



Editorial

Induced Sputum as a Method for the Study of Bronchial Inflammation[☆]

El esputo inducido como método para el estudio de la inflamación bronquial

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Cytological testing of the sputum samples obtained from patients with inflammatory, infectious or tumor pathologies of the respiratory tract has been used in clinical practice for more than a century. Findings such as Charcot–Leyden crystals, Curschmann's spirals or Creola bodies have been considered characteristic in patients with asthma. In addition, it has been postulated that the determination of the number of eosinophils in sputum samples is a useful parameter for the diagnosis of asthma and the follow-up of its evolution.^{1,2} However, many patients with chronic inflammatory pathology of the respiratory tract do not present spontaneous expectoration, which obviously represents an important drawback for the clinical implementation of this type of studies.

In 1989, Gibson et al.³ reported a method for obtaining sputum specimens, even in individuals that did not present spontaneous expectoration, based on the inhalation of hypertonic saline solution. The study of sputum specimens obtained in this manner (induced sputum) created great expectation, as it represented a method for accessing the study of lung inflammation by means of a nearly non-invasive test.⁴ Particularly, the cytological study of induced sputum samples and eosinophilic or neutrophilic inflammation markers, as well as certain cytokines and chemokines, offered for the first time the possibility to advance, with non-invasive methods, in the understanding of the mechanisms and monitoring of the evolution of inflammatory diseases of the respiratory tract, among which asthma and chronic obstructive pulmonary disease (COPD) are especially relevant.⁵

In patients with asthma or COPD, the study of induced sputum markers that facilitate the identification of groups of patients with different degrees of severity and control, as well as the prediction of the response to treatment with anti-inflammatory drugs (inhaled steroids, cysteinyl-leukotriene antagonists) and the development of therapeutic strategies for different inflammatory phenotypes, are especially attractive from a clinical standpoint. Specifically, it could be interesting to develop induced sputum sample markers that would enable us to identify the population at high risk of developing a certain process. One example could be the description of sputum markers that are useful for identifying the risk

of developing COPD in individuals with minimal symptoms or in asymptomatic smokers. A second scenario could be represented by the identification of sputum markers that could evaluate the risk of developing asthma in patients with allergic rhinitis. Some experimental data suggest that a proportion of patients with allergic rhinitis without asthma present an increase in eosinophils in induced sputum samples⁶ and may be at a high risk for developing asthma. It is undoubtable that, in these cases, only prospective studies will be able to provide relevant information from a prognostic point of view, but unfortunately the investigation of these aspects has been dealt with very infrequently.

The usefulness of identifying inflammation markers in induced sputum samples for the control of asthma has been studied by some authors during recent years. These studies^{7–9} have compared an algorithm to adjust treatments based on clinical guidelines, with adjustments in treatment being guided by the number of eosinophils in the sputum. In the therapeutic algorithm based on sputum cytology, the steroid dosage was adjusted to the degree of reduction in the eosinophils in sputum and was also reduced when eosinophilia was <3%. Bronchodilators were used when the symptoms persisted even though the percentage of eosinophils decreased to values lower than previously indicated. The results were consistent in the three studies and demonstrated a significant reduction in asthma exacerbations when the disease was managed using the sputum eosinophil count. More recently, it has been published that the concentration of interferon gamma in the induced sputum samples of asthma patients during a viral exacerbation reached its maximum level between 3 and 5 days after the beginning of the process, which allows us to hypothesize that therapeutic interventions established during this period could have been successful.¹⁰ Logically, this hypothesis should be confirmed in future prospective studies.

From another standpoint, the study of induced sputum samples has made for definitive progress in the understanding of the pathogenic mechanisms involved in the development of asthma and in the relationship between inflammation and bronchial hyperresponsiveness. Research done in Canada some years ago identified patients with chronic cough who responded to treatment with steroids, but who did not present bronchial hyperresponse. These patients did not suffer asthma but were treated successfully with antiasthmatic medication (specifically with inhaled steroids). The cytologic analysis of the induced sputum

[☆] Please cite this article as: Prieto L. El esputo inducido como método para el estudio de la inflamación bronquial. Arch Bronconeumol. 2011;47:323–4.

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samples was able to identify a marked increase in eosinophils, and the entity was called non-asthmatic eosinophilic bronchitis.¹¹ These findings raised the first doubts about the until-then firmly established relationship between eosinophilic inflammation and bronchial hyperresponsiveness. Years later, Turner et al.¹² described individuals with asthma who presented symptoms, the need for rescue β -adrenergics and bronchial hyperresponse but normal levels of eosinophils in sputum. These observations were in direct confrontation with the eosinophilic nature of asthma and led to the concept of neutrophilic asthma. Later studies have concluded that, in a considerable proportion of cases, the predominance of neutrophils in the sputum samples of these individuals with asthma could be due to the treatment with inhaled steroids. Furthermore, it seems clear that the predominance of neutrophils or eosinophils in the induced sputum samples of asthma patients is not a persistent finding and that both inflammatory patterns can develop in a same patient at different moments and clinical situations. This probably indicates that the classification of asthma in eosinophilic, neutrophilic and paucigranulocytic phenotypes, as some authors have proposed,¹³ is not realistic. However, it seems evident that the identification of a non-eosinophilic cytologic pattern in the induced sputum of a patient with asthma who remains symptomatic despite steroid treatment leads to assume that the increase in dose of steroids probably will not provide any benefit in controlling the process.

Although the majority of the research in induced sputum samples has been done in patients with asthma or COPD, the study of sputum samples obtained by means of ultrasonically nebulized hypertonic saline has been shown to be useful to progress in the understanding of the pathogeny of other lung diseases, such as tumors, infectious diseases and processes that predominantly affect the lung parenchyma.^{14–16} As for interstitial lung pathology, the analysis of induced sputum samples has been used in studies of patients with idiopathic lung fibrosis, sarcoidosis, extrinsic allergic alveolitis, interstitial lung diseases associated with connective tissue disease, Crohn's disease and bronchiolitis obliterans in lung transplant recipients. A review of the findings and of the clinical utility of studying induced sputum samples in these processes has been recently published.¹⁷

In the current issue of *Archivos de Bronconeumología*, Bellido-Casado et al.¹⁸ have studied the inflammatory profile of the induced sputum samples obtained from patients with primary Sjögren's syndrome, a systematic autoimmune process that is characterized by a lymphocytic infiltrate of the exocrine glands, with the consequent glandular atrophy and destruction. In this population, the authors have also analyzed the clinical manifestations, lung function and bronchial response to the inhalation of a direct bronchoconstrictor (methacholine). Using a cut-point for the percentage of lymphocytes in induced sputum of 2.6%, the authors found an increase in these cells in 24 of the 35 patients analyzed (69%). Curiously, cough was more frequently identified in the group of patients with normal cytological profiles in sputum (73%) than in those with pathological profiles (29%, $P=.02$). Meanwhile, the presence of bronchial lability (bronchial hyperresponsiveness or significant bronchodilation after the inhalation of 200 μ g of salbutamol) was more frequently identified in the group of patients with pathological cytological profile. Finally, the patients with cytological sputum profiles that were pathological presented an increase in residual volume when compared with the individuals with normal cytological profiles. All these findings suggest that, in many patients with primary Sjögren's syndrome, there are also lymphocytic inflammatory infiltrates in the lung that are probably responsible for

certain structural alterations in the bronchi and in the parenchyma. These alterations in the structure are manifested by means of an increase in the bronchial lability and probably by the bronchial response to direct agonists of bronchoconstriction, although the latter needs to be confirmed in studies involving a greater number of patients. All these findings suggest that the cytological studies of induced sputum samples can be useful for identifying the pulmonary inflammatory affection in patients with Sjögren's syndrome and that, as stated by the authors of the study,¹⁸ this entity should be included in the differential diagnosis of chronic cough.

In the light of the evident interest in the information provided by the study of induced sputum samples, it is difficult to comprehend why they have not been introduced into routine clinical practice. One important limitation of this method to study inflammation is that it is a laborious technique, which makes it difficult to include in the clinical routine. One solution to this drawback could reside in the automatization of the method for analyzing sputum specimens, but the attempts made in this direction either have not been successful or have not been universally accepted.

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