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A M E R I C A N C O L L E G E O F



P H Y S I C I A N S[®]



Clinical Applications of Induced Sputum*

Christopher E. Brightling, PhD, MRCP, FCCP

The development of standardized methods for sputum induction has improved the quality and reproducibility of sputum samples. This technique has been used to optimize samples in the investigation of pulmonary tuberculosis and lung cancer, but its clinical application as a noninvasive measure of airway inflammation has highlighted the enormous potential of this technique. Sputum induction has allowed researchers to characterize the inflammatory profiles of a variety of airway diseases including asthma, COPD, and chronic cough. To date, the identification of sputum eosinophilia has the greatest clinical value as this predicts a favorable response to corticosteroids and can therefore guide treatment. In asthma and COPD management, protocols aimed at normalizing the sputum eosinophil count have markedly reduced exacerbations without an overall increase in therapy. Currently, no other noninvasive measure of airway inflammation has demonstrated a benefit in reducing exacerbations. The value of sputum induction and analysis is not restricted to the recognition of sputum eosinophilia but also may be used to direct novel antineutrophilic therapies. Thus, it is time for sputum induction to move from the research laboratory to the clinic. (CHEST 2006; 129:1344–1348)

Key words: asthma; COPD; cough; sputum

Abbreviation: eNO = exhaled nitric oxide

Analysis of sputum in respiratory disease has a long pedigree. However, in many clinical settings induced-sputum analysis is hampered as an investigative tool by the inability to obtain an adequate sample. This problem has been overcome by inducing sputum using hypertonic saline solution.¹ This technique has now been applied to a number of diverse conditions. The improved yield and quality of sputum samples afforded by sputum induction provides a real alternative to bronchoscopy and BAL in the diagnosis of pulmonary tuberculosis, and has rekindled interest in sputum cytology in the management of lung cancer. However, the development

over the last 15 to 20 years of this technique as a noninvasive measure of airway inflammation has focused much attention on the potential clinical application of this technique in the assessment of airway disease.¹

Airway disease accounts for a significant proportion of a respiratory specialist's caseload. The main airway diseases asthma and COPD are defined in terms of typical symptoms and abnormal airway physiology, but additionally it is well recognized that airway inflammation is a key component of both diseases. Although measurement of lung function is a prerequisite for the diagnosis of asthma and COPD, assessment of airway inflammation is not routinely performed. A number of techniques are available, ranging from measurement of exhaled nitric oxide (eNO)² to performing differential cell counts and assessment of mediator concentrations in induced sputum. Induced-sputum cell and mediator measurements are particularly well validated, and normal ranges from large adult populations have been published.³ Sputum induction is a well-tolerated, safe procedure even in those with severe

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disease and during exacerbations. Details of the safety precautions and methods are outlined in the European Respiratory Society guidelines.^{4,5} The widespread application of induced sputum in a variety of airway diseases and across the spectrum of disease severity has given us an insight into the relationship between airway function and airway inflammation, identified new disease phenotypes and defined which of these phenotypes respond to current therapy, and perhaps most importantly provided us with a tool to guide the clinical management of patients with airway disease.

This article summarizes clinical studies that, when taken together, provide strong evidence in favor of the clinical utility of induced-sputum analysis. Most of these studies have been performed in asthma, but evidence to support the wider role of induced-sputum analysis in COPD, chronic cough, and other conditions is presented.

ASTHMA

Asthma is commonly associated with sputum eosinophilia. The normal value for nonsmokers has been reported as 0.4% with a 90th percentile of up to 1.1%. Up to 80% of corticosteroid-naïve subjects⁶ and approximately 50% of corticosteroid-treated subjects with currently symptomatic asthma have a sputum eosinophil count that is outside the normal range. The range of eosinophil counts in asthma is wide, from 0 to $\geq 50\%$. Taking a cutoff of 1% as indicating a raised sputum eosinophil count when compared with normal airways, sputum eosinophilia as a test for asthma (defined by a consistent history together with one or more of the following: provocative concentration of methacholine causing a 20% fall in FEV₁ < 8.0 mg/mL, a significant improvement in FEV₁ after β_2 -agonists, or abnormal peak expiratory flow) gives a sensitivity of > 80% and a specificity of 95%.⁷ Likewise, the addition of sputum cell counts to peak flow monitoring is also useful to improve the diagnosis of occupational asthma.⁸

Although sputum eosinophilia is a typical feature of asthma, the increasing application of this technique has led to the recognition that inflammation in asthma is more heterogeneous than previously believed (Fig 1) with identification of noneosinophilic asthma.^{6,9,10} Noneosinophilic asthma is common, accounting for 25 to 55% of corticosteroid-naïve asthmatics; is present across the range of disease severity; and is repeatable with few subjects with noneosinophilic asthma acquiring airway eosinophilia.¹¹ Its identification is important, as it is associated with a poor response to corticosteroids.⁹ In contrast, asthmatics with sputum eosinophilia have a favorable

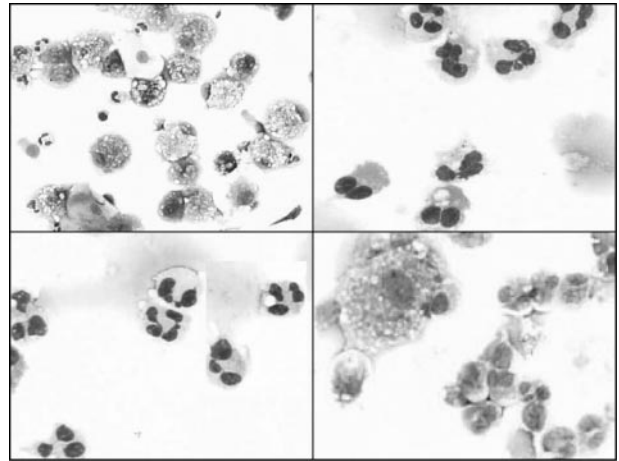


FIGURE 1. Sputum cytopsin from different subjects with asthma illustrate the heterogeneity of the airway inflammation. *Top, left:* Predominant cells are macrophages with a normal neutrophil and eosinophil count; this cytopsin cannot be distinguished from a sample from a healthy control subject (paucigranulocytic asthma, original $\times 100$). *Top, right:* Combined neutrophilic and eosinophilic inflammation (original $\times 400$). *Bottom, left:* Neutrophilic inflammation (original $\times 400$). *Bottom, right:* Eosinophilic inflammation (original $\times 400$).

response to corticosteroids, even in ex-smokers and current smokers.¹² Little et al¹³ found that in patients with asthma, a sputum eosinophil count (> 4%) had a 68% positive predictive value with a sensitivity of 59% and specificity of 76% for an improvement in FEV₁ >15% after a 2-week course of oral corticosteroids. Sputum eosinophilia was positively correlated with the degree of clinical improvement to inhaled corticosteroids and was more closely related to clinical response than eNO, sputum, or peripheral blood eosinophilic cationic protein.¹⁴ None of these earlier studies were placebo controlled, but a recent placebo-controlled cross-over trial¹⁵ of inhaled mometasone, 400 μg qd for 8 weeks, in subjects characterized as either having eosinophilic or noneosinophilic asthma prior to study entry has confirmed that a favorable response to corticosteroids was reserved to asthmatics with sputum eosinophilia.

Importantly, the value of measuring airway inflammation in sputum extends beyond these relatively short-term studies on corticosteroid responsiveness. Corticosteroid reduction studies^{16,17} have shown that sputum eosinophilia develops well before the onset of an exacerbation, suggesting that targeted therapy in asthma aimed at normalizing the sputum eosinophil count could lead to a reduction in asthma exacerbations. Indeed, there is now strong evidence from two independent studies^{11,18} supporting the importance of monitoring airway inflammation by sputum induction to reduce asthma exacerbations. In

a randomized placebo-controlled trial,¹¹ 74 subjects with asthma were assigned to either a management strategy aimed at normalizing their sputum eosinophil count or standard clinical care. Patients in the sputum management group had significantly fewer severe asthma exacerbations than patients in the control group (35 exacerbations vs 109 exacerbations, $p = 0.01$) [Fig 2], and significantly fewer patients were admitted to hospital with asthma (one patient vs six patients, $p = 0.047$). The reduction in exacerbations was achieved without an increase in the total corticosteroid dose in the sputum guidelines group, as monitoring airway inflammation in the sputum guidelines group identified a group of patients with noneosinophilic asthma whose sputum eosinophil counts remained within the normal range; and in these subjects the dose of corticosteroids was reduced without evidence of deterioration in control subjects. In a second study¹⁸ of 117 asthmatics entered into a 2-year follow-up multicenter, randomized, parallel-group effectiveness study, treatment directed at normalizing the sputum eosinophil count also led to a reduction in exacerbations (79 exacerbations vs 47 exacerbations, $p = 0.04$) and increased the time to first exacerbation by 213 days. This benefit was not at the expense of increased therapy in the intervention group. In this study, the inflam-

matory phenotype of the exacerbations were characterized; and in the sputum guidelines group, eosinophilic, but not noneosinophilic exacerbations were reduced. Interestingly, noneosinophilic exacerbations were more common (56%). The reduction in exacerbations was more apparent in those with severe disease. This suggests that it is probably most appropriate to apply this technique to the management of difficult-to-treat or refractory asthma, but that its use may not be generalizable to a primary care population.

The inclusion of sputum induction in the management of asthma is cost-effective. Economic analysis shows that the health-care-related savings as a consequence of the reduction in asthma exacerbations outweighs the cost of sputum induction and processing.¹¹ Sputum induction is relatively labor intensive and requires laboratory support, but all of the equipment is available in a routine pathology laboratory and minimal training is required. Therefore, sputum induction should be available as a routine test in specialist centers to evaluate and manage patients with severe asthma.

The value of sputum induction is greatest in those with severe asthma; thus, an alternative simpler noninvasive test for mild disease is required. eNO has been proposed as one possible alternative to

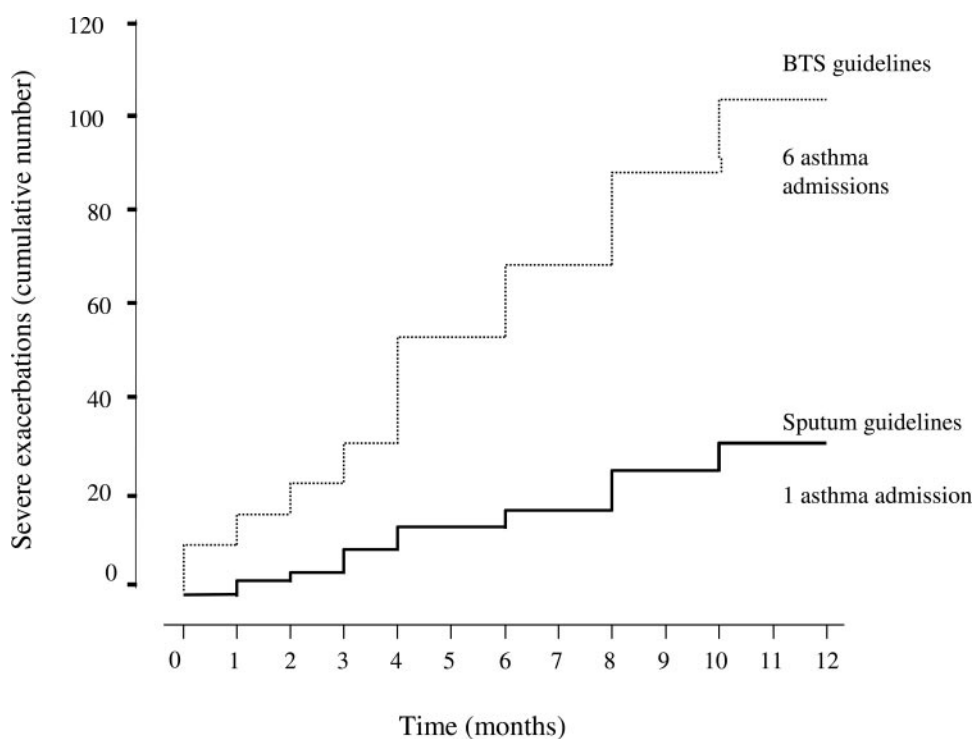


FIGURE 2. Severe exacerbations in subjects with asthma managed by standard British Thoracic Society (BTS) guidelines vs those managed by an algorithm directed at normalizing the sputum eosinophil count. Patients in the sputum management group had fewer severe exacerbations ($p < 0.01$), and fewer patients were admitted to the hospital ($p = 0.047$). Adapted from Green et al.¹¹

sputum induction, but in stark contrast to sputum induction, follow-up studies with management protocols guided by eNO have failed to demonstrate reductions in asthma exacerbations in adults¹⁹ and children.²⁰ Thus, to date sputum induction is the only noninvasive measure of airway inflammation that has a proven role in the management of moderate-to-severe asthma, and regular monitoring of airway inflammation in this disease group is required for optimal treatment.

COPD

In COPD, the sputum neutrophil count is usually raised and is related to reduced FEV₁ and the increased rate of decline in FEV₁, suggesting that neutrophilic airway inflammation is functionally important.²¹ Up to 40% of subjects with COPD have a sputum eosinophil count > 3%.^{22,23} These subjects are indistinguishable to subjects without sputum eosinophilia with regard to clinical features and lung function. There is increasing evidence that the presence of sputum eosinophilia predicts an objective response to oral^{22,23} and inhaled corticosteroid treatment in COPD.²⁴ In one study,²² the response in terms of lung function, health status, and exercise tolerance to a 2-week course of oral prednisolone increased as the baseline sputum eosinophil count increased, and was associated with a marked treatment-induced fall in the sputum eosinophil count but no change in sputum markers of neutrophilic inflammation. This suggests that eosinophilic airway inflammation is functionally important in some subjects with COPD, and that the beneficial effects of corticosteroids are due to modification of this aspect of the complex airway inflammation.

In light of the benefit achieved by monitoring the sputum eosinophil count in the management of asthma, the clinical application of sputum induction in the management of COPD has recently been applied to a group of 80 subjects with COPD. Over a 12-month period, a management approach with the additional aim of reducing the sputum eosinophil count < 3% using corticosteroids was associated with a 62% reduction in severe exacerbations of COPD requiring hospitalization when compared to traditional symptom-based management.²⁵ Again, this benefit was not at the expense of an overall increase in corticosteroid therapy. Therefore, like asthma, the measurement of a sputum eosinophil count can be used to identify COPD patients with corticosteroid responsive disease and to guide treatment. This potential role of sputum induction in the management of COPD needs to be confirmed by future studies and whether particular subgroups of

patients benefit needs to be established. For these reasons, it is premature to recommend sputum induction in the routine management of COPD.

Current therapies for COPD and asthma have little effect on the neutrophilic inflammation. Sputum induction provides an excellent research tool to assess the effect of novel therapies on neutrophilic inflammation, and it is likely that in the future sputum induction will not only direct therapies targeted at the eosinophil, but also enable clinicians to tailor treatment for patients with neutrophilic predominant inflammation.

COUGH

Up to 30% of subjects with cough have a sputum eosinophil count of > 3%.²⁶ Half of these subjects show no functional evidence of asthma but suffer from nonasthmatic eosinophilic bronchitis.^{26,27} Measurement of airway inflammation is the only way of identifying these subjects and is, therefore, an important addition to the chronic cough investigation algorithm. This has been recognized in the American College of Chest Physicians cough guideline, and sputum induction is the recommended investigation to confirm a diagnosis of nonasthmatic eosinophilic bronchitis.²⁸ Lipid-laden macrophages have been proposed as a marker of oropharyngeal reflux, but their value in the assessment of patients with chronic cough is unclear and is not currently recommended.²⁹

Sputum induction is not only valuable in the assessment of the cause of chronic cough, but like asthma and COPD can identify patients who are likely to respond to corticosteroids. Patients with cough and sputum eosinophilia exhibit an objective response to corticosteroid treatment that occurs in parallel with a treatment-associated fall in the sputum eosinophil count.²⁶ In contrast, patients without sputum eosinophilia do not respond.³⁰

OTHER CONDITIONS

Little is known about induced-sputum cell features in other respiratory conditions. In idiopathic pulmonary fibrosis, both neutrophilic and eosinophilic inflammation are reported, but one problem with using sputum induction in the assessment of interstitial lung disease is that the sputum mainly reflects inflammation in the large proximal airways and is not the best way of investigating lymphocytic alveolitis. There is emerging evidence that induced sputum may be a valuable tool to quantify environmental exposure to carbonaceous particulates, mold,

and pollen; recognize mineralogic exposure in occupational lung diseases; enumerate intracellular bacteria; and identify hemosiderin-laden macrophages in subjects with left ventricular failure. These novel applications of induced sputum deserve further study.

CONCLUSION

Sputum induction has several clinical applications. The evidence in favor of its utility in the diagnosis of chronic cough and its role in optimizing treatment in severe asthma is very strong. Evidence is emerging to support its role in COPD, and the potential for sputum induction to be of value in a number of other clinical settings is considerable. Currently, no other noninvasive measure of airway inflammation has demonstrated a benefit in reducing exacerbations by targeting treatment in patients with airway disease. Therefore, it is time to move sputum induction from the research laboratory to the clinic.

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