Original Article

Determination of the inflammatory component of airway diseases by induced sputum cell counts: use in clinical practice*

Determinação do componente inflamatório das doenças das vias aéreas através do escarro induzido: utilização na prática clínica

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Abstract

Objective: To evaluate the usefulness of determining the inflammatory component of airway diseases (inflammometry) by induced sputum cell counts, as well as its influence on treatment decisions in a tertiary facility for the treatment of respiratory diseases. **Methods:** We analyzed 151 sputum samples from 132 consecutive patients referred for clinical sputum induction by five pulmonologists between July of 2006 and February of 2007. A structured questionnaire related to the reasons for requesting the test and to the therapeutic decision making based on test results was completed by each attending physician upon receiving the test results. Induced sputum was obtained and processed according to a technique previously described. **Results:** The principal motives for ordering the test were inhaled corticosteroid dose titration in patients with moderate-to-severe asthma (in 54.3%), investigation of chronic cough (in 30.5%), and monitoring airway inflammation in patients with bronchiectasis (in 7.3%) or chronic obstructive pulmonary disease (in 6%). Of the 82 patients with asthma, 47 (57%) presented eosinophilic bronchitis (>3% eosinophils). Nonasthmatic eosinophilic bronchitis was diagnosed in 9 (19%) of the 46 patients with chronic cough. Neutrophilic bronchitis (>65% neutrophils) was found in 13 patients, of which 5 had asthma, 2 had chronic cough, and 6 had chronic obstructive pulmonary disease/bronchiectasis. Based on the induced sputum results, the corticosteroid dose was modified in 48 asthma patients (64.7%). **Conclusions:** The systematic application of inflammometry using induced sputum cell counts can be beneficial for patients with airway diseases, particularly those with asthma or chronic cough.

Keywords: Sputum; Asthma; Bronchitis.

Resumo

Objetivo: Avaliar a utilização e a influência da determinação do componente inflamatório das doenças das vias aéreas (inflamometria), através do exame do escarro induzido, nas decisões terapêuticas de um serviço terciário de pneumologia. **Métodos:** Foram analisadas 151 amostras de escarro induzido de 132 pacientes consecutivamente referidos para indução de escarro com propósitos clínicos por cinco pneumologistas, no período entre julho de 2006 e fevereiro de 2007. As indicações para a realização do teste e a conduta terapêutica adotada em função do resultado foram analisadas através de um questionário estruturado preenchido pelo médico que solicitou o escarro induzido. O escarro foi obtido e processado de acordo com uma técnica previamente descrita. **Resultados:** As principais indicações do teste foram: titulação da dose do corticosteróide inalatório na asma moderada a grave (54,3%), investigação de tosse crônica (30,5%), monitoração da inflamação em pacientes com bronquiectasias (7,3%) e monitoração da inflamação na doença pulmonar obstrutiva crônica (6%). Dos 82 pacientes com asma, 47 (57%) apresentaram bronquite eosinofílica (eosinófilos > 3%). Bronquite eosinofílica sem asma foi diagnosticada em 9 (19%) dos 46 pacientes que realizaram o exame para investigar tosse crônica. Bronquite neutrofílica (neutrófilos > 65%) foi observada em 13 pacientes; 5 com asma, 2 com tosse crônica e 6 com doença pulmonar obstrutiva crônica/bronquiectasias. Com base nos resultados do exame do escarro induzido, 48 (64,7%) pacientes com asma tiveram sua dose de corticosteróide modificada. **Conclusões:** A aplicação sistemática da inflamometria através do exame do escarro induzido pode trazer importantes benefícios aos pacientes com doenças respiratórias, principalmente àqueles portadores de asma e/ou tosse crônica.

Descritores: Escarro; Asma; Bronquite.

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Introduction

The components of airway diseases include symptoms, variable airflow limitation, airway hyperresponsiveness, chronic airflow limitation, and airway inflammation, as well as emphysema and bronchiectasis.[1,2] These components can coexist in various combinations. Airway inflammation (bronchitis) is a component that is central to all of the others, being considered the primary cause of highly prevalent respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Bronchitis is responsible for symptoms, variable airflow limitation through the release of bronchoconstrictor mediators, and chronic airway limitation through airway remodeling, with structural changes.

Currently, the methods most widely used to investigate airway diseases are spirometry, evaluation of diurnal variations in peak expiratory flow, and bronchial provocation by nonspecific stimuli (methacholine or histamine) to evaluate airway responsiveness. In contrast, methods of directly evaluating airway inflammation (inflammometry methods) are rarely used in clinical practice. These methods include fiberoptic bronchoscopy to obtain bronchoalveolar lavage or bronchial tissue specimens,[3] as well as less invasive techniques, such as examination of induced sputum cellularity,[1,4] measurement of nitric oxide in exhaled air,[5] and analysis of exhaled air condensate.[6]

The sputum induction method has been validated and standardized,[7] providing a safe and relatively noninvasive way to collect material (sputum) from the lower airways. Examination of sputum cellularity makes it possible to exclude or identify inflammation (bronchitis), which is characterized as eosinophilic, neutrophilic, or a combination of the two.[8] The importance of its use in the management of moderate-to-severe persistent asthma has grown as recent studies have demonstrated that the number of severe exacerbations and the number of hospital admissions are lower when induced sputum findings are used in order to design the anti-inflammatory treatment than when the treatment is based on the current guidelines.[9,10] The results of these studies indicate that monitoring asthma treatment guided by assessment of the eosinophilic component in sputum optimizes long-term asthma treatment, identifying and reducing the occurrence of exacerbations.

Induced sputum analysis has also allowed the recognition of nonasthmatic eosinophilic bronchitis as a treatable cause of chronic cough.[11] Therefore, airway inflammation assessment has become an important tool recently added to the investigation algorithm for chronic cough.[12,13] Regarding its applicability in COPD, a recently published randomized study[14] revealed a significant decrease in the number of severe exacerbations and hospital admissions in the group treated based on induced sputum findings, in comparison with the group treated based on the British Thoracic Society consensus.

The motivation for conducting the present study originated from two principal facts. First, the recognition that the parameters most frequently used in clinical practice to monitor patients with airway diseases, such as symptoms and pulmonary function tests, do not directly reflect the underlying inflammatory process. Second, the understanding that the different inflammatory patterns present in the airways are not exclusive to any one respiratory disease, and that the same disease can present more than one type of airway inflammation over time. Although current evidence demonstrates that determination of induced sputum cellularity provides, in a minimally invasive way, valid and specific information about the different types of inflammation present in airway diseases, the usefulness of such information in clinical practice has yet to be well established. Therefore, the objective of the present study was to evaluate the use of the method in common situations in respiratory medicine and to characterize the influence that determining the inflammatory component of airway diseases has on treatment decisions.

Methods

This was a prospective, cross-sectional study in which 132 consecutive patients capable of producing, on one or more occasions, an induced sputum sample appropriate for clinical use were selected between July of 2006 and February of 2007. The patients were referred for sputum induction by five pulmonologists with the sole objective of determining the presence and type of the inflammatory component involved so as to elucidate the nature of the respiratory symptoms or to inform treatment decisions in patients previously diagnosed with respiratory diseases. Sputum sample processing and differential cell counts were performed

in a referral laboratory, which was not provided with any information regarding the clinical characteristics of the participants from whom the samples had been collected. Upon receiving the test results, the attending physician completed a structured questionnaire related to the reasons for requesting the test and to the treatment decisions made based on those results. The study was approved by the Ethics in Research Committee of the Federal University of Santa Catarina, located in Florianópolis, Brazil.

Spirometry was performed according to the specifications of the Brazilian Thoracic Association,[15] using a computerized spirometer (Koko® Spirometer, PDS Instrumentation, Louisville, CO, USA). The predicted values of normality used were those published by Crapo et al.[16]

Sputum induction was performed using a method previously described.[1,7] In brief, the procedure was started 10 min after the administration of 200 μg of inhaled albuterol through inhalation of saline aerosol in increasing concentrations (0.9%, 3%, 4%, and 5%), each inhaled for 3 and 4 min consecutively, until enough sputum was obtained for analysis, or until there was a decrease in forced expiratory volume in one second (FEV_1) 20% in relation to the baseline value. Saline nebulization was performed using a Fisoneb ultrasonic nebulizer (Fisons, Pickering, Ontario, Canada), with an output rate of 0.87 mL/min and particles presenting a median aerodynamic mass diameter of 5.58 μm. After each inhalation period, FEV_1 was measured to ensure the safety of the test. If there was a decrease in FEV_1 ≥ 10% in relation to the baseline value, the saline concentration was not increased. Sputum samples were processed and analyzed within the first 2 h, as previously described.[1] An induced sputum sample appropriate for analysis was defined as that containing expectorated material with cellular viability greater than 50% and contamination by oropharyngeal squamous cells lower than 20%, as well as being of a quantity sufficient for differential counts of 400 cells.

The reason for requesting the test was defined as monitoring airway inflammation in patients with asthma—if the objective of the sputum cell counts was to establish the ideal inhaled corticosteroid dose, investigate the type of asthma exacerbation, or investigate the cause of symptoms in asthma patients under treatment; as monitoring airway inflammation in patients with COPD—if the objective was to investigate the type of COPD exacerbation, or to inform decisions regarding the use of inhaled corticosteroids in cases of stable disease; as monitoring airway inflammation in patients with bronchiectasis—if the objective was to determine the type of inflammation involved in the genesis of the symptoms of such patients; or as monitoring airway inflammation in patients with eosinophilic bronchitis—if the objective of the sputum cell counts was to determine whether eosinophilic bronchitis inflammation was controlled after treatment.

The following definitions were used in the analysis of sputum cellularity: eosinophilic bronchitis was defined as sputum eosinophilia ≥ 3.0%[17]; asthmatic eosinophilic bronchitis was defined as chronic cough accompanied by the physiological findings of asthma (reversibility of airway obstruction or hyperresponsiveness), together with sputum eosinophilia ≥ 3.0%; nonasthmatic eosinophilic bronchitis was defined as chronic cough without the physiological findings of asthma but accompanied by sputum eosinophilia ≥ 3.0%; and neutrophilic bronchitis was defined as sputum neutrophilia ≥ 65%, either with a total cell count (TCC) ≥ 10×10^6 , which would indicate infection, or a TCC < 10×10^6, which would indicate the absence of infection.[17]

Variables with normal distribution are summarized as mean and standard deviation, and those with non-normal distribution (e.g., eosinophils) are summarized as the median of the 5th-95th percentile

Table 1 - Clinical characteristics of the patients.

	Males	Females	Overall
Tests: success/failure, n/n	55/1	96/5	151/6
Age, years[a]	50.1 ± 19.0	48.4 ± 16.6	49.0 ± 17.5
FEV_1, % of predicted[a]	68.0 ± 23.2	72.1 ± 24.9	70.6 ± 24.3
FEV_1/FVC[a]	0.62 ± 0.1	0.66 ± 0.1	0.64 ± 0.1
Induction time, min[a]	11.0 ± 4.5	12.6 ± 5.7	12.1 ± 5.3

FEV_1: forced expiratory volume in one second; and FVC: forced vital capacity. [a]Values expressed as mean and standard deviation.

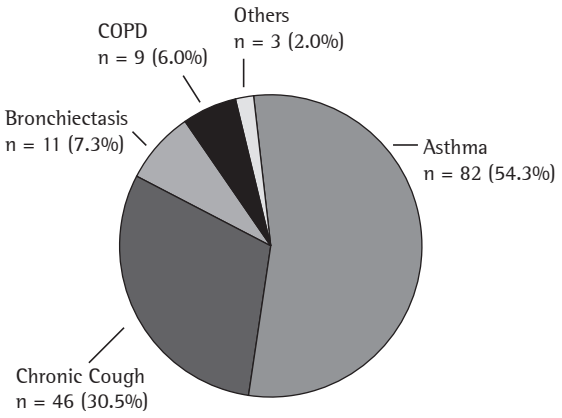

Figure 1 - The principal reasons for requesting sputum induction were monitoring airway inflammation in patients with asthma, chronic obstructive pulmonary disease (COPD), or bronchiectasis, as well as investigation of chronic cough. Others: monitoring airway inflammation in patients with eosinophilic bronchitis (n = 2) or slowly resolving pneumonia (n = 1).

range. Categorical variables are expressed as percentages. The differences among the airway diseases were initially analyzed using the Kruskal-Wallis test. The significance of the differences among the groups was analyzed using the Mann-Whitney U test. Values of $p < 0.05$ were considered significant.

Results

Of a total of 157 sputum inductions performed in the 138 patients, the material expectorated was considered appropriate for analysis on 151 occasions (in 132 patients), translating to a success rate of 96%. Eleven of the patients, all of whom had asthma, underwent the test on more than one occasion, at different stages of the disease, to treat exacerbation or to establish the ideal inhaled corticosteroid dose. All questionnaires were completed by the attending physicians upon receiving the test results. The clinical characteristics of the patients are described in Table 1. An appropriate sputum sample was obtained on 65% of the occasions using only the lower saline concentrations (0.9% and 3%). It was necessary to use the 5% concentration on only 11% of the occasions. During the induction process, the mean decrease in FEV_1, in relation to the baseline value, was 6.8%.

The most common reasons for requesting the test were monitoring airway inflammation in patients with asthma and investigation of chronic cough (Figure 1). Significant differences in TCC, percentage of neutrophils, and percentage of eosinophils were found among the different groups, divided by clinical reason for requesting the test (Table 2). The TCCs were lower in the patients with chronic cough than in the patients in any other group; the percentage of neutrophils was higher in the patients with bronchiectasis than in the patients in any other group; and the percentage of eosinophils was higher in the patients with asthma than in the patients in any other group.

Figure 2 shows the proportional results of the analysis of the induced sputum cellularity by reason for requesting the test. The frequencies of eosinophilic bronchitis, neutrophilic bronchitis, and normal cellularity varied within each group.

The treatment decisions made based on the test results are summarized in Table 3. The course of treatment was changed on 82 (55%) of the 151 occasions. On 55 (67%) of the 82 occasions in which the procedure was performed to monitor airway inflammation in patients with asthma, the treatment was changed based on the results obtained. On 37 (45.1%) of those 55 occasions, the inhaled corticosteroid dose was increased, and on 8 occasions (9.8%), oral corticosteroid treatment was instituted.

Table 2 - Induced sputum characteristics by clinical condition.

	Asthma	Chronic cough	Bronchiectasis	COPD
TCC × 10^6/mg[a]	9.6 (7.0)	5.3 (4.5)*	9.8 (9.3)	8.8 (6.8)
Neutrophils, %[a]	32.1 (25.5)	27.5 (23.2)	58.6 (24.0)*	36.8 (22.5)
Eosinophils, %[b]	3.0 (10.8)*	0.7 (3.2)	1.0 (2.0)	0.5 (3.7)
Macrophages, %[a]	51.0 (34.2)	59.0 (28.5)	35.1 (20.0)*	54.1 (20.0)
Lymphocytes, %[a]	3.5 (4.0)	3.3 (2.1)	2.8 (4.0)	4.0 (4.2)

COPD: chronic obstructive pulmonary disease; and TCC: total cell counts. [a]Values expressed as mean and interquartile range. [b]Values expressed as median and interquartile range. *Significant difference ($p < 0.05$) in relation to all the other groups.

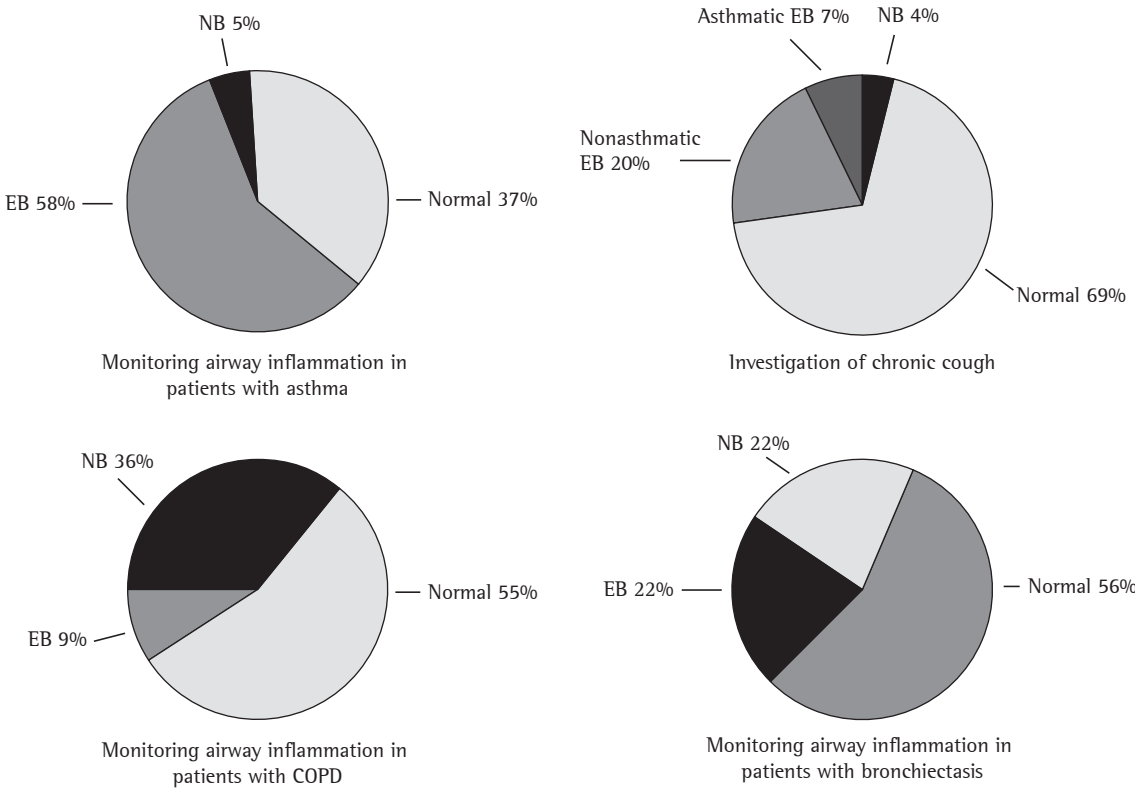

Figure 2 - Results of the examination of induced sputum cellularity by reason for requesting the test. EB: eosinophilic bronchitis; NB: neutrophilic bronchitis; and COPD: chronic obstructive pulmonary disease.

Discussion

Our results demonstrate that the characterization of the inflammatory component of common airway diseases usually resulted in a change in the treatment of the patients included in the present study. This change in the treatment occurred especially in two situations: in patients with asthma and in patients with chronic cough, in whom the diagnosis of nonasthmatic eosinophilic bronchitis was responsible for the introduction of specific treatment with an inhaled corticosteroid. These results are original and expand upon previous observations regarding the applicability of sputum induction in clinical practice.[18]

The principal reason for requesting the procedure, in the present study, was monitoring airway inflammation in patients with asthma, probably due to the fact that this is the area in which the clinical applicability of sputum cell counts has been the most widely studied.[18] The principal objective of the pulmonologists who requested the sputum cell counts, in the present study, was the characterization of the inflammatory process to establish the ideal inhaled corticosteroid dose in patients with moderate-to-severe asthma. Although these patients were being treated according to the previous consensuses of the Brazilian Thoracic Association and the American Thoracic Society, the majority presented induced sputum findings of eosinophilic bronchitis, characterizing inadequate control of the inflammatory component of the disease.

These results confirm the well established notion that clinical evaluation based only on symptoms and pulmonary function tests is not sufficient to determine the presence of airway inflammation in patients with moderate-to-severe asthma.[19] This is in accordance with the findings of two clinical trials that compared strategies of asthma management based on Canadian or British guidelines with a treatment strategy guided by induced sputum cellularity (eosinophilia). The two studies demonstrated

Table 3 - Therapeutic decision making in view of the results of the induced sputum cell counts.

Reason	Treatment	n	%
Monitoring airway inflammation in patients with asthma	Increase in the inhaled corticosteroid dose	37	45.1
	Oral corticosteroid	8	9.8
	Antibiotics	4	4.9
	Reduction in the inhaled corticosteroid dose	3	3.7
	Measures to improve compliance	3	3.7
	No change	27	33.0
Investigation of chronic cough	Inhaled or oral corticosteroid	12	26.1
	Antibiotics	2	4.3
	Discontinuation of the inhaled corticosteroid	2	4.3
	Referred for other tests	30	65.2
Monitoring airway inflammation in patients with bronchiectasis	Antibiotics	3	27.3
	Introduction of an inhaled corticosteroid	1	9.1
	No change	7	63.6
Monitoring airway inflammation in patients with COPD	Introduction of an inhaled corticosteroid	2	22.2
	Reduction in the inhaled corticosteroid dose	1	11.1
	Discontinuation of the inhaled corticosteroid	1	11.1
	Antibiotics	1	11.1
	No change	4	44.0

COPD: chronic obstructive pulmonary disease.

that the latter strategy resulted in adequate asthma control, as well as influencing two important asthma control outcomes: a significant decrease in the occurrence of exacerbations[9,10]; and the determination of the minimum treatment required in order to obtain and maintain asthma control.[10]

Persistent cough for more than eight weeks, accompanied by normal chest X-ray results, characterizes cough as chronic, a common condition in the clinical practice of respiratory medicine. Patients with chronic cough who present normal imaging study results, normal spirometry results, and normal airway responsiveness but present an increased proportion of sputum eosinophils (≥3%), are diagnosed with nonasthmatic eosinophilic bronchitis.[20] In this situation, inhaled corticosteroid use, or, in some cases, oral corticosteroid use, normalizes sputum eosinophilia, with reduction or elimination of cough. In our study, 19% of the patients who underwent sputum induction for the investigation of chronic cough were diagnosed with this condition. These findings are similar to those described in the literature, which shows that nonasthmatic eosinophilic bronchitis is the cause in 10% to 30% of the cases of chronic cough referred to pulmonologists for investigation.[20-22] It is possible that, in daily clinical practice, normal spirometry results and normal airway responsiveness induce many pulmonologists to investigate alternative causes for the cough, such as gastroesophageal reflux disease or rhinosinusitis. Therefore, there can be a delay in diagnosis, and unnecessary tests can be conducted. The recognition that chronic cough can be caused by eosinophilic bronchitis and that the diagnosis can only be made through the analysis of the cellularity of the material obtained through sputum induction or bronchoscopy has been well established.

The use of therapeutic tests with inhaled corticosteroids in clinical practice could be questioned. However, in our view, therapeutic tests with inhaled corticosteroids, despite being used in many occasions, have no diagnostic usefulness due to the

placebo effect. We studied patients with chronic cough without eosinophilic bronchitis in a tertiary care clinic using a double-blind, placebo-controlled clinical trial to evaluate the efficacy of the use of inhaled budesonide (1600 μg/day) vs. placebo for one month.[23] The primary outcome measure was cough intensity measured using a visual analog scale. The results of that clinical trial showed no differences between the intervention with budesonide and the use of a placebo. Nevertheless, at the study endpoint, all patients received a dose of inhaled budesonide (1600 μg/day/2 weeks). It was interesting to observe that, in this phase of the study, a considerable proportion of patients in both groups presented clinical improvement, clearly demonstrating the placebo effect. This is just one of the reasons that the current guidelines on chronic cough management include sputum induction and sputum cell counts in the recommendations for the investigation of the causes of cough.[12,13]

In the present study, eosinophilic bronchitis, considered one of the principal characteristics of asthma, was found in patients with COPD or bronchiectasis. Although there is evidence that approximately 30% of patients with COPD present sputum eosinophilia,[24] the frequency of this finding in patients with bronchiectasis is unknown. However, neutrophilic bronchitis, which is a characteristic of COPD, as well as of bronchiectasis and respiratory infections,[4] was found in 6% of the patients with asthma. These findings reinforce the idea that the different inflammatory patterns present in the airways are not exclusive to any one respiratory disease, and that the same disease can present more than one type of airway inflammation over time. In addition, it should be borne in mind that smoking-related asthma and COPD are common diseases that can occur in conjunction.

Of the 151 sputum inductions performed during the study, only 9 were requested for monitoring airway inflammation in patients with COPD. This might reflect the scarcity of studies investigating the usefulness of the induced sputum method in such patients. Although COPD is traditionally considered to be characterized by a neutrophilic inflammatory response, eosinophilic inflammation seems to play an important role in patients with stable disease[25] and in those presenting exacerbations.[26] In such patients, the characterization of the airway inflammatory component can predict the response to the treatment with systemic or inhaled corticosteroids, which controls eosinophilic bronchitis and is accompanied by a reduction in dyspnea, as well as by improvements in FEV_1 and quality of life.[25,26] More recently, researchers have demonstrated that the use of induced sputum findings to guide the treatment of eosinophilic inflammation in patients with COPD, similar to what happens in patients with asthma, is capable of reducing the frequency of severe exacerbations and hospital admissions.[14] Studies on the use of the method in patients with bronchiectasis are even scarcer than are those on its use in patients with COPD. However, the heterogeneity of the inflammatory component observed in the cases included in the present study corroborates the hypothesis of bronchitis, and, similar to what happens in patients with asthma or COPD, eosinophilic bronchitis might be associated with an additional response to inhaled or oral corticosteroids. In order to prove this hypothesis, a greater number of mechanistic studies are needed.

Sputum cell counts present several advantages over more invasive methods of obtaining material from the lower airways, such as bronchoscopy. Safety and practicality are the most obvious. In addition, since it is a relatively noninvasive procedure, sputum induction can be performed randomly[27] and repeatedly in individuals with diseases with different degrees of severity.[28] The success rate of 96% in the induction process found in our study confirms and expands the feasibility of the test reported in the literature.[29] Although evaluating the safety and side effects of the method was not the objective of the present study, no significant adverse events were reported during the tests, even in patients with significant airflow limitation. However, collecting induced sputum samples for cell counts requires the involvement of trained professionals, and the cell counts should be performed at a referral laboratory for sputum processing and differential counts. This process is laborious and intensive, making the method hardly applicable in terms of primary care to the population. Nevertheless, for a tertiary care referral center, the availability of this method seems to be quite important, although appropriate studies on the cost-benefit ratio of its use are needed.

The present study has some limitations that should be taken into account in the interpretation of the results. The first is the absence of a more detailed characterization of the study participants,

especially of those with asthma. A subgroup analysis of the different scenarios in which patients with asthma present themselves might bring extremely relevant information on the usefulness of the method. Second, our sample of patients with COPD was small, which limited the analysis of the results considerably. Studies involving larger samples might be able to confirm the benefits described in the literature on the use of the induced sputum method in patients with COPD. Finally, the pulmonologists who referred most of the patients for sputum induction were already accustomed to using it as a tool in clinical practice. It is possible that the patient sample and the treatment decisions made based on the test results would have been different if these patients had been referred for sputum induction by pulmonologists who had never used the method clinically.

In summary, the results of the present study demonstrate that the inflammatory component of airway diseases is heterogeneous and is not exclusive to any disease in particular. Therefore, the systematic application of inflammometry using induced sputum cell counts can be beneficial for patients with airway diseases, particularly those with asthma or chronic cough.

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