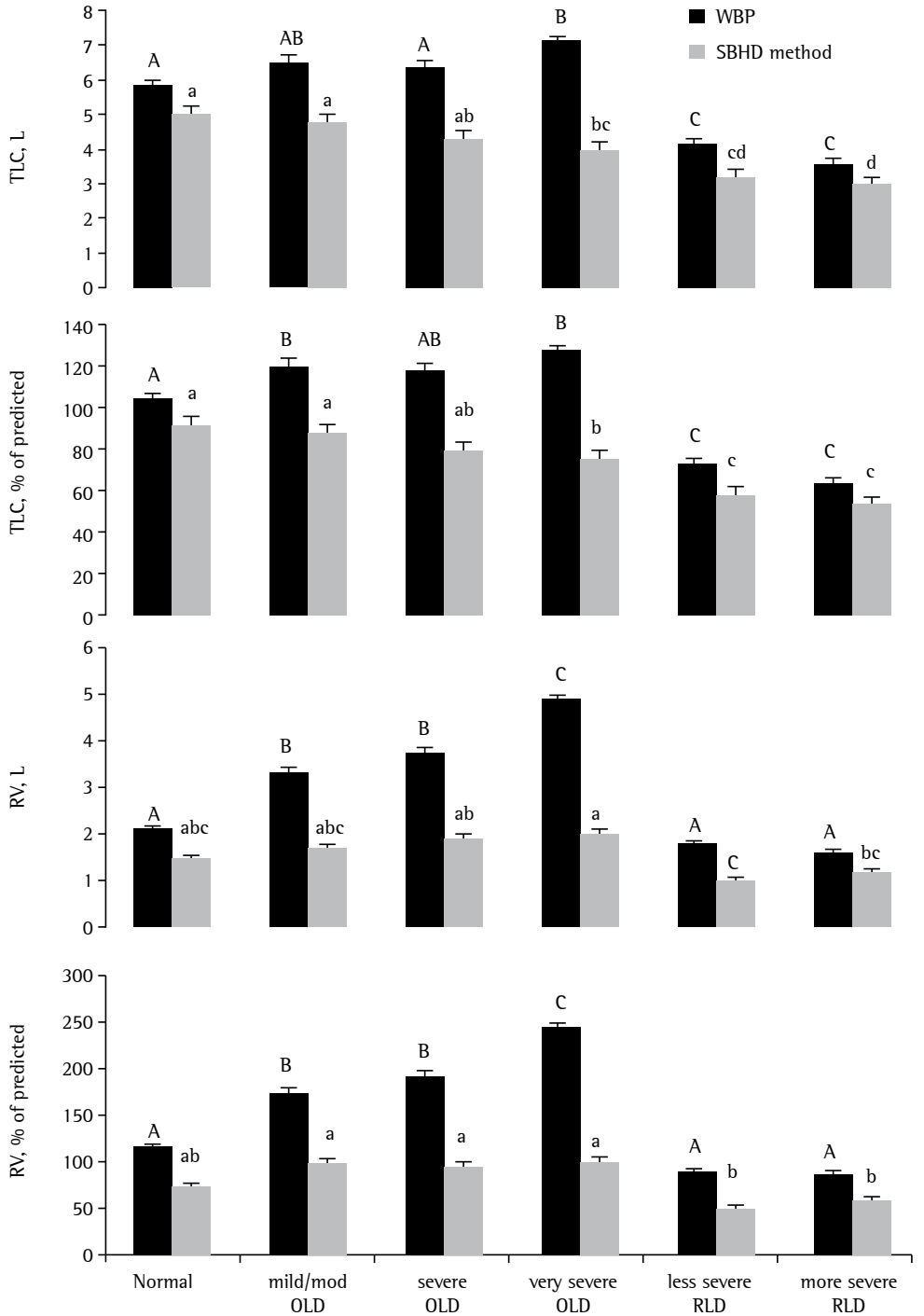

Figure 1 - Comparison of TLC and RV values as measured by whole-body plethysmography (WBP) with TLC and RV values as measured by the single-breath helium dilution (SBHD) method (in absolute values and in percentage of predicted) in different groups of patients, stratified by lung function status. In all groups, the TLC and RV values obtained by WBP were higher than those obtained by the SBHD method ($p < 0.01$). OLD: obstructive lung disease; mild/mod: mild/moderate; and RLD: restrictive lung disease. Capital letters represent comparisons of values obtained by WBP, whereas lower-case letters represent comparisons of values obtained by the SBHD method. Matching letters indicate absence of statistically significant differences. Two-way ANOVA (methods and groups); $p < 0.05$.

Table 2 - Differences between TLC and RV values as measured by whole-body plethysmography and TLC and RV values as measured by the single-breath helium dilution method in 169 individuals stratified by lung function status.

Variable	Lung function status					
	Normal	Obstructive lung disease			Restrictive lung disease	
		Mild/moderate	Severe	Very severe	Less severe	More severe
	(n = 27)	(n = 29)	(n = 29)	(n = 35)	(n = 25)	(n = 24)
$\Delta TLC_{WBP-SBHD}$, L	0.74 (0.36-0.74)[a]	1.58 (1.27-2.25)[b]	2.00 (0.95-3.31)[b]	3.09 (2.25-4.01)[c]	0.80 (0.61- 1.04)[a]	0.61 (0.35-0.95)[a]
$\Delta TLC_{WBP-SBHD}$, % of predicted	13.12 (8.40-21.20)[ab]	30.50 (22.42-38.85)[bc]	38.30 (19.70-50.40)[c]	50.40 (40.13-65.90)[d]	16.13 (11.00-20.00)[ab]	10.85 (7.15-16.90)[a]
$\Delta RV_{WBP-SBHD}$, L	0.62 (0.13-0.92)[a]	1.46 (1.07-1.97)[b]	2.03 (0.84-2.83)[b]	2.89 (2.03-3.82)[c]	0.75 (0.49-0.93)[a]	0.52 (0.23-0.79)[a]
$\Delta RV_{WBP-SBHD}$, % of predicted	34.70[a] (11.40-53.30)	80.70 (54.39-103.45)[b]	99.38 (53.20-137.15)[b]	139.50 (109.20-167.30)[c]	43.40 (28.60-56.35)[a]	30.80 (19.88-44.20)[a]

WBP: whole-body plethysmography; and SBHD: single-breath helium dilution method. Data presented as median delta and 25-75% interquartile range. Matching letters indicate absence of significant difference between groups, whereas non-matching letters indicate significant difference between groups. One-way ANOVA (groups); $p < 0.05$.

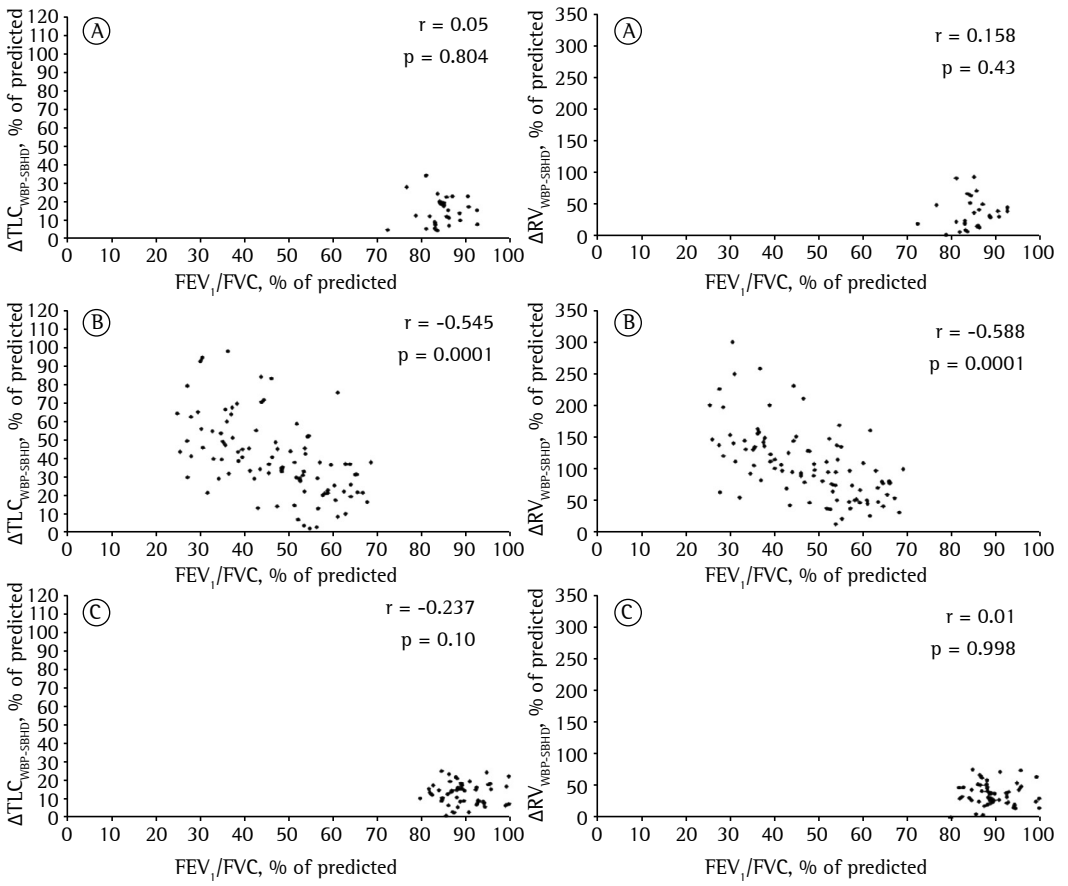

Figure 2 - Correlations of the difference between TLC as measured by whole-body plethysmography (WBP) and TLC as measured by the single-breath helium dilution (SBHD) method (left column), as well as of the difference between RV as measured by WBP and RV as measured by the SBHD method (right column), with the FEV_1/FVC ratio in % in individuals with normal lung function (A), in patients with obstructive lung disease (B), and in patients with restrictive lung disease (C).

of correlation between TLC_{WBP} and TLC_{SBHD} and between RV_{WBP} and RV_{SBHD} were, respectively, 0.92 and 0.51 ($p < 0.001$), whereas in the group of patients with OLD, those correlation coefficients were, respectively, 0.55 and 0.36 ($p < 0.001$).

We used Bland-Altman plots in order to compare TLC_{WBP} with TLC_{SBHD} and RV_{WBP} with RV_{SBHD}. The data are shown separately for each group, by lung function status, in Figure 3. The group of patients with RLD showed the smallest difference between the two methods for both variables; the largest differences were observed in the patients with OLD and higher lung volumes.

For the sample as a whole (n = 169), the difference in TLC values between the two methods was associated with the FEV_1/FVC ratio ($r = -0.75$; $p < 0.001$), FEV_1 ($r = -0.51$; $p < 0.001$), and DLCO ($r = -0.39$; $p < 0.001$). Likewise, the difference in RV values between the two methods correlated with the FEV_1/FVC ratio ($r = -0.75$; $p < 0.0001$), FEV_1 ($r = -0.53$; $p < 0.0001$), and DLCO ($r = -0.41$; $p < 0.0001$). There was no significant correlation between the difference in lung volumes and FVC ($p > 0.05$). For the 93 patients with OLD, the coefficient of correlation between TLC_{WBP} and TLC_{SBHD} was 0.57 ($p < 0.001$) and the coefficient of correlation between RV_{WBP} and RV_{SBHD} was 0.56 ($p < 0.001$). In the patients with OLD, we used a regression equation in order to predict TLC_{WBP} and RV_{WBP} on the basis of TLC_{SBHD} and RV_{SBHD}. The FEV_1/FVC ratio (%),

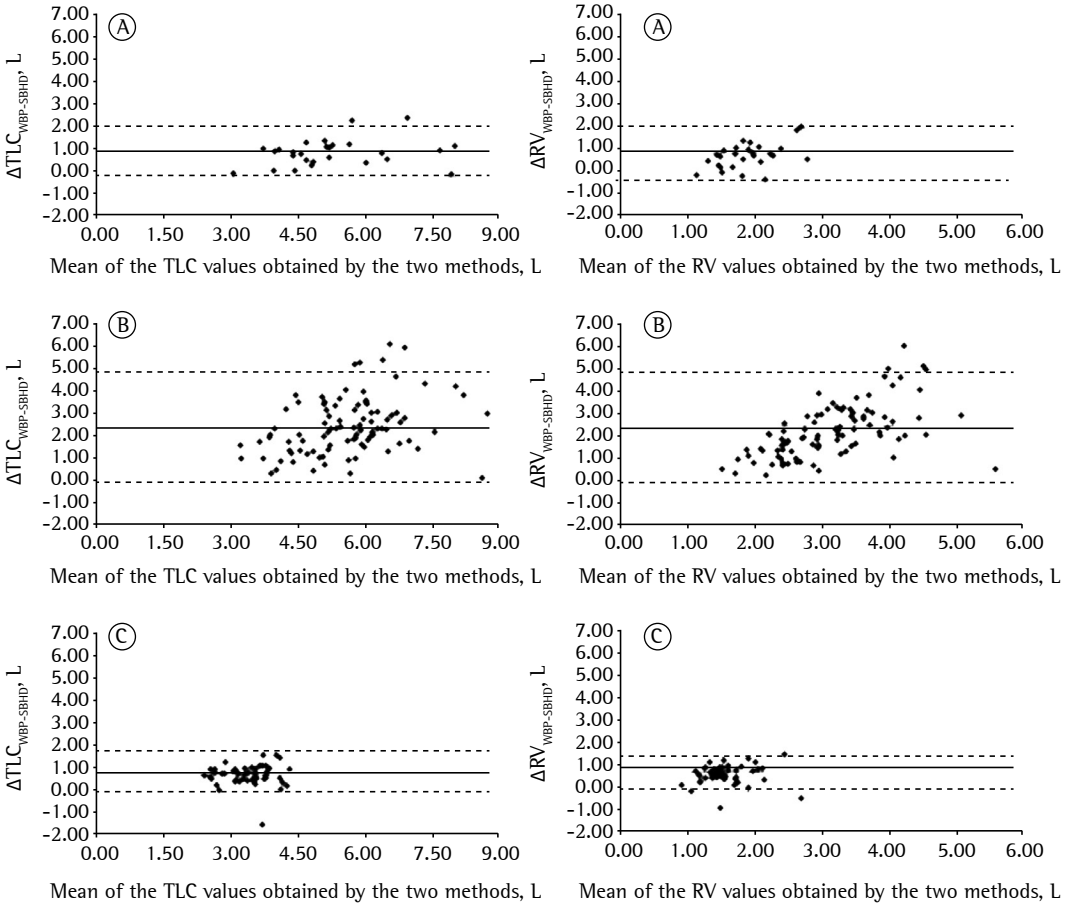

Figure 3 - Bland-Altman plots of the difference between TLC as measured by whole-body plethysmography (WBP) and TLC as measured by the single-breath helium dilution (SBHD) method (left column), as well as of the difference between RV as measured by WBP and RV as measured by the SBHD method (right column), in function of the mean of the TLC values (in L) obtained by the two methods (left column) and of the mean of the RV values (in L) obtained by the two methods (right column) in individuals with normal lung function (A), in patients with obstructive lung disease (B), and in patients with restrictive lung disease (C). The solid line represents the mean, and the dashed lines represent the SD (± 1.96).

FEV_1 (L), and DLCO (mL . min^{-1} . $mmHg^{-1}$) were included in the models. In the univariate analysis for $\Delta TLC_{WBP-SBHD}$, the adjusted coefficients for FEV_1/FVC, FEV_1, and DLCO were, respectively, $r^2 = 0.32$, $r^2 = 0.14$, and $r^2 = 0.07$, whereas for $\Delta RV_{WBP-SBHD}$, the adjusted coefficients for the same variables were, respectively, $r^2 = 0.30$, $r^2 = 0.14$, and $r^2 = 0.07$. In the multivariate analysis, FEV_1 and DLCO lost significance and were excluded from the models. The regression equation to predict the difference in TLC between the two methods in the patients with OLD was as follows:

$$y = 5.264 - 0.060x$$

where $y = \Delta TLC_{WBP-SBHD}$ in L and $x = FEV_1/FVC$ in % ($r^2 = 0.33$; adjusted $r^2 = 0.32$).

The regression equation to predict the difference in RV between the two methods was as follows:

$$Y = 4.862 - 0.055x$$

where $Y = \Delta RV_{WBP-SBHD}$ in L and $x = FEV_1/FVC$ in % ($r^2 = 0.31$; adjusted $r^2 = 0.30$).

We were unable to develop equations for individuals with normal lung function and those with RLD.

Discussion

Our results showed the following: 1) TLC_{WBP} and RV_{WBP} values were higher than TLC_{SBHD} and RV_{SBHD} values, regardless of the lung function status; 2) the magnitude of the difference in lung volumes between the two methods was associated with the FEV_1/FVC ratio, progressively increasing with the degree of airflow obstruction; 3) lung volumes as measured by WBP can be estimated on the basis of the values obtained by the SBHD method provided that the values are corrected for the severity of airflow obstruction.

Our study showed that, in the individuals with normal lung function and in those with RLD, the SBHD method underestimated lung volumes when compared with WBP. Comparable values between the two techniques[7] or a difference of 0.21 L in TLC in individuals with normal spirometry results have previously been described.[12] In another study, when compared with the multiple-breath helium dilution method, WBP overestimated TLC by 0.47 L or 7.2% in normal individuals.[19]

In the OLD patients in the present study, the difference between the lung volumes obtained by WBP and those obtained by the SBHD method increased or decreased proportionally to the increase in airflow obstruction and air trapping. Various studies have compared lung volumes as measured by different methods in patients with OLD. Garfield et al.[20] compared TLC as measured by plethysmography with TLC as measured by chest HRCT in patients with COPD and found a difference of 1.12 L (17.3%) between the two. Similarly, O'Donnell et al.[5] showed that, in patients with COPD, WBP systematically overestimates lung volumes in comparison with the multiple-breath helium dilution method and CT, and that the discrepancy is most pronounced in individuals with $FEV_1 < 30\%$ of the predicted value. In contrast, in 815 males with mild airflow obstruction, the SBHD method underestimated TLC_{WBP} by 0.75 L.[19] A difference of up to 1.08 L has been reported between TLC_{WBP} and TLC as measured by the multiple-breath helium dilution method.[5,19,21,22] Major discrepancies (of up to 2.25 L) have been observed between TLC_{SBHD} and TLC as measured by the multiple-breath helium dilution method in the presence of OLD.[12,23]

The different results across studies might be related to the cause of OLD, the varying degrees of airflow obstruction, and, in particular, the methods used in order to measure lung volumes. In addition to measuring the ventilated volume, plethysmography measures areas of air trapping. Conversely, the helium dilution technique measures only the air that is ventilated. In patients with airflow obstruction, there are variations in time constants of the respiratory system and in the distribution of ventilation, and there is early collapse of the airways during exhalation, which impairs lung emptying and causes air trapping. These physiological abnormalities help to explain the difference between the values obtained by WBP and those obtained by the SBHD method or the multiple-breath helium dilution method in individuals with OLD.[7,24]

The two helium dilution methods differ in terms of lung volume measurements. The SBHD method is a fast and simple technique in which only one ventilatory maneuver is used (in order to determine DLCO).[25] In addition, the SBHD method requires less effort from patients during pulmonary function testing.[7,23,26] In contrast, the multiple-breath helium dilution method requires a longer test time, allowing a more even distribution of the inhaled gas so that it is in equilibrium with the alveolar air.[7,26] A comparison between

the two techniques showed that they provide comparable results in individuals without airflow obstruction but show differences of up to 34% in patients with more severe obstruction.[7,12,19]

One of the statistical resources that we used in order to compare the findings of WBP with those of the SBHD method was the linear correlation test. The test showed a correlation of 0.92 in the individuals with normal spirometry results or RLD and of 0.55 in the patients with OLD. A correlation of 0.98 between TLC_{WBP} and TLC_{SBHD} in 32 normal individuals and of 0.70 between TLC_{WBP} and TLC_{SBHD} in patients with OLD had previously been reported.[27] However, in our study, Bland-Altman plots[18] were used for analysis of the lung volumes as measured by the two methods and showed that, despite a strong correlation between the two in the individuals with normal spirometry results, there was discordance between the two methods in that group of patients. The concordance between the two methods for determining lung volumes was better in the group of patients with RLD, as evidenced by a narrower 95% CI and a higher concentration of data around the mean. In contrast, in the group of patients with OLD, the difference between the two methods was more pronounced, especially in the patients with high lung volumes. The limitations of using linear correlation in order to evaluate the performance of two methods for measuring the same variable have previously been described, as have the advantages of using Bland-Altman plots in such cases.[22]

The linear regression equations that allow us to estimate TLC_{WBP} and RV_{WBP} values on the basis of TLC_{SBHD} and RV_{SBHD} values adjusted for the degree of airflow obstruction constitute an important contribution of our study. These equations can be used in patient care in situations in which only spirometry and pulmonary diffusing capacity testing are available. Although equations for TLC adjusted for the degree of airflow obstruction have previously been described,[7] the gold standard used was the multiple-breath helium dilution technique rather than WBP. Considering the coefficients of determination for the regression equations developed in our study, we emphasize that most of the difference between the two methods remains unexplained.

One limitation of our study is its retrospective nature. The number of patients with mild OLD in the present study was very small, reflecting the profile of patients treated at a referral university hospital. Likewise, the number of patients with severe RLD was small, which limited the stratification of RLD patients. In addition, RV_{SBHD} was obtained by subtracting FVC from TLC_{SBHD}; the use of FVC instead of slow VC possibly contributed to the lower accuracy of the method, especially in the patients with OLD. Furthermore, the prediction equations should be validated in other patient populations in order to increase the external validity of the study.

In conclusion, our study demonstrated that TLC_{WBP} and RV_{WBP} values were higher than TLC_{SBHD} and RV_{SBHD} values in normal individuals and in patients with RLD or OLD. The discrepancy between the two methods for measuring lung volumes was most pronounced in the group of patients with OLD caused by COPD, the magnitude of the difference being directly associated with the degree of airflow obstruction. The linear regression equations described in the present study allow us to adjust TLC_{SBHD} and RV_{SBHD} values for airflow, predicting the lung volumes as measured by WBP. Therefore, the relatively simple, faster, and more widely available SBHD method, used in order to determine pulmonary diffusing capacity, has potential for expanded use. However, additional studies are needed in order to validate the equations before they can be used in clinical practice.

References

1. Martinez FJ, de Oca MM, Whyte RI, Stetz J, Gay SE, Celli BR. Lung-volume reduction improves dyspnea, dynamic hyperinflation, and respiratory muscle function. Am J Respir Crit Care Med. 1997;155(6):1984-90. http://dx.doi.org/10.1164/ajrccm.155.6.9196106 PMid:9196106
2. Casanova C, Cote C, de Torres JP, Aguirre-Jaime A, Marin JM, Pinto-Plata V, et al. Inspiratory-to-total lung capacity ratio predicts mortality in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2005;171(6):591-7. http://dx.doi.org/10.1164/rccm.200407-867OC PMid:15591470
3. Menna Barreto SS. Volumes pulmonares. J Pneumol. 2002;28(Suppl3):S83-S94.
4. Cazzola M, Rogliani P, Curradi G, Segreti A, Ciaprini C, Pezzuto G, et al. A pilot comparison of helium dilution and plethysmographic lung volumes to assess the impact of a long-acting bronchodilator on lung hyperinflation in COPD. Pulm Pharmacol Ther. 2009;22(6):522-5. http://dx.doi.org/10.1016/j.pupt.2009.05.005 PMid:19481169
5. O'Donnell CR, Bankier AA, Stiebellehner L, Reilly JJ, Brown R, Loring SH. Comparison of plethysmographic and helium dilution lung volumes: which is best for COPD? Chest. 2010;137(5):1108-15. http://dx.doi.org/10.1378/chest.09-1504 PMid:20022972 PMCid:PMC2862399

6. Pappas GP, Brodkin CA, Sheppard L, Balmes J, Horike M, Barnhart S. The validity of radiographic estimation of total lung capacity in patients with respiratory disease. Chest. 1998;114(2):513-20. http://dx.doi.org/10.1378/chest.114.2.513 PMid:9726739
7. Punjabi NM, Shade D, Wise RA. Correction of single-breath helium lung volumes in patients with airflow obstruction. Chest. 1998;114(3):907-18. http://dx.doi.org/10.1378/chest.114.3.907 PMid:9743183
8. Wanger J, Clausen JL, Coates A, Pedersen OF, Brusasco V, Burgos F, et al. Standardisation of the measurement of lung volumes. Eur Respir J. 2005;26(3):511-22. http://dx.doi.org/10.1183/09031936.05.00035005 PMid:16135736
9. Menna Barreto SS, Cavalazzi AC. Determinação dos volumes pulmonares: métodos de mensuração dos volumes pulmonares. J Pneumol. 2002;28(Suppl3):S95-S100.
10. Crapo RA. Pulmonary function testing. In: Baum GL, Crapo JD, GlassRoth J, Karlinsky JB, Celli BR. Textbook of Pulmonary Disease. Philadelphia: Lippincott-Raven; 1998. p. 199-218.
11. Kilburn KH, Miller A, Warshaw RH. Measuring lung volumes in advanced asbestosis: comparability of plethysmographic and radiographic versus helium rebreathing and single breath methods. Respir Med. 1993;87(2):115-20. http://dx.doi.org/10.1016/0954-6111(93)90138-P
12. Milite F, Lederer DJ, Weingarten JA, Fani P, Mooney AM, Basner RC. Quantification of single-breath underestimation of lung volume in emphysema. Respir Physiol Neurobiol. 2009;165(2-3):215-20. http://dx.doi.org/10.1016/j.resp.2008.12.009 PMid:19150417 PMCid:PMC2759678
13. Pereira CA, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007;33(4):397-406. http://dx.doi.org/10.1590/S1806-37132007000400008 PMid:17982531
14. Crapo RO, Morris AH. Standardized single breath normal values for carbon monoxide diffusing capacity. Am Rev Respir Dis. 1981;123(2):185-9. PMid:7235357
15. Crapo RO, Morris AH, Clayton PD, Nixon CR. Lung volumes in healthy nonsmoking adults. Bull Eur Physiopathol Respir. 1982;18(3):419-25. PMid:7074238
16. Vestbo J, Hurd SS, Agustí AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med. 2013;187(4):347-65. http://dx.doi.org/10.1164/rccm.201204-0596PP PMid:22878278
17. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, et al. Interpretative strategies for lung function tests. Eur Respir J. 2005;26(5):948-68. http://dx.doi.org/10.1183/09031936.05.00035205 PMid:16264058
18. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986;1(8476):307-10. http://dx.doi.org/10.1016/S0140-6736(86)90837-8
19. Cliff IJ, Evans AH, Pantin CF, Baldwin DR. Comparison of two new methods for the measurement of lung volumes with two standard methods. Thorax. 1999;54(4):329-33. http://dx.doi.org/10.1136/thx.54.4.329 PMid:10092694 PMCid:PMC1745479
20. Garfield JL, Marchetti N, Gaughan JP, Steiner RM, Criner GJ. Total lung capacity by plethysmography and high-resolution computed tomography in COPD. Int J Chron Obstruct Pulmon Dis. 2012;7:119-26. http://dx.doi.org/10.2147/COPD.S26419 PMid:22399851 PMCid:PMC3292389
21. Schünemann HJ, Dmochowski J, Campbell LA, Grant BJ. A novel approach for quality control of total lung capacity in the clinical pulmonary function laboratory: a study in a veteran population. Respirology. 2003;8(3):365-70. http://dx.doi.org/10.1046/j.1440-1843.2003.00487.x PMid:12911832
22. Spence DP, Kelly YJ, Ahmed J, Calverley PM, Pearson MG. Critical evaluation of computerised x ray planimetry for the measurement of lung volumes. Thorax. 1995;50(4):383-6. http://dx.doi.org/10.1136/thx.50.4.383 PMid:7785011 PMCid:PMC474286
23. Roberts CM, MacRae KD, Seed WA. Multi-breath and single breath helium dilution lung volumes as a test of airway obstruction. Eur Respir J. 1990;3(5):515-20. PMid:2376248
24. Dykstra BJ, Scanlon PD, Kester MM, Beck KC, Enright PL. Lung volumes in 4,774 patients with obstructive lung disease. Chest. 1999;115(1):68-74. http://dx.doi.org/10.1378/chest.115.1.68 PMid:9925064
25. Garcia-Rio F, Dorgham A, Galera R, Casitas R, Martinez E, Alvarez-Sala R, et al. Prediction equations for single-breath diffusing capacity in subjects aged 65 to 85 years. Chest. 2012;142(1):175-84. http://dx.doi.org/10.1378/chest.11-2021 PMid:22194584
26. Brugman TM, Morris JF, Temple WP. Comparison of lung volume measurements by single breath helium and multiple breath nitrogen equilibration methods in normal subjects and COPD patients. Respiration. 1986;49(1):52-60. http://dx.doi.org/10.1159/000194859 PMid:3513268
27. Mitchell MM, Renzetti AD Jr. Evaluation of a single-breath method of measuring total lung capacity. Am Rev Respir Dis. 1968;97(4):571-80. PMid:5644321

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Barium swallow study in routine clinical practice: a prospective study in patients with chronic cough*,**

Estudo radiográfico com ingestão de bário na rotina clínica: um estudo prospectivo em pacientes com tosse crônica

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Abstract

Objective: To assess the routine use of barium swallow study in patients with chronic cough. **Methods:** Between October of 2011 and March of 2012, 95 consecutive patients submitted to chest X-ray due to chronic cough (duration > 8 weeks) were included in the study. For study purposes, additional images were obtained immediately after the oral administration of 5 mL of a 5% barium sulfate suspension. Two radiologists systematically evaluated all of the images in order to identify any pathological changes. Fisher's exact test and the chi-square test for categorical data were used in the comparisons. **Results:** The images taken immediately after barium swallow revealed significant pathological conditions that were potentially related to chronic cough in 12 (12.6%) of the 95 patients. These conditions, which included diaphragmatic hiatal hernia, esophageal neoplasm, achalasia, esophageal diverticulum, and abnormal esophageal dilatation, were not detected on the images taken without contrast. After appropriate treatment, the symptoms disappeared in 11 (91.6%) of the patients, whereas the treatment was ineffective in 1 (8.4%). We observed no complications related to barium swallow, such as contrast aspiration. **Conclusions:** Barium swallow improved the detection of significant radiographic findings related to chronic cough in 11.5% of patients. These initial findings suggest that the routine use of barium swallow can significantly increase the sensitivity of chest X-rays in the detection of chronic cough-related etiologies.

Keywords: Barium sulfate; Cough; Contrast media; Radiography, thoracic.

Resumo

Objetivo: Investigar o uso rotineiro do estudo radiográfico com ingestão de bário em pacientes com tosse crônica. **Métodos:** Entre outubro de 2011 e março de 2012, 95 pacientes consecutivos submetidos a radiografia de tórax devido a tosse crônica (duração > 8 semanas) foram incluídos no estudo. Como propósito do estudo, radiografias de tórax adicionais foram obtidas imediatamente após a administração oral de 5 mL de uma suspensão de sulfato de bário a 5%. Dois radiologistas avaliaram todas as imagens de forma sistemática para identificar alterações patológicas. O teste exato de Fisher e o teste do qui-quadrado para dados categóricos foram utilizados nas comparações. **Resultados:** As imagens obtidas imediatamente após a ingestão de bário revelaram patologias significativas potencialmente relacionadas a tosse crônica em 12 (12,6%) dos 95 pacientes. Essas patologias, incluindo hérnia diafragmática, neoplasia de esôfago, acalasia, divertículo esofágico e dilatação anormal do esôfago, não foram detectadas nas imagens obtidas sem a administração do contraste. Após o tratamento adequado, os sintomas desapareceram em 11 pacientes (91,6%), enquanto o tratamento foi ineficaz em 1 (8,4%). Não foram observadas complicações relacionadas à ingestão de bário, como aspiração. **Conclusões:** A ingestão de bário melhorou a detecção de achados radiológicos significantes relacionados a tosse crônica em 11,5% dos pacientes. Esses resultados iniciais sugerem que a utilização rotineira da ingestão de bário aumenta significantemente a sensibilidade de radiografias de tórax na detecção de etiologias relacionadas a tosse crônica.

Descritores: Sulfato de bário; Tosse; Meios de contraste; Radiografia torácica.

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Introduction

Chest X-ray is performed widely as the first-choice imaging modality for the investigation of thoracic complaints.[1] The method is globally available and has been proven to be cost-effective in general practice.[1,2] Frontal and lateral images are routinely acquired; however, some authors have contested the value of the lateral view in initial examinations, especially in patients under 40 years of age.[3] Radiographic images provide a significant amount of valuable and sometimes vital information[1,4] and thus have constituted the method of choice for the initial evaluation of patients for decades.

Barium sulfate is often used medically as a radiocontrast agent. It is prepared as an aqueous suspension and administered by enema or orally. Because it has a relatively high atomic number, its radiation absorption tends to be greater than that in other thoracic structures, providing better anatomic detail.[5] Three types of barium studies can be performed, each requiring a different suspension preparation: barium swallow for the evaluation of the gastroesophageal junction, barium meal for the study of the antrum and duodenum, and barium follow-through for the assessment of the small bowel.[6]

The most common causes of chronic cough in adults are upper airway cough syndrome, asthma, and gastroesophageal reflux disease.[7,8] The initial evaluation of a patient with chronic cough (duration > 8 weeks) typically includes a focused history, physical examination, and chest X-rays. These are firstly suggested in chronic cough because CT has a higher radiation dose exposure, higher costs, and a similar negative predictive value.[7,8] To our knowledge, however, no study to date has investigated the benefits or drawbacks of the routine use of the barium swallow study in these patients. Therefore, the aim of our study was to assess the routine use of the barium swallow study in patients with chronic cough. We believe that the oral administration of barium enhances the evaluation not only of the esophagus and stomach but also of the heart chambers, great vessels, and airways.[9]

Methods

The institutional review board approved the study, which was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines (Protocol no. 221-12). All participants gave written informed consent before being included in the study. Between October of 2011 and March of 2012, 95 consecutive patients submitted to CXR due to chronic cough were included in the study. The inclusion criterion was a history of chronic cough with duration > 8 weeks. All the patients underwent clinical examination and posteroanterior and lateral chest X-rays without the use of the oral barium sulfate suspension. These images were included in the control group.

For study purposes, additional posteroanterior and lateral images were obtained immediately after the oral administration of 5 mL of a 5% barium sulfate suspension (Bariogel; Cristália, São Paulo, Brazil). These images were included in the study group. The patients underwent both procedures on the same day. All of the radiographic images were acquired using a computed radiography system (CR 3110 Kodak Ektascan Storage Phosphor Reader; Kodak, Rochester, NY, USA) at a tube voltage of 125 kV and 2.5 mAs. The radiation doses were 0.1 mSv per examination.

Two thoracic radiologists with 12 and 7 years of experience, respectively, systematically evaluated the radiographic images from both groups in order to identify any pathological changes using a medical image displaying software (Advantage Workstation 4.4; GE Healthcare, Milwaukee, WI, USA). The radiologists, who were blinded to the history of the patients, were allowed to change the window width and level and to use pan and zoom functions. The images taken after the oral barium administration were also examined carefully for any complications related to the contrast agent. When consensus was not reached, a third radiologist was consulted. The images taken with barium swallow were evaluated 7 days after the assessment of the control images using the same parameters.

True positive results were defined as the images in the study group that revealed a significant pathological condition potentially related to the chronic cough that responded to the treatment of that condition. False positive results were defined as the images in the study group that revealed a significant pathological condition potentially related to the chronic cough that was unresponsive to the treatment of that condition. No patients were excluded from the study.

The analysis of the data collected was performed with the Statistical Analysis System, version 6 (SAS Institute, Cary, NC, USA). Fisher's exact test and the chi-square test for categorical data were used for the comparisons between the control and the study groups, and the level of significance was set at 5% ($p < 0.05$).

Results

The study sample comprised 95 patients, with a mean age of 51.4 years (range, 15-88 years). Among these, 31 (32.6%) were male and 64 (67.4%) were female. Of the 95 patients, 23 were current or former smokers, with a mean smoking history of 10 pack-years (range, 1-20 pack-years).

In 12 patients (12.6%), the images obtained with barium swallow revealed a significant pathological condition potentially related to chronic cough that was not detected on the images taken without contrast. These conditions included diaphragmatic hiatal hernia (confirmed by endoscopy), in 6 patients (5.2%; Figure 1); esophageal neoplasm, in 1 (1.1%; Figure 2); achalasia, in 2 (2.1%); esophageal diverticulum, in 2 (2.1%; Figure 3); and abnormal esophageal dilatation, in 1 (1.05%), which was probably achalasia but unproven by endoscopy. Tertiary esophageal contractions and one case of a retroesophageal right subclavian artery (dysphagia lusoria) were observed only on the images after barium administration but were not recorded as pathological conditions. According to the literature, the majority of patients with these findings have no clinical signs or symptoms.[8-10] None of these patients were diagnosed with cancer or lung infection.

Among the 95 patients, a final diagnosis of pulmonary tuberculosis was made in 5. The radiographic findings in these patients were not affected by barium swallow.

Patients with diaphragmatic hiatal hernias received antireflux therapy (omeprazole, antacid, and changes in lifestyle). Surgery was performed in the patients with esophageal neoplasm, achalasia, and esophageal diverticulum. The patient with abnormal esophageal dilatations also underwent surgical treatment. After appropriate treatment, the symptoms disappeared in 11 (91.6%) of the 12 patients (Table 1). Antireflux therapy was ineffective in 1 patient with an esophageal hiatal hernia. In the cohort of 12 patients with a significant pathological condition detected with barium swallow, 4 were smokers, with a mean smoking history of 7 pack-years (range, 3-15 pack-years). Among the patients diagnosed with hiatal hernia on the X-ray, 1 had reflux-related symptoms and was part of the group in which antireflux therapy was effective. In addition, 1 patient diagnosed with achalasia retrospectively presented with dysphagia. The other 10 patients reported only chronic cough as a symptom. All of the comparisons in the study were statistically significant ($p < 0.05$).

No complications related to barium swallow, such as contrast aspiration, were observed. In addition, the procedure had no effect on the quality of the images.

Discussion

Chronic cough is a very common and nonspecific symptom of almost all chronic respiratory (and some non-respiratory) conditions.[7,8] Clinical assessment, spirometry, and

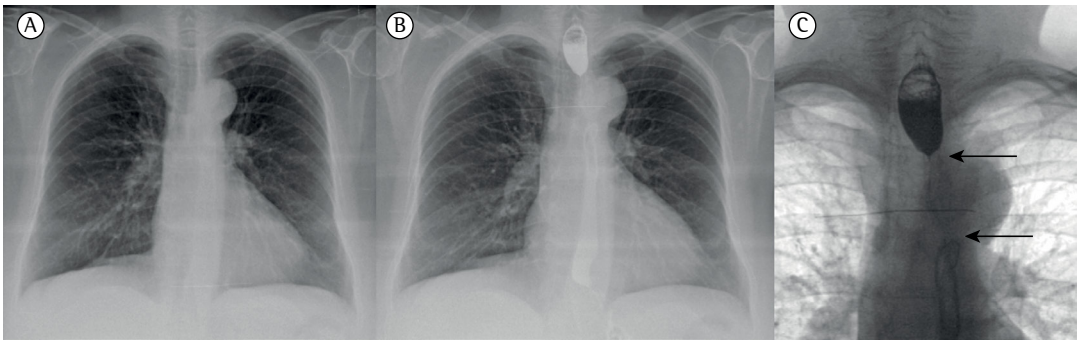

Figure 1 - Chest X-rays of a 61-year-old woman with a 3-month history of chronic cough. In A, posteroanterior chest X-ray showing no significant alterations. In B and C, respectively, posteroanterior and lateral chest X-rays taken after barium swallow and showing the gastroesophageal junction (black arrow) above the esophageal hiatus of the diaphragm, consistent with hiatal hernia.

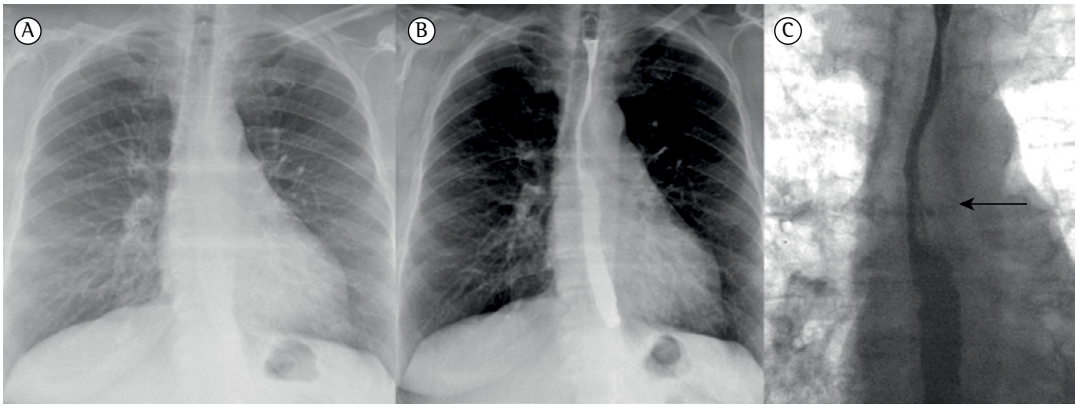

Figure 2 - Chest X-rays of a 65-year-old man with a 4-month history of chronic cough. In A, a posteroanterior chest X-ray showing no mediastinal alterations. In B and C, posteroanterior chest X-rays taken after barium swallow and showing an irregular area of narrowing (black arrows) that was subsequently diagnosed as esophageal cancer.

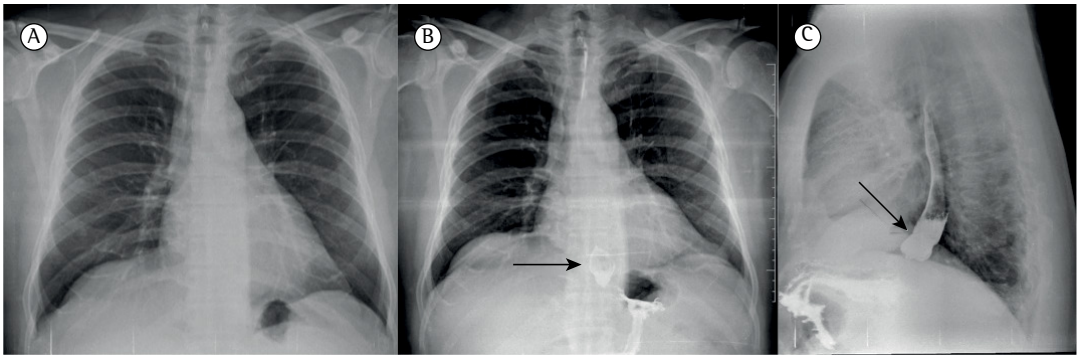

Figure 3 - Chest X-rays of a 47-year-old man with a 3-month history of chronic cough. In A, a posteroanterior chest X-ray showing no significant alterations. In B and C, chest X-rays taken after barium swallow and showing a diverticulum in the mid-esophagus (black arrow) that was diagnosed as a traction diverticulum caused by tubercular scarring in the perihilar lymph nodes.

Table 1 - Clinical characteristics of the 95 patients included in the study and diagnoses based on barium swallow studies.a

Characteristic	Result
Patients	95
Male gender	31 (32.6)
Smokers	23 (24.2)
Age, years	52.4 (15-88)[b]
Diagnosis	11 (11.5)
Diaphragmatic hiatal hernia	6 (5.2)
Achalasia	2 (2.1)
Esophageal diverticulum	2 (2.1)
Abnormal esophageal dilatation	1 (1.1)
Esophageal neoplasia	1 (1.1)

[a]Values expressed as n (%) of patients, except where otherwise indicated. [b]Value expressed as median (range).

chest X-rays can readily detect various recognizable causes of chronic cough, such as COPD, chronic bronchitis, lung cancer, foreign body aspiration, pulmonary tuberculosis, sarcoidosis, idiopathic pulmonary fibrosis, and heart failure.[7,8]

From the opposite perspective, an extensive list of conditions can be suspected or confirmed with simple chest X-rays.[1,7] An evaluation of the respiratory system might reveal lung infiltration due to various pathologies, such as consolidations and neoplasms; parenchymal destruction; lymph node enlargement; pneumothorax; and pleural effusion. Cardiovascular imaging can reveal cardiomegaly, pericardial effusion, calcification foci causing atheromatosis, acute aortic syndromes, aneurysms, and congenital malformations. Bones, muscles, and breasts are also visible, enabling the diagnosis of numerous conditions, such as fractures, spinal diseases, and breast nodules. Groups of authors have indirectly reported that barium swallow studies enable the early diagnosis of various conditions, such as gastric and esophageal diseases,[10-19] cardiovascular abnormalities,[20-23] and even respiratory

conditions,[24,25] suggesting that the routine use of this method is justified and beneficial.

Our literature review found no studies evaluating the use of barium swallow studies in patients with chronic cough. Therefore, the parameters used in the present study were selected on the basis of those used in previous studies,[4,9,14,16,24,25] taking into consideration the major anatomic relationships between the esophagus and other thoracic structures.

Barium aspiration is always a concern in the general practice. It is a well described and benign complication of barium swallow, although it is known to have harmful effects in the lung parenchyma.[26] Fatal outcomes have been reported even after a low volume intake[27] and after the use of low-dose barium suspensions.[27,28] Although no consensus has been reached on the effects of barium sulfate administration in bronchographic studies, most authors have argued that simultaneous gastric content aspiration plays a greater role in causing parenchymal tissue reaction than does barium aspiration. In our study, no complications of this or any other nature were observed, which was probably related to the small study sample.

When considering the routine utilization of barium sulfate as contrast, the potential risk for false-positive findings, which could lead to unnecessary further examinations or interventions, is an important concern. The potential increase of the dose with the addition of this routine method was negligible. Few publications have reported information about overlooked conditions, such as gastric carcinoma and diaphragmatic hernia, which represent the most likely pitfalls of barium studies; however, these issues are related primarily to the barium meal technique.[29] Since the X-ray is one of the most commonly performed imaging modalities, achieving greater sensitivity of the method and diagnostic accuracy for various diseases is perhaps a more important concern than is the potential risk for occasional false-positive diagnoses. In our study, only 1 patient (1.1% of the study sample) had a false-positive diagnosis of hiatal hernia, since the chronic cough symptoms did not resolve after the treatment. In the present study, the response to treatment was the standard of truth employed in order to identify true-positive and false-positive findings were.

Our study has some limitations. First, the specificity of the proposed method was determined by the assumption that the lack of response to the treatment would mean that the chronic cough was motivated by another cause. In addition, we were unable to determine the sensitivity of the method. The diagnoses of esophageal cancer and esophageal diverticulum may represent a geographical bias, given the high incidence of tuberculosis (causing traction diverticula) and esophageal cancer in our region.[30]

In conclusion, the use of barium swallow improved the detection of significant radiographic findings related to chronic cough in 11.5% of patients. These initial findings suggest that the routine use of barium swallow can significantly increase the sensitivity of a widely available imaging modality. No complications associated with the procedure, such as contrast aspiration, were identified. However, further studies with a larger sample are necessary for the assessment of complication risks, false-positive findings, and the cost-effectiveness of this method before its use in daily practice can be securely recommended.

References

1. Speets AM, van der Graaf Y, Hoes AW, Kalmijn S, Sachs AP, Rutten MJ, et al. Chest radiography in general practice: indications, diagnostic yield and consequences for patient management. Br J Gen Pract. 2006;56(529):574-8. PMid:16882374 PMCid:PMC1874520
2. Geitung JT, Skjaerstad LM, Göthlin JH. Clinical utility of chest roentgenograms. Eur Radiol. 1999;9(4):721-3. http://dx.doi.org/10.1007/s003300050741 PMid:10354893
3. Eisenberg RL, Hedgcock MW, Williams EA, Lyden BJ, Akin JR, Gooding GA, et al. Optimum radiographic examination for consideration of compensation awards: I. General methodology and application to chest examination. AJR Am J Roentgenol. 1980;135(5):1065-9. http://dx.doi.org/10.2214/ajr.135.5.1065 PMid:6778148
4. Gibbs JM, Chandrasekhar CA, Ferguson EC, Oldham SA. Lines and stripes: where did they go?--From conventional radiography to CT. Radiographics. 2007;27(1):33-48. http://dx.doi.org/10.1148/rg.271065073 PMid:17234997
5. Wiberg N, Holleman AF, Wiberg E, editors. Inorganic Chemistry. San Diego: Academic Press; 2001. p. 1044-342.
6. Kupershmidt M, Varma D. Radiological tests in investigations of atypical chest pain. Aust Fam Physician. 2006;35(5):282-7. PMid:16680204
7. Pavord ID, Chung KF. Management of chronic cough. Lancet. 2008;371(9621):1375-84. http://dx.doi.org/10.1016/S0140-6736(08)60596-6
8. Jacomelli M, Souza R, Pedreira Júnior WL. Abordagem diagnóstica da tosse crônica em pacientes não-tabagistas. J Pneumol. 2003;29(6):413-20. http://dx.doi.org/10.1590/S0102-35862003000600016
9. Ugalde PA, Pereira ST, Araujo C. Correlative anatomy for the esophagus. Thorac Surg Clin. 2011;21(2):307-17, x. http://dx.doi.org/10.1016/j.thorsurg.2010.12.007 PMid:21477780

10. Kahrilas PJ, Kim HC, Pandolfino JE. Approaches to the diagnosis and grading of hiatal hernia. Best Pract Res Clin Gastroenterol. 2008;22(4):601-16. http://dx.doi.org/10.1016/j.bpg.2007.12.007 PMid:18656819 PMCid:PMC2548324
11. II Brazilian guidelines for the management of chronic cough [Article in Portuguese]. J Bras Pneumol. 2006;32(Suppl 6):S403-46. http://dx.doi.org/10.1590/S1806-37132006001000002 PMid:17420904
12. Kostic SV, Rice TW, Baker ME, Decamp MM, Murthy SC, Rybicki LA, et al. Timed barium esophagogram: A simple physiologic assessment for achalasia. J Thorac Cardiovasc Surg. 2000;120(5):935-43. http://dx.doi.org/10.1067/mtc.2000.110463 PMid:11044320
13. Davies HA, Evans KT, Butler F, McKirdy H, Williams GT, Rhodes J. Diagnostic value of "bread-barium" swallow in patients with esophageal symptoms. Dig Dis Sci. 1983;28(12):1094-100. http://dx.doi.org/10.1007/BF01295808 PMid:6653300
14. Campbell C, Levine MS, Rubesin SE, Laufer I, Redfern G, Katzka DA. Association between esophageal dysmotility and gastroesophaeal reflux on barium studies. Eur J Radiol. 2006;59(1):88-92. http://dx.doi.org/10.1016/j.ejrad.2006.02.002 PMid:16530370
15. Asghar M. Radiological investigation of dysphagia at D. H. Q. teaching hospital D. I. Khan. J Ayub Med Coll Abbottabad. 2004;16(2):70-2. PMid:15455623
16. Levine MS, Rubesin SE, Ott DJ. Update on esophageal radiology. AJR Am J Roentgenol. 1990;155(5):933-41. http://dx.doi.org/10.2214/ajr.155.5.2120962 PMid:2120962
17. Aly YA. Digital radiography in the evaluation of oesophageal motility disorders. Clin Radiol. 2000;55(7):561-8. http://dx.doi.org/10.1053/crad.1999.0509 PMid:10924382
18. Thomas PS, Carré IJ. Findings on barium swallow in younger siblings of children with hiatal hernia (partial thoracic stomach). J Pediatr Gastroenterol Nutr. 1991;12(2):174-7. http://dx.doi.org/10.1097/00005176-199102000-00006 PMid:2051268
19. Soila P, Palmgren O, Thomander K. The value of barium filling of the oesophagus in radiophotography (rp) of the chest. Acta Tuberc Pneumol Scand. 1964;45:1-13. PMid:14209267
20. Tonkin IL, Elliott LP, Bargeron LM Jr. Concomitant axial cineangiography and barium esophagography in the evaluation of vascular rings. Radiology. 1980;135(1):69-76. PMid:7360983
21. Hogg K, Teece S. Best evidence topic report. The sensitivity of a normal chest radiograph in ruling out aortic dissection. Emerg Med J. 2004;21(2):199-200. PMid:14988349 PMCid:PMC1726310
22. Skinner LJ, Ryan S, Russell JD. Complete vascular ring detected by barium esophagography. Ear Nose Throat J. 2002;81(8):554-5. PMid:12199173
23. Burch M, Balaji S, Deanfield JE, Sullivan ID. Investigation of vascular compression of the trachea: the complementary roles of barium swallow and echocardiography. Arch Dis Child. 1993;68(2):171-6. http://dx.doi.org/10.1136/adc.68.2.171 PMid:8481037 PMCid:PMC1029228
24. Gatewood OM, Vanhoutte JJ. The role of the barium swallow examination in evaluation of pediatric pneumonias. Am J Roentgenol Radium Ther Nucl Med. 1966;97(1):203-10. http://dx.doi.org/10.2214/ajr.97.1.203
25. Baghdassarian OM, Gatewood WM. Barium swallow in evaluation of chronic or recurrent pneumonias in infancy and childhood. Md State Med J. 1965;14:51-6. PMid:14258949
26. Varatharaj A, Roome C, Allsup S. Barium aspiration. QJM. 2012;105(9):903-4. http://dx.doi.org/10.1093/qjmed/hcr146 PMid:21865311
27. Buschmann C, Schulz F, Tsokos M. Fatal aspiration of barium sulfate. Forensic Sci Med Pathol. 2011;7(1):63-4. http://dx.doi.org/10.1007/s12024-010-9180-y PMid:20607457
28. Chiu CY, Wong KS, Tsai MH. Massive aspiration of barium sulfate during an upper gastrointestinal examination in a child with dysphagia. Int J Pediatr Otorhinolaryngol. 2005;69(4):541-4. http://dx.doi.org/10.1016/j.ijporl.2004.11.008 PMid:15763294
29. Shindoh N, Nakagawa T, Ozaki Y, Kyogoku S, Sumi Y, Katayama H. Overlooked gastric carcinoma: pitfalls in upper gastrointestinal radiology. Radiology. 2000;217(2):409-14. http://dx.doi.org/10.1148/radiology.217.2.r00oc29409 PMid:11058636
30. de Barros SG, Ghisolfi ES, Luz LP, Barlem GG, Vidal RM, Wolff FH, et al. High temperature "matè" infusion drinking in a population at risk for squamous cell carcinoma of the esophagus [Article in Portuguese]. Arq Gastroenterol. 2000;37(1):25-30. PMid:10962624

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Immunohistochemical and morphometric evaluation of COX-1 and COX-2 in the remodeled lung in idiopathic pulmonary fibrosis and systemic sclerosis*,**

Avaliação imuno-histoquímica e morfométrica de COX-1 e COX-2 no remodelamento pulmonar na fibrose pulmonar idiopática e na esclerose sistêmica

Edwin Roger Parra, Flavia Lin, Vanessa Martins, Maristela Peres Rangel, Vera Luiza Capelozzi

Abstract

Objective: To study the expression of COX-1 and COX-2 in the remodeled lung in systemic sclerosis (SSc) and idiopathic pulmonary fibrosis (IPF) patients, correlating that expression with patient survival. **Methods:** We examined open lung biopsy specimens from 24 SSc patients and 30 IPF patients, using normal lung tissue as a control. The histological patterns included fibrotic nonspecific interstitial pneumonia (NSIP) in SSc patients and usual interstitial pneumonia (UIP) in IPF patients. We used immunohistochemistry and histomorphometry to evaluate the expression of COX-1 and COX-2 in alveolar septa, vessels, and bronchioles. We then correlated that expression with pulmonary function test results and evaluated its impact on patient survival. **Results:** The expression of COX-1 and COX-2 in alveolar septa was significantly higher in IPF-UIP and SSc-NSIP lung tissue than in the control tissue. No difference was found between IPF-UIP and SSc-NSIP tissue regarding COX-1 and COX-2 expression. Multivariate analysis based on the Cox regression model showed that the factors associated with a low risk of death were younger age, high DLCO/alveolar volume, IPF, and high COX-1 expression in alveolar septa, whereas those associated with a high risk of death were advanced age, low DLCO/alveolar volume, SSc (with NSIP), and low COX-1 expression in alveolar septa. **Conclusions:** Our findings suggest that strategies aimed at preventing low COX-1 synthesis will have a greater impact on SSc, whereas those aimed at preventing high COX-2 synthesis will have a greater impact on IPF. However, prospective randomized clinical trials are needed in order to confirm that.

Keywords: Scleroderma, systemic; Idiopathic pulmonary fibrosis; Inflammation; Survival rate.

Resumo

Objetivo: Estudar a expressão de COX-1 e COX-2 em áreas pulmonares remodeladas em pacientes com esclerose sistêmica (ES) ou fibrose pulmonar idiopática (FPI) e correlacioná-la com a sobrevida desses pacientes. **Métodos:** Examinamos espécimes de biópsia pulmonar a céu aberto de 24 pacientes com ES e de 30 pacientes com FPI, utilizando-se tecido pulmonar normal como controle. Os padrões histológicos incluíram pneumonia intersticial não específica (PINE) fibrótica em pacientes com ES e pneumonia intersticial usual (PIU) nos pacientes com FPI. Imuno-histoquímica e histomorfometria foram usadas para avaliar a expressão celular de COX-1 e COX-2 em septos alveolares, vasos e bronquíolos, sua correlação com provas de função pulmonar e seu impacto na sobrevida. **Resultados:** A expressão de COX-1 e COX-2 em septos alveolares foi significativamente maior em FPI-PIU e ES-PINE do que no tecido controle. Não houve diferença entre FPI-PIU e ES-PINE quanto à expressão de COX-1 e COX-2. A análise multivariada baseada no modelo de regressão de Cox mostrou que os fatores associados a baixo risco de morte foram ter idade menor, valores elevados de DLCO/volume alveolar, FPI, e alta expressão de COX-1 em septos alveolares, ao passo que os fatores associados a alto risco de morte foram ter idade maior, valores baixos de DLCO/volume alveolar, ES (com PINE) e baixa expressão de COX-1 em septos alveolares. **Conclusões:** Nossos resultados sugerem que estratégias de prevenção de baixa síntese de COX-1 terão maior impacto sobre a ES, ao passo que as de prevenção de alta síntese de COX-2 terão maior impacto sobre a FPI. Porém, são necessários ensaios clínicos randomizados prospectivos para confirmar essa hipótese.

Descritores: Escleroderma sistêmico; Fibrose pulmonar idiopática; Inflamação; Taxa de sobrevida.

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Introduction

Lung remodeling is a common end-stage sequela of idiopathic pulmonary fibrosis (IPF) and systemic sclerosis (SSc), resulting in disruption of lung architecture, leading to progressive respiratory failure.[1-4] Histologically, the remodeling process is characterized by diffuse chronic interstitial inflammation and increased fibroblast proliferation, as well as by increased extracellular matrix synthesis and collagen deposition.[2,5,6] Therefore, modulation of inflammation, fibroblast proliferation, and collagen synthesis by effector mediators in IPF and SSc is very important. Despite the characterization of a variety of key participants, the mediators and mechanisms involved in the pathogenesis of IPF and SSc have yet to be fully defined, which might explain the limited number of therapeutic approaches, with little impact on long-term survival.[7,8]

It is known that COX is the key enzyme in the conversion of arachidonic acid to prostaglandin E_2 (PGE_2), the precursor of a diverse family of bioactive lipid mediators including prostaglandins, thromboxane, and prostacyclin. It exists in two isoforms, namely COX-1 and COX-2. The former is constitutively expressed in most tissues and acts as a housekeeping enzyme regulating vascular homeostasis, protecting the gastric mucosa, and maintaining renal integrity,[9,10] whereas the latter has lower levels of expression in most tissues but is inducible in response to growth factors, cytokines, and other proinflammatory molecules.[11-13]

Regarding the proinflammatory and anti-inflammatory roles of COX-1 and COX-2, immunohistochemistry can be a useful tool to detect COX-1 and COX-2 in the remodeled lung in patients with SSc and IPF. Data on the assessment of COX-1 and COX-2 in the remodeled lung have previously been reported in serum[14,15] and bronchoalveolar lavage fluid[16] from patients with SSc, as well as in fibroblast cultures[4] and biopsies[17,18] from patients with IPF. However, the roles of COX-1 and COX-2 in the mechanisms involved in the remodeled lung in IPF and SSc patients are still unclear, and there has been uncertainty regarding the best way to detect COX-2. The aim of the present study was to study the expression of COX-1 and COX-2 in lung biopsy specimens (COX-1 and COX-2 expression being separately evaluated in alveolar septa, bronchioles, and vessels) and correlate it with patient survival.

Methods

Between January of 2002 and July of 2008, 24 consecutive patients with SSc and interstitial lung disease and 30 patients suspected of having IPF on the basis of HRCT findings were submitted to open lung biopsy at the University of São Paulo School of Medicine *Hospital das Clínicas*, located in the city of São Paulo, Brazil. All patients fulfilled the diagnostic criteria for SSc[19] and IPF[1] Open lung biopsy was performed by formal thoracotomy, areas of honeycombing being avoided. All patients gave written informed consent, and the study was approved by the local research ethics committee (Protocol no. 0960/08).

We analyzed the clinical records of all patients. Disease duration was determined on the basis of the onset of the first symptom. Pulmonary function testing and HRCT were performed within up to 3 months before the biopsy. Pulmonary function testing included VC, FEV_1, FVC, FEV_1/FVC, TLC, RV, and DLCO. Physiological assessment was performed before open lung biopsy and before the initiation of treatment. All pulmonary function tests, including spirometry, determination of lung volumes, and measurement of DLCO, were performed on the same day. All spirometric tests were performed with a calibrated pneumotachograph (Medical Graphics Co., St. Paul, MN, USA), all values being expressed as a percentage of their respective predicted value, the reference values having been established by Pereira et al.[20] Lung volumes were measured with a whole-body plethysmograph (Medical Graphics Co.), all values being expressed as a percentage of the predicted values.[21] Diffusing capacity was expressed as a percentage of the predicted values.[22] Diffusing capacity was expressed as a percentage of the predicted values.[23] All patients were followed regularly after treatment until death, blood and lung function tests being regularly performed. The primary endpoint was to evaluate the impact of COX-1 and COX-2 changes on survival and analyze differences between SSc and IPF. Table 1 shows the demographic data. As a control, normal lung tissue was obtained from 10 individuals (6 males and 4 females) whose median age was 46.6 ± 5.8 years and who had died suddenly of nonpulmonary causes.

Regarding open lung biopsy findings, usual interstitial pneumonia (UIP), the histological pattern of IPF, was characterized by patchy subpleural

Table 1 - Clinical data of the patients with systemic sclerosis and of those with idiopathic pulmonary fibrosis.[a]

Variable	SSc patients	IPF patients
Number	24	30
Males/females	0/24	16/14
Age at biopsy, years	45.0 ± 9.0	64.7 ± 7.9
Spirometry		
FEV_1, % of predicted	70.50 ± 14.42	77.58 ± 20.06
FVC, % of predicted	65.00 ± 13.85	70.87 ± 16.88
FEV_1/FVC	107.96 ± 8.70	92.75 ± 18.55
TLC, % of predicted	81.00 ± 11.57	77.55 ± 20.32
RV, % of predicted	117.5 ± 35.52	98.21 ± 61.14
DLCO, % of predicted	66.86 ± 21.68	56.27 ± 23.18
DLCO/AV, % of predicted	77.76 ± 37.28	55.66 ± 31.62
Follow-up period, months	70.75 (96)[b]	46.32 (69)[b]
Patients censored for survival analysis at the last follow-up visit	19	15

SSc: systemic sclerosis; IPF: idiopathic pulmonary fibrosis; and AV: alveolar volume. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as median (range).

and paraseptal distribution of parenchymal injury. Temporal heterogeneity was seen at low magnification, areas of normal lung parenchyma alternating with alveolar collapse, interstitial mononuclear infiltrates, septal fibromyxoid tissue (fibroblastic foci), and honeycomb lung.[2] All of the patients with SSc had histological patterns consistent with fibrotic nonspecific interstitial pneumonia (NSIP), as defined by temporally homogeneous septal thickening and interstitial fibrosis.[19]

For immunohistochemistry analysis, a standard peroxidase technique was used (Harris's hematoxylin being used as the counterstain) in order to identify COX-1 and COX-2 expression in alveolar septa, bronchiolar walls, and vascular walls in normal lung tissue (the control tissue), in lung tissue showing the UIP pattern (the UIP tissue), and in lung tissue showing the NSIP pattern (the NSIP tissue). All antibodies used were biotinylated goat polyclonal antibodies. Anti-COX-1 and anti-COX-2 antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) were incubated with tissue sections at dilutions of 1:50 and 1:100, respectively. The Novolink Max Polymer amplification kit (Leica Biosystems Newcastle Ltd, Newcastle upon Tyne, UK) was used for signal amplification, and 3,3'-diaminobenzidine tetrahydrochloride (0.25 mg dissolved in 1 mL of 0.02% hydrogen peroxide) was used as a precipitating substrate for signal detection. The specificity of primary antibodies was confirmed by appropriate reagent controls (the primary antibody being omitted or nonimmune serum being substituted for the primary antibody in the staining protocol), which revealed no staining.

Regarding histomorphometry, COX-1 expression and COX-2 expression were assessed by a point-counting technique in 50 and 30 fields in alveolar septa, bronchiolar walls, and vascular walls in the control tissue, in the UIP tissue, and in the NSIP tissue. The technique was performed with a 100-point grid (area, 187,500 μm^2[21]; magnification, ×400) attached to the microscope eyepiece.[23] At a magnification of ×400, the septal, bronchiolar, and vascular areas in each field were calculated on the basis of the number of points overlying connective tissue, as a proportion of the total grid area. Subsequently, the number of immunostained cells within the septal, bronchiolar, and vascular areas was counted. The areal fraction of immunostained cells represents the percentage ratio of the area of labeled cells in relationship to the total area covered by the grid in the eyepiece.

In order to assess interobserver variability, we compared the results obtained by two observers in 20% of the slides. The coefficient of variation for the interobserver error of the cell count was 5%.

Data are presented as mean ± SD and 95% CI. The Student's t-test for independent samples was used in order to test the relationship between continuous variables, and the residuals were examined to ensure that they were approximately normally distributed. The relationship between cellularity (as determined by immunostaining) and pulmonary function test results was evaluated by Pearson's correlation coefficient. For all cases, measured variable values were arranged

in ascending order and divided into two groups on the basis of the median value of each variable. For each variable, the groups were designated low degree and high degree, as follows: alveolar septal COX-1 (low degree, < 2.35%; high degree, 2.35%); vascular COX-1 (low degree, < 2.91%; high degree, 2.91%); bronchiolar COX-1 (low degree, < 2.88%; high degree, 2.88%); total COX-1 (low degree, < 2.77%; high degree, 2.77%); alveolar septal COX-2 (low degree, < 2.04%; high degree, 2.04%); vascular COX-2 (low degree, < 2.34%; high degree, 2.34%); bronchiolar COX-2 (low degree, < 2.34%; high degree, 2.34%); and total COX-2 (low degree, < 2.16%; high degree, 2.16%).

Overall survival analysis was performed in two steps. First, we performed a univariate analysis relating overall follow-up to each of the measured variables by means of the Kaplan-Meier method and then analyzed survival using the log-rank test. The variables that were found to be significant in the univariate analysis were included in the multivariate analysis based on the Cox proportional hazards regression model. A positive event was defined as any death caused by IPF or SSc. Deaths from causes other than IPF or SSc and living patients were included in the models as censored cases.

All statistical procedures were performed with the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). For all tests, the significance level was set at 5%.

Results

Table 1 summarizes the clinical features of the patients with SSc (n = 24) and those of those with IPF (n = 30). Six of 17 SSc patients (35.29%) and 13 of 19 IPF patients (68.42%) had restrictive lung disease. Respiratory function test results were as follows: FVC < 80% in 18 (75%) of the 24 SSc patients and in 19 of 22 IPF patients (86.36%); TLC < 80% in 6 of 17 SSc patients (35.9%) and in 13 of 19 IPF patients (68.42%); DLCO < 80% in 12 of 15 SSc patients (80%) and in 8 of 9 IPF patients (88.88%); and DLCO/alveolar volume < 80% in 11 of 18 SSc patients (61.11%) and in 11 of 14 IPF patients (78.57%). A significant negative correlation was found between COX-2 expression in vessels and FVC ($r = -0.28$; $p = 0.05$), as well as between COX-2 expression in alveolar septa and DLCO ($r = -0.80$; $p = 0.009$).

Figure 1 shows alveolar septa, vessels, and bronchioles in the control tissue, in the NSIP tissue, and in the UIP tissue immunostained for COX-1 (in A, C, E, G, I, K, M, O, and Q) and COX-2 (in B, D, F, H, J, L, N, P, and R). The NSIP and UIP tissues differed from the control tissue in terms of the immunostaining intensity of epithelial cells, endothelial cells, myofibroblasts, and smooth muscle cells in the alveolar septa, vessels, and bronchioles.

Table 2 summarizes the morphometric results. The proportion of alveolar septal cells immunostained for COX-1 and COX-2 was significantly higher in the UIP and NSIP tissues than in the control tissue. In other words, high proportions of alveolar septal cells staining for COX-1 and COX-2 were associated with the UIP and NSIP patterns. As can be seen in the bar plots in Figure 1 (S and T) the relationship of COX-1 and COX-2 with IPF (the UIP pattern) was stronger than was that of COX-1 and COX-2 with SSc (the NSIP pattern). Although the proportion of bronchiolar cells immunostained for COX-2 was lower in the NSIP and UIP tissues than in the control tissue (Figure 1W), the difference was not statistically significant. In addition, although the proportion of bronchiolar cells immunostained for COX-1 was higher in the UIP and NSIP tissues than in the control tissue (Figure 1W), the difference was not significant. No differences were found among the tissues in terms of the COX-1 or COX-2 immunostaining, for vessels or for the total parenchyma (Table 2).

A preliminary analysis of the Kaplan-Meier survival curves showed that survival was better in the patients with SSc (the fibrotic NSIP pattern) and COX-2 expression > 2.25% (median survival, 70.75 months) than in those with IPF (the UIP pattern) and COX-2 expression < 2.25% (median survival, 46.32 months; Figure 2). Therefore, we coded the fibrotic NSIP pattern as a single dummy variable with a value of 1 and the UIP pattern with a value of 2. The results of the multivariate analysis based on the Cox proportional hazards regression model are shown in Table 3. After controlling for age, pulmonary function test results, the UIP pattern, and the fibrotic NSIP pattern, we found that only two variables were significantly associated with survival time: the fibrotic NSIP pattern and alveolar septal COX-2 ($p = 0.02$). Once these two variables were accounted for, none of the others were related

Figure 1 - Cellular expression of COX-1 and COX-2 in alveolar septa, intrapulmonary vessels, and bronchioles in normal lung tissue (control tissue); in lung tissue obtained from patients with systemic sclerosis (SSc) and showing fibrotic nonspecific interstitial pneumonia (NSIP); and in lung tissue obtained from patients with idiopathic pulmonary fibrosis (IPF) and showing usual interstitial pneumonia (UIP). The intensity of COX-1 immunostaining of epithelial cells, endothelial cells, myofibroblasts, and smooth muscle cells in SSc-NSIP and IPF-UIP tissue alveolar septa (G and M, respectively), vessels (I and O, respectively), and bronchioles (K and Q, respectively) was higher than was that of COX-1 immunostaining of those cells in control tissue alveolar septa (A), vessels (C), and bronchioles (E). Likewise, the intensity of COX-2 immunostaining of those cells in SSc-NSIP and IPF-UIP tissue alveolar septa (H and N, respectively), vessels (J and P, respectively), and bronchioles (L and R, respectively) was higher than was that of COX-2 immunostaining of those cells in control tissue alveolar septa (B), vessels (D), and bronchioles (F). The bar plots show the quantification of COX-1 and COX-2 immunostaining of cells in alveolar septa (S), total lung parenchyma (T), and bronchioles (W) in the control tissue, in the SSc-NSIP tissue, and in the IPF-UIP tissue (immunohistochemical staining; magnification, ×400).

Table 2 - Morphometric results in normal lung tissue (control tissue), in lung tissue showing the usual interstitial pneumonia pattern (from patients with idiopathic pulmonary fibrosis), and in lung tissue showing the nonspecific interstitial pneumonia pattern (from patients with systemic sclerosis).[a]

Variable	Control	IPF-UIP	SSc-NSIP
COX-1			
Septal	1.14 ± 0.94*	4.09 ± 1.33*	2.74 ± 0.98
Vascular	3.55 ± 1.20	2.71 ± 1.33	2.13 ± 0.90
Bronchiolar	1.70 ± 1.38	2.20 ± 0.92	1.70 ± 0.62
Total	2.20 ± 1.27	3.20 ± 0.86	2.20 ± 0.59
COX-2			
Septal	1.55 ± 1.26*	2.90 ± 1.68*	1.82 ± 1.18
Vascular	2.88 ± 2.02	2.42 ± 1,25	1.80 ± 1.65
Bronchiolar	1.95 ± 1.90	2.10 ± 3.40	0.34 ± 0.38
Total	2.18 ± 1.15	2.17 ± 0.98	1.16 ± 0.66

IPF-UIP: idiopathic pulmonary fibrosis (with the usual interstitial pneumonia pattern); and SSc-NSPI: systemic sclerosis (with the nonspecific interstitial pneumonia pattern). [a]The values presented correspond to the percentage ratio of the area of labeled cells in relationship to the total area covered by the grid in the eyepiece. Results obtained by one-way ANOVA and post hoc analysis with the Bonferroni test for multiple comparisons (control, IPF-UIP, and SSc-NSPI) and the Student's t-test for between-group comparisons. The level of significance was set at 0.05. *Statistically significant difference between groups.

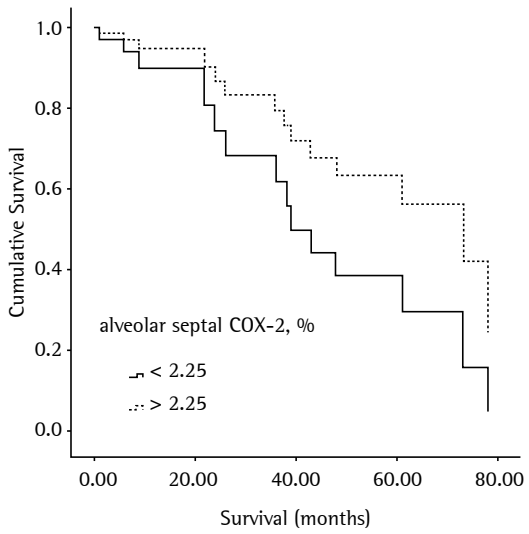

Figure 2 - Cox regression plots for risk of death risk versus duration of follow-up (in months) in young patients with low DLCO/alveolar volume, systemic sclerosis (and a histological pattern of cellular nonspecific interstitial pneumonia), high-degree total COX-1, and low-degree alveolar septal COX-2. The top curve represents the group of patients with systemic sclerosis and cellular nonspecific interstitial pneumonia. The bottom curve represents two groups of patients: those with systemic sclerosis and fibrotic nonspecific interstitial pneumonia; and those with idiopathic pulmonary fibrosis and the usual interstitial pneumonia pattern.

to survival. The multivariate analysis showed a low risk of death for young patients with low FEV_1/FVC, fibrotic NSIP pattern, and high-degree alveolar septal COX-2.

Discussion

The limited number of therapeutic approaches that have any impact on long-term survival in patients with IPF-UIP and in those with SSc and fibrotic NSIP is due to the lack of definition regarding the mediators and mechanisms involved in the pathogenesis of IPF and SSc. Therefore, the question of interest is whether additional mediators can provide a better understanding of the pathogenesis of these diseases. The repair process involves two distinct stages: a regenerative, inflammatory phase, in which the microenvironment attempts to replace injured cells; and a fibrotic phase, in which connective tissue replaces normal parenchymal tissue.[24-26] In the repair process, PGE_2 production by fibroblasts is increased,[27,28] which constitutes further evidence of the antiproliferative, anti-inflammatory and antifibrotic properties of COX-2/PGE_2.[15] Therefore, our finding that immunohistochemistry staining for COX provides important information on the repair processes in pulmonary fibrosis is not surprising, and our results confirm that the expression of COX-2 is increased in IPF and SSc, with improved outcome in a group of patients. We found that the proportion of alveolar septal cells immunostained for COX-1 and COX-2 was significantly higher in lung tissue showing the UIP pattern and the fibrotic NSIP pattern than in normal lung tissue. Increased COX-1 expression was expected because COX-1 is constitutively expressed in most cells and tissues, whereas COX-2 is induced by inflammatory or

Table 3 - Cox proportional hazards regression to ascertain the individual contribution of the histological pattern and morphological factors associated with survival and to compare adjusted survival between the two groups.

	β	SE	Wald	Significance	Exp (β)	95% CI for Exp (β)	
						Lower	Upper
Age	0.09	0.09	1.00	0.31	1.10	0.91	1.32
DLCO/AV	−0.08	0.05	2.09	0.14	0.92	0.82	1.03
IPF-UIP			0.01	0.99			
SSc-NSIP	0.12	1.37	0.008	0.92	1.13	0.07	6.68
Septal COX-1	2.27	1.06	4.52	0.03	9.65	1.19	8.13

B: beta coefficient; Exp(β): exponential beta; AV: alveolar volume; IPF-UIP: idiopathic pulmonary fibrosis (with the usual interstitial pneumonia pattern); and SSc-NSIP: systemic sclerosis (with the nonspecific interstitial pneumonia pattern). −2 log likelihood = 24.17; chi-square = 12.2; p = 0.001.

mitogenic stimuli.[9] These results contrast with those of previous studies investigating IPF.[4,17,18] Those studies showed reduced COX-2 expression in pulmonary fibroblasts secondary to decreased COX-2 production. However, in those studies, COX-2 expression was measured only in fibroblasts, whereas in our study it was measured in the alveolar septa, including epithelial cells and fibroblasts in normal areas, collapsed areas, and fibroblast foci. Other studies, including a study by Lappi-Blanco et al.,[3] found increased expression of COX-2 in metaplastic epithelium and fibroblasts from fibrotic areas in IPF-UIP. These contradictory findings suggest that COX-2 plays a dual role in IPF-UIP. First, reduced COX-2 expression in normal areas, collapsed areas, and fibroblastic foci suggests an anti-inflammatory role for COX-2 in early-stage IPF-UIP. Second, the presence of progressive fibrosis even in the presence of increased COX-2 expression suggests that fibroblasts are unable to respond to stimulation by COX-2 and its main product (PGE_2) so as to inhibit fibroblast proliferation, myofibroblastic transformation, and increased production of collagen and other extracellular matrix molecules.

In the present study, the proportion of alveolar septal cells immunostained for COX-1 and COX-2 was found to be lower in fibrotic NSIP tissue (from SSc patients) than in UIP tissue (from IPF patients). This finding contrasts with those of previous studies showing that COX-2 levels are higher in SSc patients.[14-16] In addition, COX-2 production has been shown to be much greater in the inflammatory resolution phase than in the early phase.[29] These contradictory findings suggest that COX-2 has a dual role in a normal inflammatory process, playing a proinflammatory role in the early phase and an anti-inflammatory role in the resolution phase.[29] Therefore, in view of the abovementioned evidence and of the latent inflammation in patients with SSc and lung involvement, our results emphasize the idea that COX-2 does not exert its anti-inflammatory effect properly, because there is inflammation even when COX-2 expression is increased in patients with SSc and fibrotic NSIP. However, further studies are needed in order to clarify the real reason why the COX-2 mechanism is deficient. We hypothesize that this is due to an inability of COX-2 to stimulate the production of PGE_2 or other anti-inflammatory mediators in opposition to its own proinflammatory effects or an inability of the cells to respond appropriately to COX-2.

Our study has clinical and functional impact. We sought to establish a correlation between COX-2 and patient survival controlled for age, pulmonary function test results, the UIP pattern (in patients with IPF) and the NSIP pattern (in patients with SSc). Our multivariate analysis showed a low risk of death for younger patients with low DLCO/alveolar volume, SSc (and the NSIP histological pattern), high-degree total COX-2, and high-degree alveolar septal COX-1.

In conclusion, the expression of COX-1 and COX-2 in the lung parenchyma offers us the potential to control repair processes involved in the progression of SSc-NSIP and IPF-UIP, suggesting that strategies aimed at preventing low COX-1 synthesis will have a greater impact on SSc, whereas those aimed at preventing high COX-2 synthesis will have a greater impact on IPF. Prospective randomized trials are required in order to confirm that.

References

1. Sociedade Brasileira de Pneumologia e Tisiologia. Diretrizes de Doenças Pulmonares Intersticiais da Sociedade Brasileira de Pneumologia e Tisiologia. J Bras Pneumol. 2012;38(Suppl 2):S1-S133.

2. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. American Thoracic Society; European Respiratory Society. Am J Respir Crit Care Med. 2002;165(2):277-304. Erratum in: Am J Respir Crit Care Med. 2002;166(3):426. http://dx.doi.org/10.1164/ajrccm.165.2.ats01 PMid:11790668
3. Lappi-Blanco E, Kaarteenaho-Wiik R, Maasilta PK, Anttila S, Pääkkö P, Wolff HJ. COX-2 is widely expressed in metaplastic epithelium in pulmonary fibrous disorders. Am J Clin Pathol. 2006;126(5):717-24. http://dx.doi.org/10.1309/PFGXCLNG2N17PJX9 PMid:17050069
4. Wilborn J, Crofford LJ, Burdick MD, Kunkel SL, Strieter RM, Peters-Golden M. Cultured lung fibroblasts isolated from patients with idiopathic pulmonary fibrosis have a diminished capacity to synthesize prostaglandin E2 and to express cyclooxygenase-2. J Clin Invest. 1995;95(4):1861-8. http://dx.doi.org/10.1172/JCI117866 PMid:7706493 PMCid:PMC295728
5. Gabbiani G. The myofibroblast: a key cell for wound healing and fibrocontractive diseases. Prog Clin Biol Res. 1981;54:183-94. PMid:7015359
6. Gross TJ, Hunninghake GW. Idiopathic pulmonary fibrosis. N Engl J Med. 2001;345(7):517-25. http://dx.doi.org/10.1056/NEJMra003200 PMid:11519507
7. Dempsey OJ. Clinical review: idiopathic pulmonary fibrosis--past, present and future. Respir Med. 2006;100(11):1871-85. http://dx.doi.org/10.1016/j.rmed.2006.08.017 PMid:16987645
8. Downey DG, Brockbank S, Martin SL, Ennis M, Elborn JS. The effect of treatment of cystic fibrosis pulmonary exacerbations on airways and systemic inflammation. Pediatr Pulmonol. 2007;42(8):729-35. http://dx.doi.org/10.1002/ppul.20646 PMid:17588254
9. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, et al. Cyclooxygenase in biology and disease. FASEB J. 1998;12(12):1063-73. PMid:9737710
10. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998;38:97-120. http://dx.doi.org/10.1146/annurev.pharmtox.38.1.97 PMid:9597150
11. Xie WL, Chipman JG, Robertson DL, Erikson RL, Simmons DL. Expression of a mitogen-responsive gene encoding prostaglandin synthase is regulated by mRNA splicing. Proc Natl Acad Sci U S A. 1991;88(7):2692-6. http://dx.doi.org/10.1073/pnas.88.7.2692 PMid:1849272 PMCid:PMC51304
12. O'Banion MK, Sadowski HB, Winn V, Young DA. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J Biol Chem. 1991;266(34):23261-7. PMid:1744122
13. Hla T, Neilson K. Human cyclooxygenase-2 cDNA. Proc Natl Acad Sci U S A. 1992;89(16):7384-8. http://dx.doi.org/10.1073/pnas.89.16.7384
14. Bassyouni IH, Talaat RM, Salem TA. Serum concentrations of cyclooxygenase-2 in patients with systemic sclerosis: association with lower frequency of pulmonary fibrosis. J Clin Immunol. 2012;32(1):124-30. http://dx.doi.org/10.1007/s10875-011-9601-z PMid:21979415
15. Xiao R, Kanekura T, Yoshida N, Higashi Y, Yan KL, Fukushige T, et al. 9-Cis-retinoic acid exhibits antifibrotic activity via the induction of cyclooxygenase-2 expression and prostaglandin E2 production in scleroderma fibroblasts. Clin Exp Dermatol. 2008;33(4):484-90. http://dx.doi.org/10.1111/j.1365-2230.2008.02727.x PMid:18462443
16. Kowal-Bielecka O, Kowal K, Distler O, Rojewska J, Bodzenta-Lukaszyk A, Michel BA, et al. Cyclooxygenase- and lipoxygenase-derived eicosanoids in bronchoalveolar lavage fluid from patients with scleroderma lung disease: an imbalance between proinflammatory and antiinflammatory lipid mediators. Arthritis Rheum. 2005;52(12):3783-91. http://dx.doi.org/10.1002/art.21432 PMid:16320329
17. Xaubet A, Roca-Ferrer J, Pujols L, Ramírez J, Mullol J, Marin-Arguedas A, et al. Cyclooxygenase-2 is up-regulated in lung parenchyma of chronic obstructive pulmonary disease and down-regulated in idiopathic pulmonary fibrosis. Sarcoidosis Vasc Diffuse Lung Dis. 2004;21(1):35-42. PMid:15127973
18. Petkova DK, Clelland CA, Ronan JE, Lewis S, Knox AJ. Reduced expression of cyclooxygenase (COX) in idiopathic pulmonary fibrosis and sarcoidosis. Histopathology. 2003;43(4):381-6. http://dx.doi.org/10.1046/j.1365-2559.2003.01718.x PMid:14511257
19. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr, et al. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol. 1988;15(2):202-5. PMid:3361530
20. Pereira CA, Sato T, Rodrigues SC. New reference values for forced spirometry in white adults in Brazil. J Bras Pneumol. 2007;33(4):397-406. http://dx.doi.org/10.1590/S1806-37132007000400008 PMid:17982531
21. Neder JA, Andreoni S, Castelo-Filho A, Nery LE. Reference values for lung function tests. I. Static volumes. Braz J Med Biol Res. 1999;32(6):703-17. PMid:10412549
22. Andreoni S, Lerario MC, Nery LE. Reference values for lung function tests. II. Maximal respiratory pressures and voluntary ventilation. Braz J Med Biol Res. 1999;32(6):719-27. PMid:10412550
23. Hsia CC, Hyde DM, Ochs M, Weibel ER; ATS/ERS Joint Task Force on Quantitative Assessment of Lung Structure. An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. Am J Respir Crit Care Med. 2010;181(4):394-418. http://dx.doi.org/10.1164/rccm.200809-1522ST PMid:20130146
24. Meneghin A, Hogaboam CM. Infectious disease, the innate immune response, and fibrosis. J Clin Invest. 2007;117(3):530-8. http://dx.doi.org/10.1172/JCI30595 PMid:17332880 PMCid:PMC1804377
25. Oikonomou N, Harokopos V, Zalevsky J, Valavanis C, Kotanidou A, Szymkowski DE, et al. Soluble TNF mediates the transition from pulmonary inflammation to fibrosis. PLoS One. 2006;1:e108. http://dx.doi.org/10.1371/journal.pone.0000108 PMid:17205112 PMCid:PMC1762410
26. Pardo A, Selman M. Molecular mechanisms of pulmonary fibrosis. Front Biosci. 2002;7:d1743-61.
27. Kobayashi T, Narumiya S. Function of prostanoid receptors: studies on knockout mice. Prostaglandins Other Lipid Mediat. 2002;68-69:557-73. http://dx.doi.org/10.1016/S0090-6980(02)00055-2
28. McCann MR, Monemdjou R, Ghassemi-Kakroodi P, Fahmi H, Perez G, Liu S, et al. mPGES-1 null mice are resistant to bleomycin-induced skin fibrosis. Arthritis Res Ther. 2011;13(1):R6. http://dx.doi.org/10.1186/ar3226 PMid:21266028 PMCid:PMC3546456
29. Gilroy DW, Colville-Nash PR, Willis D, Chivers J, Paul-Clark MJ, Willoughby DA. Inducible cyclooxygenase may have anti-inflammatory properties. Nat Med. 1999;5(6):698-701. http://dx.doi.org/10.1038/9550 PMid:10371510

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CT densitovolumetry in children with obliterative bronchiolitis: correlation with clinical scores and pulmonary function test results*,**

Densitovolumetria pulmonar por TC em crianças com bronquiolite obliterante: correlação com escores clínicos e testes de função pulmonar

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Abstract

Objective: To determine whether air trapping (expressed as the percentage of air trapping relative to total lung volume [AT%]) correlates with clinical and functional parameters in children with obliterative bronchiolitis (OB). **Methods:** CT scans of 19 children with OB were post-processed for AT% quantification with the use of a fixed threshold of −950 HU (AT%950) and of thresholds selected with the aid of density masks (AT%DM). Patients were divided into three groups by AT% severity. We examined AT% correlations with oxygen saturation (SO_2) at rest, six-minute walk distance (6MWD), minimum SO_2 during the six-minute walk test (6MWT_SO_2), FVC, FEV_1, FEV_1/FVC, and clinical parameters. **Results:** The 6MWD was longer in the patients with larger normal lung volumes (r = 0.53). We found that AT%950 showed significant correlations (before and after the exclusion of outliers, respectively) with the clinical score (r = 0.72; 0.80), FVC (r = 0.24; 0.59), FEV_1 (r = −0.58; −0.67), and FEV_1/FVC (r = −0.53; r = −0.62), as did AT%DM with the clinical score (r = 0.58; r = 0.63), SO_2 at rest (r = −0.40; r = −0.61), 6MWT_SO_2 (r = −0.24; r = −0.55), FVC (r = −0.44; r = −0.80), FEV_1 (r = −0.65; r = −0.71), and FEV_1/FVC (r = −0.41; r = −0.52). **Conclusions:** Our results show that AT% correlates significantly with clinical scores and pulmonary function test results in children with OB.

Keywords: Multidetector computed tomography; Respiratory function tests; Bronchiolitis obliterans.

Resumo

Objetivo: Determinar as correlações entre o volume de aprisionamento aéreo em relação ao volume pulmonar total (AA%) e parâmetros clínicos e funcionais em crianças com bronquiolite obliterante (BO). **Métodos:** Técnicas de pós-processamento de imagem foram usadas em imagens de TC de 19 crianças com BO para quantificar AA% por meio de um limiar fixo de −950 UH (AA%950) e de limiares selecionados por meio de máscaras de densidade (AA%MD). Os pacientes foram divididos em três grupos, de acordo com a gravidade de AA%. Foram examinadas as correlações entre AA% e a saturação de oxigênio (SO_2) em repouso, a distância percorrida no teste de caminhada de seis minutos (DTC6), a SO_2 mínima durante o teste de caminhada de seis minutos (SO_2_TC6), a CVF, o VEF_1, a relação VEF_1/CVF e parâmetros clínicos. **Resultados:** A DTC6 foi maior nos pacientes com maiores volumes pulmonares normais (r = 0,53). Na amostra como um todo, encontramos (antes e depois da exclusão de valores extremos, respectivamente), correlações estatisticamente significativas entre AA%950 e o escore clínico (r = 0,72; 0,80), a CVF (r = 0,24; 0,59), o VEF_1 (r = −0,58; −0,67) e a relação VEF_1/CVF (r = −0,53; r = −0,62), bem como entre AA%MD e o escore clínico (r = 0,58; r = 0,63), a SO_2 em repouso (r = −0,40; r = −0,61), a SO_2_TC6 (r = −0,24; r = −0,55), a CVF (r = −0,44; r = −0,80), o VEF_1 (r = −0,65; r = −0,71) e a relação VEF_1/CVF (r = −0,41; r = −0,52). **Conclusões:** Os resultados deste estudo mostram que AA% correlaciona-se significativamente com escores clínicos e testes de função pulmonar em crianças com BO.

Descritores: Tomografia computadorizada multidetectores; Testes de função respiratória; Bronquiolite obliterante.

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Introduction

Postinfectious obliterative bronchiolitis (OB) is an uncommon and severe form of chronic obstructive lung disease that occurs in children after lower respiratory tract injury.[1] In terms of diagnosis, there are no specific signs or symptoms of postinfectious OB. Clinical and imaging findings are used in combination with laboratory test results in order to identify the cause and rule out other forms of chronic lung disease. The diagnostic criteria for postinfectious OB are as follows: severe acute bronchiolitis/viral pneumonia during the first 3 years of life in previously healthy children; evidence of persistent airway obstruction after the acute event, identified by physical examination, pulmonary function testing, or both; chest X-ray findings of obstructive lung disease; a mosaic pattern and air trapping (AT) on chest CT scans; and exclusion of other chronic lung diseases progressing to permanent respiratory symptoms.[2,3]

Histologically, OB is characterized by the presence of granulation tissue plugs in the small airway lumen, complete small airway destruction, or both.[4] Mauad et al.[5] showed that OB is histologically characterized by a constrictive pattern with varying degrees of inflammation and airway obliteration, ranging from minimal bronchiolar inflammation to complete obliteration of the bronchioles and bronchi by fibrotic tissue. Indirect signs of obstruction, such as macrophage accumulation, bronchiectasis, mucus accumulation, and hyperinflation, are always present.

Although there have been several reviews of studies examining long-term sequelae of adenovirus pneumonia,[5,6] few studies have examined CT findings in children with OB.[6,7] Some of these studies have reported that abnormal CT findings in such children can predict abnormal lung function later in life.[7]

It has been reported that HRCT is an important diagnostic tool for the evaluation of pulmonary damage in patients with OB.[2] The volume of an organ (or the volume of abnormal parts of an organ) can be determined by the use of helical CT images and attenuation coefficient values or densities on the Hounsfield scale, expressed in Hounsfield units (HU). This method is known as volumetric CT densitometry or CT densitovolumetry,[8,9] and it can be used in order to measure the volume of areas of lung with low attenuation (i.e., AT), as well as total lung volume (TLV), in children with OB. In addition, it can be used in order to measure the percentage of AT relative to TLV (AT%) with the use of thresholds to differentiate normal lung areas from OB areas. It has been shown that AT% is a major contributing factor to chronic persistent airflow obstruction in asthma.[10]

Pulmonary function remains abnormal for long periods after an episode of OB.[11,12] The clinical course following the onset of OB is variable and depends on the volume of affected lung tissue.[1] Measures of clinical status and of the ability to perform physically demanding activities—such as the number of hospital admissions or missed school days, walk test distance, nutritional status, and oxygen saturation (SO_2) at rest or during exercise—facilitate the selection of appropriate clinical interventions.[2,3] Because the prognosis of OB patients depends not only on patient behavior but also on functional impairment and the extent of anatomical damage, clinicians should gather information regarding the extent and type of anatomical abnormalities. Bronchiectasis, atelectasis, lobar collapse, and areas of low density are known consequences of postinfectious OB, and HRCT is the best method of examining such lesions,[4,6,7] all of which can influence the clinical status of patients and their pulmonary function test results.[4,6,7] Previous studies have shown that low attenuation areas correlate well with pulmonary function test results in patients with AT of various causes.[13-15] However, to our knowledge, no studies have shown a correlation of CT densitovolumetry findings with pulmonary function in children with OB.[4,6,7]

Our objective was to describe the correlation between the volume of areas of AT and pulmonary function test results in children with OB. In addition, we compared AT% values with SO_2 values, pulmonary function test results, and clinical scores in those children in an attempt to obtain objective measurement criteria for OB.

Methods

This was a prospective study including all of the children treated at our postinfectious OB clinic for more than 5 years and having clinically stable OB and symptom onset before the age of 2 years. The study protocol was approved by the local research ethics committee, and the parents or legal guardians of all participants gave written informed consent.

The diagnosis of OB was based on the identification of chronic obstructive respiratory symptoms appearing after an episode of lower respiratory tract infection before the age of 2 years in previously healthy children.[2,3] All clinical diagnoses were confirmed by characteristic findings on contemporary HRCT scans. Differential diagnoses were excluded.

The inclusion criteria were as follows[3]: acute bronchiolitis/viral pneumonia before the age of 2 years in previously healthy children; evidence of persistent airway obstruction after the acute event, identified by physical examination, pulmonary function testing, or both, the obstruction being unresponsive to at least 2 weeks of treatment with systemic corticosteroids and bronchodilators; radiological findings of obstructive lung disease, including hyperinflation, atelectasis, bronchial wall thickening, and bronchiectasis, as well as a mosaic perfusion pattern and AT on CT scans; and absence of other chronic lung diseases progressing to persistent respiratory symptoms, including tuberculosis, cystic fibrosis, bronchopulmonary dysplasia, immunodeficiency, asthma, and severe alpha-1 antitrypsin deficiency. The exclusion criteria were as follows: being unable to undergo pulmonary function testing or CT without sedation; being unable to hold breath for the duration of CT scanning; Having disease exacerbation < 30 days before CT or pulmonary function testing; having other lung diseases; and requiring continuous oxygen therapy.

A total of 25 patients with OB met the inclusion criteria. Of those 25 patients, 6 were excluded (because they were unable to perform the required respiratory maneuvers). The final sample consisted of 19 children (14 males and 5 females) in the 7-15 year age bracket (mean age, 10 ± 2.5 years).

All patients performed spirometry and six-minute walk tests (6MWTs) in accordance with previous reports.[12,16] Spirometry was performed with a Vitalograph ALPHA spirometer (Vitalograph, Buckingham, UK) before and 10 min after the administration of an inhaled dose of albuterol (300 µg; Aerolin®; GlaxoSmithKline plc, Ware, UK) with a valve spacer (Fisonair®; GlaxoSmithKline plc). All children performed a 6MWT with SO_2 control. Because the 6MWT was performed after spirometry (and on the same day as the latter), all patients used albuterol before the 6MWT.

Clinical scores included the following: 1) nutritional status[17] (Z score: 0 = good nutritional status; 1 = mild malnutrition; 2 = moderate malnutrition; and 3 = severe malnutrition); 2) cough during remission (0 = absent and 1 = present); 3) cough within 2 weeks before the examination (0 = absent and 1 = present); 4) wheezing during remission (0 = absent and 1 = present); 5) wheezing on most days of the week (0 = absent and 1 = present); 6) wheezing in the last 2 weeks (0 = absent and 1 = present); 7) difficulty breathing in the 2 weeks preceding the examination (0 = absent and 1 = present); 8) frequency of exacerbations in the last 6 months (0 = no exacerbations; 1 = sporadic exacerbations; 2 = exacerbations every 2 months; 3 = exacerbations every month; and 4 = exacerbations every week); 9) increased anteroposterior chest diameter (0 = absent and 1 = present); 10) SO_2 at rest (≥ 95% = 0; 90-94% = 1; and < 90% = 2); 11) minimum SO_2 during the 6MWT (6MWT_SO_2; ≥ 95% = 0; 90-94% = 1; and < 90% = 2), mean desaturation during exercise being measured by calculating the difference between SO_2 at rest and 6MWT_SO_2; 12) desaturation > 4% during the 6MWT (0 = negative and 1 = positive); 13) the FEV_1/FVC ratio; and 14) percent predicted FEV_1 (FEV_1%; > 80% = 0; 61-80% = 1; 41-60% = 2; < 41% = 3).

All CT images were acquired with the use of the lowest possible radiation dose and a commercially available helical CT scanner (XVision EX; Toshiba Medical Systems Corporation, Otawara, Japan), being post-processed on a workstation (O2®; SGI, Fremont, CA, USA) running three-dimensional (3D) rendering software (ALATOVIEW; Toshiba Medical Systems Corporation). The CT scanner was calibrated periodically, as recommended by the manufacturer. An initial set of nine axial HRCT scans (with 1-mm collimation at increments of 20 mm) were acquired with the use of a high-frequency algorithm. Those HRCT images were evaluated by two thoracic radiologists with more than 10 years of experience in chest CT. A final decision was reached by consensus.

Two additional sets of images were acquired by the helical CT scanner during single breath-hold maneuvers (during inhalation and exhalation). To minimize respiratory motion artifacts, helical CT scans were taken in the caudocranial direction. On the basis of previous studies,[18-21] the following parameters were used: collimation, 10 mm; table

speed, 14 mm/rotation (pitch, 1.4); and low radiation dose (120 kV and 50 mAs). The mean total radiation dose was 5 ± 1.3 mSv. We used helical CT scans of 10 mm, low mAs, and high pitch in order to reduce radiation exposure. A standard reconstruction algorithm was used in order to avoid the effects of edge-enhancing filters on tissue density.[22] All scans were taken without intravenous contrast medium.

The first step of CT densitovolumetry was lung segmentation for measuring TLV. Two segmentation steps were applied to each set of helical CT images before the calculation of TLV. The lungs were isolated by eliminating from the image data any structure with a density exceeding −250 HU. Subsequently, we eliminated the air within the abdomen and outside the patient (nonpulmonary air) by selecting regions of interest with the 3D rendering software. The regions of interest were selected by drawing a line between the lung and the nonpulmonary air on each slice. Different 3D software, unavailable to us during the study period, might include different tools for lung segmentation. In order to minimize operator-dependent variability, we considered the air within the trachea or main bronchi to be pulmonary air rather than excluding it. The operator assessed segmentation accuracy by reviewing the 3D lung image generated by volume rendering (Figure 1). The software then automatically calculated TLV.

For measuring the volume of lung parenchyma with abnormally low attenuation values, areas of extremely low attenuation or density were considered to be abnormal, because of the disproportion between the volume of lung parenchyma (including interstitial tissue, vessels, blood, lymph, interstitial fluid, and airway walls) and the air in those regions. In order to simplify measurement, we defined "AT volume" as the total volume of lung zones showing extremely low density values.

We calculated AT% using a fixed threshold. We first calculated the lung volume affected by AT using a fixed threshold of −950 HU (AT%950). This threshold was first proposed by Gevenois et al.[23] and has been used by many others in order to quantify emphysema.[18] We considered any portion of the lungs with a density below −950 HU to be affected by AT. We calculated AT%950 by dividing the AT volume for that threshold by the TLV. These data were also calculated for expiratory scans.

The next step was to calculate AT% for thresholds selected by using density masks. No validated threshold is available for determining the volume of hyperinflation, AT, or emphysema in children. Because the −950 HU threshold can underestimate the extent of disease in this population, we also calculated AT volume using a threshold selected for each patient with the aid of a density mask. The threshold level was adjusted until the mask corresponded to our

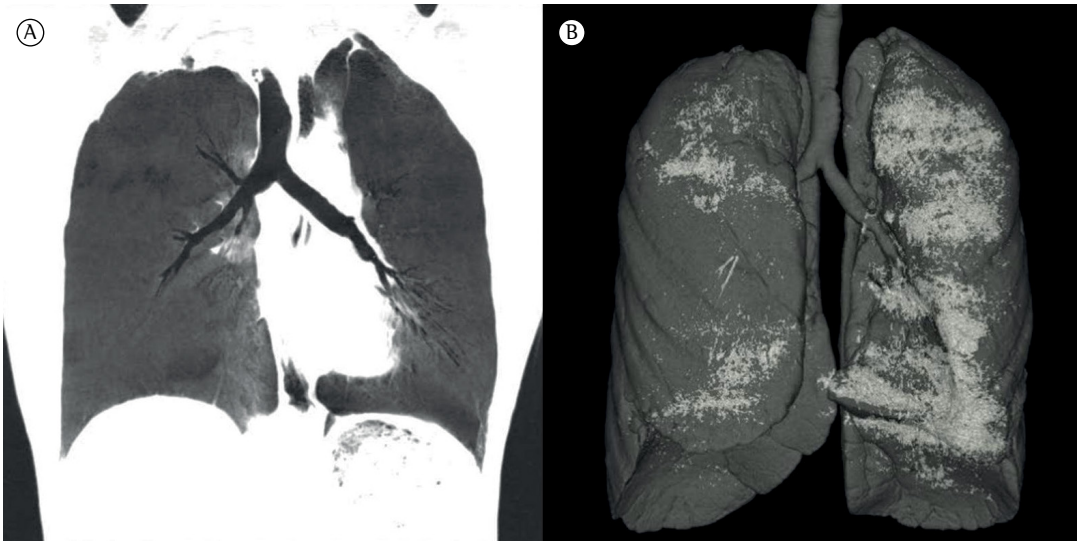

Figure 1 - In A, coronal reformatted CT image (minimum intensity projection) showing air trapping. In B, three-dimensional CT reconstruction showing low attenuation volumes, which represent air trapping volumes.

subjective visual impression of affected lung portions (Figure 2). That threshold was selected, and TLV was then segmented. Any portions of the lungs with densities below the selected HU were considered abnormal. We then calculated AT% using that density mask (AT%DM). These data were also calculated for expiratory scans.

The shrink (deflation) volume of the lungs was calculated by subtracting the TLV as measured on images acquired during exhalation from the TLV as measured on images acquired during inhalation. The percentage of shrink volume was considered excellent if it was > 50%, reasonable/good if it was 30-50%, and poor if it was < 30%.

For the statistical analysis, test results were entered into a Microsoft Excel database and processed by means of Excel tools, the analysis being performed with the Statistical Package for the Social Sciences, version 11 (SPSS Inc., Chicago, IL, USA). Statistical significance was set at $p < 0.05$. We assumed a power of 90% for a sample size of 19 patients and statistical significance of $p < 0.05$, on the basis of a previous study.[7]

Scatter plots (Figure 3) were used in order to classify patients according to the severity of AT%. For AT%950 and AT%DM, AT% < 1% was considered to indicate normality or minimal disease expression. In addition, AT%950 values of 1-5% were considered to indicate moderate disease expression, and AT%950 values > 5% were considered to indicate severe disease expression. Moreover, AT%DM values of 1-10% were considered to indicate moderate disease expression, and AT%DM values > 10% were considered to indicate severe disease expression. The magnitude of variability in AT%950 and AT%DM guided the selection of values to differentiate between moderate and severe disease expression (Figure 3).

All variables were analyzed by Pearson's product-moment correlation coefficient. Correlations were determined before and after the exclusion of outliers (Figure 4). Values of r and p were calculated separately for censored and uncensored data. Correlations were also calculated for pulmonary function test results and clinical scores.

Results

Of the 19 patients, 2 had normal clinical scores, 7 had clinical scores < 5 (including the two patients with clinical scores = 0), 6 had scores of 5-10, and 6 had scores > 10. In the

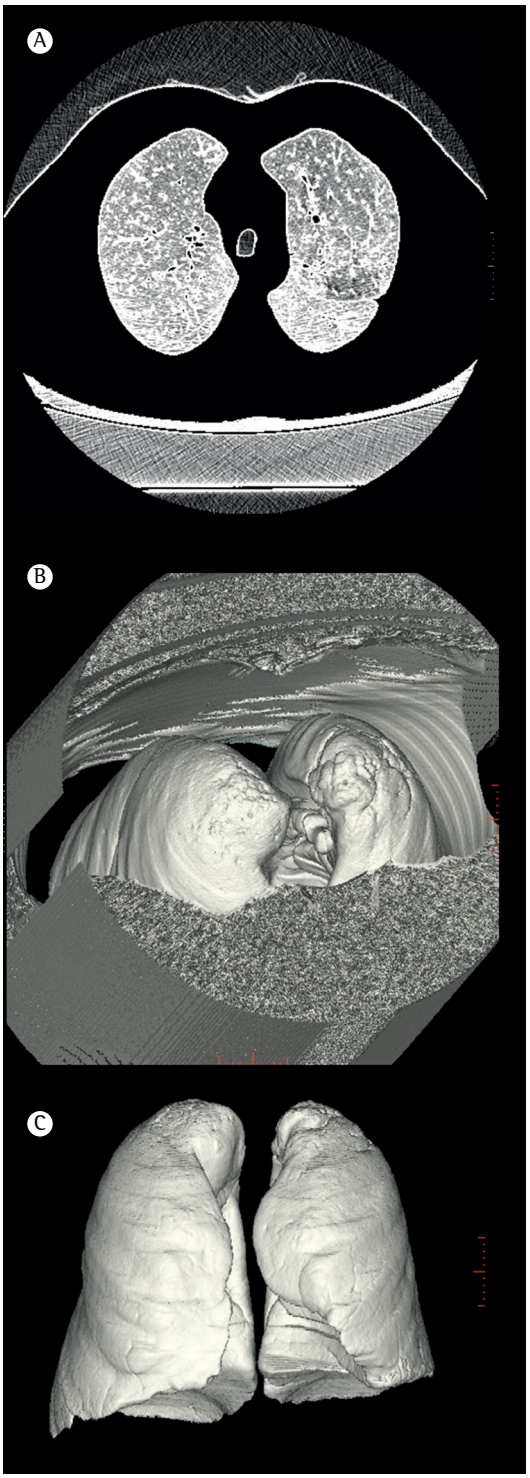

Figure 2 - Post-processing tools. After the use of a threshold, the air inside and outside the lungs is isolated. In A, axial CT scan showing air inside and outside the lungs. In B, three-dimensional (3D) volume rendering of the same data. In C, 3D volume rendering of total lung volume after the exclusion of air outside the lungs.

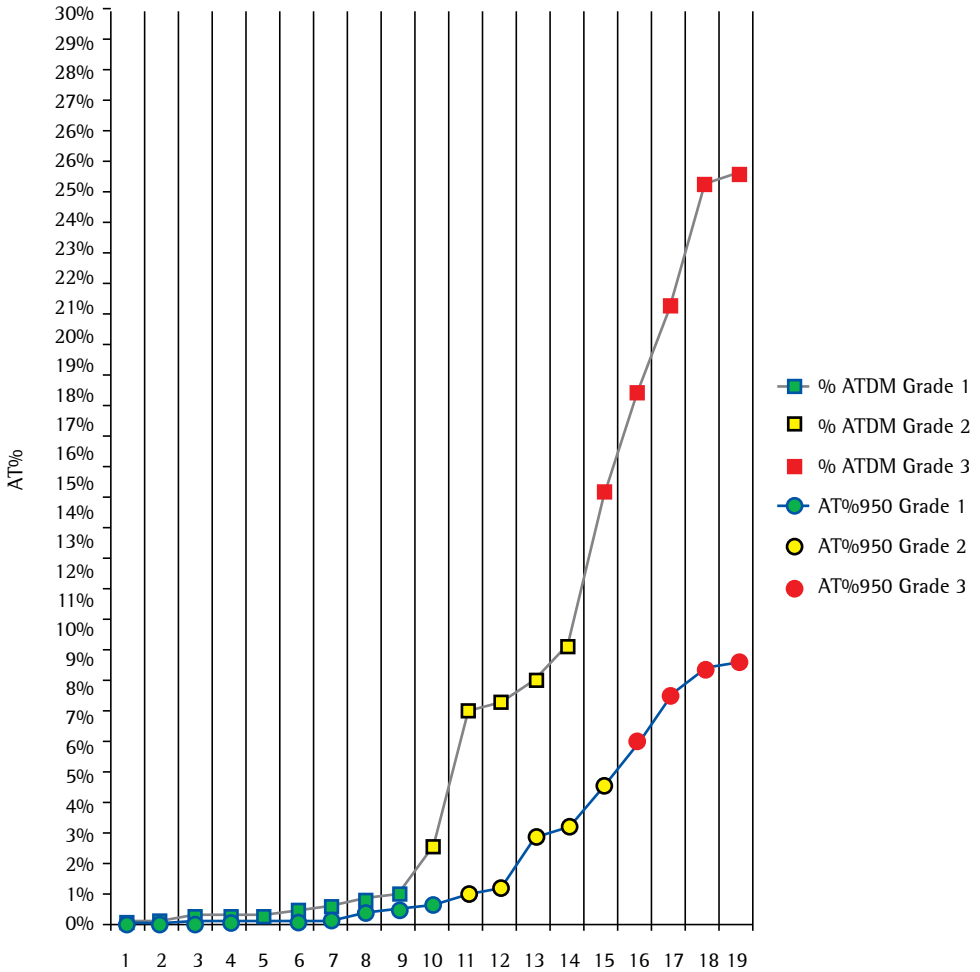

Figure 3 - Scatter plot illustrating the distribution of patients according to the percentage of air trapping relative to total lung volume (AT%), which was calculated for a fixed threshold of –950 HU (AT%950) and for thresholds set by subjective analysis based on density masks (AT%DM). Note that AT%DM allows better discrimination among the grades of disease severity (i.e., grade 1, normal/mild; grade 2, moderate; and grade 3, severe), especially for moderate disease (AT% > 1%) and severe disease (AT% > 10%). Note also that the stratification of disease severity changed from normal/mild to moderate in 1 patient (case 10) and from moderate to severe in 1 patient (case 14) depending on the method for selecting the threshold.

evaluation of the nutritional status, 8 patients had Z scores < 0.

Regarding 6MWT parameters, mean SO_2 at rest was 96 ± 2% (range, 92-99%), and mean 6MWT_SO_2 was 92 ± 4% (range, 83-99%). We found no correlation between SO_2 at rest and SO_2 during exercise (r = 0.00). Mean desaturation during exercise (measured by calculating the difference between SO_2 at rest and 6MWT_SO_2) was 4 ± 4% (range, –2% to 15%). The mean six-minute walk distance (6MWD) was 552 ± 131 m (range, 90-705 m). The 6MWD correlated significantly with 6MWT_SO_2 (r = 0.52; p < 0.05) and desaturation during exercise (r = 0.58; p < 0.05) but not with SO_2 at rest (r = 0.26; p > 0.05). Regarding pulmonary function parameters, mean FVC was 75 ± 20% (range, 43-106%), mean FEV_1% was 58 ± 20% (range, 36-100%), and mean FEV_1/FVC was 72 ± 16% (range, 49-107%).

Regarding CT densitovolumetry parameters, mean TLV was 3,009 ± 1,184 mL (range, 1,252-6,673 mL). The 6MWD was longer in those with larger normal lung volumes (r = 0.53). Mean shrink volume was 1,174 ± 789 mL (range, 182-3,471 mL), and mean percentage of shrink volume was 36 ± 13% (range, 9-63%). Mean AT%DM was 7.28 ± 9% (range, 0.03-24.67%), and mean AT%950 was 2.4 ± 3% (range, 0.03-8.67%). In

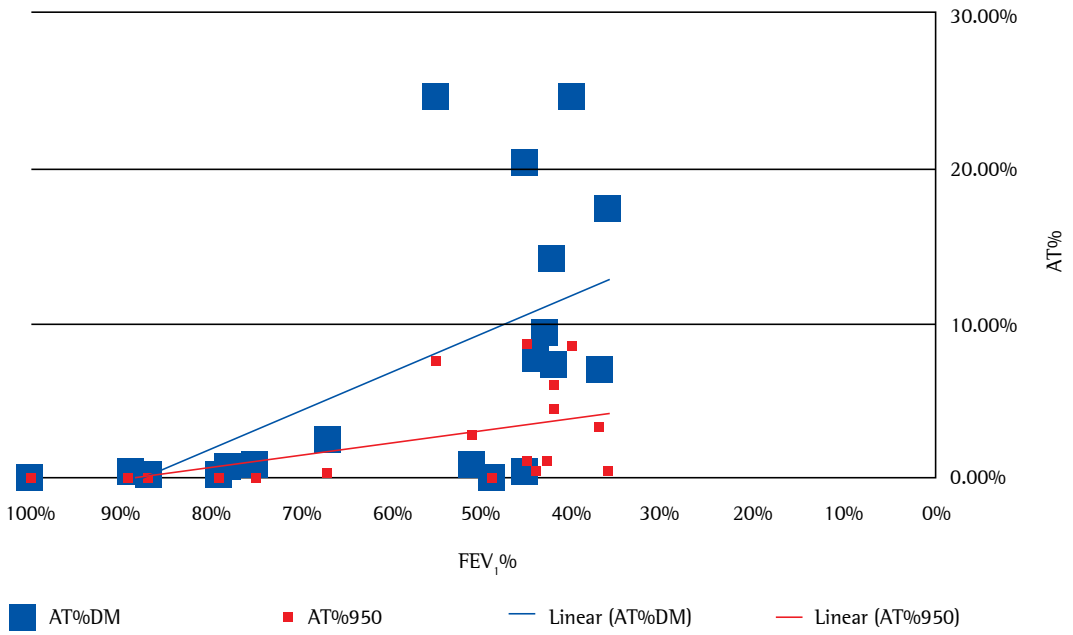

Figure 4 - Distribution of the observations of the percentage of air trapping relative to total lung volume (AT%), calculated for a fixed threshold of −950 HU—AT%950—(red squares) and for thresholds selected with the aid of density masks—AT%DM—(blue squares), including the corresponding linear regression lines. The distribution suggests that patients with FEV_1% above 70% had only mild anatomical damage as measured by quantification of areas of air trapping or hyperinflation. Some patients with FEV_1% in the range of 50% or less had only mild disease as measured by AT%950, a finding that highlights the limitations of a threshold of −950 HU for the assessment of patients with obliterative bronchiolitis. The correlation between FEV_1% and AT%DM was much better, as shown by the regression line.

9, 5, and 5 of the 19 patients, AT%DM was classified as normal or mild, moderate, and severe, respectively (points above the gray line in Figure 3). In 10, 5, and 4 of the 19 patients, AT%950 was classified as normal or mild, moderate, and severe, respectively (points above the blue line in Figure 3). We found a correlation between AT%DM and AT%950, as evidenced by r = 0.83 (or r = 0.93 after the exclusion of one outlier). No significant correlation was found between the percentage of shrink volume and AT%DM or between the percentage of shrink volume and AT%950.

The correlations of CT densitovolumetry parameters with clinical scores, pulmonary function test results, and 6MWT parameters are summarized in Table 1. Table 1 shows the correlation values for each parameter before and after the exclusion of outliers. Figure 4 illustrates the correlations of AT%DM and AT%950 with nondensitometric parameters.

Discussion

An uncommon and severe form of chronic obstructive lung disease in children and adults, OB results from lower respiratory tract injury.[6] The diagnosis of postinfectious OB in children is based on a history of lower respiratory tract infection (usually an acute viral infection), followed by persistent chronic obstructive lung disease.[2,3] HRCT is an excellent method for the identification of anatomical damage following the onset of the disease, such damage including areas of low attenuation, areas of consolidation/atelectasis, bronchial wall thickening, bronchiectasis, and mosaic perfusion. In addition, expiratory HRCT scans can assist in confirming the presence of AT. However, HRCT allows only a subjective assessment of the extent of the disease and is dependent on the experience and skill of radiologists.[6,9] Our study demonstrated that the 6MWD was longer in patients with larger normal lung (r = 0.53). In addition, we found that AT%950 showed significant correlations (before and after the exclusion of outliers, respectively) with the clinical score (r = 0.72; 0.80), FVC (r = 0.24; 0.59), FEV1 (r = −0.58; −0.67), and FEV1/FVC (r = −0.53; r = −0.62), as did AT%DM with the clinical score (r = 0.58; r = 0.63), SO2 at rest

Table 1 - Correlations between CT findings and functional data.

INSPIRATORY CT SCANS						
Correlations	Clinical score	SO_2 at rest	6MWT_SO_2	FVC%	FEV_1%	FEV_1/FVC%
AT%DM	0.58*				−0.65*	
AT%950	0.72*				−0.58*	−0.53*
cAT%DM	0.63*	−0.61*	−0.55*	−0.80*	−0.71*	−0.52*
cAT%950	0.80*			−0.59*	−0.67*	−0.62*
EXPIRATORY CT SCANS						
Correlations	Clinical score	SO_2 at rest	6MWT_SO_2	FVC%	FEV_1%	FEV_1/FVC%
AT%950	0.40	0.10	−0.20	−0.30	−0.30	−0.10
AT%DM	0.40	−0.17	−0.17	−0.45	−0.44	−0.06
cAT%DM	0.57	−0.28	−0.48	−0.60	−0.67	−0.29

AT%950: percentage of air trapping relative to total lung volume, calculated for a fixed threshold of −950 HU; AT%DM: percentage of air trapping relative to total lung volume, calculated for thresholds selected with the aid of density masks; cAT%950: censored AT%950 (i.e., AT%950 after the exclusion of outliers); cAT%DM: censored AT%DM (i.e., AT%DM after the exclusion of outliers); SO_2: oxygen saturation; 6MWT_SO_2: minimum oxygen saturation during the six-minute walk test. *$p < 0.05$.

(r = −0.40; r = −0.61), 6MWT_SO2 (r = −0.24; r = −0.55), FVC (r = −0.44; r = −0.80), FEV1 (r = −0.65; r = −0.71), and FEV1/FVC (r = −0.41; r = −0.52). These data suggest that objective CT measurements adequately represent clinical scores and functional impairment in OB.

CT densitovolumetry has been proven to overcome this limitation and is a standard recommendation for the quantification of other lung diseases in which the proportion between pulmonary air and the lung parenchyma is increased, therefore decreasing lung density.[20,21] Areas of decreased attenuation can also result from decreased perfusion of hypoventilated alveoli distal to obstructed bronchioles. The main finding on expiratory CT scans is a geographic heterogeneity of lung attenuation (mosaic attenuation pattern), which is seen in 40-80% of patients.[6,19] The abnormalities can be subtle on inspiratory CT scans, being usually easier to detect on expiratory CT scans.[24] In a previous study of the correlation between pulmonary function abnormalities and the extent of HRCT features of OB, significant relationships were found only between FEV_1 and the number of bronchopulmonary segments affected by bronchiectasis.[14] Hansell et al.[15] confirmed that the extent of decreased attenuation was independently associated with a reduction in FEV_1. In contrast, bronchial wall thickening was independently associated with the presence of AT (as measured by RV/TLC). In patients with Sauropus androgynus-associated OB, pulmonary function test results were more closely correlated with AT than with any other CT parameter.[16] To our knowledge, the present study is the first to demonstrate significant correlations of AT% with clinical scores and pulmonary function test results. The quantification of areas of abnormally low attenuation is an important diagnostic tool for OB, and the technique has substantial advantages over the traditional subjective assessment of HRCT images.[19-21] The quantification of anatomical damage is important in patients with OB; CT densitovolumetry can measure lung volumes directly and therefore aid clinicians in making decisions regarding patient quarantining and the aggressiveness of treatment.

In the present study, clinical scores were moderately correlated with FVC and FEV_1/FVC ($r \sim 0.5$). Although there was a stronger correlation between clinical scores and FEV_1 (r = 0.8), these findings are possibly biased because pulmonary function test results were included in the clinical score parameters. Additionally, both measures of AT% were significantly correlated with clinical scores ($r \geq 0.6$); the strength of those correlations increased when outliers were eliminated (censored AT%950, r = 0.8), which suggests that it might increase further in larger series. We found that inspiratory CT scans were much more informative than expiratory CT scans regarding the presence of AT. The extent of AT areas might have been underestimated on the expiratory CT scans because of the higher expiratory lung density. This is important because atelectasis is more common in children.[24]

Our study has some limitations. In order to calculate AT%, we used a threshold that has yet to be validated. The −950 HU threshold was validated for emphysema quantification in adult

patients, and we used it in the present study despite our conviction that segmentation at this level would result in an underestimation of areas of low attenuation in children, especially in the absence of hyperinflation/emphysema.[23] Although histopathological findings were unavailable for our patients, previous studies[2,3] have examined correlations between imaging findings and histopathological findings in patients with OB. In addition, our clinical score has yet to be validated, its clinical application requiring further investigation.

In conclusion, anatomical damage to the lungs as measured by CT (i.e., AT%) correlated significantly with clinical scores and pulmonary function test results. After our censoring of extreme values, AT%DM showed stronger correlations than did AT%950.

References

1. Ryu JH, Myers JL, Swensen SJ. Bronchiolar disorders. Am J Respir Crit Care Med. 2003;168(11):1277-92. http://dx.doi.org/10.1164/rccm.200301-053SO PMid:14644923
2. Hardy K. Childhood bronchiolitis obliterans. In: Epler G, editor. Diseases of the bronchioles. New York: Raven Press; 1994. p. 415-26.
3. Fischer GB, Sarria EE, Mattiello R, Mocelin HT, Castro-Rodriguez JA. Post infectious bronchiolitis obliterans in children. Paediatr Respir Rev. 2010;11(4):233-9. http://dx.doi.org/10.1016/j.prrv.2010.07.005 PMid:21109182
4. Muller NL, Silva CI. Obliterative Bronchiolitis. In: Boiselle PM, Lynch DA, editors. CT of the airways. Totowa: Humana Press; 2008. p. 75-120.
5. Mauad T, Dolhnikoff M; São Paulo Bronchiolitis Obliterans Study Group. Histology of childhood bronchiolitis obliterans. Pediatr Pulmonol. 2002;33(6):466-74. http://dx.doi.org/10.1002/ppul.10097 PMid:12001281
6. Zhang L, Irion K, da Silva Porto N, Abreu e Silva F. High-resolution computed tomography in pediatric patients with postinfectious bronchiolitis obliterans. J Thorac Imaging. 1999;14(2):85-9. http://dx.doi.org/10.1097/00005382-199904000-00003 PMid:10210478
7. Mattiello R, Sarria EE, Mallol J, Fischer GB, Mocelin H, Bello R, et al. Post-infectious bronchiolitis obliterans: can CT scan findings at early age anticipate lung function? Pediatr Pulmonol. 2010;45(4):315-9. http://dx.doi.org/10.1002/ppul.21115 PMid:20205267
8. Mergo PJ, Williams WF, Gonzalez-Rothi R, Gibson R, Ros PR, Staab EV, et al. Three-dimensional volumetric assessment of abnormally low attenuation of the lung from routine helical CT: inspiratory and expiratory quantification. AJR Am J Roentgenol. 1998;170(5):1355-6. http://dx.doi.org/10.2214/ajr.170.5.9574615 PMid:9574615
9. Müller NL, Staples CA, Miller RR, Abboud RT. "Density mask". An objective method to quantitate emphysema using computed tomography. Chest. 1988;94(4):782-7. http://dx.doi.org/10.1378/chest.94.4.782 PMid:3168574
10. Park SW, Park JS, Jeong SH, Lee YN, Hwangbo Y, Park JS, et al. Air trapping is a major determinant of persistent airway obstruction in asthmatics. Respir Med. 2012;106(6):786-93. http://dx.doi.org/10.1016/j.rmed.2012.02.012 PMid:22445772
11. de Blic J, Deschildre A, Chinet T. Post-infectious bronchiolitis obliterans [Article in French]. Rev Mal Respir. 2013;30(2):152-60. http://dx.doi.org/10.1016/j.rmr.2012.10.600 PMid:23419446
12. Mattiello R, Mallol J, Fischer GB, Mocelin HT, Rueda B, Sarria EE. Pulmonary function in children and adolescents with postinfectious bronchiolitis obliterans. J Bras Pneumol. 2010;36(4):453-9. http://dx.doi.org/10.1590/S1806-37132010000400010 PMid:20835592
13. Mets OM, Zanen P, Lammers JW, Isgum I, Gietema HA, van Ginneken B, et al. Early identification of small airways disease on lung cancer screening CT: comparison of current air trapping measures. Lung. 2012;190(6):629-33. http://dx.doi.org/10.1007/s00408-012-9422-8 PMid:23064488
14. Padley SP, Adler BD, Hansell DM, Müller NL. Bronchiolitis obliterans: high resolution CT findings and correlation with pulmonary function tests. Clin Radiol. 1993;47(4):236-40. http://dx.doi.org/10.1016/S0009-9260(05)81129-8
15. Hansell DM, Rubens MB, Padley SP, Wells AU. Obliterative bronchiolitis: individual CT signs of small airways disease and functional correlation. Radiology. 1997;203(3):721-6. PMid:9169694
16. Rodrigues JC, Cardieri JM, Bussamra MH, Nakaie CM, Almeida MB, Silva FL, et al. Provas de função pulmonar em crianças e adolescentes. J Pneumol. 2002;28(Suppl 3):S207-S221.
17. de Almeida CA, Ricco RG, Nogueira MP, Del Ciampo LA, Mucillo G. Comparison of four anthropometric methods of nutritional assessment and evaluation of the agreement between two reference populations. J Trop Pediatr. 1999;45(6):345-50. http://dx.doi.org/10.1093/tropej/45.6.345 PMid:10667003
18. Mattiello R, Sarria EE, Stein R, Fischer GB, Mocelin HT, Barreto SS, et al. Functional capacity assessment in children and adolescents with post-infectious bronchiolitis obliterans. J Pediatr (Rio J). 2008;84(4):337-43. http://dx.doi.org/10.2223/JPED.1807
19. Kim CK, Chung CY, Kim JS, Kim WS, Park Y, Koh YY. Late abnormal findings on high-resolution computed tomography after Mycoplasma pneumonia. Pediatrics. 2000;105(2):372-8. http://dx.doi.org/10.1542/peds.105.2.372 PMid:10654958
20. Kauczor HU, Heussel CP, Fischer B, Klamm R, Mildenberger P, Thelen M. Assessment of lung volumes using helical CT at inspiration and expiration: comparison with pulmonary function tests. AJR Am J Roentgenol. 199;171(4):1091-5.
21. Arakawa A, Yamashita Y, Nakayama Y, Kadota M, Korogi H, Kawano O, et al. Assessment of lung volumes in pulmonary emphysema using multidetector helical CT: comparison with pulmonary function tests. Comput Med Imaging Graph. 2001;25(5):399-404. http://dx.doi.org/10.1016/S0895-6111(01)00004-0
22. Johnston C, Carvalho WB. Atelectasis: mechanisms, diagnosis and treatment in the pediatric patient [Article in Portuguese]. Rev Assoc Med Bras. 2008;54(5):455-60. http://dx.doi.org/10.1590/S0104-42302008000500021 PMid:18989568
23. Gevenois PA, De Vuyst P, de Maertelaer V, Zanen J, Jacobovitz D, Cosio MG, et al. Comparison of computed density and microscopic morphometry in pulmonary emphysema. Am J Respir Crit Care Med. 1996;154(1):187-92. http://dx.doi.org/10.1164/ajrccm.154.1.8680679 PMid:8680679

24. Schulz KF, Grimes DA. Sample size calculations in randomised trials: mandatory and mystical. Lancet. 2005;365(9467):1348-53. http://dx.doi.org/10.1016/S0140-6736(05)61034-3

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Detection of *Mycobacterium tuberculosis* complex by nested polymerase chain reaction in pulmonary and extrapulmonary specimens*,**

Detecção do complexo *Mycobacterium tuberculosis* por *nested polymerase chain reaction* em espécimes pulmonares e extrapulmonares

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Abstract

Objective: To compare the performance of nested polymerase chain reaction (NPCR) with that of cultures in the detection of the *Mycobacterium tuberculosis* complex in pulmonary and extrapulmonary specimens. **Methods:** We analyzed 20 and 78 pulmonary and extrapulmonary specimens, respectively, of 67 hospitalized patients suspected of having tuberculosis. An automated microbial system was used for the identification of *Mycobacterium* spp. cultures, and *M. tuberculosis* IS*6110* was used as the target sequence in the NPCR. The kappa statistic was used in order to assess the level of agreement among the results. **Results:** Among the 67 patients, 6 and 5, respectively, were diagnosed with pulmonary and extrapulmonary tuberculosis, and the NPCR was positive in all of the cases. Among the 98 clinical specimens, smear microscopy, culture, and NPCR were positive in 6.00%, 8.16%, and 13.26%, respectively. Comparing the results of NPCR with those of cultures (the gold standard), we found that NPCR had a sensitivity and specificity of 100% and 83%, respectively, in pulmonary specimens, compared with 83% and 96%, respectively, in extrapulmonary specimens, with good concordance between the tests (kappa, 0.50 and 0.6867, respectively). **Conclusions:** Although NPCR proved to be a very useful tool for the detection of *M. tuberculosis* complex, clinical, epidemiological, and other laboratory data should also be considered in the diagnosis and treatment of pulmonary and extrapulmonary tuberculosis.

Keywords: Tuberculosis/diagnosis; Tuberculosis/microbiology; Mycobacterium tuberculosis; Polymerase chain reaction.

Resumo

Objetivo: Comparar o desempenho da técnica *nested polymerase chain reaction* (NPCR) com aquele de culturas na detecção do complexo *Mycobacterium tuberculosis* em espécimes pulmonares e extrapulmonares. **Métodos:** Analisamos 20 e 78 espécimes pulmonares e extrapulmonares, respectivamente, de 67 pacientes hospitalizados com suspeita de tuberculose. Um sistema automatizado foi utilizado na identificação de culturas de *Mycobacterium* spp., e *M. tuberculosis* IS*6110* foi utilizada como sequência alvo na NPCR. A estatística kappa foi utilizada para verificar a concordância entre os resultados.**Resultados:** Entre os 67 pacientes, 6 e 5, respectivamente foram diagnosticados com tuberculose pulmonar e extrapulmonar, e a NPCR foi positiva em todos os casos. Entre os 98 espécimes clínicos, a baciloscopia, cultura e NPCR foram positivas em 6,00%, 8,16% e 13,26%, respectivamente. Comparando-se os resultados da NPCR com aqueles da cultura (padrão ouro) nos espécimes pulmonares, a sensibilidade e a especificidade foram 100% e 83%, respectivamente, enquanto essas nos espécimes extrapulmonares foram 83% e 96% respectivamente, com boa concordância entre os testes (kappa, 0,50 e 0,6867, respectivamente). **Conclusões:** Embora a NPCR tenha se mostrado uma ferramenta muito útil na detecção do complexo *M. tuberculosis*, No entanto, os resultados positivos da NPCR devem ser associados à clínica, dados clínicos, epidemiológicos e outros dados laboratoriais devem também ser considerados no diagnóstico e tratamento da tuberculose pulmonar e extrapulmonar.

Descritores: Tuberculose/diagnóstico; Tuberculose/microbiologia; Mycobacterium tuberculosis; Reação em cadeia da polimerase.

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Introduction

The World Health Organization estimates that there were between 8.8 and 9.2 million new tuberculosis cases in 2010.[1] In addition, it is estimated that 1.2 million of those cases are infected by HIV. Furthermore, it is estimated that 1.1 million deaths occurred in HIV-negative tuberculosis patients, which equates to 15 deaths per 100,000 population. Mortality is the most sensitive indicator of tuberculosis control measures[2]; tuberculosis remains among the top ten causes of death worldwide. The goal of curing 85% of tuberculosis cases, set by the World Health Assembly in 1991, was not achieved in 2009 in 7 of the 22 countries with the highest burden of the disease, including Brazil (at 72%).[3]

Diagnosis in an early stage of the disease is of paramount importance for treatment initiation, with direct consequences for individual disease control and, therefore, for public health initiatives aimed at the prevention of tuberculosis transmission. Therefore, it is necessary that clinical microbiology laboratories be able to quickly identify mycobacteria by means of microscopy and culture. However, sputum smear tests, although rapid and cost-effective, have low sensitivity and specificity, particularly in paucibacillary samples, and culture, even though it is considered the gold standard due to its high sensitivity, requires several weeks to produce a result.[4-8]

Currently, Brazil is one of the countries that, together, accounts for 80% of tuberculosis cases, with an incidence of 70,977 cases in 2010.[4,6] For the majority of those cases, the diagnosis was based only on sputum smear test results; chest X-rays, cultures, and biochemical tests for *Mycobacterium* spp. were only carried out in patients with negative sputum smear results but with respiratory symptoms.[8,9] These diagnostic limitations have encouraged the use of molecular tools with improved sensitivity, specificity, and speed, in order to detect mycobacteria in all clinical specimens.[5-8,10] The new technologies that are being developed have recently redefined the diagnosis of tuberculosis, providing a basis for diagnostic laboratory techniques.[5,8] The molecular diagnosis of tuberculosis by polymerase chain reaction (PCR) and primers with high specificity (98%), with high variations in sensitivity (20-100%), has been used in order to identify genetic targets in the bacillus.[7,11,12]

Despite the widespread use of conventional PCR, modifications in the technique, such as the addition of one extra reaction (nested PCR), have increased its sensitivity and specificity.[6] This might be due to the fact that it dilutes potential PCR inhibitors, which are commonly present in biological samples.[11] Therefore, the possibility of having access to a molecular tool that leads to a more rapid diagnosis and that is effective for the detection of cases that are difficult to elucidate by conventional tests certainly helps decrease morbidity and improve tuberculosis control. The aim of the present study was to evaluate the technique of nested PCR targeting the insertion sequence IS*6110* in *Mycobacterium tuberculosis* and to compare the results with those obtained in cultures of samples from patients suspected of having pulmonary or extrapulmonary tuberculosis.

Methods

This study was carried out between February and December of 2009. The patients included in the study were submitted to physical evaluation and sample collection at the *Hospital de Base*, a referral center for the diagnosis and treatment of tuberculosis located in the city São José do Rio Preto, Brazil. Epidemiological and clinical data were obtained from medical records in accordance with a protocol approved by the Research Ethics Committee of *Faculdade de Medicina de São José do Rio Preto* (São José do Rio Preto School of Medicine; Protocol no. 064/2009). The presence of HIV antibodies, identified by ELISA and confirmed by Western blot, indicated HIV seropositivity.

All of the patients included in the study were over 18 years of age, were immunosuppressed (due to immunosuppression therapy, autoimmune disease, organ transplantation, or HIV-positivity), and presented with clinical symptoms and signs suggestive of pulmonary or extrapulmonary tuberculosis. Our sample comprised 67 hospitalized patients, and 98 clinical specimens were collected, of which 20 were pulmonary specimens (sputum, BAL fluid, or gastric lavage fluid), and 78 were extrapulmonary specimens (blood, cerebrospinal fluid, lymph node aspirate, urine, pleural fluid, secretion from ganglia, pleura fragment, liver fragment, ascitic fluid, bone marrow aspirate, or biopsy specimens). The number of specimens collected from the patients ranged from one

to three, according to physician requests. The diagnostic confirmation of tuberculosis was based on the following criteria: clinical and radiological evidence of tuberculosis confirmed by laboratory tests, isolation of *M. tuberculosis* in clinical specimens by direct smear microscopy or culture (gold standard), and evident clinical improvement after antimycobacterial treatment.

In brief, direct smear microscopy was performed using Ziehl-Neelsen staining, and an automated microbial system (BacT/ALERT MP; Organon Teknika Corp., Durham, NC, USA) was used for the identification of *Mycobacterium* spp. in cultures. The strains were identified by phenotypic methods.[13] Genotyping was carried out by PCR-restriction enzyme analysis in accordance with Chimara et al.,[14] although with modifications.

Blood samples were collected in 5-mL tubes containing EDTA, and peripheral blood mononuclear cells were isolated by density gradient centrifugation (Ficoll-Histopaque) for future extraction of the DNA.[15-17] For solid organs, 2.0-mm punch biopsy samples were collected. All clinical samples were kept at −20°C until DNA extraction, which was performed in accordance with the method described by Rossetti et al.[18] with modifications by Lima et al.[11,18,19] In brief, a 500-µL aliquot of the sample was centrifuged at 13,000 rpm for 10 min and washed three times in Tris-EDTA (TE). The pellet was resuspended in 100 µL of TE and heated at 100°C for 10 min. The supernatant was transferred to a different tube, and 5 µL of resin were added (Sephaglas BandPrep Kit; Amersham-Pharmacia Biotech, Uppsala, Sweden); an aliquot of sodium iodide solution (0.9 g/mL) was added to the final volume. The tube was shaken for 5 min and incubated at room temperature for 5 min. After centrifuging the tube for 1 min and discarding the supernatant, we added 200 µL of iced 70% ethanol; the tube was then shaken, after which it was centrifuged for 1 min. The resulting pellet was kept at room temperature for 60 min, in order to complete the drying process, and resuspended in 40 µL of 1×TE. The tube was incubated in a water bath at 50°C for 10 min. Subsequently, the tube was centrifuged for 1 min, and the supernatant was transferred to another tube and stored at −20°C until processing.[11,18]

For the nested PCRs, IS*6110* of *M. tuberculosis* was used as the target sequence (GenBank accession no. 215310.1). The reactions were performed in accordance with Ritis et al.[17] The following primers were used: sense (TJ3 5'-ATC CCC TAT CCG TAT GGT G-3'); antisense (TJ5 5'-CCG CAA AGT GTG GCT AAC-3'); sense (*STAN3* 5'-GTC GAG TAC GCC TTC TTG TT-3'); and antisense (*OLI 5* 5'-AAC GGC TGA TGA CCA AAC-3'). The PCR used a final volume of 50 µL (1× buffer, 50 mM $MgCl_2$, 10 pmol/µL of each oligonucleotide, 0.2 µM dNTP, and 2 U Taq DNA polymerase [Invitrogen Life Technologies, Carlsbad, CA, USA]). The amplification process consisted of an initial denaturation step of 94°C for 3 min, 30 denaturing cycles (at 94°C for 1 min, 57°C for 1 min, and 72°C for 1 min), followed by a final extension at 72°C for 5 min. The second PCR was performed using 3 µL of the product of the first PCR under similar conditions to those described above, but with an annealing temperature of 60°C. DNA of the H37Rv strain of *M. tuberculosis* and PCR mix alone were used as positive and negative controls, respectively. The result was analyzed by electrophoresis on 2% agarose gel, stained with ethidium bromide, and visualized in an ultraviolet transilluminator (Fisher-Biotech, Fairlawn, NJ, USA), resulting in a 316-bp fragment.

Statistical analyses were performed with the Epi Info statistical package, version 6.0. The kappa statistic was used in order to assess the level of agreement among the results.[20] The level of significance was set at 5%.

Results

Our sample comprised 67 individuals, 63.7% being male. The mean age of the patients was 40.10 ± 3.66 years (range, 18-87 years). The most common comorbidity was HIV/AIDS, in 41 patients.

Tuberculosis was diagnosed in 11 individuals (16.41%), 5 being diagnosed with pulmonary tuberculosis and the remaining with extrapulmonary tuberculosis (pleural tuberculosis in 3, meningeal tuberculosis in 2, and miliary tuberculosis in 1). All culture isolates were confirmed by *M. tuberculosis* genotyping. Nested PCR was positive in all of the cases of confirmed tuberculosis, as shown in Table 1. In 4 of the tuberculosis patients, positive laboratory results were obtained only by the molecular technique (p = 0.110; Fisher's exact test).

Of the 98 clinical specimens analyzed, 6.00%, 8.16%, and 13.26%, respectively, showed positive

results in smear microscopy, culture, and nested PCR, as summarized in Table 1. Culture was negative in 2 of the samples with positive smear microscopy results, whereas smear microscopy was negative in 4 of the samples with positive culture results.

By comparing the results of nested PCR with those of cultures in pulmonary samples (Table 2), we found that the sensitivity and specificity of nested PCR were 100% and 83%, respectively. The positive and negative predictive values were 40% and 100%, respectively, with good concordance between the tests (kappa = 0.50; p = 0.25; McNemar's test). Regarding extrapulmonary samples (Table 2), sensitivity, specificity, positive predictive value, and negative predictive value were, respectively, 83.0%, 96.0%, 62.5%, and 98.5%, with good concordance between the tests (kappa = 0.6867; p = 0.625; McNemar's test).

Discussion

The early diagnosis of tuberculosis is essential for prompt treatment and effective control of the disease.[5] This is particularly true in cases of extrapulmonary tuberculosis, because various factors can complicate the diagnosis of the disease. Due to the paucibacillary nature of extrapulmonary tuberculosis, studies have shown a variability in positive culture results (from 12% to 80%) and a variety of clinical samples/biological tissues, which implies a non-uniform distribution of the bacillus, as well as the presence of nonspecific signs and symptoms.[10,12,19-21] In our study, pleural tuberculosis was diagnosed in 2 HIV-negative

Table 1 - Clinical, epidemiological, and laboratory data of the eleven patients with confirmed pulmonary or extrapulmonary tuberculosis.

Patient[a]	Form of tuberculosis	Type of sample	Results			Outcome
			Smear microscopy	Culture	Nested PCR	
1	Pulmonary	Blood	–	–	+	Death/NTB
2	Pulmonary	Blood	–	–	+	Cure
3	Pleural	Blood	–	–	+	Death/NTB
4	Pleural	Pleural fluid	+	–	+	Cure
5	Pulmonary	Blood	+	+	+	Death/TB
6	Pulmonary	Sputum	+	–	+	Death/NTB
7	Pulmonary	Sputum	+	+	+	Cure
		CSF	+	+	+	
		Blood	–	–	–	
8	Meningeal	CSF	–	+	+	Cure
9	Meningeal	CSF	–	+	+	Cure
10	Miliary	Sputum	+	+	+	Death/NTB
		CSF	–	+	–	
		Blood	–	+	+	
11	Pleural	Pleural fluid	–	–	+	Abandonment

PCR: polymerase chain reaction; Death/NTB: death due to causes other than tuberculosis; Death/TB: death due to tuberculosis; and CSF: cerebrospinal fluid. [a]Patients 1 to 4 were HIV-negative, and patients 5 to 11 were HIV-positive. Culture vs. nested PCR (negative/positive): 7 × 8/2 × 13 (p = 0.110; Fisher's exact test).

Table 2 - Culture and nested polymerase chain reaction for the detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary samples.

Nested PCR	Culture				Total
	Extrapulmonary samples (n = 78)		Pulmonary samples (n = 20)		
	(+)	(-)	(+)	(-)	
(+)	5	3	2	3	13
(-)	1	69	0	15	85
Total	6	72	2	18	98

PCR: polymerase chain reaction.

patients, corroborating the findings of a study reporting that pleural tuberculosis is prevalent in extrapulmonary cases in HIV-negative patients.[22] Approximately 50% of HIV-positive individuals with tuberculosis develop extrapulmonary forms.[12,20,21,23] Despite the small number of HIV-positive patients included in the present study, our results show a predominance of extrapulmonary tuberculosis (57%).

Various tests based on PCR techniques using commercial kits and in-house tests are being evaluated. The nested PCR technique, by targeting IS*6110* in order to identify the *M. tuberculosis* complex,[4] provides variable sensitivity and specificity, depending on the laboratory, clinical specimen, bacillary load, cell lysis, and technical parameters.[10] Molecular techniques have greatly improved the detection of mycobacteria in lymph nodes and in various body fluids (aspirates, cerebrospinal fluid, ascitic fluid, and pleural fluid). However, due to the variable sensitivity and specificity in different studies, positive results should be interpreted in conjunction with clinical findings.[21] In the present study, the proportion of positive results in the individuals diagnosed with pulmonary or extrapulmonary tuberculosis using nested PCR (100%) was higher than in other studies using the same target gene and carried out in Brazil,[6] India,[21] and Greece.[17]

The discordance between the molecular method and smear microscopy in pulmonary and extrapulmonary samples in our study (in 1 and in 6 samples, respectively) might be directly related to the low sensitivity of the phenotyping technique, the paucibacillary nature of samples, or even the absence of infection by AFB, as observed by other authors.[12,15,20] Negi et al.[19] reported that a molecular technique targeting IS*6110* showed high positivity in pulmonary and extrapulmonary samples (90% and 77%, respectively), whereas smear microscopy showed low positivity (49% and 24%, respectively). Similarly, Barani et al.,[10] when studying 19 pulmonary and 104 extrapulmonary samples, obtained higher positivity with a molecular technique targeting IS*6110* and TCR4 than with the smear method (17 similar and 12 divergent results). It is of note that a reported 50% of tuberculosis cases are classified as negative on the basis of smear microcopy results.[24] In addition, smear microcopy was not performed in 32.43% of the reported cases of tuberculosis in Brazil in 2010.[1]

When we compared nested PCR and culture results in pulmonary samples, we found that 3 of the samples showed positive nested PCR results but negative culture results. The negative results in the cultures might be due to co-infection with HIV in 2 of the patients and kidney transplantation in 1 (Table 1) or to the characteristics of paucibacillary infections.[6,12] Various factors can influence the result of cultures, such as the number of organisms present in the specimen, the methods of sample collection, previous treatments, and the processing method. In addition, the solutions used for digestion/decontamination of the samples can damage the mycobacteria.[4,25]

In the present study, the molecular results supported the clinical and diagnostic criteria widely accepted for the diagnosis of tuberculosis. The sensitivity and specificity found for nested PCR in our study (100% and 83%, respectively) are higher than those reported in previous studies using molecular techniques targeting the IS*6110* gene in sputum samples (88-98% and 15-100%, respectively).[5,12,23] This difference might be attributable to the volume and type of samples, as well as to the different molecular typing protocols employed in different laboratories.[11,26,27] Hence, the correlation of the results with the clinical profile of the patient is essential for the diagnosis of tuberculosis, and the definition of the disease can therefore be established from negative cultures after the therapeutic test.[26] This was found in 3 of our patients who had positive nested PCR results and negative culture results in their pulmonary samples, 2 of whom were cured after treatment and 1 of whom was noncompliant with the treatment. In addition, 2 patients died before the beginning of the recommended tuberculosis treatment. As to the concordance between nested PCR and culture, although the kappa coefficient (0.50) was lower than that reported in other studies, carried out in India (kappa = 0.6-0.8) and in Brazil (kappa = 0.78), the concordance was good.

Regarding extrapulmonary samples, nested PCR was positive in 3 of the blood samples with negative culture results, and negative in 1 of the cerebrospinal fluid samples with a positive culture result. This false-negative result in a cerebrospinal fluid sample (Table 1) might be

related to the absence of copies of the IS*6110* gene, as previously described in studies conducted in southeast Asia, Denmark, Tunisia, India, and Vietnam, indicating the need to incorporate additional targets, such as TRC4, in order to improve molecular detection.[7,10] However, to our knowledge, no study to date has reported the absence of this element in *M. tuberculosis* strains in Brazil, and various studies have reported that this sequence is the most sensitive in order to detect the *M. tuberculosis* complex.[9,17] Another factor could be the presence of molecular reaction inhibitors in up to 18.6% of extrapulmonary specimens.[5]

In Brazil, the prevalence of *M. tuberculosis* isolated in cultures ranges from 15.0% to 25.6%,[28,29] the highest values being obtained prior to the highly active antiretroviral therapy era. Positive nested PCR results in blood samples led to the diagnosis of pulmonary tuberculosis in 2 HIV-negative patients and in 1 HIV-positive patient, as well as to the diagnosis of extrapulmonary tuberculosis in 1 HIV-negative patient and in 1 HIV-positive patient. In those blood samples, smear microscopy and culture were positive in only 1 and 2 samples, respectively; this might be due to the small number of bacilli in the circulation.[6,7] In fact, the sensitivity of PCR in peripheral blood mononuclear cells, as used here, has been reported to be better than is that of culture, both having similar specificity.[11,16,29] In addition, clinical specimens, such as cerebrospinal fluid, blood, and sputum, have been described as good substrates for PCR[26] with good concordance (kappa = 0.6867; p = 0.625; McNemar's test), as in our results, in which the level of positivity in extrapulmonary samples was higher when nested PCR was used (p = 0.0042), thus corroborating the findings of Noussair et al.[21] Different rates of sensitivity were described using the molecular methodology for this type of sample in studies conducted in France[21] and India[12] (86.6% and 90%, respectively). If standardization studies using the same molecular target, DNA extraction method, and PCR optimization were validated in different laboratories, the sensitivity of the test could be improved and, consequently, so could its concordance.

In the present study, a positive nested PCR result, associated with the clinical features, was used as the single laboratory criterion for the diagnosis of 4 patients (2 with pulmonary tuberculosis and 2 with extrapulmonary tuberculosis), because it was the only test producing positive results (Table 1); the lack of isolates in cultures might be mainly due to the paucibacillary character of the samples. Although the difference is not significant in this situation, diagnosis is often established only by clinical and radiological data, even if the culture and sputum smear testing are negative. In such cases, molecular analysis can help establish specific antimycobacterial therapy and, consequently, contribute to reduce empirical treatment, which has been currently used in almost 27% of suspected pulmonary tuberculosis cases. In addition, it can help control the spread of the bacillus[11] and prevent more severe clinical evolution, mainly in HIV-positive patients. However, the current Brazilian guidelines on tuberculosis do not include positive molecular results in the definition of tuberculosis cases; as a rule, the use of this method has been restricted to certain referral and research centers in Brazil.[9]

It is worth mentioning that clinical screening that is carefully designed to study the disease caused by mycobacteria, together with similar attention to the collection and transport of biological samples, are factors that contributed to the isolation of a higher rate of *M. tuberculosis* strains than was expected at our hospital. The present study corroborates the results obtained at a referral center for the diagnosis of tuberculosis in the city of São José do Rio Preto (XV Subdivision of the São Paulo State Health Department), in which a 50% increase in the number of isolates was observed during the study period.[25]

The results of the nested PCR assay revealed good agreement with those of the culture. The specificity and sensitivity achieved with this relatively simple molecular approach can be seen as an important contribution to the future establishment of a protocol for the molecular detection of *M. tuberculosis* complex in pulmonary and extrapulmonary samples. In addition, this methodology could reduce the time required for the appropriate diagnosis of tuberculosis.

References

1. World Health Organization [homepage on the Internet]. Geneva: World Health Organization. [cited 2012 May 10]. Brazil - Tuberculosis profiles. Available from: https://extranet.who.int/sree/Reports?op=Replet&name=%2FWHO_HQ_Reports%2FG2%2FPROD%2FEXT%2FTBCountryProfile&ISO2=BR&outtype=html

2. World Health Organization [homepage on the Internet]. Geneva: World Health Organization. [cited 2012 Mar 18]. HIV/AIDS. Available from: http://www.who.int/hiv/topics/tb/en
3. REDE-TB - Rede Brasileira de Pesquisas em Tuberculose [homepage on the Internet]. Rio de Janeiro: Rede Brasileira de Pesquisas em Tuberculose. [cited 2012 Jul 10]. Tuberculose, o 'mal do século' XXI. Available from: http://redetb.org/noticias/155-noticias-abr2012/1681-tuberculose-o-mal-do-seculo-xxi
4. Ani A, Okpe S, Akambi M, Ejelionu E, Yakubu B, Owolodun O, et al. Comparison of a DNA based PCR method with conventional methods for the detection of M. tuberculosis in Jos, Nigeria. J Infect Dev Ctries. 2009;3(6):470-5. http://dx.doi.org/10.3855/jidc.420 PMid:19762962
5. Cheng VC, Yew WW, Yuen KY. Molecular diagnostics in tuberculosis. Eur J Clin Microbiol Infect Dis. 2005;24(11):711-20. http://dx.doi.org/10.1007/s10096-005-0039-1 PMid:16283213
6. da Cruz HL, de Albuquerque Montenegro R, de Araújo Lima JF, da Rocha Poroca D, da Costa Lima JF, Maria Lapa Montenegro L, et al. Evaluation of a nested-PCR for mycobacterium tuberculosis detection in blood and urine samples. Braz J Microbiol. 2011;42(1):321-9. http://dx.doi.org/10.1590/S1517-83822011000100041 PMid:24031638 PMCid:PMC3768939
7. Ereqat S, Bar-Gal GK, Nasereddin A, Said S, Greenblatt CL, Azmi K, et al. Pulmonary tuberculosis in the West Bank, Palestinian Authority: molecular diagnostic approach. Trop Med Int Health. 2011;16(3):360-7. http://dx.doi.org/10.1111/j.1365-3156.2010.02697.x PMid:21159079
8. Lawn SD, Zumla AI. Tuberculosis. Lancet. 2011;378(9785):57-72. http://dx.doi.org/10.1016/S0140-6736(10)62173-3
9. Conde MB, Melo FA, Marques AM, Cardoso NC, Pinheiro VG, Dalcin Pde T, et al. III Brazilian Thoracic Association Guidelines on tuberculosis. J Bras Pneumol. 2009;35(10):1018-48. PMid:19918635
10. Barani R, Sarangan G, Antony T, Periyasamy S, Kindo AJ, Srikanth P. Improved detection of Mycobacterium tuberculosis using two independent PCR targets in a tertiary care centre in South India. J Infect Dev Ctries. 2012;6(1):46-52. PMid:22240428
11. Lima JF, Montenegro LM, Montenegro Rde A, Cabral MM, Lima AS, Abath FG, et al. Performance of nested PCR in the specific detection of Mycobacterium tuberculosis complex in blood samples of pediatric patients. J Bras Pneumol. 2009;35(7):690-7. http://dx.doi.org/10.1590/S1806-37132009000700011 PMid:19669008
12. Sekar B, Selvaraj L, Alexis A, Ravi S, Arunagiri K, Rathinavel L. The utility of IS6110 sequence based polymerase chain reaction in comparison to conventional methods in the diagnosis of extra-pulmonary tuberculosis. Indian J Med Microbiol. 2008;26(4):352-5. http://dx.doi.org/10.4103/0255-0857.43575 PMid:18974489
13. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. Atlanta: U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control; 1985.
14. Chimara E, Ferrazoli L, Ueky SY, Martins MC, Durham AM, Arbeit RD, et al. Reliable identification of mycobacterial species by PCR-restriction enzyme analysis (PRA)-hsp65 in a reference laboratory and elaboration of a sequence-based extended algorithm of PRA-hsp65 patterns. BMC Microbiol. 2008;8:48. http://dx.doi.org/10.1186/1471-2180-8-48 PMid:18366704 PMCid:PMC2323382
15. Gopinath K, Singh S. Multiplex PCR assay for simultaneous detection and differentiation of Mycobacterium tuberculosis, Mycobacterium avium complexes and other Mycobacterial species directly from clinical specimens. J Appl Microbiol. 2009;107(2):425-35. http://dx.doi.org/10.1111/j.1365-2672.2009.04218.x PMid:19302308
16. Rebollo MJ, San Juan Garrido R, Folgueira D, Palenque E, Díaz-Pedroche C, Lumbreras C, et al. Blood and urine samples as useful sources for the direct detection of tuberculosis by polymerase chain reaction. Diagn Microbiol Infect Dis. 2006;56(2):141-6. http://dx.doi.org/10.1016/j.diagmicrobio.2006.03.018 PMid:16698218
17. Ritis K, Tzoanopoulos D, Speletas M, Papadopoulos E, Arvanitidis K, Kartali S, et al. Amplification of IS6110 sequence for detection of Mycobacterium tuberculosis complex in HIV-negative patients with fever of unknown origin (FUO) and evidence of extrapulmonary disease. J Intern Med. 2000;248(5):415-24. http://dx.doi.org/10.1046/j.1365-2796.2000.00750.x PMid:11123506
18. Rossetti ML, Jardim SB, Rodrigues VF, Moura AR, Oliveira H, Zaha A. Improvement of Mycobacterium tuberculosis detection in clinical samples using DNA purified by glass matrix. J Microbiol Methods.1997;28(2):139-46. http://dx.doi.org/10.1016/S0167-7012(97)00978-0
19. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, et al. Diagnostic potential of IS6110, 38kDa, 65kDa and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. Indian J Med Microbiol. 2007;25(1):43-9. http://dx.doi.org/10.4103/0255-0857.31061 PMid:17377352
20. Noussair L, Bert F, Leflon-Guibout V, Gayet N, Nicolas-Chanoine MH. Early diagnosis of extrapulmonary tuberculosis by a new procedure combining broth culture and PCR. J Clin Microbiol. 2009;47(5):1452-7. http://dx.doi.org/10.1128/JCM.00066-09 PMid:19321729 PMCid:PMC2681850
21. Purohit MR, Mustafa T, Wiker HG, Sviland L. Rapid diagnosis of tuberculosis in aspirate, effusions, and cerebrospinal fluid by immunocytochemical detection of Mycobacterium tuberculosis complex specific antigen MPT64. Diagn Cytopathol. 2012;40(9):782-91. http://dx.doi.org/10.1002/dc.21637 PMid:21416644
22. Portal Saúde [homepage on the Internet]. Brasília: Ministério da Saúde. [cited 2011 Jun 06]. Informe Técnico de Tuberculose. Novo sistema de tratamento da tuberculose para adultos e adolescentes no Brasil. [Adobe Acrobat document, 8p.]. Available from: http://portal.saude.gov.br/portal/arquivos/pdf/informe_tb_julho10_certo_22_07_2010.pdf
23. Sharma SK, Mohan A. Extrapulmonary tuberculosis. Indian J Med Res. 2004;120(4):316-53. PMid:15520485
24. Haldar S, Chakravorty S, Bhalla M, De Majumdar S, Tyagi JS. Simplified detection of Mycobacterium tuberculosis in sputum using smear microscopy and PCR with molecular beacons. J Med Microbiol. 2007;56(Pt 10):1356-62. http://dx.doi.org/10.1099/jmm.0.47265-0 PMid:17893174
25. Pedro Hda S, Pereira MI, Goloni Mdo R, Ueki SY, Chimara E. Nontuberculous mycobacteria isolated in São José do Rio Preto, Brazil between 1996 and 2005. J Bras Pneumol. 2008;34(11):950-5. http://dx.doi.org/10.1590/S1806-37132008001100010 PMid:19099102

26. Bollela VR, Sato DN, Fonseca BA. Problems in the standardization of the polymerase chain reaction for the diagnosis of pulmonary tuberculosis [Article in Portuguese]. Rev Saude Publica. 1999;33(3):281-6. http://dx.doi.org/10.1590/S0034-89101999000300009 PMid:10457001
27. Runa F, Yasmin M, Hoq MM, Begum J, Rahman AS, Ahsan CR. Molecular versus conventional methods: clinical evaluation of different methods for the diagnosis of tuberculosis in Bangladesh. J Microbiol Immunol Infect. 2011;44(2):101-5. http://dx.doi.org/10.1016/j.jmii.2010.05.001 PMid:21439511
28. Oplustil CP, Leite OH, Oliveira MS, Sinto SI, Uip DE, Boulos M, et al. Detection of mycobacteria in the bloodstream of patients with acquired immunodeficiency syndrome in a university hospital in Brazil. Braz J Infect Dis. 2001;5(5):252-9. http://dx.doi.org/10.1590/S1413-86702001000500003 PMid:11779451
29. Nakatani SM, Burger M, Assef MC, Brockelt SR, Cogo LL, Messias-Reason IJ. Efficient method for mycobacterial DNA extraction in blood cultures aids rapid PCR identification of Mycobacterium tuberculosis and Mycobacterium avium. Eur J Clin Microbiol Infect Dis. 2004;23(11):851-4. http://dx.doi.org/10.1007/s10096-004-1236-z PMid:15558344

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Inflammatory and immunogenetic markers in correlation with pulmonary tuberculosis*

Marcadores inflamatórios e imunogenéticos e sua relação com tuberculose pulmonar

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Abstract

Objective: To describe serum levels of the cytokines IL-10, TNF-α, and IFN-γ, as well as polymorphisms in the genes involved in their transcription, and their association with markers of the acute inflammatory response in patients with pulmonary tuberculosis. **Methods:** This was a descriptive, longitudinal study involving 81 patients with pulmonary tuberculosis treated at two referral hospitals. We collected data on sociodemographic variables and evaluated bacteriological conversion at the eighth week of antituberculosis treatment, gene polymorphisms related to the cytokines studied, and serum levels of those cytokines, as well as those of C-reactive protein (CRP). We also determined the ESR and CD4+ counts. **Results:** The median age of the patients was 43 years; 67 patients (82.7%) were male; and 8 patients (9.9%) were infected with HIV. The ESR was highest in the patients with high IFN-γ levels and low IL-10 levels. IFN-γ and TNF-α gene polymorphisms at positions +874 and −238, respectively, showed no correlations with the corresponding cytokine serum levels. Low IL-10 levels were associated with IL-10 gene polymorphisms at positions −592 and −819 (but not −1082). There was a negative association between bacteriological conversion at the eighth week of treatment and CRP levels. **Conclusions:** Our results suggest that genetic markers and markers of acute inflammatory response are useful in predicting the response to antituberculosis treatment.

Keywords: Tuberculosis; Cytokines; Immune system; Polymorphism, single nucleotide.

Resumo

Objetivo: Descrever os níveis séricos das citocinas IL-10, TNF-α e IFN-γ, assim como polimorfismos presentes em genes envolvidos na sua transcrição, e sua associação com marcadores de resposta inflamatória aguda em pacientes com tuberculose. **Métodos:** Estudo descritivo e longitudinal realizado em 81 pacientes com tuberculose pulmonar atendidos em dois hospitais de referência. Foram coletadas informações sociodemográficas, conversão bacteriológica na oitava semana de tratamento antituberculose, polimorfismos relacionados às citocinas estudadas, níveis séricos dessas citocinas, assim como de proteína C reativa (PCR). Também foram avaliados VHS e contagem de CD4+. **Resultados:** A mediana de idade dos pacientes era de 43 anos, sendo 67 (82,7%) do sexo masculino e 8 (9,9%) infectados por HIV. Os pacientes com níveis elevados de IFN-γ e baixos níveis de IL-10 apresentaram valores mais elevados de VHS. Não houve associação dos polimorfismos do gene IFN-γ na posição +874 e do gene TNF-α na posição −238 com os níveis das citocinas correspondentes. Houve uma associação entre polimorfismos do gene IL-10 nas posições −592 e −819 (mas não −1082) e baixos níveis de IL-10. Houve uma associação negativa entre a taxa de conversão bacteriológica na oitava semana de tratamento e níveis de PCR. **Conclusões:** Nossos resultados sugerem que marcadores genéticos e de resposta inflamatória aguda podem ser úteis na predição da resposta ao tratamento antituberculose.

Descritores: Tuberculose; Citocinas; Sistema imunológico; Polimorfismo de nucleotídeo único.

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Introduction

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, the most common clinical manifestation of which is pulmonary involvement; however, tuberculosis can affect other anatomical sites (extrapulmonary tuberculosis) or present as disseminated disease.[1]

Despite being a curable disease, tuberculosis remains a major public health problem worldwide. According to the World Health Organization, Brazil ranks 19th among the 22 countries that collectively account for 80% of all cases of tuberculosis worldwide and 108th among those in which the incidence of tuberculosis is highest. According to the Brazilian National Ministry of Health, 71,000 new cases of tuberculosis were added to the Brazilian Case Registry Database in 2010, corresponding to an incidence rate of 37.2/100,000 population.[2]

The systemic inflammation observed in patients with tuberculosis is mediated by the activation of the immune system, with excessive production of cytokines, such as IL-1, IL-2, IFN-γ, and TNF-α.[3] After the inflammatory process, there is an increase in the hepatic synthesis and serum levels of acute phase proteins, such as C-reactive protein (CRP), as well as in the ESR, which have been used in the diagnosis and follow-up of patients, given that their plasma levels directly reflect the intensity of the pathological process.[4]

Genetic factors have been associated with susceptibility to or protection against infection with *M. tuberculosis*.[5] In the immune response to *M. tuberculosis*, allele frequencies in cytokine gene polymorphisms vary considerably across populations, as reported in meta-analyses evaluating IFN-γ, IL-10, and TNF-α gene polymorphisms.[6,7] It has been proposed that serum cytokine levels and their role as markers of response to antituberculosis treatment be evaluated.[8] The maintenance of initially low serum levels of IFN-γ or high serum levels of TNF-α and of increased serum levels of IL-17 is associated with a worse prognosis, including a higher mortality rate and lower bacteriological conversion at the end of the 8th week of antituberculosis treatment. Recently, Lago et al.[9] described a possible association between recurrent tuberculosis and maintenance of high serum levels of IL-10 during antituberculosis treatment. The study of the genes involved in these processes and their interactions with the immune and inflammatory responses can aid in identifying better markers of protection against tuberculosis.

There have been few studies simultaneously evaluating the genotypic and phenotypic aspects of the human host immune response to infection with *M. tuberculosis*.[10,11] Given the paucity of data on the simultaneous evaluation of genetic, immunological, and inflammatory biomarkers in patients with pulmonary tuberculosis, we conducted the present study in order to determine the prevalence of IL-10 gene polymorphisms at positions −592, −819, and −1082; the prevalence of TNF-α gene polymorphisms at position −238; and the prevalence of IFN-γ gene polymorphisms at position +874. The study involved a sample of active pulmonary tuberculosis patients admitted to and treated at either of two referral hospitals in the city of Rio de Janeiro, Brazil. In addition, we measured the serum levels of the corresponding cytokines and analyzed the acute inflammatory response by determining CRP levels and CD4+ counts, as well as the ESR.

Methods

This was a longitudinal descriptive study involving 81 patients diagnosed with pulmonary tuberculosis and admitted to either of two referral hospitals for the treatment of tuberculosis in the state of Rio de Janeiro, Brazil (the *Hospital Estadual Santa Maria* and the *Instituto Estadual de Doenças do Tórax Ary Parreiras*), between March 23, 2007 and August 7, 2009. We included patients with positive smear microscopy and culture for mycobacteria, the presence of *M. tuberculosis* being subsequently confirmed by biochemical tests. We analyzed the following variables: CRP, ESR, CD4+, and bacteriological conversion at the 8th week of antituberculosis treatment.

For DNA extraction, a commercial kit (DNAzol; Gibco BRL/Life Technologies, Gaithersburg, MD, USA) was used in accordance with the manufacturer instructions. After DNA extraction, a DNA sample was analyzed by electrophoresis on 1% agarose gel in order to determine integrity and concentration, the sample being subsequently stored at −20°C.

For the analysis of TNF-α gene polymorphisms at position −238, 100 ng of DNA, 1× buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) were added to 15 pmol of each primer for polymerase chain reaction, which

was performed as follows: one cycle at 94°C for 1 min, followed by 5 cycles at 94°C, 67°C, and 72°C (60 s each), and 25 cycles at 94°C, 62°C, and 72°C (60 s each). For the genotyping of IFN-γ gene polymorphism at position +874, we used 200 mL of dNTP, 1.5 mM $MgCl_2$, 8.5% sucrose, 0.25 U of ThermoPrime Plus DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 5 mL of each specific primer, 0.5 mL of internal control primer, and 100 ng of DNA. The mixture was incubated at 95°C for 1 min; subsequently, 10 cycles were performed at 95°C for 15 s, followed by 10 cycles at 62°C for 50 s, 10 cycles at 72°C for 40 s, 20 cycles at 95°C for 20 s, 20 cycles at 56°C for 50 s, and 20 cycles at 72°C for 50 s. For the detection of IL-10 promoter gene polymorphisms at positions −819, −1082, and −592, the following steps were taken: for the −592 position, a 480-bp fragment was amplified and subsequently digested with the enzyme RsaI. For the −1082 and −819 positions, a 360-bp fragment was amplified and subsequently digested with the enzymes BseRI and MslI, respectively. In brief, 100 ng of DNA were added to each polymerase chain reaction, resulting in a final volume of 40 µL (−819 and −1082) or 30 µL (−592), consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTP, 1.25 U AmpliTaq Gold DNA Polymerase (Perkin-Elmer Cetus, Norwalk, CT, USA) and specific primers for each mutation (10 pmol for the −592 position and 12.5 pmol for the −819/−1082 positions). All mixtures were incubated at 95°C for 10 min and submitted to amplification at 94°C for 30 s, at 60°C for 30 s, at 72°C for 40 s, and at 72°C for 7 min (IL-10 at position −592), followed by 35 cycles at 94°C for 30 s, at 58°C for 30 s, and at 72°C for 45 s, plus a final cycle at 72°C for 5 min (positions −819 and −1082). The amplified products were electrophoresed on 2% agarose gel containing ethidium bromide (0.5 mL/mL).

In the determination of cytokine levels, bead populations were visualized on the basis of their fluorescence intensities. In the cytometric bead array system, cytokine capture beads are mixed with detection antibody conjugated to the fluorochrome phycoerythrin and then incubated with the samples for the "conjugate" assay. The acquisition tubes were prepared with 50 µL of sample, 50 µL of the bead mixture, and 50 µL of detection reagent human Th1/Th2 phycoerythrin. The same procedure was performed in order to obtain the standard curve. The tubes were homogenized and incubated for three hours at room temperature in the dark. Subsequently, the reading was performed with a BD™ Cytometric Bead Array system (Thermo Fisher Scientific).[12]

Serum levels of CRP were used as a marker of the acute phase response (APR), i.e., as a marker of the systemic response to severe inflammation. A positive APR was defined as CRP levels > 0.3 mg/dL, whereas a negative APR was defined as CRP levels < 0.3 mg/dL. Serum CRP levels were measured by nephelometry.

The ESR was also used as a marker of the APR, a positive APR being defined as an ESR > 2 mm/h for females and as an ESR > 7 mm/h for males. The ESR was measured by the Westergren method.

We used descriptive statistics, including range (minimum and maximum values), mean, standard deviation, and 95% CI. We used the Kolmogorov-Smirnov test in order to test the normality of the variables and the Levene test in order to determine the equality of variances. Variables with non-normal distribution were log-transformed. For means with normal distribution, we used the Student's t-test. We used ANOVA in order to analyze the differences among quantitative variables. We used the chi-square test in order to identify associations among categorical variables. A value of $p < 0.05$ was considered statistically significant. We used the Statistical Package for the Social Sciences, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). In the analyses, we used the bacteriological conversion coefficient, which was calculated as the number of cases of patients who converted from a negative test result to a positive test result divided by the total number of patients at the beginning of treatment, and the mutation coefficient for polymorphisms, which was calculated as the number of cases of a given mutation divided by the total number of cases.

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

The median age of the patients was 43 years (range, 20-60 years). Of the 81 patients studied, 67 (82.7%) were male, 54 (66.7%) were non-White,

8 (9.9%) were co-infected with HIV, 52 (64.2%) reported regular alcohol use, 55 (67.9%) were smokers or former smokers, 20 (24.7%) reported illicit drug use, and 64 (79.0%) had normal CD4+ counts. All patients had smear-positive pulmonary tuberculosis.

In the analysis of the prevalence of IFN-γ gene polymorphisms at position +874, of TNF-α gene polymorphisms at position −238, and of IL-10 gene polymorphisms at positions −592, −819, and −1082, the mutant allele frequency was found to be 0.56, 0.56, 0.29, 0.43, and 0.68, respectively.

Table 1 shows the distribution of polymorphisms in the patients under study, by serum cytokine levels. Serum IFN-γ levels were found to range from 0 (zero) pg/mL to 20.5 pg/ml, and there was no relationship between low serum levels of IFN-γ and the presence of mutations. Regarding TNF-α, although we found no homozygous mutations, we found a trend toward low serum levels of TNF-α among heterozygotes. We found a negative relationship between serum IL-10 levels and IL-10 gene polymorphisms at positions −592 and −819 ($p < 0.001$; Figure 1).

As can be seen in Table 2, there was a trend ($p = 0.08$) toward lower CRP production in the patients in whom serum IFN-γ levels were low (0.0-4.9 pg/mL) when compared with those in whom serum IFN-γ levels were higher (> 5.0 pg/mL). In the patients in whom serum TNF-α levels were low (0.0-4.9 pg/mL), there was a trend toward a higher ESR ($p = 0.04$). Low serum levels of IL-10 (i.e., serum IL-10 levels of 0.0-4.9 pg/

Table 1 - Distribution of the polymorphisms found in the patients under study, by serum cytokine levels.

Polymorphism	Patients, n (%)		
	Serum cytokine levels		
	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL
IFN-γ			
TT	1 (1.2)	6 (7.4)	2 (2.4)
TA	14 (17.2)	29 (35.8)	10 (12.3)
AA	5 (6.1)	10 (12.3)	4 (4.9)
TA/AA	19 (23.3)	39 (48.1)	14(17.2)
TNF-α			
GG	10 (12.3)	1 (1.2)	0 (0.0)
GA	57 (70.3)	13 (16)	0 (0.0)
AA	0 (0.0)	0 (0.0)	0 (0.0)
GA/AA	57 (70.3)	13 (16)	0 (0.0)
IL-10 at position −592			
CC	22 (27.1)	12 (14.8)	0 (0.0)
CA	22 (27.1)	22 (27.1)	1 (1.2)
AA	1 (1.2)	1 (1.2)	0 (0.0)
CA/AA	23 (28.3)	23 (28.3)	1 (1.2)
IL-10 at position −819			
CC	7 (8.6)	4 (4.9)	0 (0.0)
CT	25 (30.8)	23 (28.3)	1 (1.2)
TT	1 (1.2)	8 (9.8)	0 (0.0)
CT/TT	26 (32)	31 (38.1)	1 (1.2)
IL-10 at position −1082			
GG	2 (2.4)	6 (7.4)	0 (0.0)
GA	22 (27.1)	13 (16)	0 (0.0)
AA	2 (2.4)	16 (19.7)	1(1.2)
GA/AA	24 (29.5)	29 (35.7)	1(1.2)

(IFN-γ): TT: wild-type homozygous genotype; TA: heterozygous genotype; and AA: mutant homozygous genotype. (TNF-α): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −592): CC: wild-type homozygous genotype; CA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −819): CC: wild-type homozygous genotype; CT: heterozygous genotype; and TT: mutant homozygous genotype. (IL-10 at position −1082): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype.

mL) were not associated with a higher ESR or with higher CRP levels. However, the ESR was negatively correlated with serum IL-10 levels (p = 0.03) and was positively correlated with serum IFN-γ levels (p = 0.008; Table 3).

Table 4 shows that lower bacteriological conversion was associated only with high serum levels of CRP. However, by applying the bacteriological conversion coefficient, we found a negative correlation between serum TNF-α levels and bacteriological conversion (r = −0.43; p < 0.001).

Discussion

To our knowledge, this is the first study to examine the relationships among biochemical markers, inflammatory markers, and immunogenetic markers in pulmonary tuberculosis patients in Brazil. The clinical features of the patients in our sample were similar to those of patients admitted to tuberculosis hospitals in developing countries.[13]

The genetic component of the host response to infection with *M. tuberculosis* in restricted ethnic groups is evident in the literature.[5] In the present study, the frequency of the mutant allele for IFN-γ gene polymorphisms at position +874 was 0.56, which is similar to that reported by other authors in various countries[14-16] but different from that reported by Fitness et al. in Africa.[17] In addition, the frequency of the mutant allele for TNF-α gene polymorphisms at position +238 was 0.56, which is similar

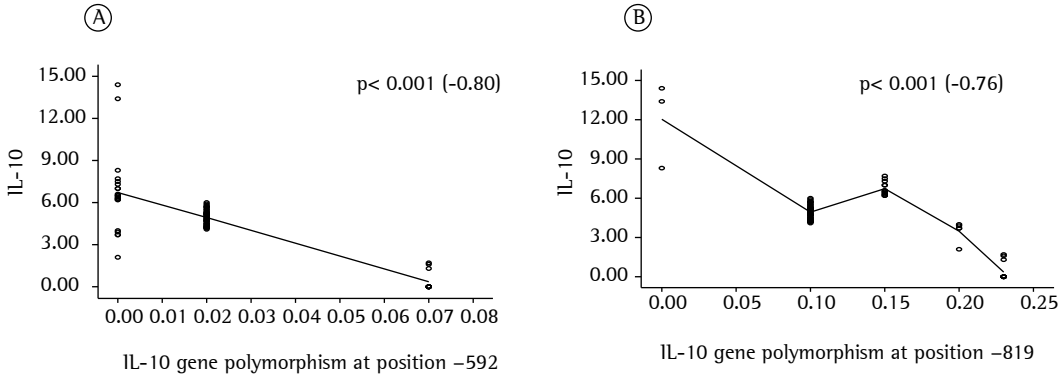

Figure 1 - Regression coefficient for IL-10 gene polymorphisms at positions −592 (in A) and −819 (in B).

Table 2 - Distribution of serum levels of IFN-γ, TNF-α, and IL-10 in the patients under study, by laboratory test results.

Results	Patients, n (%)								
	Serum cytokine levels								
	IFN-γ			TNF-α			IL-10		
	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL
ESR									
Low	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal	1 (1.3)	6 (7.6)	1 (1.3)	4 (5.1)	4 (5.1)	0 (0.0)	4 (5.1)	3 (3.8)	0 (0.0)
High	19 (24.1)	37 (46.8)	15 (19)	61 (77.2)	10 (12.7)	0 (0.0)	41 (51.9)	30 (38)	1 (1.3)
CD4+									
Low	5 (6.6)	9 (11.8)	3 (4)	13 (17.1)	4 (5.3)	0 (0.0)	9 (11.8)	8 (10.5)	0 (0.0)
Normal	15 (19.7)	31 (40.8)	10 (13.2)	47 (61.8)	9 (11.8)	0 (0.0)	30 (39.57)	25 (32.9)	1 (1.3)
High	0 (0.0)	2 (2.6)	1 (1.3)	3 (3.9)	0 (0.0)	0 (0.0)	3 (3.9)	0 (0.0)	0 (0.0)
CRP									
Normal	1 (1.3)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)
High	19 (23.8)	45 (56.3)	15 (18.8)	66 (82.5)	13 (16.9)	0 (0.0)	45 (56.3)	33 (41.3)	1 (1.3)

CRP: C-reactive protein.

to that reported in other studies.[18-20] In our sample, the allele frequencies for IL-10 gene polymorphisms at positions −592, −819, and −1082 were, respectively, 0.29, 0.43, and 0.68, being similar to those reported in most of the studies included in a meta-analysis.[6] Although our results are consistent with those of various studies, any differences regarding the frequency of these polymorphisms can be explained by ethnic differences among the study populations.

Table 3 - Correlation between serum cytokine levels and laboratory test results.

Biochemical variable	p		
	IFN-γ	TNF-α	IL-10
CD4+	0.59	0.11	0.47
CRP	0.43	0.32	0.33
ESR	0.008 (+)	0.31	0.03 (−)

C-reactive protein; (+): positive correlation; and (−): negative correlation.

The functional role of allele −238A (TNF-α) in the regulation of TNF-α gene expression was described by Kaluza et al.,[21] whose in vitro studies showed an association between allele −238A and a downregulation of the TNF-α gene (and, consequently, a reduction in TNF-α protein production). Although we found no homozygous mutations, we observed a trend toward low serum levels of TNF-α in patients with a heterozygous genotype, as did Abhimanyu et al.[10] in a population of individuals from India whose ethnic characteristics were quite different from those of our study population. However, Haroon et al.[22] found no association between mutation and cytokine expression in a population of White individuals.

The presence of single-nucleotide polymorphisms in the first intron of the IFN-γ gene (at position +874) has been associated with tuberculosis[8,14] and severe tuberculosis.[23] The

Table 4 - Serum levels of C-reactive protein, ESR, CD4+, and cytokines, as well as frequency of genotypes, by bacteriological conversion.[a]

Variable	Bacteriological conversion		p
	Yes	No	
CRP, mg/dL	5.37 ± 3.96	8.6 ± 4.7	0.004
ESR, mm/h	60.3 ± 43.3	53.8 ± 37.6	0.57
CD4+, cells/mm^3	784.09 ± 699.44	657.5 ± 311.49	0.46
IL-10, pg/mL	4.6 ± 2.8	4.8 ± 1.21	0.73
TNF-α, pg/mL	3.6 ± 1.93	3.99 ± 1.36	0.42
IFN-γ, pg/mL	7.17 ± 4.84	7.69 ± 3.99	0.68
IL-10 at position −592[b]			
AA	2 (3.4)	0 (0.0)	0.42
CA/CC	57 (96.6)	18 (100.0)	
IL-10 at position −819[b]			
TT	8 (13.6)	2 (11.1)	0.78
CT/CC	51 (86.4)	16 (88.9)	
IL-10 at position −1082[b]			
AA	27 (45.8)	9 (50.0)	0.75
GA/GG	32 (54.2)	9 (50.0)	
TNF-α[b]			
AA	0 (0.0)	0 (0.0)	--
GA/GG	59 (100.0)	18 (100.0)	
IFN-γ[b]			
AA	11 (18.6)	6 (33.3)	0.18
TA/TT	48 (81.4)	12 (66.7)	

CRP: C-reactive protein; (IFN- γ): TT: wild-type homozygous genotype; TA: heterozygous genotype; and AA: mutant homozygous genotype. (TNF-α): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −592): CC: wild-type homozygous genotype; CA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −819): CC: wild-type homozygous genotype; CT: heterozygous genotype; and TT: mutant homozygous genotype. (IL-10 at position −1082): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as n (%).

gene encoding IFN-γ is highly conserved, and few polymorphisms are found in the intragenic region. In our sample, we found no association between IFN-γ gene polymorphisms at position +874 and serum IFN-γ levels, a finding that is similar to those of Abhimanyu et al.[10] and Vidyarani et al.[24] but different from those of Vallinoto et al.,[11] who found low serum levels of IFN-γ in patients with a homozygous mutant genotype at position +874A/A.

We found a significant relationship between high IFN-γ levels and a high ESR, a finding that is consistent with those of Peresi et al.[4] This is possibly due to the fact that the presence of this mutation has been associated with decreased production of IFN-γ (a cytokine that plays an important role in controlling the defense against the pathogen) and, therefore, a diminished acute inflammatory response.

In our study, low serum levels of IL-10 were found to be associated with IL-10 gene polymorphisms at positions −592 and −819 (but not −1082). This finding is similar to those of Abhimanyu et al.[10] and Edwards-Smith et al.,[25] who investigated IL-10 gene polymorphisms at position −1082 and showed that individuals carrying the AA genotype are low IL-10 producers, those carrying the GA genotype are intermediate IL-10 producers, and those carrying the GG genotype are high IL-10 producers; however, the ATA haplotype is associated with low IL-10 production. These discrepant results can be partly explained by the distinct and heterogeneous ethnic characteristics of the study populations.

We observed a trend toward a higher ESR among carriers of IL-10 gene polymorphisms at positions −592 (CA/AA) and −819 (CT/TT). The authors of a recent meta-analysis including 18 studies (none of which included patients from Latin America) were unable to confirm a higher risk of tuberculosis among patients with IL-10 gene polymorphisms at positions −592, −819, or −1082 but found a higher risk of tuberculosis among Europeans with IL-10 gene polymorphisms at position −1082.[6] In that meta- analysis, one of the studies assessing serum IL-10 levels also assessed serum levels of IFN-γ and IL-10. The authors demonstrated that a stronger relationship translated to less severe tuberculosis.

Jamil et al.[26] and Lago et al.[9] suggested that the maintenance of high serum levels of IL-10 during antituberculosis treatment is associated with an increased risk of recurrence, whereas low serum levels of IL-10 usually occur in mild forms of tuberculosis. The results obtained in the present study do not allow us make inferences regarding this issue, given that serum IL-10 levels were assessed only at time point zero and not during clinical follow-up (after completion of antituberculosis treatment).

In the present study, acute inflammatory response markers (CRP levels) were found to be higher in the patients in whom serum TNF-α levels were low (0.0-4.9 pg/mL) than in those in whom serum TNF-α levels were above 5.0 pg/mL. These data suggest that the presence of low concentrations of TNF-α at the time of the initial response against the disease is associated with a worse prognosis and clinical course; however, studies involving larger samples, as well as correlation studies, should be conducted in order to test these hypotheses in the Brazilian population, as mentioned in a review article by Wallis et al.[8] The role of TNF-α in the pathophysiology of tuberculosis has been associated with defense via macrophage activation and the subsequent inflammatory reaction.[3] Our findings reinforce the importance of this cytokine in the host response to *M. tuberculosis*.

In the present study, an association was found between elevated CRP levels and lower bacteriological conversion at the 8th week of antituberculosis treatment, showing the potential role of this mediator as a marker for monitoring the clinical course of the disease. Some authors have reported that ESR normalization is a marker of good response to treatment in subacute and chronic diseases, such as tuberculosis.[27,28] Various studies have shown increased levels of immune response markers, CRP, and ESR in the initial phase, all of which decrease during treatment.[29,30] Similar results were reported in a study by Peresi et al.,[4] in which CRP levels were significantly decreased only in the 3rd and 6th months of treatment. These findings suggest that CRP can be used in order to evaluate the APR in tuberculosis patients and as a marker of response to antituberculosis treatment, together with the clinical and epidemiological history of such patients.

In our study, there was no association between serum IFN-γ levels and bacteriological conversion, a finding that is similar to those of another study.[8] However, there was an association

between initially low serum levels of TNF-α and higher bacteriological conversion. These data are similar to those reported by Su et al.[30] Regarding bacteriological conversion (or lack thereof) and immunological and biochemical variables, we found a positive correlation between the inflammatory marker CRP and the absence of conversion. However, no such correlation was found for the remaining inflammatory markers (ESR and CD4+).

The limitations of the present study include the fact that we did not analyze IL-10 haplotypes, the fact that we did not include other cytokines that play a relevant role in the immune response to active tuberculosis, and the fact that we did not monitor the clinical and bacteriological response of the patients throughout the antituberculosis treatment period.

It is of note that, to our knowledge, this is the first study in Brazil to investigate the presence of proinflammatory and anti-inflammatory cytokines, acute inflammatory response mediators (by measuring serum CRP levels and the ESR), and the genetic background of patients in an attempt to elucidate certain mechanisms of the immunopathogenesis of tuberculosis. Given that this was a descriptive study, there was no control group, which is why we were careful to present the statistical associations without referring to the variables as "risk factors" for any given event.

References

1. World Health Organization. Global tuberculosis control - epidemiology, strategy, financing - WHO Report 2009. Geneva: World Health Organization; 2009.
2. Ministério da Saúde. PNCT – Tuberculose - Situação no Brasil. Brasília: Ministério da Saúde; 2012.
3. Ottenhoff TH. New pathways of protective and pathological host defense to mycobacteria. Trends Microbiol. 2012;20(9):419-28. http://dx.doi.org/10.1016/j.tim.2012.06.002 PMid:22784857
4. Peresi E, Silva SM, Calvi SA, Marcondes-Machado J. Cytokines and acute phase serum proteins as markers of inflammatory regression during the treatment of pulmonary tuberculosis. J Bras Pneumol. 2008;34(11):942-9. http://dx.doi.org/10.1590/S1806-37132008001100009 PMid:19099101
5. Takiff HE. Host Genetics and Susceptibility. In: Palomino JC, Leao SC, Ritacco V, editors. Tuberculosis 2007 - From Basic Science to Patient Care. Amedeo Challenge; 2007. p. 207-62.
6. Zhang J, Chen Y, Nie XB, Wu WH, Zhang H, Zhang M, et al. Interleukin-10 polymorphisms and tuberculosis susceptibility: a meta-analysis. Int J Tuberc Lung Dis. 2011;15(5):594-601. http://dx.doi.org/10.5588/ijtld.09.0703 PMid:21756510
7. Wang Q, Zhan P, Qiu LX, Qian Q, Yu LK. TNF-308 gene polymorphism and tuberculosis susceptibility: a meta-analysis involving 18 studies. Mol Biol Rep. 2012;39(4):3393-400. http://dx.doi.org/10.1007/s11033-011-1110-x PMid:21735105
8. Wallis RS, Kim P, Cole S, Hanna D, Andrade BB, Maeurer M, et al. Tuberculosis biomarkers discovery: developments, needs, and challenges. Lancet Infect Dis. 2013;13(4):362-72. http://dx.doi.org/10.1016/S1473-3099(13)70034-3
9. Lago PM, Boéchat N, Migueis DP, Almeida AS, Lazzarini LC, Saldanha MM, et al. Interleukin-10 and interferon-gamma patterns during tuberculosis treatment: possible association with recurrence. Int J Tuberc Lung Dis. 2012;16(5):656-9. PMid:22410761 PMCid:PMC3729476
10. Abhimanyu, Mangangcha IR, Jha P, Arora K, Mukerji M, Banavaliker JN, et al. Differential serum cytokine levels are associated with cytokine gene polymorphisms in north Indians with active pulmonary tuberculosis. Infect Genet Evol. 2011;11(5):1015-22. http://dx.doi.org/10.1016/j.meegid.2011.03.017 PMid:21463712
11. Vallinoto AC, Graça ES, Araújo MS, Azevedo VN, Cayres-Vallinoto I, Machado LF, et al. IFNG +874T/A polymorphism and cytokine plasma levels are associated with susceptibility to Mycobacterium tuberculosis infection and clinical manifestation of tuberculosis. Hum Immunol. 2010;71(7):692-6. http://dx.doi.org/10.1016/j.humimm.2010.03.008 PMid:20353805
12. BD™Cytometric Bead Array (CBA). Human Th1/Th2 Cytokine Kit II - Instruction Manual. San Jose: Becton, Dickinson and Company BD Biosciences; 2008.
13. Oliveira HM, Brito RC, Kritski AL, Ruffino-Netto A. Epidemiological profile of hospitalized patients with TB at a referral hospital in the city of Rio de Janeiro, Brazil. J Bras Pneumol. 2009;35(8):780-7. http://dx.doi.org/10.1590/S1806-37132009000800010 PMid:19750331
14. Amim LH, Pacheco AG, Fonseca-Costa J, Loredo CS, Rabahi MF, Melo MH, et al. Role of IFN-gamma +874 T/A single nucleotide polymorphism in the tuberculosis outcome among Brazilians subjects. Mol Biol Rep. 2008;35(4):563-6. http://dx.doi.org/10.1007/s11033-007-9123-1 PMid:17682837
15. Sallakci N, Coskun M, Berber Z, Gürkan F, Kocamaz H, Uysal G, et al. Interferon-gamma gene+874T-A polymorphism is associated with tuberculosis and gamma interferon response. Tuberculosis (Edinb). 2007;87(3):225-30. http://dx.doi.org/10.1016/j.tube.2006.10.002 PMid:17276141
16. Lio D, Marino V, Serauto A, Gioia V, Scola L, Crivello A, et al. Genotype frequencies of the +874T-->A single nucleotide polymorphism in the first intron of the interferon-gamma gene in a sample of Sicilian patients affected by tuberculosis. Eur J Immunogenet. 2002;29(5):371-4. http://dx.doi.org/10.1046/j.1365-2370.2002.00327.x PMid:12358843
17. Fitness J, Floyd S, Warndorff DK, Sichali L, Malema S, Crampin AC, et al. Large-scale candidate gene study of tuberculosis susceptibility in the Karonga district of northern Malawi. Am J Trop Med Hyg. 2004;71(3):341-9. PMid:15381817
18. Oh JH, Yang CS, Noh YK, Kweon YM, Jung SS, Son JW, et al. Polymorphisms of interleukin-10 and tumour necrosis factor-alpha genes are associated with newly diagnosed and recurrent pulmonary tuberculosis. Respirology. 2007;12(4):594-8. http://dx.doi.org/10.1111/j.1440-1843.2007.01108.x PMid:17587429

19. Oliveira MM, Da Silva JC, Costa JF, Amim LH, Loredo CC, et al. Single Nucleotide Polymorphisms (SNPs) of the TNF-α(-238/-308) gene among TB and nom TB patients: Susceptibility markers of TB occurrence? J Bras Pneumol. 2004;30(4):461-7.
20. Ates O, Musellim B, Ongen G, Topal-Sarikaya A. Interleukin-10 and tumor necrosis factor-alpha gene polymorphisms in tuberculosis. J Clin Immunol. 2008;28(3):232-6. http://dx.doi.org/10.1007/s10875-007-9155-2 PMid:18071881
21. Kaluza W, Reuss E, Grossmann S, Hug R, Schopf RE, Galle PR, et al. Different transcriptional activity and in vitro TNF-alpha production in psoriasis patients carrying the TNF-alpha 238A promoter polymorphism. J Invest Dermatol. 2000;114(6):1180-3. http://dx.doi.org/10.1046/j.1523-1747.2000.00001.x PMid:10844563
22. Haroon N, Tsui FW, Chiu B, Tsui HW, Inman RD. Serum cytokine receptors in ankylosing spondylitis: relationship to inflammatory markers and endoplasmic reticulum aminopeptidase polymorphisms. J Rheumatol. 2010;37(9):1907-10. http://dx.doi.org/10.3899/jrheum.100019 PMid:20595269
23. Paletta RM. Fatores de risco associados a ocorrência de tuberculose. [dissertation]. Rio de Janeiro: Universidade Federal do Rio de Janeiro; 2010.
24. Vidyarani M, Selvaraj P, Prabhu Anand S, Jawahar MS, Adhilakshmi AR, Narayanan PR. Interferon gamma (IFNgamma) & interleukin-4 (IL-4) gene variants & cytokine levels in pulmonary tuberculosis. Indian J Med Res. 2006;124(4):403-10. PMid:17159260
25. Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. Hepatology. 1999;30(2):526-30. http://dx.doi.org/10.1002/hep.510300207 PMid:10421663
26. Jamil B, Shahid F, Hasan Z, Nasir N, Razzaki T, Dawood G, et al. Interferon gamma/IL10 ratio defines the disease severity in pulmonary and extra pulmonary tuberculosis. Tuberculosis (Edinb). 2007;87(4):279-87. http://dx.doi.org/10.1016/j.tube.2007.03.004 PMid:17532265
27. Dubost JJ, Soubrier M, Meunier MN, Sauvezie B. From sedimentation rate to inflammation profile [Article in French]. Rev Med Interne. 1994;15(11):727-33. http://dx.doi.org/10.1016/S0248-8663(05)81398-0
28. Sahiratmadja E, Alisjahbana B, de Boer T, Adnan I, Maya A, Danusantoso H, et al. Dynamic changes in pro- and anti-inflammatory cytokine profiles and gamma interferon receptor signaling integrity correlate with tuberculosis disease activity and response to curative treatment. Infect Immun. 2007;75(2):820-9. http://dx.doi.org/10.1128/IAI.00602-06 PMid:17145950 PMCid:PMC1828524
29. Suzuki K, Takashima Y, Yamada T, Akiyama J, Yagi K, Kawashima M, et al. The sequential changes of serum acute phase reactants in response to antituberculous chemotherapy [Article in Japanese]. Kekkaku. 1992;67(4):303-11. PMid:1376373
30. Su WL, Perng WC, Huang CH, Yang CY, Wu CP, Chen JH. Association of reduced tumor necrosis factor alpha, gamma interferon, and interleukin-1beta (IL-1beta) but increased IL-10 expression with improved chest radiography in patients with pulmonary tuberculosis. Clin Vaccine Immunol. 2010;17(2):223-31. http://dx.doi.org/10.1128/CVI.00381-09 PMid:20007364 PMCid:PMC2815523

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Interpretation of autoantibody positivity in interstitial lung disease and lung-dominant connective tissue disease*

Interpretação da positividade de autoanticorpos na doença pulmonar intersticial e colagenose pulmão dominante

Daniel Antunes Silva Pereira, Alexandre de Melo Kawassaki, Bruno Guedes Baldi

Abstract

The initial evaluation of patients with interstitial lung disease (ILD) primarily involves a comprehensive, active search for the cause. Autoantibody assays, which can suggest the presence of a rheumatic disease, are routinely performed at various referral centers. When interstitial lung involvement is the condition that allows the definitive diagnosis of connective tissue disease and the classical criteria are met, there is little debate. However, there is still debate regarding the significance, relevance, specificity, and pathophysiological role of autoimmunity in patients with predominant pulmonary involvement and only mild symptoms or formes frustes of connective tissue disease. The purpose of this article was to review the current knowledge of autoantibody positivity and to discuss its possible interpretations in patients with ILD and without clear etiologic associations, as well as to enhance the understanding of the natural history of an allegedly new disease and to describe the possible prognostic implications. We also discuss the proposition of a new term to be used in the classification of ILDs: lung-dominant connective tissue disease.

Keywords: Idiopathic interstitial pneumonias; Autoantibodies; Connective tissue diseases; Autoimmune diseases; Diagnosis, differential.

Resumo

A avaliação inicial de pacientes com doença pulmonar intersticial (DPI) envolve primordialmente a busca ativa e detalhada por uma etiologia. A pesquisa rotineira de autoanticorpos é comum em diferentes centros e permite sugerir a presença de alguma doença do espectro reumatológico. Quando o acometimento pulmonar intersticial é a condição que permite o diagnóstico firmado de uma colagenose bem estabelecida, preenchendo os critérios clássicos, há pouco debate. Entretanto, ainda existe muita discussão sobre o significado, a relevância, a especificidade e o papel fisiopatológico da autoimunidade nos pacientes que tenham prioritariamente acometimento respiratório e apenas algum indício leve ou frustro de colagenose. O propósito dessa revisão foi apresentar o conhecimento atual e discutir possibilidades de interpretação da positividade de autoanticorpos em pacientes com DPI que não tenham associações etiológicas inequívocas, assim como aumentar o entendimento da história natural de uma possível nova doença e descrever possíveis implicações prognósticas. Discutimos ainda a proposição de uma nova terminologia na classificação das DPIs, a colagenose pulmão dominante.

Descritores: Pneumonias intersticiais idiopáticas; Autoanticorpos; Doenças do tecido conjuntivo; Autoimunidade; Diagnóstico diferencial.

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Introduction

Interstitial lung diseases (ILDs) are heterogeneous diseases that affect the lung parenchyma in a diffuse and multicompartmental manner, being characterized by different combinations of inflammation and fibrosis; the understanding of ILDs has increased dramatically in recent years.[1] On the basis of histological and CT patterns, ILDs can be subdivided into categories, as follows: granulomatous diseases; lymphoid diseases; miscellaneous; idiopathic diseases; and diseases of known cause, which include connective tissue diseases (CTDs).[1,2] There is considerable radiological, functional, and histological overlap among the abovementioned categories, especially between the last two.

Among ILDs, CTDs are of great importance, and pulmonary involvement causes significant functional limitation, which is the leading cause of death in such patients.[3] However, other factors determining ILD can be observed in patients with rheumatic diseases, and these factors should be taken into consideration in the differential diagnosis: i) a reasonable proportion of such patients were smokers at some point; ii) various drugs that are potentially toxic to the lungs are used in the treatment of CTDs; and iii) there is an increased susceptibility to infections.[1,4] In addition, the ILD patterns that are most commonly identified in this population can be quite similar to idiopathic forms.[2-10]

In the initial evaluation of patients with clinical and CT signs of ILD, serum autoantibody positivity is not uncommon.[1,11-14] In addition, routine ANF and serum autoantibody testing for the detection of occult CTD is recommended in several consensus guidelines for ILDs.[1,10-13,15]

Since 1994, when Katzenstein and Fiorelli first described the histological pattern of nonspecific interstitial pneumonia (NSIP), the understanding of idiopathic interstitial pneumonias (IIPs) and the role of CTDs in their etiology has changed.[5,16,17] It is well established that CTD-associated ILD has a better prognosis than does IIP.[7,8] However, many questions remain unanswered, including those regarding patients with ILD and positive ANF or serum autoantibodies.[12,13] Do such patients have formes frustes of CTD, which initially or exclusively manifest as ILD?[10,17,18] Will such patients present with well-defined CTD in the follow-up period?[12,19] Are there prognostic or therapeutic peculiarities?[17]

The purpose of this article was to review the current knowledge of autoantibody positivity and to discuss its possible interpretations in patients with ILD without etiologic associations, as well as to standardize recognition of these diseases, enhance the understanding of their natural history, and describe the prognostic implications. We also discuss the proposition of a new term to be used in the classification of ILDs: lung-dominant CTD (LDCTD).

CTD-associated ILD and undifferentiated CTD

The challenge of answering the question of whether IIP is actually a pulmonary manifestation of occult CTD increases when the criteria for defining CTDs are taken into consideration.[20,21] In the context of CTD-associated ILD, two scenarios are possible: one in which a patient with CTD subsequently develops respiratory symptoms due to new interstitial involvement; and one in which ILD-related respiratory symptoms precede or coincide with clinical and laboratory findings of autoimmune diseases.[19,21]

In the first scenario, when there is no doubt about the association between ILD and CTD, the causal relationship must be confirmed,[1,9,11] and it is essential to exclude potential causes of ILD, such as drug use, smoking, and infection. [22-25] In addition, the functional consequences of lung involvement and the need for treatment modification in the presence of ILD must be evaluated.[1] Lung biopsy is rarely indicated in this scenario, being reserved for cases having atypical presentations or for those in which there is the possibility of another cause for ILD.[4,18,23,26]

In the second scenario, in which pulmonary involvement predominates and there is no definite CTD, the difficulties increase significantly, which is mainly due to the fact that definitive rheumatologic criteria have yet to be established.[12,19,21] Historically, although the lung interstitium is a major target of CTD-associated autoimmunity, the lung is traditionally overlooked in the diagnostic criteria for these diseases.[10,12] As an example, there is the case of a 56-year-old female patient who presented with dyspnea, dry cough, moderate restrictive lung disease, Raynaud's phenomenon, telangiectases of the hands, a pattern of NSIP (as determined by HRCT), esophageal dilation with an air-fluid level (as determined by HRCT), homogeneous ANF (titer, 1:1,280), and positive anti-Ro antibodies (Figure 1). Although the patient showed clear

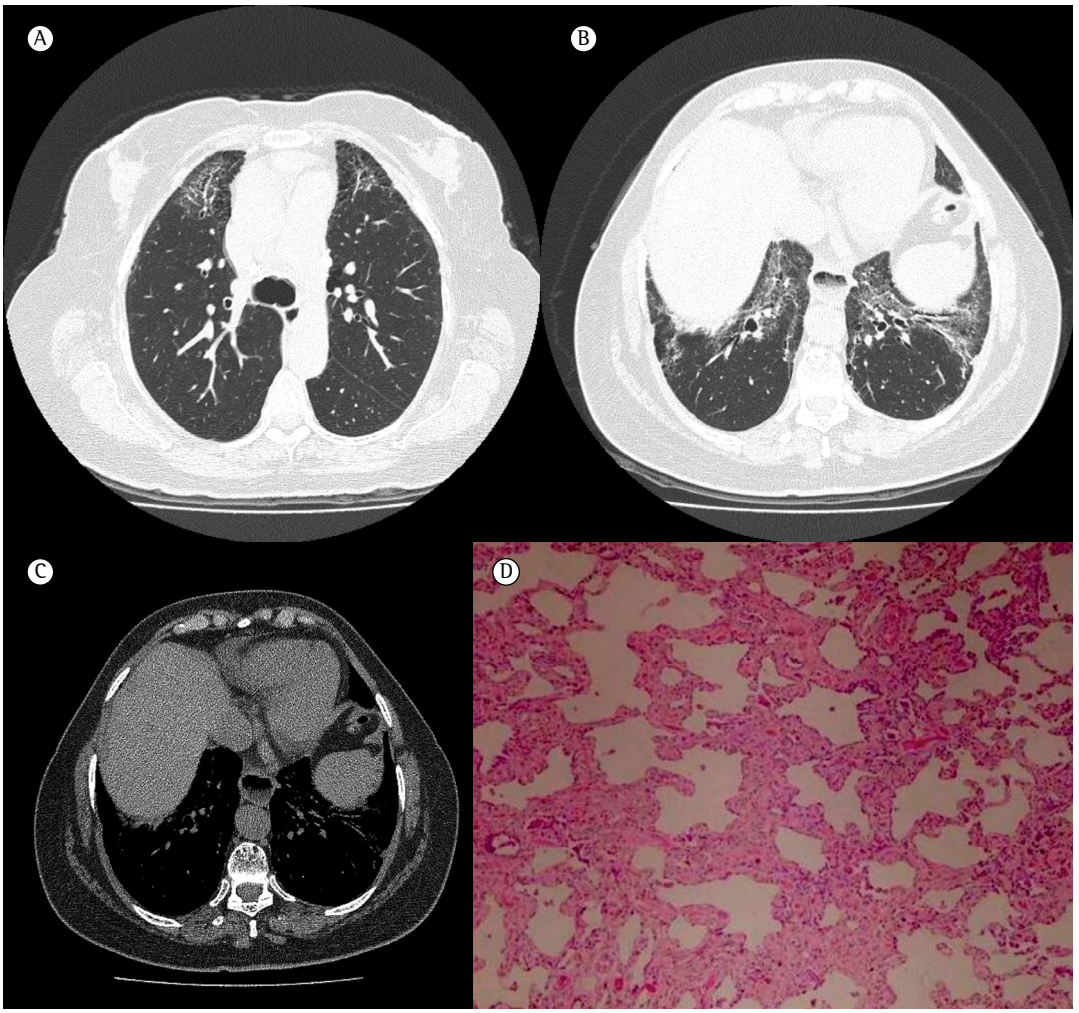

Figure 1 - Lung-dominant connective tissue disease. In A, HRCT scan showing reticular opacities, permeated by areas of ground-glass attenuation, and paracicatricial emphysema in the upper lobes. In B, HRCT scan showing peribronchovascular reticular opacities, ground-glass opacities, and traction bronchiectasis in the lower lobes. In C, mediastinal CT scan showing esophageal dilation with an air-fluid level. In D, histological examination of an open lung biopsy specimen showing inflammatory infiltrate in the alveolar septa, consistent with a nonspecific interstitial pneumonia pattern.

signs of autoimmune disease, they were not sufficient to establish a specific rheumatologic diagnosis, such as progressive systemic sclerosis (PSS).[14] If the patient had presented with a history of large joint arthritis for at least three years, the rheumatology team might have considered a diagnosis of undifferentiated CTD (UCTD).[27] Therefore, various rheumatologists seem to believe that predominant pulmonary involvement is not enough to recognize an autoimmune etiology.

The term UCTD is used in order to define patients with unclassifiable CTD, and the criteria presented in Chart 1 are currently used for the diagnosis of UCTD.[27]

The main characteristics of patients with UCTD are as follows: being female; being younger than 50 years of age; testing positive for ANF (approximately 80% of cases); and testing positive for various autoantibodies, especially anti-Ro (SSA) autoantibodies.[28-31] Patients with UCTD have predominant joint involvement that is mild to moderate in intensity and that resolves spontaneously or responds quite well to prednisone.[32] Commonly reported symptoms include Raynaud's phenomenon, xerophthalmia, xerostomia, joint pain, and morning stiffness. It is possible that up to one third of patients will meet the diagnostic criteria for a specific CTD (in

Chart 1 - Diagnostic criteria for undifferentiated connective tissue disease.[a]

1. Signs and symptoms suggestive of connective tissue disease, patients, however, not meeting established criteria for connective tissue disease;
2. positive ANF, positive autoantibodies, or both;
3. Symptom duration greater than three years

[a]Adapted from Mosca et al.[27]

particular, systemic lupus erythematosus) up to 2 years after the onset of symptoms; thereafter, this possibility is reduced, and most patients will persist with mild impairment (as observed at the onset of the disease), without meeting the diagnostic criteria for a specific CTD.[30-32]

In patients with UCTD, extra-articular involvement (including respiratory involvement) is rare.[29-31] These findings stand in contrast to the finding of severe pulmonary involvement habitually seen in patients with ILD and unclassifiable CTD.[13,15,33-35] In the practice of pulmonology, autoantibody-positive ILD patients who do not meet the diagnostic criteria for any established CTD present with pulmonary involvement whose intensity is markedly different from that of pulmonary involvement in relevant cohorts of patients with UCTD. This is the main reason why some authors avoid using the term UCTD to refer to cases of ILD and autoantibody positivity. In 2010, Fischer et al. proposed that the term LDCTD be used in order to characterize patients with any known pattern of ILD (usual interstitial pneumonia—UIP—NSIP, lymphocytic interstitial pneumonia, organizing pneumonia, desquamative interstitial pneumonia, etc.) associated with the presence of at least one CTD-specific autoantibody, two histological findings classically associated with pulmonary involvement secondary to CTD, or both, these findings, however, not characterizing a well-established rheumatic disease or an alternative etiology for ILD (Chart 2).[12] These stricter criteria, which include clinical, laboratory, CT, and histological findings, are intended for use in studies aimed at understanding the natural history, prognosis, and therapeutic response of this allegedly new form of ILD.

Importance of autoantibody positivity in the evaluation of patients with ILD

In patients with ILD and suspected occult CTD, specific laboratory tests are needed in order to determine the presence or absence of a systemic disease. Although most tests are not highly specific or sensitive for the diagnosis of CTD, a positive result in conjunction with clinical changes can indicate the presence of a CTD with a high degree of confidence.[3,14,36]

Homma et al. were the first to show the possibility of ILD as the sole presentation of occult CTD, having followed (for up to 11 years) 68 patients in whom the initial evaluation showed no clinical or serological evidence of CTD.[37] Of the 68 patients, 13 (19%) developed a definite CTD after an average of 25 months of follow-up: rheumatoid arthritis, in 5; polymyositis/dermatomyositis, in 5; systemic lupus erythematosus, in 1; Sjögren's syndrome (SS), in 1; and mixed CTD, in 1. In comparison with the remaining patients, those who met diagnostic criteria for a given CTD were younger, most being female. Creatine phosphokinase and ESR were the biochemical markers that distinguished between the two groups of patients, together with discoid atelectasis in the lower lung fields on chest X-rays.[37,38] At a time when autoantibody assays were not available in clinical practice, those results shed light on the possibility of predominant pulmonary manifestation of occult CTD or even the currently recognized LDCTD.

In a recent study aimed at evaluating the prevalence of occult CTD in all ILD patients presenting exclusively with respiratory symptoms, careful multidisciplinary evaluation and a comprehensive autoimmune panel showed that 34 (29%) of the 114 patients with confirmed ILD met diagnostic criteria for CTD.[39] Of those 34 patients, half had met diagnostic criteria for CTD in the initial evaluation and the other half were diagnosed during the follow-up period, the incidence of CTD in the cohort being 15%. The most common autoantibodies were ANF, in 56%, rheumatoid factor (RF), in 31%, and anti-Ro, in 15%. Inflammatory myopathies were present in 50% of cases, and the histological pattern most commonly associated with CTD was NSIP. An independent analysis of the data from that study shows that, of the 80 patients who showed no association with CTD at the end of the study period, 34 (42.5%) tested positive for ANF (7 of whom had ANF titer greater than or equal to 1:640) and 11 (16.3%) had a nucleolar pattern, which is highly specific for PSS.[14,40] These findings strongly suggest the presence of occult CTD.

Chart 2 - Provisional criteria for lung-dominant connective tissue disease.[a]

1. NSIP, UIP, LIP, OP, and DAD (or DIP) as determined by histology or HRCT;	
2. Extrathoracic findings that are insufficient to establish a definitive diagnosis of CTD	
3. ILD of unknown cause or association	
4. Any of the autoantibodies below or at least 2 of the histological findings below:	
Autoantibodies	Histological findings
a. ANF ≥ 1:320 or RF > 60	a. Lymphoid aggregates with germinal centers
b. Nucleolar ANF	b. Extensive pleurisy
c. anti-CCP	c. Prominent lymphoplasmacytic infiltrate
d. anti-Scl-70	d. Dense perivascular collagen
e. anti-Ro	
f. anti-La	
g. anti-nDNA	
h. anti-Sm	
i. anti-RNP	
j. anti-tRNA synthetase (anti-Jo-1, anti-PL-7, anti-PL-12)	
k. anti-PM/Scl	
l. anticentromere	

NSIP: nonspecific interstitial pneumonia; UIP: usual interstitial pneumonia; LIP: lymphocytic interstitial pneumonia; OP: organizing pneumonia; DAD: diffuse alveolar damage; DIP: desquamative interstitial pneumonia; CTD: connective tissue disease; ILD: interstitial lung disease; and RF: rheumatoid factor. [a]Adapted from Fischer et al.[12]

Although the diagnoses of those 80 patients were not reported, the serological evidence of autoimmunity in those patients is curious, given that idiopathic pulmonary fibrosis (IPF) is the most prevalent ILD. Although autoantibody positivity in the absence of clinical evidence of CTD should be interpreted with great caution, screening for autoantibodies in patients with diffuse parenchymal lung disease (as recommended by national and international guidelines) is justified.[1,11] It is of note that a rigorous evaluation is required in order to diagnose inflammatory myopathies.[38,41] Given that these conditions are commonly accompanied by interstitial lung involvement, recognition of occult manifestations requires a high degree of suspicion, an active search for desquamative lesions on the hands and increased serum muscle enzymes being therefore justified.

Autoantibodies and IPF

A specific form of chronic interstitial pneumonia, IPF is a disease of unknown etiology that is limited to the lungs and that occurs primarily in adults in their sixth decade of life, being associated with the histological and CT patterns of UIP.[1,42] The most widely accepted theory for the pathogenesis of IPF involves an interaction between alveolar epithelial injury and apoptosis, followed by abnormal mesenchymal repair.[43] The damaged epithelial cells adopt a mesenchymal behavior during the tissue repair process, producing fibrogenic cytokines.[1,44] Because IPF is a disease that is limited to the lungs, there is much debate about the presence of autoantibodies and clinical manifestations of occult CTD in patients with IPF. According to the recently published Brazilian Thoracic Association Guidelines for ILDs, up to 25% of patients have low-titer ANF and RF. However, this is considered to have no clinical relevance if CTD is excluded, determination of anti-cyclic citrullinated peptide (anti-CCP) antibodies being recommended in order to confirm rheumatoid arthritis in RF-positive patients.[1] It remains unknown whether the association between IPF and serum markers of immunity is only fortuitous or whether the identification of autoantibodies in such patients has any pathophysiological relevance.

Of all known rheumatic diseases, PSS is the disease in which fibrotic involvement is most pronounced.[45] The classic cutaneous manifestations of PSS (sclerodactyly, scleroderma facies, and morphea) cause significant thickening and tightening of the skin, which are markers of multisystem fibrotic involvement.[3] Interstitial lung injury is more closely related to the limited form of systemic sclerosis, which is known as CREST syndrome (CREST being an acronym for Calcinosis, Raynaud's phenomenon, Esophageal dysfunction, Sclerodactyly, and Telangiectasia). Although NSIP

is the pattern that is most commonly associated with PSS, UIP is quite common.[46] Because of the difficulty in excluding occult CTD in patients with ILD, Fischer et al. reported the serological findings in patients with a clinical diagnosis of IPF.[40] Of the 285 patients who were tested for ANF, 25 had titers greater than 1:40 and a nucleolar-staining pattern. Among the various antinuclear antibodies found in patients with PSS, antinucleolar antibodies are found in 10-15%. Antinucleolar antibodies constitute a group of heterogeneous autoantibodies that cause ANF to have an antinucleolar pattern of immunofluorescence, being highly specific markers of PSS. Of the 25 IPF patients who had a positive ANF, 13 tested positive for anti-Th/To antibody—an antinucleolar antibody that is strongly related to systemic sclerosis-associated ILD—PSS being therefore confirmed. This underscores the need for comprehensive serological testing for the identification of occult CTD in patients with IPF, with therapeutic and prognostic implications.

The results of a recently published study investigating the prevalence and clinical significance of circulating autoantibodies in 67 patients with IPF[47] add weight to the abovementioned conclusion. Autoantibody positivity was found in 22% of the patients with IPF and in 21% of the healthy controls. There were no differences between the IPF patients with circulating antibodies and those without in terms of clinical characteristics, CT findings, histological features, or treatment response. However, the subgroup of patients with circulating autoantibodies had a longer transplant-free survival time. The authors also compared the group of IPF patients who tested positive for circulating autoantibodies (n = 15) with a group of patients with UCTD (n = 22) and found that the latter were younger and had higher titers of ANF.

Therefore, the significance of positive autoantibodies in patients with IPF depends on the presence of systemic signs of rheumatic disease and on the specificity of the technique used for screening, as well as on autoantibody titers and patterns. In the presence of these factors, an alternative diagnosis of CTD (rather than IPF) is possible. However, studies have recognized the role of autoimmunity in the pathogenesis of IPF, the identification of circulating autoantibodies and the direct association of circulating autoantibodies with lung epithelial injury suggesting the presence of autoimmunity. Takahashi et al. assessed autoantibody positivity in 22 patients with IPF and compared those patients with 37 healthy individuals.[48] Through techniques of immunohistochemistry and immunoprecipitation of epithelial lung cells, the authors identified in the sera from IPF patients an antibody that precipitated in the cytoplasm of those cells but not in that of cells of mesenchymal origin (ovary). Spectrophotometry of the precipitated immunocomplex allowed the isolation of the antigen, which was a protein consistent with alanyl-tRNA synthetase (also known as PL-12) in 50% of the patients with IPF. This antibody is associated with inflammatory myopathies that affect the pulmonary interstitium (such as antisynthetase syndrome), although it is present in only a small proportion of such patients.[38] Despite a potential systematic error in the diagnosis of IPF in that study, the presence of that autoantibody in half of the sample adds validity to the findings and shows that autoimmunity plays a relevant role in the pathogenesis of IPF (in some patients, at least). In addition, ensuring the distinction between IPF and CTD, the antigen described was present in less than 5% of the patients with CTD-associated ILD in whom the techniques described above were performed.

There is a tendency in the literature to interpret the presence of nonspecific autoantibodies or low-titer ANF in patients with IPF as positivity that is similar to that in the general population (20-30%) and that generally has no clinical consequences.[1,35,39,40,42,47,48] However, in a subgroup of patients, the presence of associated autoimmunity can have an impact on prognosis and response to treatment. In the case of diseases for which there is currently insufficient scientific evidence to endorse most of the treatments that are currently available, identifying patients with stigmata of autoimmunity can stimulate pharmacological innovations. In addition, formes frustes of rheumatic diseases and occult forms of CTD should not be forgotten.

Autoantibodies and idiopathic NSIP

Early in the last decade, an international panel of experts in ILDs met, with the support of the American Thoracic Society, in order to gain a better understanding of idiopathic NSIP in light of the scientific knowledge available at the time.[17]

Researchers who had previously reported cases of idiopathic NSIP were invited to submit cases for review. Patients who, at the onset of pulmonary symptoms, met diagnostic criteria for CTD (or other diseases) were excluded from the analysis. The selected cases were considered idiopathic after a detailed evaluation performed by the multidisciplinary expert panel (including clinicians, radiologists, and pathologists), who subsequently studied the epidemiological, histological, and CT features. Of the 67 cases that were considered idiopathic, 43% had positive ANF and 23% had increased RF; Raynaud's phenomenon and arthritis had been reported in 8% and 3%, respectively. The authors considered and confirmed the possibility of interstitial lung involvement as the initial or sole manifestation of CTD, or as a manifestation of LDCTD. In a report published in 2006, the expert panel concluded that NSIP is a distinct form of ILD; however, because of a possible association between NSIP and other forms, especially hypersensitivity pneumonitis and CTD, a comprehensive multidisciplinary approach is required in order to establish a diagnosis of idiopathic NSIP.[17]

LDCTD

On the basis of the abovementioned report from the American Thoracic Society, various authors attempted to characterize patients presenting with histological features of NSIP and meeting the previously discussed diagnostic criteria for UCTD. Kinder et al. sought to determine whether idiopathic NSIP was actually the pulmonary manifestation of a systemic autoimmune disease and, consequently, the pulmonary manifestation of UCTD.[13] By applying their own criteria (which were considered permissive for including findings that have low specificity for autoimmunity, such as gastroesophageal reflux and ESR), which combined CTD symptoms with evidence of systemic inflammation (autoantibodies, inflammatory activity, or both), the authors identified, in a population of 285 patients with ILD, 28 cases without any other apparent cause. Most were female, were younger than 50 years of age, and complained of arthralgia, joint swelling, Raynaud's phenomenon, and dysphagia more often than did patients who met no diagnostic criteria for UCTD. With the exception of digital clubbing, which was more prevalent in those with the idiopathic form (26% vs. 7%), there were no differences between the groups in terms of physical examination findings. Regarding CT findings, ground-glass opacities and consolidations were more common in the UCTD group, whereas honeycombing was more common in the IIP group (being more consistent with the UIP pattern). In the histological analysis, most of the patients with UCTD were found to have the NSIP pattern, which was rarely found in the IIP group. The UIP pattern (as determined by pathological examination) was positively associated with IIP (OR = 111) and negatively associated UCTD (OR = 0.009). The authors concluded that the NSIP pattern is strongly associated with patients with CTD symptoms, especially Raynaud's phenomenon, arthralgia, and gastrointestinal manifestations, when associated with positive serum autoantibodies. In addition, the authors suggested that meeting diagnostic criteria for UCTD in this context constitutes a predictor of NSIP, which is actually a pulmonary manifestation of systemic autoimmunity.[13] Suda et al.[49] evaluated 47 patients with idiopathic NSIP and, by applying the same criteria as those applied by Kinder et al.,[13] identified 22 cases of UCTD (47%), which were compared with those of patients who did not meet those criteria. In addition to having found clinical and laboratory features that were quite similar to those found in the previous study, the authors demonstrated that cases considered idiopathic showed a higher mortality rate than did those with UCTD, as well as showing a lower 5-year survival rate (58% vs. 100%). For the first time, it was suggested that the prognosis of such patients follows the pattern of that of other patients with CTD-associated ILD, progression being more favorable.

In contrast, in another recent study, in which stricter diagnostic criteria were applied, 63 (32%) of 200 patients with ILD met diagnostic criteria for autoimmunity, and 58 (29%) met diagnostic criteria for IPF.[35] In the autoimmunity group, the UIP pattern was the most common CT finding (62%), correlating well with histological findings. There were no differences in survival between the autoimmunity and IPF groups, and both had worse survival than did those with known CTD. In yet another study, stringent criteria (CTD-specific symptoms and autoantibodies) were applied, and their accuracy in predicting NSIP was evaluated.[34] Of 100 patients with NSIP or UIP (as determined by surgical biopsy), 21 had LDCTD, which accounted for 31% of all

cases of PINE and only 13% of all cases of UIP. Therefore, those strict criteria were three times more likely to be associated with NSIP histology, their sensitivity being 31% and their specificity being 88%. A poor accuracy in predicting this pattern is probably due to the fact that LDCTD can be associated with any known pattern of ILD.

The two studies cited above[34,35] seem to characterize a population that is different from those in the studies by Kinder et al.[13] and Suda et al.[49] Can this difference be explained solely by differences among the study populations? Do these patients actually have UCTD and will rheumatologic criteria apply in this context of predominant ILD?[35,50] When this association was first recognized, NSIP was the most prevalent pattern. However, relevant data suggest that, if there are cases in which ILD is a pulmonary manifestation of systemic autoimmunity, the patterns of response to epithelial injury do vary. This constitutes an obstacle to the use of classifications or terms that originate from other specialties (such as UCTD), if not because such classifications and terms are well established then at least because of the clinical and epidemiological distinction described herein. In this context, we propose that the term LDCTD be used in order to aid in distinguishing such patients from those with UCTD and predominant joint involvement, as well as to aid in standardizing criteria that will allow pulmonologists to characterize such patients more precisely. Chart 2 presents stringent criteria for the diagnosis of LDCTD. The proposed criteria give weight to autoantibodies that are more specific to CTD and to higher titers of highly sensitive markers of autoimmunity. In addition, other causes for ILD or even well-established CTD should be ruled out. However, the major difference between these and other criteria is that they recognize the possibility of classic patterns other than NSIP and allow the inclusion of histological findings suggestive of pulmonary involvement secondary to CTD. Figure 1 shows CT and histological findings in a female patient with LDCTD after a 6-year follow-up period, during which she met no diagnostic criteria for CTD.

Occult CTD

It is clearly difficult to distinguish between CTD-associated ILD and formes frustes of CTD. Rheumatic diseases are classified in accordance with clinical criteria established by leading rheumatology societies worldwide, chief among which is the American College of Rheumatology. The primary criticism directed toward the use of these criteria is that they do not reflect the clinical reality.[19] Chief among formes frustes of CTD are PSS sine scleroderma and amyopathic dermatomyositis, which are characterized by the absence of clinical findings that primarily characterize the diseases to which they are related.[36,38,51,52] The different criteria used in the LDCTD studies reviewed above do not escape this criticism, and LDCTD can often be characterized as occult CTD.

Next, we discuss the major rheumatic diseases that can present as formes frustes and mimic ILD, and that can be mistaken for LDCTD in the absence of a high degree of clinical suspicion. Fischer et al. evaluated 37 patients with clinical features of antisynthetase syndrome, negative ANF, and negative anti-Jo-1 antibodies. In order to test the hypothesis that antisynthetase syndrome is an unrecognized cause of ILD, those patients were screened for other anti-tRNA synthetase antibodies, and 9 (24%) tested positive for anti-PL-7 or anti-PL-12 antibodies.[41] In the presence of a high degree of clinical suspicion, a provisional diagnosis of antisynthetase syndrome can be established in those with Raynaud's phenomenon, desquamation on the sides of the fingers (mechanic's hands), arthritis, Gottron's papules, and pulmonary fibrosis, even if they have tested negative for ANF and anti-Jo-1 antibodies. Therefore, screening for other anti-tRNA synthetase antibodies, basically anti-PL-7 and anti- PL-12 antibodies, increases the chance of a diagnosis of occult CTD.[39,53]

In another study, 38 patients with ILD and sicca syndrome (keratoconjunctivitis sicca or xerostomia) or abnormal levels of SS-associated autoantibodies (anti-Ro and anti-La) underwent labial salivary gland biopsy.[54] When biopsy was requested, none of the patients had a definable CTD. Thirteen patients (34%) had chronic sialadenitis, which, together with sicca syndrome, is diagnostic of SS.[55] Of those 13 patients, 3 tested negative for ANF, RF, anti-Ro antibodies, and anti-La antibodies, a finding that increases the possibility of CTD even in the absence of autoantibodies.

The difficulty in and importance of identifying patients with CTD-associated ILD are therefore clear. All ILD patients should be screened for occult or early CTD in order to decrease the diagnosis of IIP, with prognostic and therapeutic

implications. A multidisciplinary approach, involving pulmonologists, radiologists, pathologists, and rheumatologists, is recommended in order to overcome the limitations of current diagnostic criteria and the unavailability of autoantibody assays.

How should patients with ILD be evaluated in terms of the presence of features of rheumatic disease? Chart 3 presents the main clinical, laboratory, radiological, and histological findings for an active search for patients with CTD-associated ILD. The presence of positive ANF titers ≥ 320 strongly suggests an association with rheumatic disease; however, as is the case with any other autoantibody, a positive ANF alone loses specificity unless certain highly specific immunofluorescence patterns (including a nucleolar pattern, a centromere pattern, and a cytoplasmic pattern) are found.[56] The hands should be routinely examined for changes suggestive of rheumatic disease. Mechanic's hands and Gottron's papules are associated with inflammatory myopathies and antisynthetase syndrome.[3] The presence of sclerodactyly, Raynaud's phenomenon, digital pitting scars, and fingertip ulcers is suggestive of PSS, whereas the presence of Raynaud's phenomenon, digital pitting scars, and fingertip ulcers without sclerodactyly is suggestive of PSS sine scleroderma.[3,51,52] Nailfold capillaroscopy is a technique that is used in order to evaluate microvascular involvement (which is quite common in patients with PSS), polymyositis/dermatomyositis, and mixed CTD.[36]

What is the importance of this discussion? What is the advantage of identifying rheumatic features in patients with ILD?

There are various reasons why patients with ILD should be screened for features of CTD.[12] Initially, the identification of a CTD allows patients to gain a better understanding of their health problems and allows health professionals to seek lesions in organs that can also be affected by systemic autoimmunity.[12] Standardization of criteria allows researchers to conduct epidemiological studies that are more reproducible, allowing a better understanding of the natural history and prognosis of the disease in question. Regarding the treatment of ILDs, robust data regarding the efficacy or inefficacy of certain drugs have only recently been obtained.[19,42] In patients with PSS-associated ILD, the use of cyclophosphamide for 12 months, followed by maintenance therapy with azathioprine or mycophenolate, slows functional deterioration.[57,58] The therapeutic response in patients with LDCTD remains unclear. It remains unknown whether it is similar to the refractoriness in those with IPF or to the stability in those with PSS, or whether the possibility of partial or complete resolution exists.

Another important point is patient prognosis. Most of the studies reviewed here showed that the prognosis of patients with CTD-associated ILD is better than is that of those with idiopathic forms. Park et al.[7] showed that survival was better in the group of patients with CTD-associated ILD than in that of those with IIP, the groups of patients with CTD-associated NSIP, idiopathic NSIP, and CTD-associated UIP having a similar prognosis. The group of patients with IPF had the worst prognosis. The authors concluded that patients with CTD-associated ILD (including those with CTD-associated UIP) had a better prognosis, screening for occult CTD or even frustes formes of CTD (such as LDCTD) being therefore justified. Suda et al. demonstrated that patients with NSIP and LDCTD have better 5-year survival than do those with idiopathic NSIP.[49] In another study, in which the diagnostic criteria for LDCTD were adopted, lung histology revealed that the proportion of patients with UIP was much higher than was that of those with NSIP, and, despite the low mortality in the study population, their survival was better than was that of those with IPF.[35] However, these results were not reproduced in another study, in which strict criteria were also adopted.[34]

Therefore, prospective studies should be conducted in order to establish the real prognosis and the best treatment strategy for patients with LDCTD.

Final considerations

The initial evaluation of patients with ILD involves a comprehensive, active search for the cause. Routine screening for autoantibodies allows the diagnosis of rheumatic diseases ranging from UCTD (a fruste forme of CTD) to a definable CTD. However, autoantibody positivity should be interpreted with caution, given that autoantibody assays are laboratory tests whose accuracy is limited in many cases. Autoantibody positivity

Chart 3 - Findings suggestive of connective tissue disease as the cause of interstitial lung disease.

Points for consideration	Findings	
Demographics	Female gender	
	Age < 50 years	
Symptoms	Change in the color of hands in response to cold exposure (Raynaud's phenomenon)	
	Xerophthalmia/xerostomia	
	Arthralgia/arthritis/joint swelling	
	Gastroesophageal reflux	
Physical examination of the hands	Raynaud's phenomenon	
	Desquamation on the sides of the fingers ("mechanic's hands")	
	Telangiectasia	
	Sclerodactyly	
	Thickening of the interphalangeal joints	
	Gottron's papules	
General physical examination	Ocular hyperemia	
	Photosensitivity	
	Digital clubbing	
	Skin sclerosis	
	Arthritis	
Ancillary tests		Possible diagnosis
ANF	Titter ≥ 320	Strongly suggestive of CTD
	Nucleolar/centromere pattern	PSS
	Homogeneous nuclear pattern	SLE, PSS, autoimmune hepatitis
	Speckled nuclear pattern	SLE, PSS, MCTD, SS
	Cytoplasmic pattern	PM/DM, SS, SLE, primary biliary cirrhosis, PSS
FR, anti-CCP		RA
Anti-Ro (SSA), Anti-La (SSB)		Highly sensitive; SS, RA, PSS, UCTD
Anti-RNP		MCTD
Anti-Scl-70		PSS (diffuse form)
Anti-DNA, Anti-Sm		SLE
Antisynthetase antibodies (Jo-1, PL-7, PL-12, etc.)		PM/DM, antisynthetase syndrome
Nailfold capillaroscopy	Any microvascular change	Raynaud's phenomenon, SD, inconclusive
Barium esophagography	Esophageal dysmotility	
Chest HRCT	Typical patterns	NSIP, LIP, OP, DIP
	Atypical or mixed patterns	Biopsy is required
	Cystic honeycombing	Suggestive of RA
	Diffuse esophageal dilation	
	Esophageal air-fluid level	
	Pleural and pericardial effusion	Serositis
	Dilation of the pulmonary trunk	Suggestive of PH but lacks specificity
Histology	Lymphoid aggregates with germinal centers	
	Extensive pleurisy	
	Prominent lymphoplasmacytic infiltrate	
	Dense perivascular collagen	

RA: rheumatoid arthritis; UCTD: undifferentiated connective tissue disease; LDCTD: lung-dominant connective tissue disease; MCTD: mixed connective tissue disease; PM/DM: polymyositis/dermatomyositis; CTD: connective tissue disease; PSS: progressive systemic sclerosis; RF: rheumatoid factor; SLE: systemic lupus erythematosus; DIP: desquamative interstitial pneumonia; LIP: lymphocytic interstitial pneumonia; NSIP: nonspecific interstitial pneumonia; UIP: usual interstitial pneumonia; OP: organizing pneumonia; SS: Sjögren's syndrome; SD: schleroderma/dermatomyositis; and PH: pulmonary hypertension.

in the general population, technical difficulties, and analytical errors are typical. Therefore, the diagnosis or suspicion of CTD should be associated with typical signs and symptoms.

The terms LDCTD and UCTD are often treated as synonyms. However, studies investigating patients with UCTD show little joint morbidity and virtually no pulmonary involvement. The criteria used in the studies investigating patients with predominant respiratory disease and using the term UCTD lacked specificity, as well as having differed from study to study. Despite conflicting data, different terminology, varying inclusion criteria, and different views on the same issue, it seems that patients with LDCTD constitute a distinct population of patients with ILD. The hypothesis that the lung is the organ that is predominantly affected by systemic autoimmunity in such cases is now more likely than not. Although this hypothesis emerged from studies of idiopathic NSIP, it is possible that other patterns of ILD are involved. The immune system is highly complex, and very little is known regarding the orchestration of the distinction between self and non-self in innate and acquired immunity. What is the explanation for the finding of anti-PL-12 antibodies in patients with IPF, given that PL-12 is highly specific to rheumatic disease and that IPF is a highly fibrotic and poorly cellular disease? How can patients with well-defined CTD have prognostic features that are different from those of patients with LDCTD?

Many questions remain unanswered, and many others will arise. However, this knowledge must be extrapolated to current daily practice. In approaching ILDs, it is essential to consider the possibility of CTD as the etiologic agent. Patients with ILD should be actively screened for extrapulmonary manifestations, such as Raynaud's phenomenon, arthralgia, sicca symptoms, and gastrointestinal symptoms. In addition, they should be routinely screened for rheumatoid antibodies, such as ANF, anti-Ro, anti-La, anti-RNP, anti-Sm, anti-DNA, anti-Scl-70, RF, anti-CCP, and antisynthetase antibodies (especially anti-Jo-1, given that the tests for the remaining antisynthetase antibodies are available only in the USA). In the presence of negative test results and a high degree of clinical suspicion of CTD, the possibility of repeating the tests should be taken into consideration because of the possibility of formes frustes.[14,56] In the presence of positive test results, the possibility of a false association of indeterminate clinical significance (which is due to the considerable sensitivity of some of these tests) should not be discarded. The clinical context, titers, and patterns, as well as which specific antibody test results were abnormal, are factors that will jointly contribute to the relevance of positive test results.

Finally, in addition to recommending a multidisciplinary approach to patients with LDCTD, we suggest that LDCTD be defined as a distinct phenotype and as a classification of disease so that future studies can examine its prognostic, therapeutic, and biopathological implications.

References

1. Baldi BG, Pereira CA, Rubin AS, Santana AN, Costa AN, Carvalho CR, et al. Highlights of the Brazilian Thoracic Association guidelines for interstitial lung diseases. J Bras Pneumol. 2012;38(3):282-91. http://dx.doi.org/10.1590/S1806-37132012000300002 PMid:22782597
2. Elicker B, Pereira CA, Webb R, Leslie KO. High-resolution computed tomography patterns of diffuse interstitial lung disease with clinical and pathological correlation. J Bras Pneumol. 2008;34(9):715-44. http://dx.doi.org/10.1590/S1806-37132008000900013 PMid:18982210
3. Tzelepis GE, Toya SP, Moutsopoulos HM. Occult connective tissue diseases mimicking idiopathic interstitial pneumonias. Eur Respir J. 2008;31(1):11-20. http://dx.doi.org/10.1183/09031936.00060107 PMid:18166591
4. Antoniou KM, Margaritopoulos G, Economidou F, Siafakas NM. Pivotal clinical dilemmas in collagen vascular diseases associated with interstitial lung involvement. Eur Respir J. 2009;33(4):882-96. http://dx.doi.org/10.1183/09031936.00152607 PMid:19336591
5. de Lauretis A, Veeraraghavan S, Renzoni E. Review series: Aspects of interstitial lung disease: connective tissue disease-associated interstitial lung disease: how does it differ from IPF? How should the clinical approach differ? Chron Respir Dis. 2011;8(1):53-82. PMid:21339375
6. Song JW, Do KH, Kim MY, Jang SJ, Colby TV, Kim DS. Pathologic and radiologic differences between idiopathic and collagen vascular disease-related usual interstitial pneumonia. Chest. 2009;136(1):23-30. http://dx.doi.org/10.1378/chest.08-2572 PMid:19255290
7. Park JH, Kim DS, Park IN, Jang SJ, Kitaichi M, Nicholson AG, et al. Prognosis of fibrotic interstitial pneumonia: idiopathic versus collagen vascular disease-related subtypes. Am J Respir Crit Care Med. 2007;175(7):705-11. http://dx.doi.org/10.1164/rccm.200607-912OC PMid:17218621
8. Flaherty KR, Colby TV, Travis WD, Toews GB, Mumford J, Murray S, et al. Fibroblastic foci in usual interstitial pneumonia: idiopathic versus collagen vascular disease. Am J Respir Crit Care Med. 2003;167(10):1410-5. http://dx.doi.org/10.1164/rccm.200204-373OC PMid:12615630
9. Lynch DA. Lung disease related to collagen vascular disease. J Thorac Imaging. 2009;24(4):299-309. http://dx.doi.org/10.1097/RTI.0b013e3181c1acec PMid:19935226
10. Cottin V. Interstitial lung disease: are we missing formes frustes of connective tissue disease?

Eur Respir J. 2006;28(5):893-6. http://dx.doi.org/10.1183/09031936.00101506 PMid:17074915
11. American Thoracic Society/European Respiratory Society International Multidisciplinary Consensus Classification of the Idiopathic Interstitial Pneumonias. This joint statement of the American Thoracic Society (ATS), and the European Respiratory Society (ERS) was adopted by the ATS board of directors, June 2001 and by the ERS Executive Committee, June 2001. Am J Respir Crit Care Med. 2002;165(2):277-304. Erratum in: Am J Respir Crit Care Med. 2002;166(3):426. http://dx.doi.org/10.1164/ajrccm.165.2.ats01 PMid:11790668
12. Fischer A, West SG, Swigris JJ, Brown KK, du Bois RM. Connective tissue disease-associated interstitial lung disease: a call for clarification. Chest. 2010;138(2):251-6. http://dx.doi.org/10.1378/chest.10-0194 PMid:20682528 PMCid:PMC3662187
13. Kinder BW, Collard HR, Koth L, Daikh DI, Wolters PJ, Elicker B, et al. Idiopathic nonspecific interstitial pneumonia: lung manifestation of undifferentiated connective tissue disease? Am J Respir Crit Care Med. 2007;176(7):691-7. http://dx.doi.org/10.1164/rccm.200702-220OC PMid:17556720 PMCid:PMC1994238
14. Papiris SA, Kagouridis K, Bouros D. Serologic evaluation in idiopathic interstitial pneumonias. Curr Opin Pulm Med. 2012;18(5):433-40. http://dx.doi.org/10.1097/MCP.0b013e3283560840 PMid:22699420
15. Romagnoli M, Nannini C, Piciucchi S, Girelli F, Gurioli C, Casoni G, et al. Idiopathic nonspecific interstitial pneumonia: an interstitial lung disease associated with autoimmune disorders? Eur Respir J. 2011;38(2):384-91. http://dx.doi.org/10.1183/09031936.00094910 PMid:21273390
16. Katzenstein AL, Fiorelli RF. Nonspecific interstitial pneumonia/fibrosis. Histologic features and clinical significance. Am J Surg Pathol. 1994;18(2):136-47. http://dx.doi.org/10.1097/00000478-199402000-00003 PMid:8291652
17. Travis WD, Hunninghake G, King TE Jr, Lynch DA, Colby TV, Galvin JR, et al. Idiopathic nonspecific interstitial pneumonia: report of an American Thoracic Society project. Am J Respir Crit Care Med. 2008;177(12):1338-47. Erratum in: Am J Respir Crit Care Med.2008;178(2):211. http://dx.doi.org/10.1164/rccm.200611-1685OC PMid:18388353
18. Leslie KO. My approach to interstitial lung disease using clinical, radiological and histopathological patterns. J Clin Pathol. 2009;62(5):387-401. http://dx.doi.org/10.1136/jcp.2008.059782 PMid:19398592 PMCid:PMC2668105
19. Fischer A, du Bois R. Interstitial lung disease in connective tissue disorders. Lancet. 2012;380(9842):689-98. http://dx.doi.org/10.1016/S0140-6736(12)61079-4
20. Coletta EN. Padrões histológicos dos processos infiltrativos difusos em colagenoses. J Pneumol. 1997;23(4):197-207.
21. Fischer A. Interstitial lung disease: a rheumatologist's perspective. J Clin Rheumatol. 2009;15(2):95-9. http://dx.doi.org/10.1097/RHU.0b013e31819b715d PMid:19265357
22. Rodrigues SC, Rodrigues MM, Colleta EN, Rocha NS, Pereira CA. Respiratory bronchilitis-associated interstitial lung disease. J Bras Pneumol. 2004;30(6):574-80.
23. Antin-Ozerkis D, Rubinowitz A, Evans J, Homer RJ, Matthay RA. Interstitial lung disease in the connective tissue diseases. Clin Chest Med. 2012;33(1):123-49. http://dx.doi.org/10.1016/j.ccm.2012.01.004 PMid:22365251
24. Reichert J, Reichert A, Bendhack LI, Noronha E. Pneumonite intersticial em paciente sob tratamento com leflunomide: toxicidade da droga? J Pneumol. 2003;29(6):395-400. http://dx.doi.org/10.1590/S0102-35862003000600013
25. Dias OM, Pereira DA, Abensur A, Rodrigo K, Ronaldo A, Carvalho CR, et al. Pneumonite intersticial aguda induzida por adalimumabe em paciente com artrite reumatoide: relato de caso. J Bras Pneumol. 2013. [epub ahead of print]. PMid:23670508
26. Leslie KO, Trahan S, Gruden J. Pulmonary pathology of the rheumatic diseases. Semin Respir Crit Care Med. 2007;28(4):369-78. http://dx.doi.org/10.1055/s-2007-985609 PMid:17764055
27. Mosca M, Tani C, Carli L, Bombardieri S. Undifferentiated CTD: a wide spectrum of autoimmune diseases. Best Pract Res Clin Rheumatol. 2012;26(1):73-7. http://dx.doi.org/10.1016/j.berh.2012.01.005 PMid:22424194
28. LeRoy EC, Maricq HR, Kahaleh MB. Undifferentiated connective tissue syndromes. Arthritis Rheum. 1980;23(3):341-3. http://dx.doi.org/10.1002/art.1780230312 PMid:7362686
29. Mosca M, Tavoni A, Neri R, Bencivelli W, Bombardieri S. Undifferentiated connective tissue diseases: the clinical and serological profiles of 91 patients followed for at least 1 year. Lupus. 1998;7(2):95-100. http://dx.doi.org/10.1191/096120398678919787 PMid:9541093
30. Vaz CC, Couto M, Medeiros D, Miranda L, Costa J, Nero P, et al. Undifferentiated connective tissue disease: a seven-center cross-sectional study of 184 patients. Clin Rheumatol. 2009;28(8):915-21. http://dx.doi.org/10.1007/s10067-009-1175-2 PMid:19390908
31. Bodolay E, Csiki Z, Szekanecz Z, Ben T, Kiss E, Zeher M, et al. Five-year follow-up of 665 Hungarian patients with undifferentiated connective tissue disease (UCTD). Clin Exp Rheumatol. 2003;21(3):313-20. PMid:12846049
32. Mosca M, Tani C, Neri C, Baldini C, Bombardieri S. Undifferentiated connective tissue diseases (UCTD). Autoimmun Rev. 2006;6(1):1-4. http://dx.doi.org/10.1016/j.autrev.2006.03.004 PMid:17110308
33. Kinder BW, Shariat C, Collard HR, Koth LL, Wolters PJ, Golden JA, et al. Undifferentiated connective tissue disease-associated interstitial lung disease: changes in lung function. Lung. 2010;188(2):143-9. http://dx.doi.org/10.1007/s00408-009-9226-7 PMid:20069430 PMCid:PMC2837880
34. Corte TJ, Copley SJ, Desai SR, Zappala CJ, Hansell DM, Nicholson AG, et al. Significance of connective tissue disease features in idiopathic interstitial pneumonia. Eur Respir J. 2012;39(3):661-8. http://dx.doi.org/10.1183/09031936.00174910 PMid:21920896
35. Vij R, Noth I, Strek ME. Autoimmune-featured interstitial lung disease: a distinct entity. Chest. 2011;140(5):1292-9. http://dx.doi.org/10.1378/chest.10-2662 PMid:21565966 PMCid:PMC3205845
36. Cutolo M, Sulli A, Secchi ME, Paolino S, Pizzorni C. Nailfold capillaroscopy is useful for the diagnosis and follow-up of autoimmune rheumatic diseases. A future tool for the analysis of microvascular heart involvement? Rheumatology (Oxford). 2006;45(Suppl 4):iv43-6. http://dx.doi.org/10.1093/rheumatology/kel310 PMid:16980724
37. Homma Y, Ohtsuka Y, Tanimura K, Kusaka H, Munakata M, Kawakami Y, et al. Can interstitial pneumonia as the sole presentation of collagen vascular diseases be differentiated from idiopathic interstitial pneumonia? Respiration.

1995;62(5):248-51. http://dx.doi.org/10.1159/000196457 PMid:8560090
38. Solomon J, Swigris JJ, Brown KK. Myositis-related interstitial lung disease and antisynthetase syndrome. J Bras Pneumol. 2011;37(1):100-9. http://dx.doi.org/10.1590/S1806-37132011000100015 PMid:21390438 PMCid:PMC3676869
39. Mittoo S, Gelber AC, Christopher-Stine L, Horton MR, Lechtzin N, Danoff SK. Ascertainment of collagen vascular disease in patients presenting with interstitial lung disease. Respir Med. 2009;103(8):1152-8. http://dx.doi.org/10.1016/j.rmed.2009.02.009 PMid:19304475
40. Fischer A, Pfalzgraf FJ, Feghali-Bostwick CA, Wright TM, Curran-Everett D, West SG, et al. Anti-th/to-positivity in a cohort of patients with idiopathic pulmonary fibrosis. J Rheumatol. 2006;33(8):1600-5. PMid:16783860
41. Fischer A, Swigris JJ, du Bois RM, Lynch DA, Downey GP, Cosgrove GP, et al. Anti-synthetase syndrome in ANA and anti-Jo-1 negative patients presenting with idiopathic interstitial pneumonia. Respir Med. 2009;103(11):1719-24. http://dx.doi.org/10.1016/j.rmed.2009.05.001 PMid:19497723 PMCid:PMC2857337
42. Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med. 2011;183(6):788-824. http://dx.doi.org/10.1164/rccm.2009-040GL PMid:21471066
43. Barbas-Filho JV, Ferreira MA, Sesso A, Kairalla RA, Carvalho CR, Capelozzi VL. Evidence of type II pneumocyte apoptosis in the pathogenesis of idiopathic pulmonary fibrosis (IFP)/usual interstitial pneumonia (UIP). J Clin Pathol. 2001;54(2):132-8. http://dx.doi.org/10.1136/jcp.54.2.132 PMid:11215282 PMCid:PMC1731356
44. Gonçalves JJ, Leão LE, Ferreira RG, Oliveira R, Ota LH, dos Santos RS. Semiquantitative analysis of surgical biopsies of distinct lung lobes of patients with usual interstitial pneumonia/idiopathic pulmonary fibrosis. J Bras Pneumol. 2009;35(7):676-82. http://dx.doi.org/10.1590/S1806-37132009000700009 PMid:19669006
45. Jezler SF, Santiago MB, Andrade TL, Araujo Neto C, Braga, H, Cruz AA. Interstitial lung disease in patients with progressive systemic sclerosis: a study of 58 cases. J Bras Pneumol. 2005;31(4):300-6.
46. Parra ER, Otani LH, de Carvalho EF, Ab'Saber A, Capelozzi VL. Systemic sclerosis and idiopathic interstitial pneumonia: histomorphometric differences in lung biopsies. J Bras Pneumol. 2009;35(6):529-40. http://dx.doi.org/10.1590/S1806-37132009000600006 PMid:19618033
47. Lee JS, Kim EJ, Lynch KL, Elicker B, Ryerson CJ, Katsumoto TR, et al. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. Respir Med. 2013;107(2):249-55. http://dx.doi.org/10.1016/j.rmed.2012.10.018 PMid:23186614
48. Takahashi T, Wada I, Ohtsuka Y, Munakata M, Homma Y, Kuroki Y. Autoantibody to alanyl-tRNA synthetase in patients with idiopathic pulmonary fibrosis. Respirology. 2007;12(5):642-53. http://dx.doi.org/10.1111/j.1440-1843.2007.01140.x PMid:17875050
49. Suda T, Kono M, Nakamura Y, Enomoto N, Kaida Y, Fujisawa T, et al. Distinct prognosis of idiopathic nonspecific interstitial pneumonia (NSIP) fulfilling criteria for undifferentiated connective tissue disease (UCTD). Respir Med. 2010;104(10):1527-34. http://dx.doi.org/10.1016/j.rmed.2010.04.022 PMid:20483576
50. Pereira DA, Dias OM, Almeida GE, Araujo MS, Kawano-Dourado L, Kairalla RA, et al. Features of a Brazilian cohort of patients with lung-dominant connective tissue disease (LD-CTD). Progress in Connective Tissue Disease Related to Interstitial Lung Disease. American Thoracic Society International Conference. 2013 May 1; Philadelphia, USA. p. A2928.
51. Kairalla RA. Interstitial lung disease in scleroderma. J Bras Pneumol. 2005;31(4):i-iii.
52. Marangoni RG, Rocha LF, Del Rio AP, Yoshinari NH, Marques-Neto JF, Sampaio-Barros PD. Systemic sclerosis sine scleroderma: distinct features in a large Brazilian cohort. Rheumatology (Oxford). 2013;52(8):1520-4. http://dx.doi.org/10.1093/rheumatology/ket163 PMid:23661427
53. Castelino FV, Goldberg H, Dellaripa PF. The impact of rheumatological evaluation in the management of patients with interstitial lung disease. Rheumatology (Oxford). 2011;50(3):489-93. http://dx.doi.org/10.1093/rheumatology/keq233 PMid:20685802
54. Fischer A, Swigris JJ, du Bois RM, Groshong SD, Cool CD, Sahin H, et al. Minor salivary gland biopsy to detect primary Sjogren syndrome in patients with interstitial lung disease. Chest. 2009;136(4):1072-8. http://dx.doi.org/10.1378/chest.08-2839 PMid:19429722 PMCid:PMC3662204
55. Deheinzelin D, Capelozzi VL, Kairalla RA, Barbas Filho JV, Saldiva PH, de Carvalho CR. Interstitial lung disease in primary Sjögren's syndrome. Clinical-pathological evaluation and response to treatment. Am J Respir Crit Care Med. 1996;154(3 Pt 1):794-9. http://dx.doi.org/10.1164/ajrccm.154.3.8810621 PMid:8810621
56. Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. Autoimmun Rev. 2007;7(1):77-84. http://dx.doi.org/10.1016/j.autrev.2007.08.003 PMid:17967730
57. Tashkin DP, Elashoff R, Clements PJ, Goldin J, Roth MD, Furst DE, et al. Cyclophosphamide versus placebo in scleroderma lung disease. N Engl J Med. 2006;354(25):2655-66. http://dx.doi.org/10.1056/NEJMoa055120 PMid:16790698
58. Fischer A, Brown KK, Du Bois RM, Frankel SK, Cosgrove GP, Fernandez-Perez ER, et al. Mycophenolate mofetil improves lung function in connective tissue disease-associated interstitial lung disease. J Rheumatol. 2013;40(5):640-6. http://dx.doi.org/10.3899/jrheum.121043 PMid:23457378 PMCid:PMC3676865

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Assessment of regional lung ventilation by electrical impedance tomography in a patient with unilateral bronchial stenosis and a history of tuberculosis*

Avaliação da ventilação pulmonar regional por tomografia de impedância elétrica em paciente com estenose brônquica unilateral pós-tuberculose

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Abstract

Bronchial stenosis can impair regional lung ventilation by causing abnormal, asymmetric airflow limitation. Electrical impedance tomography (EIT) is an imaging technique that allows the assessment of regional lung ventilation and therefore complements the functional assessment of the lungs. We report the case of a patient with left unilateral bronchial stenosis and a history of tuberculosis, in whom regional lung ventilation was assessed by EIT. The EIT results were compared with those obtained by ventilation/perfusion radionuclide imaging. The patient was using nasal continuous positive airway pressure (CPAP) for the treatment of obstructive sleep apnea syndrome. Therefore, we studied the effects of postural changes and of the use of nasal CPAP. The EIT revealed heterogeneous distribution of regional lung ventilation, the ventilation being higher in the right lung, and this distribution was influenced by postural changes and CPAP use. The EIT assessment of regional lung ventilation produced results similar to those obtained with the radionuclide imaging technique and had the advantage of providing a dynamic evaluation without radiation exposure.

Keywords: Tomography; Electric impedance; Positive-pressure respiration; Pulmonary ventilation; Airway obstruction; Tuberculosis, pulmonary.

Resumo

A estenose brônquica pode comprometer a ventilação pulmonar regional devido a limitações anormais e assimétricas ao fluxo aéreo. A tomografia de impedância elétrica (TIE) é uma técnica que possibilita a avaliação da ventilação pulmonar regional por imagem e, portanto, pode complementar a avaliação funcional dos pulmões. Relatamos o caso de uma paciente com estenose brônquica unilateral à esquerda, pós-tuberculose, em que se avaliou a ventilação pulmonar regional através da TIE, relacionando-a com a cintilografia de ventilação/perfusão. Foram estudados os efeitos das mudanças posturais e da aplicação de *continuous positive airway pressure* (CPAP, pressão positiva contínua nas vias aéreas) nasal, uma vez que a paciente usava esse tratamento para síndrome da apneia obstrutiva do sono. A TIE demonstrou distribuição heterogênea da ventilação pulmonar regional com maior ventilação no pulmão direito, sendo essa distribuição influenciada pelas mudanças de decúbitos e pela aplicação de CPAP. A análise da ventilação pulmonar regional pela TIE se mostrou similar aos achados da cintilografia pulmonar de ventilação com a vantagem de possibilitar uma avaliação dinâmica e sem exposição à radiação.

Descritores: Tomografia; Impedância elétrica; Respiração com pressão positiva; Ventilação pulmonar; Obstrução das vias respiratórias; Tuberculose pulmonar.

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* Study carried out at the Federal University of Ceará, Fortaleza, Brazil.
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Introduction

Tuberculosis remains a serious public health problem worldwide and can lead to various sequelae in the respiratory tract, one of which is bronchial stenosis.[1-5] Bronchial stenosis can lead to dyspnea due to airflow obstruction, with the aggravating factor that it is often characterized by asymmetric, proximal airway lesions. The left main bronchus is particularly susceptible to this complication.[6,7]

Pulmonary function tests evaluate lung function as a whole, but tell nothing about regional changes in ventilation. These changes are usually evaluated using radioisotope scintigraphy, which is costly and not widely available in Brazil.

Electrical impedance tomography (EIT) is a technique that allows safe, real-time, dynamic image reconstruction of axial slices of the lung, making it possible to assess regional lung ventilation.[8] This tool is particularly promising in cases of asymmetric lung disease, such as unilateral bronchial stenosis.

We report the case of a female patient with stenosis of the left main bronchus and obstructive sleep apnea syndrome (OSAS), who was being treated with nasal continuous positive airway pressure (CPAP) and in whom regional lung ventilation was assessed by EIT. We highlight the effects of postural changes and of the use of CPAP on regional lung ventilation.

Case report

A 53-year-old female nonsmoker who had been treated for tuberculosis at the age of 20 presented with a three-year history of progressive dyspnea. Pulmonary auscultation revealed rhonchi and wheezing in the left hemithorax.

Spirometry showed the following: FVC, 2.56 L (88%); FEV_1, 1.81 L (77%); FEV_1/FVC, 0.71 (88%); and $FEF_{25-75\%}$, 1.15 L/s (50%). These results are consistent with mild obstructive lung disease. No significant bronchodilator response was observed.

Digital reconstruction of CT scans of the chest showed narrowing of the left main bronchus, beginning at its origin and reaching 4.8 cm along its length, with a width of 0.5 cm (Figure 1A-D). Forced exhalation increased the degree of bronchial stenosis, resulting in almost complete collapse and causing air trapping in the left lung. Ventilation/perfusion radionuclide imaging (Figure 1E and 1F) showed that the blood flow and ventilation were directed to the right lung (68%). Bronchoscopy confirmed the chest CT finding of left bronchial stenosis.

We used an EIT device with 32 electrodes (DX 1800, Dixtal Biomédica, Manaus, Brazil)[6,7] to assess regional lung ventilation, as well as the effects of different postures—supine position (SP), right lateral decubitus position (RLDP), and left lateral decubitus position (LLDP)—and of CPAP use (10 cmH_2O). In all situations, the patient lay for 10 min with the head elevated 30°, and the measurements under the different conditions were taken 5 min apart.

Figure 2 shows functional maps of the distribution of regional lung ventilation in the three postures during spontaneous breathing and during the use of nasal CPAP, all of which were constructed on the basis of EIT.

Video 1 (available online at ***http://www.jornaldepneumologia.com.br/detalhe_artigo_pre_visualizar.asp?id=2236***) features a dynamic demonstration of regional lung ventilation in the three postures during spontaneous breathing and during CPAP use. During spontaneous breathing, it is possible to see that the ventilation was almost entirely directed to the right side, being represented by a light-blue color; a lighter shade of blue translates to higher ventilation in that area. During CPAP use, a portion of the ventilation was distributed to the left lung.

Discussion

In the present case, the relative distribution of regional lung ventilation, during spontaneous breathing, was always higher in the right lung, with values of 91%, 82%, and 58% for the SP, the RLDP, and the LLDP, respectively, which shows the effect of bronchial stenosis on the distribution of regional lung ventilation and the influence of postural changes (Figure 2). The images obtained by EIT are consistent with those obtained with the radionuclide imaging technique.

In healthy individuals breathing spontaneously and placed in the SP, regional lung ventilation is higher in the dependent regions than in the nondependent regions, a physiological phenomenon that has been well documented by studies using EIT. Studies using EIT in healthy volunteers have shown the marked influence of postural changes on the distribution of lung ventilation. Studies analyzing regional lung ventilation in healthy

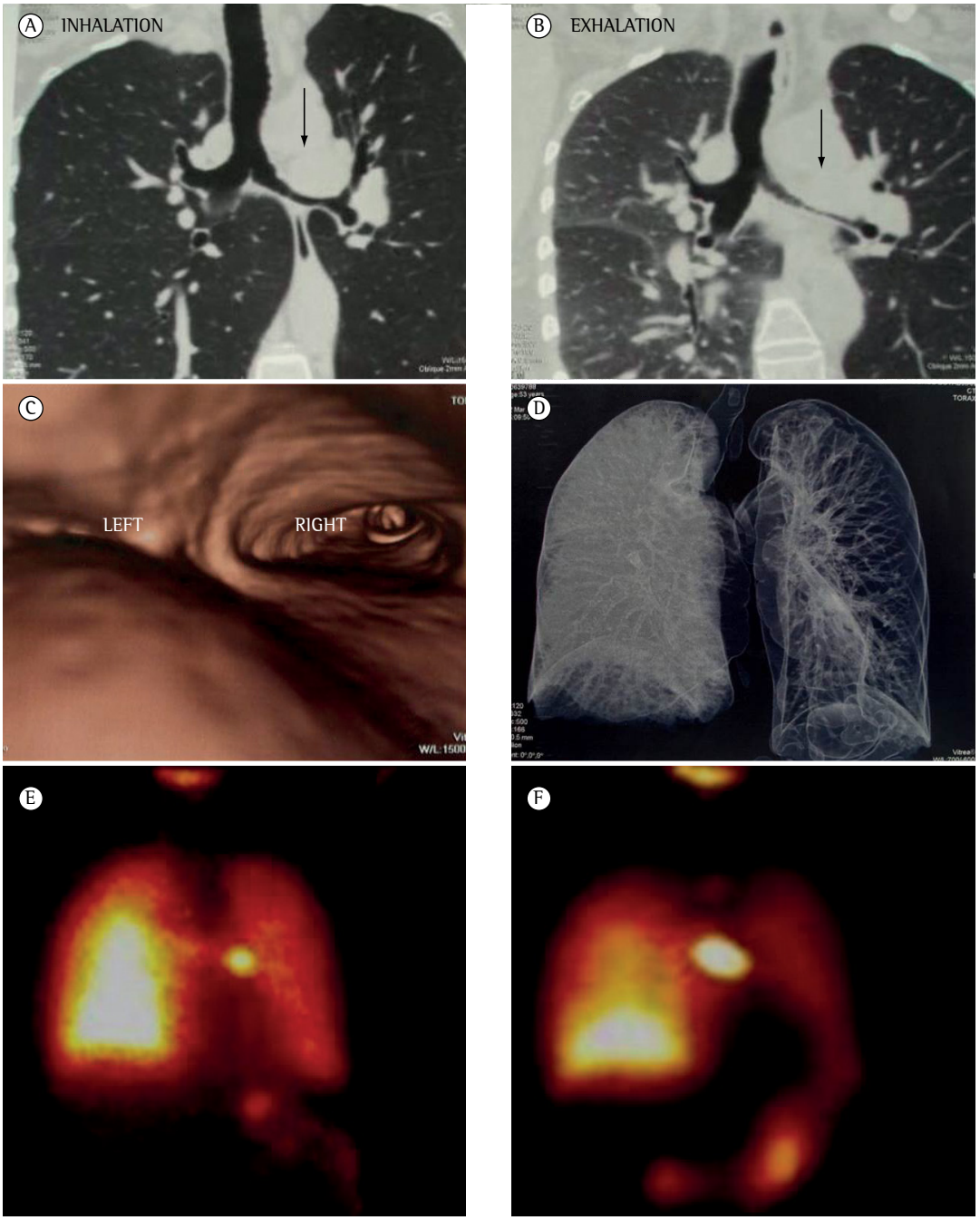

Figure 1 - Coronal reconstruction of multidetector chest CT scans acquired during inhalation (A) and exhalation (B), endobronchial reconstruction of a multidetector chest CT scan (C), and volumetric reconstruction of a multidetector chest CT scan acquired at end-exhalation showing air trapping in the left lung (D); as well as radionuclide lung perfusion (E) and lung ventilation (F) images. The arrows show the site of the bronchial stenosis.

volunteers have shown it to be invariably higher toward the dependent lung in the lateral decubitus positions.[9,10]

During CPAP use (10 cmH_2O), the differences in the regional distribution of ventilation increased. In the postures studied, the gravitational distribution of ventilation occurred mostly toward the dependent regions. In the SP, the use of CPAP caused a slight redistribution of regional ventilation to the left lung, predominantly to its dorsal portion. In contrast, the RLDP resulted in an increase in ventilation asymmetry, concentrating

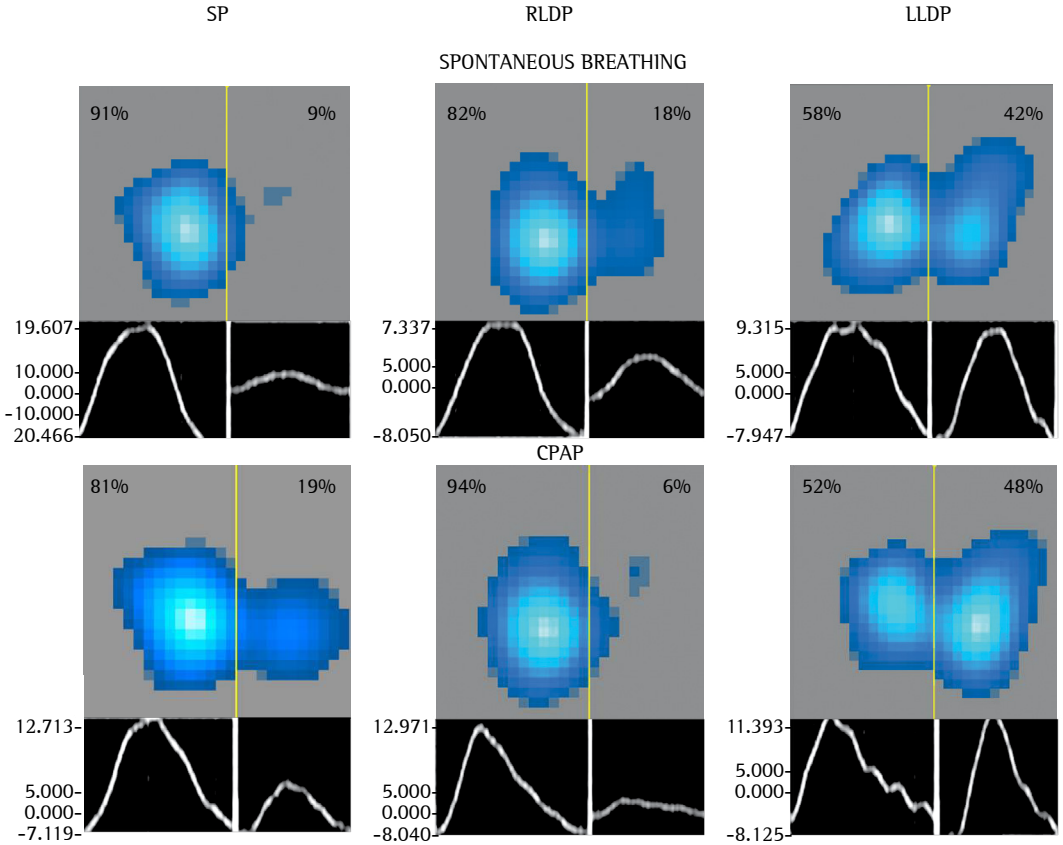

Figure 2 - Functional map of the distribution of regional lung ventilation as assessed by electrical impedance tomography in the supine position (SP), in the right lateral decubitus position (RLDP), and in the left lateral decubitus position (LLDP). The three first images were obtained during spontaneous breathing. The three last images were obtained during the use of continuous positive airway pressure (CPAP; 10 cmH_2O). The percentages represent the distribution of tidal volume to each lung. At the bottom of each figure, there is the plethysmographic curve showing the variation in electrical impedance.

ventilation almost entirely in the right lung. In the LLDP, ventilation to the dependent lung increased from 42% to 48%, possibly because of mechanical bronchodilation of the left bronchus caused by CPAP use.[8]

The present case strengthens the interest in using EIT to assess regional lung ventilation, especially in cases of asymmetric airway lesions. In addition, when compared with radionuclide imaging, EIT has the advantage of being a high-temporal resolution method that dynamically evaluates the effects of postural changes and of CPAP use.

References

1. Bombarda S, Figueiredo CM, Funari MB, Soares Jr J, Seiscento M, Filho MT. Imagem em tuberculose pulmonar. J Pneumol. 2001;27(6):329-40. http://dx.doi.org/10.1590/S0102-35862001000600007
2. Barreira D, Grangeiro A. Evaluation of tuberculosis control strategies in Brazil. Foreword [Article in Portuguese]. Rev Saude Publica. 2007;41 Suppl 1:4-8. http://dx.doi.org/10.1590/S0034-89102007000800002 PMid:18038085
3. McConkey M, Greenberg S. Persistent rhonchi in the diagnosis of bronchial stenosis complicating pulmonary tuberculosis. Trans Am Clin Climatol Assoc. 1934;50:218-23. PMid:21407480 PMCid:PMC2441225
4. Anjos DM, Junqueira JJ, Gomes DB, Terra RM, Minamoto H, Jatene FB. Endopróteses: opção terapêutica para estenoses traqueobrônquicas adquiridas. Rev Med (Sao Paulo). 2007;86(3):174-84.
5. Lyons HA. The diagnosis of bronchial stenosis. Dis Chest. 1950;18(1):16-26. http://dx.doi.org/10.1378/chest.18.1.16 PMid:15421236
6. Costa EL, Lima RG, Amato MB. Electrical impedance tomography. Curr Opin Crit Care. 2009;15(1):18-24. http://dx.doi.org/10.1097/MCC.0b013e3283220e8c PMid:19186406
7. Victorino JA, Borges JB, Okamoto VN, Matos GF, Tucci MR, Caramez MP, et al. Imbalances in regional lung ventilation: a validation study on electrical impedance tomography. Am J Respir Crit Care Med. 2004;169(7):791-

800. http://dx.doi.org/10.1164/rccm.200301-133OC PMid:14693669
8. Frerichs I, Dudykevych T, Hinz J, Bodenstein M, Hahn G, Hellige G. Gravity effects on regional lung ventilation determined by functional EIT during parabolic flights. J Appl Physiol (1985). 2001;91(1):39-50.
9. Reifferscheid F, Elke G, Pulletz S, Gawelczyk B, Lautenschläger I, Steinfath M, et al. Regional ventilation distribution determined by electrical impedance tomography: reproducibility and effects of posture and chest plane. Respirology. 2011;16(3):523-31. http://dx.doi.org/10.1111/j.1440-1843.2011.01929.x PMid:21261780
10. Matias MS, Barros CA, Monteiro LT, Sousa NP, Marinho LS, Reis RC, et al. Impacto da ventilação não invasiva (VNI) nos modos CPAP e BiLevel sobre a distribuição da ventilação pulmonar avaliada através do tomógrafo de impedância elétrica (TIE). Rev Bras Ter Intensiva. 2011;Suppl 1:S3.

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Synchronous diagnosis of primitive papillary adenocarcinomas: beyond the realm of probability

Diagnóstico simultâneo de adenocarcinomas papilares primitivos: além da probabilidade

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To the Editor:

We report the recent case of a 74-year-old female who had no relevant history of smoking or occupational history and presented to the emergency room with a two-week history of dyspnea on exertion. There was no evidence of a recent respiratory infection, peripheral edema, or orthopnea. The patient reported a history of arterial hypertension and osteoporosis and was being treated with ramipril, fluvastatin, and ibandronate.

Physical examination revealed that the patient was apyretic and breathed normally, with an SpO_2 of 98%, a blood pressure of 116/68 mmHg, and an HR of 91 bpm. In addition, heart auscultation was regular, without heart murmurs and with absent breath sounds at the right lung base, accompanied by dullness to percussion and absent vocal fremitus in this topography.

A chest X-ray showed a dense opacity in the lower half of the right lung field, consistent with massive, apparently free pleural effusion.

An initial diagnostic thoracentesis revealed a serosanguineous exudative effusion (pH, 7.33; proteins, 4.5 g/dL; lactate dehydrogenase, 728 IU/mL; glucose, 38 mg/dL; albumin, 3.0 g/dL), with a predominance of lymphocytes (60%) and an adenosine deaminase level of 10.6 IU. The first cytopathological analysis was negative, as was the microbiological study.

The patient had a neuron-specific enolase level of 16 U/mL and a cancer antigen 125 level of 124 U/mL. Blood gas analysis indicated mild hypoxemia on room air. Flexible bronchoscopy showed only thickening of the emergence of the right upper lobe bronchus, the biopsy of which showed hyperplasia of basal cells.

The second cytopathological analysis of the fluid obtained via a second thoracentesis led to the suspicion of papillary adenocarcinoma of unknown origin. A chest CT scan (Figure 1) revealed right pleural effusion without nodular pleural thickening or mediastinal/hilar adenopathy, and the assessment of the lung parenchyma was inconclusive because of effusion-related atelectasis.

By this time, the working diagnoses were papillary adenocarcinoma of the thyroid with pleural metastasis, papillary adenocarcinoma of the lung with pleural and thyroid metastasis, or synchronous papillary adenocarcinoma of the lung with pleural metastasis and papillary adenocarcinoma of the thyroid.

On the basis of those diagnoses, the pleural fluid was examined for expression of tumor markers, including squamous cell carcinoma, Cyfra 21-1, CA 19-9, and thyroglobulin (TG), and the patient was referred for medical thoracoscopy and ultrasonography of the thyroid gland.

The thyroid ultrasonography showed heterogeneous nodular hyperplasia, and aspiration biopsy of the largest nodule revealed "primitive/secondary" papillary carcinoma, which was subsequently confirmed (in a total thyroidectomy specimen) to be primitive papillary carcinoma of the thyroid with immunohistochemical expression of thyroglobulin, TTF-1, CK19, and galectin-3 (Figure 2).

The pleural fluid was negative for thyroglobulin but positive for Cyfra 21-1 and CA19-9. The medical thoracoscopy revealed nonspecific nodular lesions at the level of the parietal pleura, and the pleural biopsies revealed metastatic papillary adenocarcinoma of the lung, on the basis of positive expression of TTF-1, vimentin, and CK7 and negativity for TG.

A second CT scan, performed after drainage of the fluid, made it possible to unveil a subpleural nodular formation in the medial segment of the middle lobe, with contrast enhancement, suspected to be the primitive lung injury.

Therefore, we established the diagnosis of synchronous papillary adenocarcinoma of the

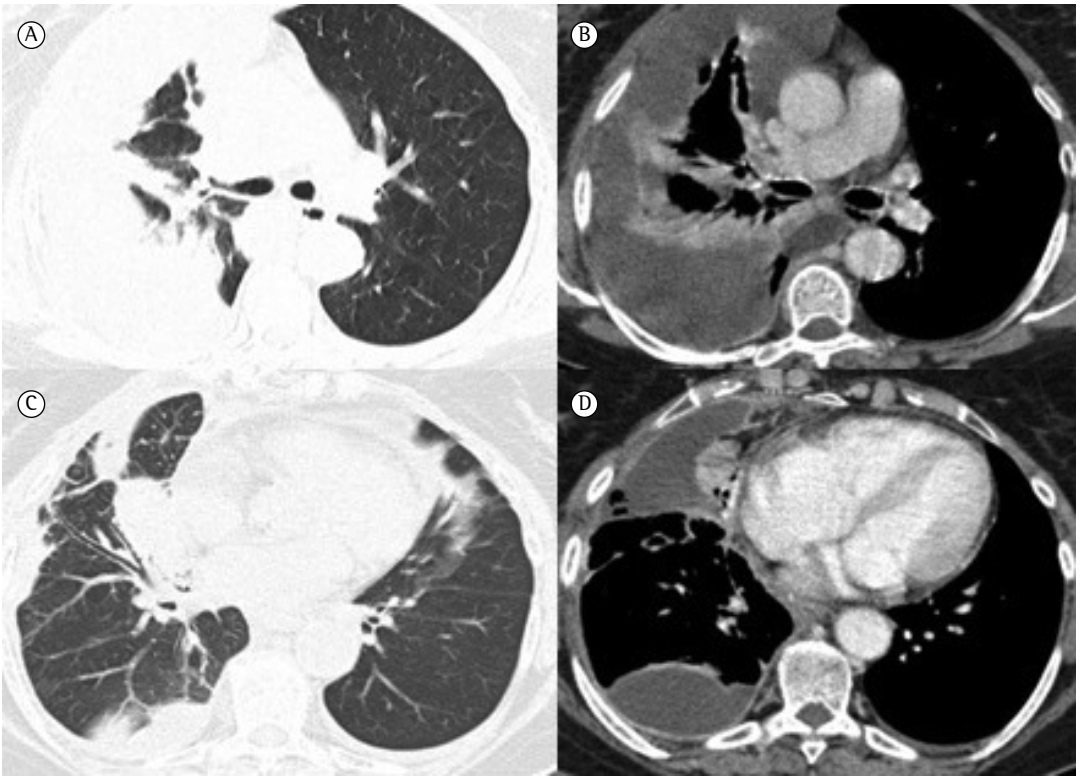

Figure 1 - Chest CT scans. In A and B, initial CT scan showing right pleural effusion with ipsilateral parenchymal atelectasis. In C and D, post-drainage CT scan showing residual sites of pleural effusion with a nodular formation in the medial segment of the middle lobe, with contrast enhancement.

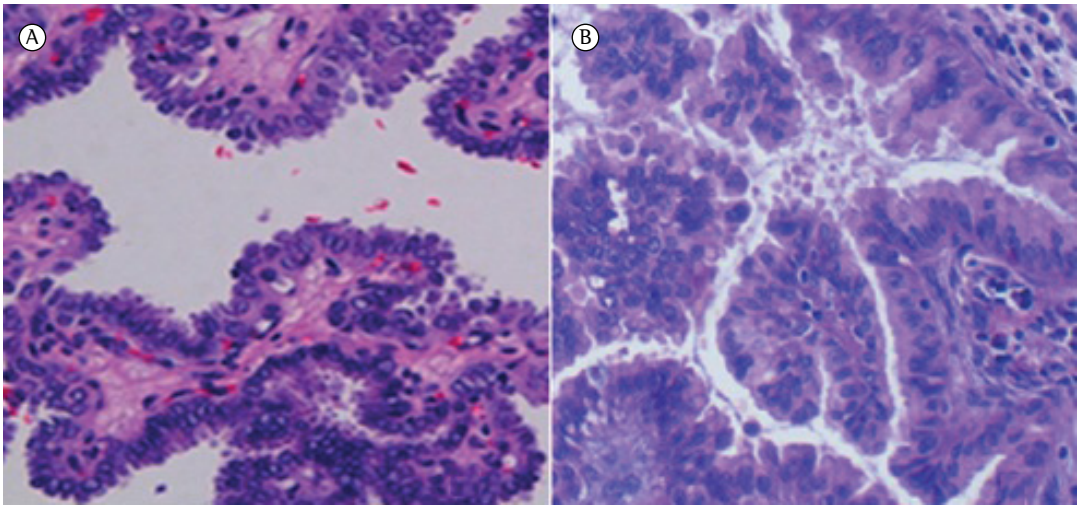

Figure 2 - Photomicrographs showing papillary adenocarcinoma of the lung in A ((H&E; magnification, ×20) and papillary adenocarcinoma of the thyroid in B (H&E; magnification, ×100).

thyroid and stage IV papillary adenocarcinoma of the lung with pleural metastasis. The patient underwent pleurodesis and began chemotherapy with cisplatin and gemcitabine, with partial response after three cycles.

Although distant metastases of papillary adenocarcinoma of the thyroid are rare, cases presenting as malignant plural effusion have been reported,[1-5] and, in some cases, the final diagnosis was only confirmed by analysis of a

total thyroidectomy specimen.[1] In contrast, lung adenocarcinoma is the type of cancer that most commonly metastasizes to the pleura, resulting in poor prognosis,[6] and its papillary variant is unusual. There are reported cases associated with thyroid metastases.[5,7,8]

We emphasize that, in cases like the present one, with diagnosis of synchronous cancers showing the same pattern of differentiation, etiologic determination of malignant pleural involvement is of utmost importance because, even in disseminated disease, the difference in mean survival between papillary adenocarcinoma of the thyroid (a 5-year survival rate of 56%) and papillary adenocarcinoma of the lung with pleural dissemination (an average of 3-5 months) is remarkable.

In circumstances in which diagnostic discrimination is poor, such as that of the present case, analysis of immunohistochemical expression is a key differentiating element. In particular, since TTF-1 is expressed in the lung and in the thyroid, the finding of TG in the pleural fluid, and especially in the histological specimen, can facilitate the differential diagnosis.[9]

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References

1. Pérez Vega C, Lecube Torelló A, Narváez García J, Vilaseca Momplet J. Pleural metastasis as presenting form of papillary thyroid microcarcinoma: an exceptional case [Article in Spanish]. Rev Clin Esp. 2003;203(4):217-8. http://dx.doi.org/10.1157/13045550 PMid:12681214
2. Vassilopoulou-Sellin R, Sneige N. Pleural effusion in patients with differentiated papillary thyroid cancer. South Med J. 1994;87(11):1111-6. http://dx.doi.org/10.1097/00007611-199411000-00010
3. Vernon AN, Sheeler LR, Biscotti CV, Stoller JK. Pleural effusion resulting from metastatic papillary carcinoma of the thyroid. Chest. 1992;101(5):1448-50. http://dx.doi.org/10.1378/chest.101.5.1448 PMid:1582317
4. Jung KH, Seo JA, Lee J, Jo WM, Kim JH, Shim C. A case of papillary thyroid cancer presenting as pleural effusion. Tuberc Respir Dis (Seoul). 2008;64(4):314-17. http://dx.doi.org/10.4046/trd.2008.64.4.314
5. Berge T, Lundberg S. Cancer in Malmö 1958-1969. An autopsy study. Acta Pathol Microbiol Scand Suppl. 1977;(260):1-235. PMid:269649
6. NCCN Clinical Practice Guidelines in Oncology. Non Small Cell Lung Cancer version 2.2010. Fort Washington: National Comprehensive Cancer Network; 2010.
7. Singh R, Lehl SS, Sachdev A, Handa U, D'Cruz S, Bhalla A. Metastasis to thyroid from lung carcinoma. Indian J Chest Dis Allied Sci. 2003;45(3):203-4. PMid:12866639
8. Nakhjavani MK, Gharib H, Goellner JR, van Heerden JA. Metastasis to the thyroid gland. A report of 43 cases. Cancer. 1997;79(3):574-8. http://dx.doi.org/10.1002/(SICI)1097-0142(19970201)79:3<574::AID-CNCR21>3.0.CO;2-
9. Porcel JM, Vives M, Esquerda A, Salud A, Pérez B, Rodríguez-Panadero F. Use of a panel of tumor markers (carcinoembryonic antigen, cancer antigen 125, carbohydrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusions. Chest. 2004;126(6):1757-63. http://dx.doi.org/10.1378/chest.126.6.1757 PMid:15596670

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Plasmodium falciparum malaria: another infection of interest to pulmonologists

Malária por *Plasmodium falciparum*: outra infecção de interesse para o pneumologista

Edson Marchiori, Gláucia Zanetti, Bruno Hochhegger, Clarissa Canella, Klaus Loureiro Irion

To the Editor:

Even though tuberculosis is the major theme on most of the recent articles concerning pulmonary infections published in the Brazilian literature,[1-4] less common infectious diseases have been described and deserve attention. Studies on parainfluenza virus 3 pneumonia,[5] pulmonary cryptosporidiosis,[6] histoplasmosis,[7] and bird fancier's lung[8] have been recently published. We would like to highlight another infection that can cause diffuse lung disease and is common in extensive tropical and subtropical regions: malaria, caused by the protozoan *Plasmodium* sp.

We report the case of a 38-year-old man born in Amazonas, Brazil, who presented with intermittent fever, chills, dyspnea, and hematuria. Laboratory tests detected anemia and transaminase elevation, and a blood smear was positive for *Plasmodium falciparum*. A chest X-ray showed bilateral pulmonary infiltrates, whereas CT showed interlobular septal thickening, areas of consolidation, and bilateral pleural effusion, suggestive of pulmonary edema (Figure 1). The patient responded well to antimalarial drugs and was discharged from the hospital after 11 days.

Malaria is a vector-borne disease caused by *Plasmodium* sp. (*P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*), being responsible for a significant global public health problem. Nearly one million people die annually due to complications of the disease, which is endemic in tropical and subtropical regions. The bite of an infected anopheline mosquito transmits the infectious agent into the bloodstream through the invasion of erythrocytes. The parasitized erythrocytes impair perfusion, nutrition, and oxygen delivery in tissues, especially the brain.[9-11] Infection with P. falciparum may result in potentially lethal complications, including cerebral malaria, acute renal failure, and pulmonary involvement.

Pulmonary manifestations occur in 3-10% of the patients and range from asymptomatic cases to fatal pulmonary edema caused by capillary leakage.[11] Although patients with uncomplicated malaria usually present with fever and nonspecific symptoms, severe and complicated malaria is characterized by multiorgan involvement, including acute lung injury and acute respiratory distress syndrome (ARDS).[9] Pulmonary edema is a major complication of severe malaria, with a high mortality rate. It is often difficult to differentiate between pulmonary edema and ARDS.[10] The development of pulmonary edema in association with malaria characteristically occurs in the absence of cardiac failure or fluid overload.

In patients with acute lung injury/ARDS due to malaria, chest X-rays may reveal bilateral opacities and increased interstitial markings mimicking the pattern observed in patients with ARDS due to other causes.[9] Small pleural effusions may be observed. Pulmonary edema may occur early due to heavy parasitemia or later due to prolonged altered capillary permeability in severe malaria.[11] Malaria is diagnosed parasitologically and is usually confirmed by thick (for parasitemia detection) and thin (for species identification) peripheral blood smear examinations.[9,11] In patients with severe complicated malaria, the early administration of specific antimalarial therapy is life-saving. Quinine and artemisinin derivatives are currently used for the parenteral treatment of severe complicated malaria.[9-11]

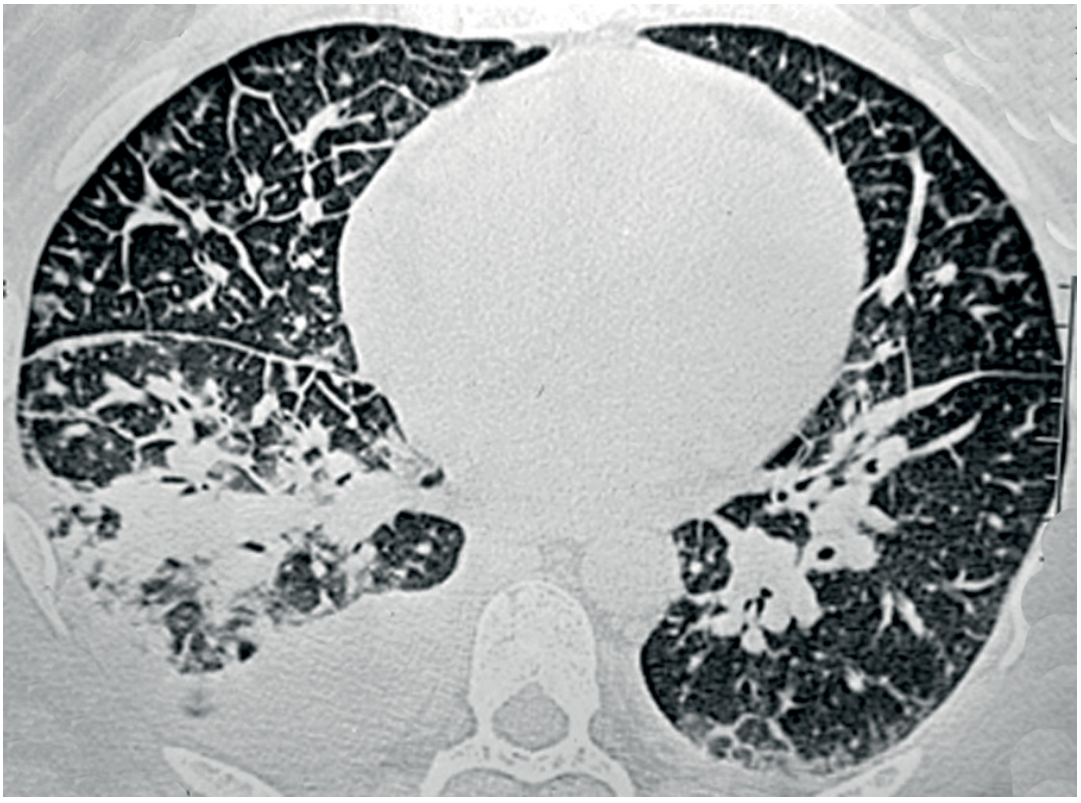

Figure 1 - Axial CT image obtained at the level of the lower lobes and showing interlobular septal thickening, consolidation in the right lung, and bilateral pleural effusion.

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References

1. Santos Neto M, da Silva FL, de Sousa KR, Yamamura M, Popolin MP, Arcêncio RA. Clinical and epidemiological profile and prevalence of tuberculosis/HIV co-infection in a regional health district in the state of Maranhão, Brazil. J Bras Pneumol. 2012;38(6):724-32. http://dx.doi.org/10.1590/S1806-37132012000600007 PMid:23288117
2. Ribeiro LB, Magalhães V, Magalhães M. Primary and acquired pyrazinamide resistance in patients with pulmonary tuberculosis treated at a referral hospital in the city of Recife, Brazil. J Bras Pneumol. 2012;38(6):740-7. http://dx.doi.org/10.1590/S1806-37132012000600009 PMid:23288119
3. Traebert J, Ferrer GC, Nazário NO, Schneider IJ, Silva RM. Temporal trends in tuberculosis-related morbidity and mortality in the state of Santa Catarina, Brazil, between 2002 and 2009. J Bras Pneumol. 2012;38(6):771-5. http://dx.doi.org/10.1590/S1806-37132012000600014 PMid:23288124

4. Telles MA, Menezes A, Trajman A. Bottlenecks and recommendations for the incorporation of new technologies in the tuberculosis laboratory network in Brazil. J Bras Pneumol. 2012;38(6):766-70. http://dx.doi.org/10.1590/S1806-37132012000600013 PMid:23288123
5. Asai N, Ohkuni Y, Kaneko N, Kawamura Y, Aoshima M. A successfully treated case of parainfluenza virus 3 pneumonia mimicking influenza pneumonia. J Bras Pneumol. 2012;38(6):810-2. http://dx.doi.org/10.1590/S1806-37132012000600020 PMid:23288130
6. Albuquerque YM, Silva MC, Lima AL, Magalhães V. Pulmonary cryptosporidiosis in AIDS patients, an underdiagnosed disease. J Bras Pneumol. 2012;38(4):530-2. http://dx.doi.org/10.1590/S1806-37132012000400017 PMid:22964939
7. Dall Bello AG, Severo CB, Guazzelli LS, Oliveira FM, Hochhegger B, Severo LC. Histoplasmosis mimicking primary lung cancer or pulmonary metastases. J Bras Pneumol. 2013;39(1):63-8. http://dx.doi.org/10.1590/S1806-37132013000100009 PMid:23503487
8. Komiya K, Ishii H, Tsubone T, Okabe E, Matsumoto B, Kadota J. Bird fancier's lung complicated by pulmonary nocardiosis. J Bras Pneumol. 2013;39(1):102-7. http://dx.doi.org/10.1590/S1806-37132013000100015 PMid:23503493
9. Mohan A, Sharma SK, Bollineni S. Acute lung injury and acute respiratory distress syndrome in malaria. J Vector Borne Dis. 2008;45(3):179-93. PMid:18807374
10. Mishra SK, Mohanty S, Mohanty A, Das BS. Management of severe and complicated malaria. J Postgrad Med. 2006;52(4):281-7. PMid:17102547
11. Tsang KW, File TM Jr. Respiratory infections unique to Asia. Respirology. 2008;13(7):937-49. http://dx.doi.org/10.1111/j.1440-1843.2008.01409.x PMid:18945321

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Sarcomatoid carcinoma of the lung with brain metastases

Carcinoma sarcomatoide de pulmão com metástases cerebrais

Matheus Fernandes de Oliveira, Sílvia Conde Watanabe, Mara Patrícia Guilhermina de Andrade, José Marcus Rotta, Fernando Campos Gomes Pinto

To the Editor:

We report the case of a 61-year-old Brazilian female who presented with cough, hemoptysis, and fever for seven days prior to evaluation. Her previous medical history revealed that she was a heavy smoker (80 pack-years) and had systemic arterial hypertension. No occupational exposures were reported. Physical examination was unremarkable. Blood workup revealed leukocytosis (white blood cell count, 15,000). Chest X-ray was also unremarkable, and the initial management was antibiotic treatment. A CT of the chest was requested, which revealed a spiculated tumor mass of 5 cm in diameter in the right upper lobe, as well as mediastinal lymphadenopathy. The patient was then submitted to bronchoscopy, which was negative for neoplasms. The decision was to perform pulmonary lobectomy.

Gross pathology showed that the right upper lobe weighed 300 g and measured 18.5 × 12.0 × 3.5 cm. The cut surface revealed a poorly circumscribed, tan-gray, hemorrhagic lesion measuring 7.0 × 6.0 × 3.8 cm that infiltrated into the visceral pleura. Histologically, the tumor was composed of spindle-shaped cells with marked pleomorphism and abnormal mitosis. These tumor cells displayed a sarcoma-like growth pattern with scarce multinucleated giant cells. Multiple foci of necrosis were present. Squamous cell carcinoma or adenocarcinoma areas were not observed. Immunohistochemical studies were performed using the avidin-biotin-peroxidase complex method. The tumor cells were positive for pan-cytokeratin (AE1/AE3), CK7, and epithelial membrane antigen (Figure 1) but negative for other antibodies. The pathology report revealed sarcomatoid carcinoma of the lung, with spindle cell features. No lymph nodes were involved. The tumor-node-metastasis staging classification, based on the findings, was T2bN0Mx.

Immediately after the surgery, the oncological evaluation was completed. Abdominal CT and bone scintigraphy resulted normal. Chest CT scans revealed pneumothorax in the right upper lobe in the site of the resected tumor. Radiotherapy and chemotherapy were proposed. However, the patient was lost to follow-up, and no adjuvant treatment was performed.

After four months without any adjuvant therapy, the patient returned to the hospital presenting with progressive, complete right hemiparesis and impaired verbal expression/aphasia for 10 days. At admission to the neurosurgery ward, the patient was alert and cooperative, with a Glasgow Coma Scale score of 15, the pupils were isochoric and reactive to light, and the patient presented with a grade-4 right hemiparesis in association with aphasia.

The patient was promptly submitted to a neuroimaging study, which revealed three images (one in the left inferior frontal gyrus and two in the right parietal cortex) of lesions with different dimensions in the brain parenchyma that resembled tumor invasion (Figure 2).

The neurosurgery staff decided to perform microsurgery for the resection of the larger lesion in the left frontal gyrus and adjuvant radiosurgery of the other lesions.

The resected specimen was sent to pathology. The microscopic findings were similar to the sarcomatoid carcinoma of the lung, characterized by the proliferation of pleomorphic spindle-shaped cells. An immunohistochemical study was performed and revealed focal positivity of the tumor cells for CK7 and diffuse positivity for epithelial membrane antigen. The microscopic and immunohistochemical findings were consistent with metastatic sarcomatoid carcinoma.

The patient was discharged without any postoperative complications; however, she was rehospitalized one week later with progressive worsening of her general health status and, two months after the surgery, she died, prior to performing any adjuvant therapy.

Sarcomatoid carcinoma of the lung is a rare tumor, showing a male-to-female ratio of 7.25:1.00. The mean and median age of the patients is 65 years, and the tumor accounts for 0.1-0.3% of all lung cancers.[1-4] It arises from the central airway in two-thirds of the patients and exhibits the morphology of polypoid airway lesions.[3]

Sarcomatoid carcinoma most often presents as solitary masses in the upper lobes, with an average size of 7 cm in diameter.[4,5] Parenchymal masses appear as cavities with marked central necrosis and peripheral rim. The association with smoking is strong.[1-4] The treatment is essentially performed with surgical resection, chemotherapy and radiotherapy being used as adjuvant therapy or in cases of poor surgical conditions, since there seems to be little benefit.[1-4,6,7] The five-year survival for patients with sarcomatoid carcinoma is approximately 20% (compared with 50% for those with non-small cell lung cancer), and the median duration of survival is three months.[1,2,5,7]

The probable pathogenesis of sarcomatoid carcinoma includes malignant transformation of hamartoma, simultaneous malignant transformation of epithelial elements and stroma, malignant transformation of cancer-derived stroma, sarcomatous change in carcinoma, and carcinomatous change in sarcoma.[2,3]

Although there are reports of systemic metastases, such as in the skin, stomach, pancreas, esophagus, jejunum, rectum, kidneys, bones, and adrenal glands,[4-10] to our knowledge, only one report documented brain metastases,[1] especially because of the aggressiveness and low survival time.

Few reports have presented and discussed the presentation of metastatic sarcomatoid carcinoma of the lung in the brain. In our case, we illustrate the presentation of brain metastases in a patient who had been previously treated for sarcomatoid carcinoma of the lung and highlight the need to consider the differential diagnosis of pulmonary cancer and the aggressive features of sarcomatoid carcinoma. The treatment, in accordance with current therapies in metastatic brain disease, consisted of the surgical resection of the larger lesion and complementary radiosurgery in the surgical cavity, with the resection of the two smaller lesions.[1,2,10] However, due to the progressive worsening of the general status of the patient, the radiosurgical treatment was not performed, and the patient died two months after being diagnosed with brain metastases.

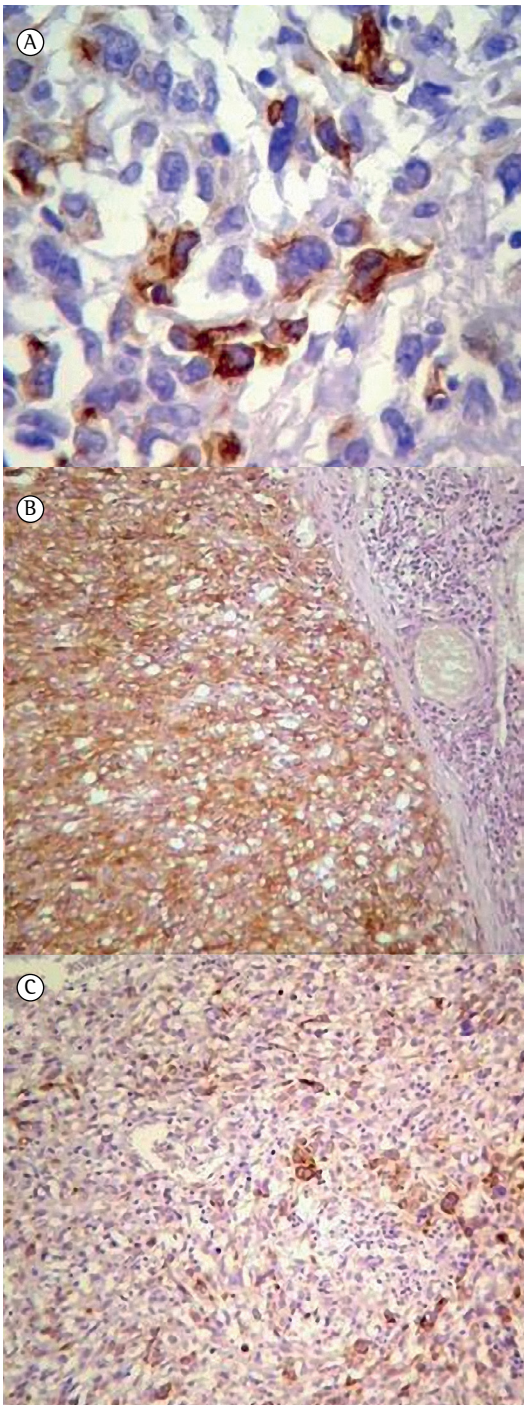

Figure 1 - Photomicrographs of pathological and immunohistochemical studies. In A, pan-cytokeratin cytoplasmic positivity of the tumor cells (AE1/AE3 immunostaining; magnification, ×400). In B, tumor cells showing diffuse positivity for CK7 (CK7 immunostaining; magnification, ×100). In C, carcinoma cells showing strong positivity for epithelial membrane antigen in contrast with inflammatory adjacent non-tumoral tissue (immunostaining; magnification, ×100).

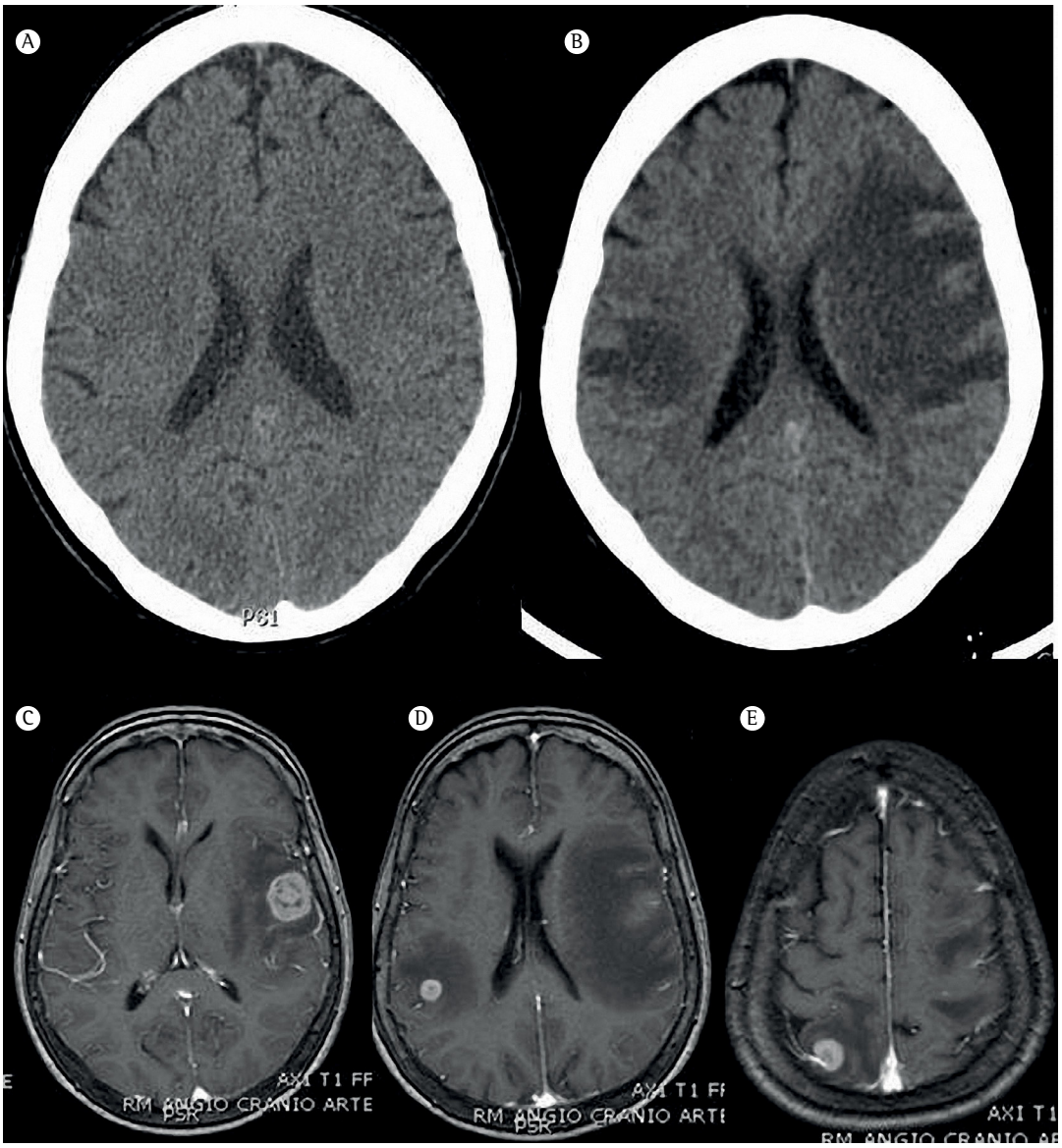

Figure 2 - Neuroimaging scans. In A, a CT scan of the skull taken when the pulmonary mass was discovered four months prior to the appearance of neurological symptoms, which is apparently normal. In B, a CT scan of the skull taken four months after readmission, when the patient presented with neurological symptoms. In C-E, magnetic resonance imaging scans revealing details of the metastatic spread and intense vasogenic edema.

The early diagnosis and proper surgical and adjuvant therapy of the primary disease might lead to prolonged survival, and physicians should be aware of the appearance of metastatic symptoms of sarcomatoid carcinoma, such as neurological symptoms, which can be prominent and misleading to other differential diagnoses.

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References

1. Franks TJ, Galvin JR. Sarcomatoid carcinoma of the lung: histologic criteria and common lesions in the differential diagnosis. Arch Pathol Lab Med. 2010;134(1):49-54. PMid:20073605
2. Goto T, Maeshima A, Tajima A, Kato R. A resected case of pulmonary carcinosarcoma. Ann Thorac Cardiovasc Surg. 2010;16(3):190-3. PMid:20930681
3. Ito K, Oizumi S, Fukumoto S, Harada M, Ishida T, Fujita Y, et al. Clinical characteristics of pleomorphic carcinoma of the lung. Lung Cancer. 2010;68(2):204-10. http://dx.doi.org/10.1016/j.lungcan.2009.06.002 PMid:19577320
4. Mochizuki T, Ishii G, Nagai K, Yoshida J, Nishimura M, Mizuno T, et al. Pleomorphic carcinoma of the lung: clinicopathologic characteristics of 70 cases. Am J Surg Pathol. 2008;32(11):1727-35. http://dx.doi.org/10.1097/PAS.0b013e3181804302 PMid:18769330
5. Rossi G, Cavazza A, Sturm N, Migaldi M, Facciolongo N, Longo L, et al. Pulmonary carcinomas with pleomorphic, sarcomatoid, or sarcomatous elements: a clinicopathologic and immunohistochemical study of 75 cases. Am J Surg Pathol. 2003;27(3):311-24. http://dx.doi.org/10.1097/00000478-200303000-00004 PMid:12604887
6. Park JY, Kim HS, Zo JI, Lee S, Choi SW. Initial presentation of lung sarcomatoid carcinoma as a metastatic lesion in the mandibular gingiva. J Periodontol. 2006;77(4):734-7. http://dx.doi.org/10.1902/jop.2006.050137 PMid:16584358
7. Robbins JR, Ryu S, Kalkanis S, Cogan C, Rock J, Movsas B, et al. Radiosurgery to the surgical cavity as adjuvant therapy for resected brain metastasis. Neurosurgery. 2012;71(5):937-43. http://dx.doi.org/10.1227/NEU.0b013e31826909f2 PMid:22806080
8. Terada T. Sarcomatoid carcinoma of the lung presenting as a cutaneous metastasis. J Cutan Pathol. 2010;37(4):482-5. http://dx.doi.org/10.1111/j.1600-0560.2009.01292.x PMid:19602061
9. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger KR, Yatabe Y, et al. International association for the study of lung cancer/american thoracic society/european respiratory society: international multidisciplinary classification of lung adenocarcinoma. J Thorac Oncol. 2011;6(2):244-85. http://dx.doi.org/10.1097/JTO.0b013e318206a221 PMid:21252716
10. Yendamuri S, Caty L, Pine M, Adem S, Bogner P, Miller A, et al. Outcomes of sarcomatoid carcinoma of the lung: a Surveillance, Epidemiology, and End Results Database analysis. Surgery. 2012;152(3):397-402. http://dx.doi.org/10.1016/j.surg.2012.05.007 PMid:22739072

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Intrapulmonary lymph node: a common and underrecognized tomography finding

Linfonodo intrapulmonar: um achado tomográfico comum e pouco reconhecido

Bruno Hochhegger, Daniela Quinto dos Reis Hochhegger, Klaus Irion, Ana Paula Sartori, Fernando Ferreira Gazzoni, Edson Marchiori

To the Editor:

We read with great interest the well-written manuscript by Melo et al.,[1] who analyzed clinical and radiological findings that influence the pathological diagnosis of solitary pulmonary nodule (SPN). They concluded that advanced age, greater maximum SPN diameter, and spiculated margins were significantly associated with the diagnosis of malignancy.

For radiologists and pulmonologists, SPNs continue to represent a major diagnostic challenge. Recent technological advances in imaging techniques and the widespread use of CT have increased the frequency of pulmonary nodule detection.[2] Small nodules (1-2 mm in diameter) are commonly detected on CT images, and their clinical importance appears to differ markedly from that of larger nodules identified on chest X-rays.[2] Thus, this enhanced detection has not affected the basic issue of distinguishing the status of a nodule, whether benign (requiring no specific approach) or indeterminate (potentially malignant),[2] and most nodules are resected for diagnosis and determination of the appropriate treatment.[3]

Pulmonary lymph nodes are a common and underrecognized cause of a peripheral SPN. These lymph nodes are usually found at the bifurcation of the bronchi, before the fourth branch, where they are referred to as peribronchial lymph nodes. Lymph nodes are occasionally present within the lung parenchyma, where they are designated intrapulmonary lymph nodes (IPLNs)[3] or perifissural nodules (PFNs).

The differentiation of IPLNs from other small pulmonary nodules on CT images is difficult although clinically important. In particular, the misinterpretation of a radiologically detected IPLN as a separate tumor nodule leads to overstaging and possible exclusion from indication for surgical treatment in patients with primary lung cancer.[3] Several tomographic characteristics may aid in the differential diagnosis of an IPLN (Figure 1). These lymph nodes are oval, round, triangular, or trapezoidal, with sharply defined borders; they are almost always located below the level of the carina, predominantly in the subpleural regions of the lower lobes. They are frequently attached to the pleura or separated from the pleural surface by a few millimeters.[3-5] IPLNs have thin linear attachments extending from the nodule to the pleura. These linear densities have been shown to represent ectatic lymphatic channels.[5] De Hoop et al.[6] recently reported that the growth rates of PFNs can reach those of malignant nodules, but no PFN in their study was malignant.

In conclusion, IPLNs are benign features that should be taken into consideration in the differential diagnosis of an SPN. Their identification might reduce the number of unnecessary surgeries and follow-up examinations.

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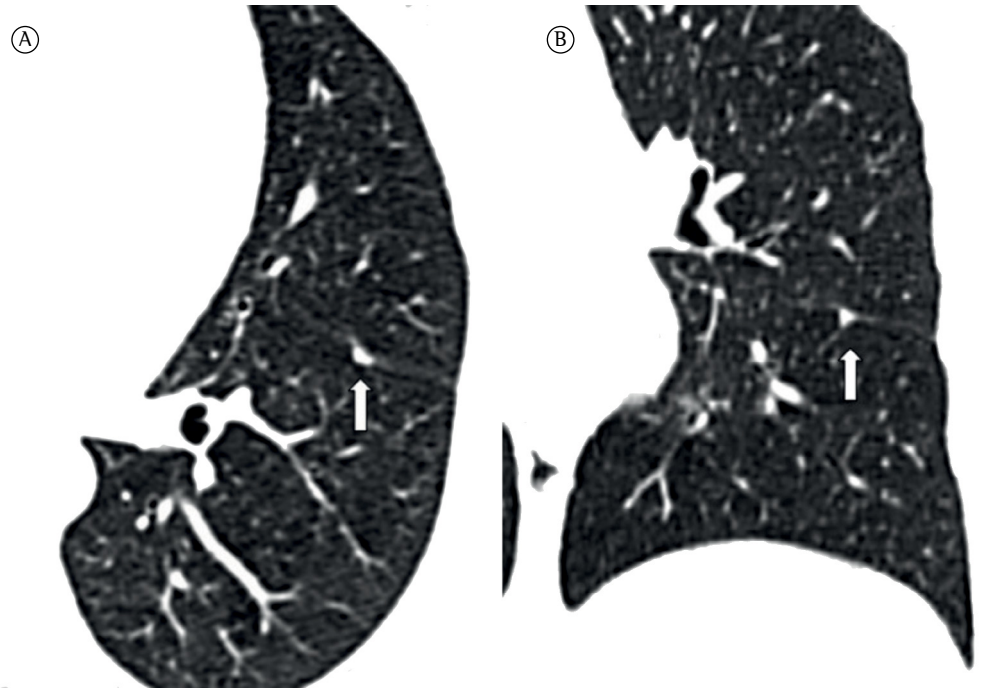

Figure 1 - In A, an axial CT image of the chest (lung window) of a 53-year-old man. A triangular intrapulmonary lymph node (3 × 4 mm) is attached to the pleural fissure. In B, a coronal CT image (lung window) showing the triangular shape and septal contact (arrow) of the intrapulmonary lymph node.

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References

1. Melo CB, Perfeito JA, Daud DF, Costa Júnior Ada S, Santoro IL, Leão LE. Analysis and validation of probabilistic models for predicting malignancy in solitary pulmonary nodules in a population in Brazil. J Bras Pneumol. 2012;38(5):559-65. http://dx.doi.org/10.1590/S1806-37132012000500004 PMid:23147047
2. Marchiori E, Irion KL. Advances in the radiological diagnosis of pulmonary nodules. J Bras Pneumol. 2008;34(1):2-3. http://dx.doi.org/10.1590/S1806-37132008000100002 PMid:18278369
3. Wang CW, Teng YH, Huang CC, Wu YC, Chao YK, Wu CT. Intrapulmonary lymph nodes: computed tomography findings with histopathologic correlations. Clin Imaging. 2013;37(3):487-92. http://dx.doi.org/10.1016/j.clinimag.2012.09.010 PMid:23102925
4. Takenaka M, Uramoto H, Shimokawa H, So T, Hanagiri T, Aoki T, et al. Discriminative features of thin-slice computed tomography for peripheral intrapulmonary lymph nodes. Asian J Surg. 2013;36(2):69-73. http://dx.doi.org/10.1016/j.asjsur.2012.11.005 PMid:23522758
5. Shaham D, Vazquez M, Bogot NR, Henschke CI, Yankelevitz DF. CT features of intrapulmonary lymph nodes confirmed by cytology. Clin Imaging. 2010;34(3):185-90. http://dx.doi.org/10.1016/j.clinimag.2009.05.005 PMid:20416482
6. de Hoop B, van Ginneken B, Gietema H, Prokop M. Pulmonary perifissural nodules on CT scans: rapid growth is not a predictor of malignancy. Radiology. 2012;265(2):611-6. http://dx.doi.org/10.1148/radiol.12112351 PMid:22929331

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The Jornal Brasileiro de Pneumologia (J Bras Pneumol, Brazilian Journal of Pulmonology) ISSN-1806-3713, published once every two months, is the official organ of the *Sociedade Brasileira de Pneumologia e Tisiologia* (Brazilian Thoracic Society) for the publication of scientific papers regarding Pulmonology and related areas.

After being approved by the Editorial Board, all articles will be evaluated by qualified reviewers, and anonymity will be preserved throughout the review process.

Articles that fail to present merit, have significant errors in methodology or are not in accordance with the editorial policy of the journal will be directly rejected by the Editorial Board, with no recourse. Articles may be written in Portuguese, Spanish or English. In the online version of the Journal (www.jornaldepneumologia.com.br, ISSN-1806-3756), all articles will be made available in Spanish or Portuguese, as well as in English. Authors may submit color figures. However, the cost of printing figures in color, as well as any related costs, will be borne by the authors.

For further clarification, please contact the Journal Secretary by e-mail or by telephone.

The *Jornal Brasileiro de Pneumologia* upholds the World Health Organization (WHO) and International Committee of Medical Journal Editors (ICMJE) policies regarding the registration of clinical trials, recognizing the importance of these initiatives for the registration and international, open-access dissemination of information on clinical trials. Therefore, as of 2007, the Journal only accepts clinical trials that have been given an identification number by one of the clinical trials registries meeting the criteria established by the WHO and the ICMJE. This identification number must be included at the end of the abstract.

Within this context, the *Jornal Brasileiro de Pneumologia* adheres to the definition of a clinical trial as described by the WHO, which can be summarized as "any study that prospectively assigns human beings to be submitted to one or more interventions with the objective of evaluation the effects that those interventions have on health-related outcomes. Such interventions include the administration of drugs, cells and other biological products, as well as surgical procedures, radiological techniques, the use of devices, behavioral therapy, changes in treatment processes, preventive care, etc

Authorship criteria

An individual may be considered an author of an article submitted for publication only if having made a significant intellectual contribution to its execution. It is implicit that the author has participated in at least one of the following phases: 1) conception and planning of the study, as well as the interpretation of the findings; 2) writing or revision of all preliminary drafts, or both, as well as the final revision; and 3) approval of the final version.

Simple data collection or cataloging does not constitute authorship. Likewise, authorship should not be conferred upon technicians performing routine tasks, referring physicians, doctors who interpret routine exams or department heads who are not directly involved in the research. The contributions made by such individuals may be recognized in the acknowledgements.

The accuracy of all concepts presented in the manuscript is the exclusive responsibility of the authors. The number of authors should be limited to six, although exceptions will be made for manuscripts that are considered exceptionally complex. For manuscripts with more than six authors, a letter should be sent to the Journal describing the participation of each.

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All manuscripts must be submitted online from the home-page of the journal. The instructions for submission are available at: www.jornaldepneumologia.com.br/sgp. Although all manuscripts are submitted online, they must be accompanied by a Copyright Transfer Statement and Conflict of Interest Statement signed by all the authors based on the models available at: www.jornaldepneumologia.com.br.

It is requested that the authors strictly follow the editorial guidelines of the journal, particularly those regarding the maximum number of words, tables and figures permitted, as well as the rules for producing the bibliography. Failure to comply with the author instructions will result in the manuscript being returned to the authors so that the pertinent corrections can be made before it is submitted to the reviewers.

Special instructions apply to the preparation of Special Supplements and Guidelines, and authors should consult the instructions in advance by visiting the homepage of the journal.

The journal reserves the right to make stylistic, grammatical and other alterations to the manuscript.

With the exception of units of measure, abbreviations should be used sparingly and should be limited only to those that are widely accepted. These terms are defined in the List of Abbreviations and Acronyms accepted without definition in the Journal. Click here (List of Abbreviations and Acronyms). All other abbreviations should be defined at their first use. For example, use "C-reactive protein (CRP)", and use "CRP" thereafter. After the definition of an abbreviation, the full term should not appear again. Other than those accepted without definition, abbreviations should not be used in titles, and their use in the abstracts of manuscripts should be avoided if possible.

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". . . ergometric treadmill (model ESD-01; FUNBEC, São Paulo, Brazil) . . ."

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". . . guinea pig liver tTg (T5398; Sigma, St. Louis, MO, USA) . . ."

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Title Page: The title page should include the title (in Portuguese and in English); the full names, highest academic degrees and institutional affiliations of all authors; complete address, including telephone number, fax number and e-mail address, of the principal author; and a declaration of any and all sources of funding.

Abstract: The abstract should present the information in such a way that the reader can easily understand without referring to the main text. Abstracts should not exceed 250 words. Abstracts should be structured as follows: Objective, Methods, Results and Conclusion. Abstracts for review articles and case reports may be unstructured.

Abstracts for brief communications should not exceed 100 words.

Summary: An abstract in English, corresponding in content to the abstract in Portuguese, should be included.

Keywords: Three to six keywords in Portuguese defining the subject of the study should be included as well as the corresponding keywords in English. Keywords in Portuguese must be based on the Descritores em Ciência da Saúde (DeCS, Health and Science Keywords), published by Bireme and available at: http://decs.bvs.br, whereas keywords in English should be based on the National Library of Medicine Medical Subject Headings (MeSH), available at: http://www.nlm.nih.gov/mesh/MBrowser.html.

Text:

Original articles: For original articles, the text (excluding the title page, abstracts, references, tables, figures and figure legends) should consist of 2000 to 3000 words. Tables and figures should be limited to a total of five. The

number of references should not exceed 30. Original articles should be divided into the following sections: Introduction, Methods, Results, Discussion, Acknowledgments, and References. The Methods section should include a statement attesting to the fact the study has been approved by the ethics in human research committee or the ethics in animal research committee of the governing institution. There should also be a section describing the statistical analysis employed, with the respective references. In the Methods and Results sections, subheadings may be used, provided that they are limited to a reasonable number. Subheadings may not be used in the Introduction or Discussion.

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Pictorial essays: Pictorial essays are also submitted only at the request of the Editors or after the authors have consulted and been granted permission by the Editorial Board. The text accompanying such essays should not exceed 3000 words, excluding the references and tables. No more than 12 illustrations (figures and tables) may be used, and the number of references may not exceed 30.

Case Reports: Case Reports should not exceed 1500 words, excluding title page, abstract, references and illustrations. The text should be composed of: Introduction, Case Report, Discussion and References. It is recommended that any and all information that might identify the patient be withheld, and that only those laboratory exams that are important for the diagnosis and discussion be presented. The total number of illustrations (figures or tables) should not exceed three, and the number of references should be limited to 20. When the number of cases presented exceeds three, the manuscript will be classified as a Case Series, and the same rules applicable to an original article will be applied.

Brief Communications: Brief communications should not exceed 1500 words, excluding references and tables. The total number of tables and figures should not exceed two, and the references should be limited to 20. The text should be unstructured.

Letters to the Editor: Letters to the Editor should be succinct original contributions, not exceeding 800 words and containing a maximum of 6 references. Comments and suggestions related to previously published materials or to any medical theme of interest will be considered for publication.

Tables and Figures: All tables and figures should be in black and white, on separate pages, with legends and captions appearing at the foot of each. All tables and figures should be submitted as files in their original format. Tables should be submitted as Microsoft Word files, whereas figures should be submitted as Microsoft Excel, TIFF or JPG files. Photographs depicting surgical procedures, as well as those showing the results of exams or biopsies, in which staining and special techniques were used will be considered for publication in color, at no additional cost to the authors. Dimensions, units and symbols should be based on the corresponding guidelines set forth by the Associação Brasileira de Normas Técnicas (ABNT, Brazilian Association for the Establishment of Technical Norms), available at: http://www.abnt.org.br.

Legends: Legends should accompany the respective figures (graphs, photographs and illustrations) and tables. Each legend should be numbered with an Arabic numeral corresponding to its citation in the text. In addition, all abbreviations, acronyms, and symbols should be defined below each table or figure in which they appear.

References: References should be listed in order of their appearance in the text and should be numbered consecutively with Arabic numerals. The presentation should follow the Vancouver style, updated in October of 2004, according to the examples below. The titles of the journals listed should be abbreviated according to the style presented by the List of Journals Indexed in the Index Medicus of the National Library of Medicine, available at: http://www.ncbi.nlm.nih.gov/entrez/journals/loftext.noprov.html. A total of six authors may be listed. For works with more than six authors, list the first six, followed by 'et al.'

Examples:

Journal Articles

1. Neder JA, Nery LE, Castelo A, Andreoni S, Lerario MC, Sachs AC et al. Prediction of metabolic and cardiopulmonary responses to maximum cycle ergometry: a randomized study. Eur Respir J. 1999;14(6):1204-13.

Abstracts

2. Singer M, Lefort J, Lapa e Silva JR, Vargaftig BB. Failure of granulocyte depletion to suppress mucin production in a murine model of allergy [abstract]. Am J Respir Crit Care Med. 2000;161:A863.

Chapter in a Book

3. Queluz T, Andres G. Goodpasture's syndrome. In: Roitt IM, Delves PJ, editors. Encyclopedia of Immunology. 1st ed. London: Academic Press; 1992. p. 621-3.

Official Publications

4. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. WHO/Tb, 1994;178:1-24.

Theses

5. Martinez TY. Impacto da dispnéia e parâmetros funcionais respiratórios em medidas de qualidade de vida relacionada a saúde de pacientes com fibrose pulmonar idiopática [thesis]. São Paulo: Universidade Federal de São Paulo; 1998.

Electronic publications

6. Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Homepages/URLs

7. Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/

Other situations:

In other situations not mentioned in these author instructions, authors should follow the recommendations given by the International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Updated October 2004. Available at http://www.icmje.org/.

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furoato de mometasona

A evolução no tratamento da asma[1-6]

Nada melhor que
máxima flexibilidade.

200 / 400 mcg

12 mcg

Mesmo dispositivo inalatório

O uso combinado com Fluir permite e facilita o Step Up e o Step Down.[7,8]

OXIMAX®:CONTRAINDICAÇÕES: contra-indicado em pacientes com hipersensibilidade conhecida ao furoato de mometasona ou à lactose. INTERAÇÕES MEDICAMENTOSAS: A co-administração de OXIMAX com o cetoconazol, um potente inibidor da enzima CYP3A4, pode aumentar os níveis plasmáticos de furoato de mometasona durante administração concomitante.

OXIMAX® (furoato de mometasona). **INDICAÇÕES:** indicado para o controle e na profilaxia da asma de qualquer intensidade, inclusive no tratamento dos pacientes asmáticos dependentes de corticosteróides inalatórios ou sistêmicos, e de pacientes asmáticos não-dependentes de corticosteróides, porém inadequadamente controlados com outros esquemas de tratamento. **PRECAUÇÕES E ADVERTÊNCIAS:** Durante os estudos clínicos, ocorreu o desenvolvimento de infecções localizadas de boca e faringe com Candida albicans. Poderá haver o desencadeamento de um episódio de broncoespasmo com aumento imediato de sibilos após a dose. É necessário cuidado especial com pacientes em processo de transição de corticosteróides sistemicamente ativos para OXIMAX. OXIMAX não é um broncodilatador e não é indicado para o alívio rápido do broncoespasmo ou de outros episódios agudos de asma. Durante esses episódios, os pacientes poderão precisar de terapia com corticosteróides orais. OXIMAX não deve ser utilizado durante a gravidez, nem por mães que estejam amamentando, a menos que o benefício justifique o risco potencial à mãe, ao feto ou ao bebê. **REAÇÕES ADVERSAS:** As reações adversas mais comuns são cefaléia, rinite alérgica, faringite, infecção do trato respiratório superior, sinusite, candidíase oral, dismenorreia, dor músculo esquelética, dor lombar e dispepsia. **POSOLOGIA:** OXIMAX destina-se ao uso em adultos e em crianças a partir de 12 anos. A dose inicial recomendada na terapia com OXIMAX para a maioria dos pacientes, independentemente de terem sido anteriormente tratados apenas com broncodilatadores ou corticosteróides inalatórios, é de 400 µg uma vez por dia, aplicados com o dispositivo. Alguns pacientes podem ser mais adequadamente controlados com 400 µg administrados em duas doses diárias (200 µg duas vezes por dia). A redução da dose para 200 µg uma vez por dia pode ser uma alternativa para a manutenção eficiente em alguns pacientes. MS 1.7287.0488. **VENDA SOB PRESCRIÇÃO MÉDICA. SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO.** Mais informações à disposição da classe médica no departamento científico da Mantecorp. Distribuição exclusiva à classe médica. (MB-OXI5). **Referências bibliográficas:** 1) Nayak AS, et al. Once-daily mometasone furoate dry powder inhaler in the treatment of patients with persistent asthma. Ann Allergy Asthma Immunol. 2000;84(4):417-24. 2) D'Urzo A. Mometasone furoate dry-powder inhaler for the control of persistent asthma Expert Opin Pharmacother. 2007;8(16):2871-84. 3) Sharpe M, Jarvis B. Inhaled mometasone furoate: a review of its use in adults and adolescents with persistent asthma. Drugs. 2001;61(9):1325-50. 4) Price D, et al. Improved adherence with once-daily versus twice-daily dosing of mometasone furoate administered via a dry powder inhaler: a randomized open-label study. BMC Pulm Med. 2010;10:1. 5) Navaratnam P, et al. The impact of adherence and disease control on resource use and charges in patients with mild asthma managed on inhaled corticosteroid agents. Patient Prefer Adherence. 2010;4:197-205. 6) Bula do produto: Oximax, 2012. 7) Global initiative for asthma. GINA. Global strategy for asthma management and prevention. Updated 2010. Available from: http://www.ginasthma.com 8) Bacharier LB, et al. Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. Allergy. 2008;63(1):5-34.

FLUIR® - fumarato de formoterol diidratado. Indicações: profilaxia e no tratamento da broncoconstrição em pacientes com doença obstrutiva reversível das vias aéreas. **CONTRAINDICAÇÕES:** Hipersensibilidade a algum dos componentes da fórmula. **PRECAUÇÕES E ADVERTÊNCIAS:** Cuidado especial e supervisão, com ênfase particular nos limites da dose, serão necessários quando coexistirem as seguintes condições: doença cardíaca isquêmica, arritmias cardíacas, especialmente bloqueio atrioventricular de terceiro grau, descompensação cardíaca grave, estenose subvalvular aórtica idiopática, cardiomiopatia obstrutiva hipertrófica, tireotoxicose, prolongamento suspeito ou conhecido do intervalo QT (QTc > 0,44 seg). Recomenda-se controle adicional de glicose sangüínea em pacientes diabéticos. O uso de Fluir durante a gravidez deve ser evitado, salvo se não existir alternativa mais segura. As mães em tratamento com FLUIR não devem amamentar. **INTERAÇÕES MEDICAMENTOSAS:** Fármacos como quinidina, disopiramida, procainamida, fenotiazínicos, anti-histamínicos e antidepressivos tricíclicos podem ser associados com prolongamento do intervalo QT e com aumento do risco de arritmia ventricular. A administração concomitante de outros agentes simpatomiméticos pode potencializar os efeitos indesejáveis. FLUIR não deve ser administrado juntamente com bloqueadores beta-adrenérgicos (incluindo-se colírios). **REAÇÕES ADVERSAS:** tremores; palpitações; cefaléia; agravamento do broncoespasmo. Outros- Reações de hipersensibilidade, como hipotensão grave, urticária, angioedema, prurido e exantema. Edemas periféricos, irritação conjuntival e edema de pálpebra, alteração do paladar e náuseas. **POSOLOGIA:** Terapia de manutenção regular: Adultos – inalação de 1 a 2 cápsulas (12 a 24 mcg), duas vezes por dia. Crianças acima de 5 anos – inalação de uma cápsula (12 mcg), duas vezes por dia. MS 1.7287.0497. **VENDA SOB PRESCRIÇÃO MÉDICA.** Julho/2011. (MB-FLU10). **SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. JUNHO/13**

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Mantecorp Farmasa
Respiratória

Vannair®

fumarato de formoterol diidratado/budesonida

ÚNICO SPRAY

formoterol/budesonida [1]

- Controle **RÁPIDO** e **SUSTENTADO** da Asma.[1,2]
- **ALCANCE DAS PEQUENAS VIAS AÉREAS,** 50% a 70% de partículas finas.[3]
- **NÃO PRECISA** ser conservado em geladeira.

Referências bibliográficas: 1. Morice AH, Peterson S, Beckman O, Kukova Z. Efficacy and safety of a new pressurised metered-dose inhaler formulation of budesonide/formoterol in children with asthma: a superiority and therapeutic equivalence study. Pulm Pharmacol Ther.2008;21(1):152-9. **2.** Noonan M, Rosenwasser LJ, Martin P, O'Brien CD, O'Dowd L. Efficacy and safety of budesonide and formoterol in one pressurised metered-dose inhaler in adults and adolescents with moderate to severe asthma: a randomised clinical trial. Drugs.2006;66(17):2235-54. **3.** Chambers F, Ludzik A. *In vitro* drug delivery performance of a new budesonide/formoterol pressurized metereddose inhaler. Journal of aerosol medicine and pulmonary drug delivery. 2009 Jun;22(2):113-20.

VANNAIR® 6/100 mcg/inalação e VANNAIR® 6/200 mcg/inalação (fumarato de formoterol di-hidratado/budesonida) VANNAIR® (fumarato de formoterol di-hidratado/budesonida) é composto por substâncias que possuem diferentes modos de ação e que apresentam efeitos aditivos em termos de redução da asma do que outros produtos isoladamente. A budesonida é um glicocorticosteróide que tem uma rápida (dentro de horas) e dose-dependente ação antiinflamatória nas vias aéreas e o formoterol é um agonista beta-2-adrenérgico seletivo de início de ação rápido (1-3 minutos) e de longa duração (pelo menos 12 horas). **Indicações: VANNAIR** está indicado no tratamento da asma nos casos em que o uso de uma associação (corticosteróide inalatório com um beta-2 agonista de ação prolongada) é apropriado. **Contra-indicações: Hipersensibilidade a budesonida, ao formoterol ou a outros componentes da fórmula. Cuidados e Advertências:** Advertências: É recomendado que a dose seja titulada quando o tratamento de longo prazo é descontinuado e este não deve ser interrompido abruptamente. Para minimizar o risco de candidíase orofaríngea, o paciente deve ser instruído a lavar a boca com água após administrar as inalações de **VANNAIR**. Uma deterioração súbita e progressiva do controle da asma é um risco potencial e o paciente deve procurar suporte médico.Pacientes que necessitaram de terapia corticosteróide de alta dose emergencial ou tratamento prolongado de altas doses recomendadas de corticosteróides inalatórios podem exibir sinais e sintomas de insuficiência adrenal quando expostos a situações de estresse grave. Administração de corticosteróide sistêmico adicional deve ser considerada durante situações de estresse ou cirurgia eletiva. **VANNAIR** deve ser administrado com cautela em pacientes com graves alterações cardiovasculares (incluindo anomalias do ritmo cardíaco), *diabetes mellitus*, hipocalemia não tratada ou tireotoxicose. Pacientes com prolongamento do intervalo QTc devem ser cuidadosamente observados (para maiores informações vide bula completa do produto). *Uso durante a gravidez e a lactação:* categoria C de risco de gravidez. Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. A administração de **VANNAIR** em mulheres lactantes deve ser apenas considerada se os benefícios esperados para a mãe superarem qualquer possível risco para a criança (para maiores informações vide bula completa do produto). Interações medicamentosas: o metabolismo da budesonida é mediado principalmente pela CYP3A4, uma subfamília do citocromo P450. Portanto, inibidores desta enzima, como o cetoconazol ou suco de *grapefruit* (pomelo), podem aumentar a exposição sistêmica à budesonida. A cimetidina apresenta um leve efeito inibidor sobre o metabolismo hepático da budesonida. Fármacos como a procainamida, fenotiazina, agentes antihistamínicos (terfenadina), inibidor da monoaminooxidase (MAO) e antidepressivos tricíclicos foram relacionados com um intervalo QTc prolongado e um aumento do risco de arritmia ventricular. **Os bloqueadores beta-adrenérgicos (incluindo os colírios oftálmicos) podem atenuar ou inibir o efeito do formoterol** (para maiores informações vide bula completa do produto). **Reações adversas:** as reações adversas que foram associadas à budesonida ou ao formoterol são apresentadas a seguir. *Comum*: palpitações, candidíase na orofaringe, cefaléia, tremor, leve irritação na garganta, tosse, rouquidão. *Incomum*: taquicardia, náusea, câibras musculares, tontura, agitação, ansiedade, nervosismo eperturbações do sono. (para outras reações adversas, vide bula completa do produto). **Posologia:** a dose de **VANNAIR** deve ser individualizada conforme a gravidade da doença. Quando for obtido o controle da asma, a dose deve ser titulada para a menor dose que permita manter um controle eficaz dos sintomas. **VANNAIR® 6/100 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Crianças (6-11 anos)*: 2 inalações duas vezes ao dia. Dose máxima diária: 4 inalações. **VANNAIR® 6/200 mcg/inalação:** *Adultos (a partir de 18 anos de idade)*: 2 inalações uma ou duas vezes ao dia. Em alguns casos, uma dose máxima de 4 inalações duas vezes ao dia pode ser requerida como dose temporária de manutenção durante a piora da asma. *Adolescentes (12-17 anos)*: 2 inalações uma ou duas vezes ao dia. Durante a piora da asma a dose pode temporariamente ser aumentada para o máximo de 4 inalações duas vezes ao dia. *Instruções de Uso*: vide bula completa do produto. **Superdose:** A superdosagem de formoterol irá provavelmente provocar efeitos típicos dos agonistas beta-2-adrenérgicos: tremor, cefaléia, palpitações e taquicardia. Poderá igualmente ocorrer hipotensão, acidose metabólica, hipocalemia e hiperglicemia. Pode ser indicado um tratamento de suporte e sintomático. A administração de uma dose de 90 mcg durante três horas em pacientes com obstrução brônquica aguda e quando administrada três vezes ao dia como um total de 54 mcg/dia por 3 dias para a estabilidade asmática não suscitou quaisquer problemas de segurança. Não é esperado que uma superdosagem aguda da budesonida, mesmo em doses excessivas, constitua um problema clínico. Quando utilizado cronicamente em doses excessivas, podem ocorrer efeitos glicocorticosteróides sistêmicos (para informações de superdosagem grave vide bula completa do produto). **Apresentações: VANNAIR® 6/100 mcg/inalação:** Aerossol bucal 6/100 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO E PEDIÁTRICO. VANNAIR® 6/200 mcg/inalação:** Aerossol bucal 6/200 mcg/inalação em embalagem com 1 tubo contendo 120 doses. **USO ADULTO. USO POR INALAÇÃO ORAL. VENDA SOB PRESCRIÇÃO MÉDICA.** Para maiores informações, consulte a bula completa do produto. (VAN005). AstraZeneca do Brasil Ltda., Rod. Raposo Tavares, Km 26,9 - Cotia - SP - CEP 06707-000 Tel.: 0800-0145578. www.astrazeneca.com.br **Vannair®. MS – 1.1618.0234**

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Eventos 2014

NACIONAIS

Curso de Ventilação e Sono
Data: 27 a 29 de março de 2014 Local: Hotel Novotel, São Paulo/SP
Informações: Secretaria da SBPT
Portal: www.sbpt.org.br / Telefone: 0800616218

Curso de Atualização 2014
Data: 24 a 26 de abril de 2014 Local: Hotel Atlântico Búzios, Búzios/RJ.
Informações: Secretaria da SBPT
Portal: www.sbpt.org.br / Telefone: 0800616218

XXXVI Congresso Brasileiro de Pneumologia e Tisiologiaa
Data: 07 a 11 de outubro de 2014 Local: Expogramado, Gramado/RS
Informações: Secretaria da SBPT
Portal: www.sbpt.org.br / Telefone: 0800616218

INTERNACIONAIS

CHEST World Congress
Data: 21 a 24 de março de 2014 Local: Madrid/ Espanha
Informações: www.chestnet.org

ATS 2014
Data: 16 a 21/05/2014
Local: San Diego/CA
Informações: www.thoracic.org

ERS 2014
Data: 06 a 10 de setembro de 2014
Local: Munique/Alemanha
Informações: www.ersnet.org

Chest 2014
Data: 25 a 30 de outubro de 2014
Local: Austin/Texas
Informações: www.chestnet.org

vacina pneumocócica 13-valente (conjugada). **Indicações:** A vacina pneumocócica 13-valente (conjugada) é indicada para a prevenção de doença invasiva, pneumonia e otite média causadas pelo *Streptococcus pneumoniae* dos sorotipos 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F e 23F em lactentes e crianças e para adultos com 50 anos ou mais para a prevenção de doença pneumocócica (incluindo pneumonia e doença invasiva), pelos mesmos sorotipos. **Contraindicações**: A vacina pneumocócica 13-valente (conjugada) está contraindicada para pacientes hipersensíveis a qualquer dos componentes da vacina, incluindo o toxoide diftérico. **Advertências:** Doenças menores, como infecção respiratória leve, com ou sem febre de baixo grau, em geral não constituem contraindicações para a vacinação. A decisão de administrar ou adiar a vacinação devido a uma doença febril atual ou recente depende em grande parte da severidade dos sintomas e de sua etiologia. A administração da vacina pneumocócica 13-valente (conjugada) deve ser adiada em indivíduos sofrendo de doença febril aguda severa. Como ocorre com qualquer vacina, a vacina pneumocócica 13-valente (conjugada) pode não proteger todos os indivíduos que receberem a vacina contra a doença pneumocócica. **Precauções:** Como ocorre com todas as vacinas pediátricas injetáveis, o possível risco de apneia deve ser considerado ao administrar a série de imunização primária em lactentes prematuros. A necessidade de monitoramento por no mínimo 48 horas após a vacinação deve ser considerada para lactentes muito prematuros (nascidos ≤ 30 semanas de gestação) que permaneçam hospitalizados no momento da administração recomendada. Uma vez que o benefício da vacinação é elevado neste grupo de lactentes, a vacinação não deve ser suspensa ou adiada. *Gravidez:* Categoria de risco C: Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. Não se sabe se os antígenos da vacina ou os anticorpos são excretados no leite materno. A segurança e a eficácia da vacina pneumocócica 13-valente (conjugada) em crianças com menos de 6 semanas ou após 6 anos não foram estabelecidas. A vacina pneumocócica 13-valente (conjugada) mostrou-se segura e imunogênica na população geriátrica. **Reações adversas:** Lactentes e Crianças com 6 Semanas a 5 Anos de Idade: Reação muito comum: diminuição do apetite, irritabilidade, sonolência/aumento do sono, sono inquieto/diminuição do sono, febre, qualquer eritema, endurecimento/tumefação ou dor/sensibilidade no local da vacinação, eritema ou endurecimento/tumefação no local da vacinação 2,5 cm – 7,0 cm (após dose em crianças entre 1 e 2 anos e crianças mais velhas [2 a 5 anos de idade]). Reação comum: diarreia, vômitos, erupção cutânea, febre acima de 39°C, eritema ou endurecimento/tumefação no local da vacinação 2,5 cm – 7,0 cm (após série em lactentes), dor / sensibilidade no local da vacinação interferindo com o movimento. Adultos com 50 Anos de Idade ou Mais: Reação muito comum: diminuição do apetite, cefaleias, diarreia, erupção cutânea, dor generalizada nas articulações recente/agravada, dor muscular generalizada recente/agravada, calafrios, fadiga, eritema no local da vacinação, endurecimento/inchaço no local da vacinação, dor/sensibilidade no local da vacinação, limitação do movimento do braço. Reação comum: vômitos, febre. **Interações Medicamentosas:** A vacina pneumocócica 13-valente (conjugada) pode ser administrada com qualquer um dos seguintes antígenos de vacina, seja de modo monovalente ou em vacinas combinadas: difteria, tétano, pertussis acelular ou de célula inteira, *Haemophilus influenzae* tipo b, poliomielite inativada, hepatite B, meningococo do sorogrupo C, sarampo, caxumba, rubéola e varicela. Estudos clínicos demonstraram que as respostas imunológicas e os perfis de segurança das vacinas administradas não foram afetados. Em estudos clínicos, quando a vacina pneumocócica 13-valente (conjugada) foi administrada concomitantemente, porém em um local ou por via diferente, com vacina de hepatite A ou de rotavírus, não foi observada alteração nos perfis de segurança para estes lactentes. A vacina pneumocócica 13-valente (conjugada) pode ser administrada com a vacina inativada trivalente contra influenza (VIT). A resposta imune para vacina pneumocócica 13-valente (conjugada) quando administrada concomitantemente com a VIT foi menor comparada à sua administração isolada. O significado clínico disto é desconhecido. Não foram realizados estudos para avaliar a resposta imune da vacina pneumocócica 13-valente (conjugada) quando administrada concomitantemente a outras vacinas além da VIT. **Posologia:** A dose é 0,5 mL, administrada por via IM, com cuidado para evitar a aplicação em nervos e vasos sanguíneos ou suas proximidades. Para lactentes até 6 meses de idade, a série de imunização recomendada consiste em três doses de 0,5 mL cada, com aproximadamente 2 meses de intervalo, seguidas por uma quarta dose de 0,5 mL aos 12-15 meses de idade, no mínimo 2 meses após a terceira dose. A idade usual para a primeira dose corresponde a 2 meses de idade, mas esta pode ser administrada mais cedo com 6 semanas de idade. A imunização para lactentes acima de 6 meses e crianças não vacinadas previamente: lactentes entre 7 e 11 meses devem receber 2 doses com intervalo mínimo de 4 semanas e uma dose de reforço entre 12 e 15 meses no mínimo 2 meses após a dose anterior; crianças entre 12 e 23 meses devem receber duas doses com intervalo de 2 meses; e crianças de 24 meses a 6 anos incompletos devem receber uma dose. Para adultos com 50 anos de idade ou mais a recomendação é uma dose única de 0,5 mL. VENDA SOB PRESCRIÇÃO MÉDICA. SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. **MS – 1.2110.0277.** Para informações completas, consulte a bula do produto (PRV13_12). Documentação científica e informações adicionais estão à disposição da classe médica mediante solicitação. Wyeth Indústria Farmacêutica Ltda. Rua Alexandre Dumas, 1.860, São Paulo – SP – CEP 04719-904 Tel.: 08000-160625. www.wyeth.com.br.

CONTRAINDICAÇÕES: A VACINA PNEUMOCÓCICA 13-VALENTE (CONJUGADA) ESTÁ CONTRAINDICADA PARA PACIENTES HIPERSENSÍVEIS A QUALQUER DOS COMPONENTES DA VACINA, INCLUINDO O TOXOIDE DIFTÉRICO. **INTERAÇÕES MEDICAMENTOSAS:** LACTENTES E CRIANÇAS COM 6 SEMANAS A 5 ANOS DE IDADE: VACINA PNEUMOCÓCICA 13-VALENTE (CONJUGADA) PODE SER ADMINISTRADA COM QUALQUER UMA DAS SEGUINTES VACINAS: VACINAS CONTRA DIFTERIA, TÉTANO E *PERTUSSIS* (DTP) OU DIFTERIA, TÉTANO E *PERTUSSIS* ACELULAR (DTPA); *HAEMOPHILUS INFLUENZAE* TIPO B (HIB); VACINA CONTRA POLIOMIELITE INATIVADA; HEPATITE B; VACINA MENINGOCÓCICA C (CONJUGADA); SARAMPO, CAXUMBA E RUBÉOLA (MMR) E VARICELA. ADULTOS COM 50 ANOS DE IDADE OU MAIS: A VACINA PNEUMOCÓCICA 13-VALENTE (CONJUGADA) PODE SER ADMINISTRADA COM A VACINA INATIVADA TRIVALENTE CONTRA INFLUENZA (VIT).

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...PARA A PREVENÇÃO DE PNEUMONIA E DOENÇA PNEUMOCÓCICA INVASIVA

Referências bibliográficas: 1. Bula do produto **2.** Centers for Disease Control and Prevention. Licensure of 13-Valent Pneumococcal Conjugate Vaccine for Adults Aged 50 Years and Older. *MMWR* 2012;61(21):394-5

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