



Induced Sputum as a Diagnostic Tactic in Pulmonary Diseases

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Abstract

The induced sputum technique allows sampling of the airways in a non-invasive manner and thus offers a unique opportunity to identify biomarkers of potential clinical utility in respiratory medicine. Sputum cells were originally examined in stained smears and the procedure was applied in both research and clinical settings from the 1950s through the 1970s. The cells, recovered from spontaneous coughing, were used to study lung cancer and respiratory infections and, later on, to diagnose *Pneumocystis carinii* pneumonia in patients infected with human immunodeficiency virus. The method was largely improved by the induction of sputum with aerosol of hypertonic saline and was extended to become part of the assessment of airway inflammation in bronchial asthma and chronic obstructive pulmonary disease. It was recently shown that induced sputum can be used to study interstitial lung diseases and, more specifically, sarcoidosis, non-granulomatous ILD, occupational lung diseases and other systemic diseases with lung involvement.

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The nature and extent of pulmonary diseases has, until recently, been assessed by direct invasive bronchoscopy with bronchial washings, biopsy, bronchoalveolar lavage, or by indirect measurements of symptoms, pulmonary function tests or peripheral blood inflammatory markers.

The ability to differentiate groups or subgroups of patients sharing similar biological patterns on clinical grounds was greatly limited before the advent of non- or minimally invasive techniques for assessing airway inflammation. The induced sputum technique is thus a major improvement as a non-invasive and reproducible technique for sampling the airways. Induced sputum is now widely used in medicine. Intensive work has been done to compare IS to BAL findings [1], to standardize normal values [2] and methods [3] and to interpret results in clinical practice [4].

The present review demonstrates the application of IS in the diagnosis and management of asthma, chronic obstructive pulmonary disease, interstitial lung disease, occupational lung disorders, systemic syndromes involving the lungs, pediatric disorders, infectious diseases, and malignancy.

Induction: processing and safety of sputum

The use of an ultrasonic nebulizer for sputum induction was reportedly more likely to be successful than other jet nebulizers [5] as well as being free of risk in patients with asthma of varying severity and in patients with COPD [6]. Although the use of hypertonic saline was first reported to be as safe as isotonic saline and to result in a relatively higher success rate, two studies have now demonstrated that induction with isotonic saline is better tolerated by patients with severe asthma and is often sufficient to induce sputum [6].

In combination with the above-mentioned safety precautions, it is recommended to pretreat patients with a beta-2 agonist since the sputum induction procedure produces a minimal fall in forced expiratory volume in 1 second, which can be inhibited by pretreatment with salbutamol. Moreover, there should be continuous monitoring of the oxygen saturation since the implied decrease of SO_2 saturation during sputum induction is still controversial: some investigators showed a slight fall in $Sa O_2$ which was not clinically significant, while others showed more significant effects [6].

Since the first attempts to use standardize methods for sampling induced sputum, two techniques for processing the expectorate have evolved. One involves collecting and analyzing the more viscid portions of mucus (plugs) extracted from a sputum sample and the second involves collecting and analyzing the entire sample, including saliva [3]. In both methods, processing must be done within 2 hours from induction. A recent study indicates that samples can be stored after induction at 4°C for 9 hours without altering cell composition [7]. Although both methods are useful in terms of eosinophils and eosinophil cationic protein content for differentiating asthmatics from healthy subjects, they are not interchangeable, and once a technique has been adopted for a given study it should always be applied [8].

Induced sputum in normal subjects

Accurate interpretation of sputum cell results requires knowledge of the normal range of cell count in the induced sputum of non-smoking healthy adults. The studies conducted in this field show that the majority of the cells are neutrophils and macrophages

ILD = interstitial lung diseases
BAL = bronchoalveolar lavage

COPD = chronic obstructive pulmonary disease

whereas eosinophils, lymphocytes and bronchial epithelial cells are scarce and metachromatic cells (basophils/mast cells) are almost totally absent [2].

The different ways of processing had no effect upon the differential cell count in both a selected portion of sputum and the entire expectorate in the same group of healthy volunteers. In addition, sputum cell counts were not affected by the tonicity of the saline used for induction as long as the duration of induction was 15–20 minutes [5].

Induced sputum in asthma

IS examination provides a better understanding of inflammation in airway diseases. Compared with sputum from healthy subjects, asthmatic sputum contains a significantly higher proportion of eosinophils [1,4].

In addition to the cellular composition of sputum, there is increasing interest in the analysis of its soluble factors, cellular subtypes or cellular markers in asthma and in chronic bronchitis. It was found that compared with healthy subjects and smokers with bronchitis, patients with asthma had a higher proportion of eosinophilic cationic protein and major basic protein and matrix metalloproteinase-9 in sputum. Although the proportion of eosinophils was found to correlate with interleukin-5, a recent report demonstrated that the validity of the measurement of this metabolite is poor due to sputum proteases, soluble receptors or autoantibodies [9].

The role of eosinophils was further studied in IS during exercise-induced bronchoconstriction: there was no influx of eosinophils during effort [10] but a strong influx of eosinophils was seen after exercise [11]. The differences may be due to the differences in the protocols. The role of other cells was also studied in asthmatic inflammation. Flow cytometric analysis of lymphocytes showed an increase in CD4+ T cells [4] and T cells with high chemotactic activity due to high levels of IL-16 [12]. Another study showed that goblet cell hyperplasia in asthmatics is accelerated due to cytokines secreted by CD4+ T cells [13]. Suppressive macrophages [14] and macrophage migration inhibitory factor [15] in IS correlated with decreased hyperresponsiveness of airways and symptomatic asthma, respectively.

Airway inflammation is considered to be the primary cause of airway diseases. Its prevention and reversal are the primary aims of treatment. Measurement of the inflammation is now possible in a relatively non-invasive and reliable manner using IS cell counts. Sputum eosinophilic count was widely investigated. It was found that a treatment targeted to normalize eosinophil cell counts in sputum together with reduction of symptoms correlated with a reduction in asthma exacerbations [16]. Another study quantified asthma using a score including control of symptoms, expiratory flow rates and eosinophil cell count in sputum [17].

Eosinophils in sputum were used in several studies to monitor response to treatment. Inhaled steroids [4], montelukast and budesonide [18] were found to reduce the number of eosinophils in IS.

Induced sputum in COPD

Extensive studies have been carried out with IS to investigate the issue of inflammation in COPD. The finding that the highest neutrophil percentages were found in patients with the worst airflow obstruction confirmed the hypothesis that neutrophilic inflammation of the small airways is a key factor in the pathogenesis of COPD. Many patients with COPD produce spontaneous sputum, but induction of sputum, being a safe [6] and well-tolerated technique in this group of patients, produces superior samples with higher proportions of viable cells.

The presence of sputum eosinophilia [19] or neutrophilia [20] in the IS of COPD patients indicates the need for treatment. However, recent studies have shown a significant degree of overlap between asthma and COPD, with raised levels of neutrophils in more severe forms of asthma and a different pathologic phenotype in COPD patients with airway eosinophilia and mast cell activation.

Induced sputum in persistent cough

On systematic investigation, patients with persistent cough are often diagnosed as having asthma, gastroesophageal reflux disease and postnasal drip. Cough variant asthma and eosinophilic bronchitis present with cough, usually associated with airway eosinophilia, and respond well to corticosteroids [21].

Gastroesophageal reflux is commonly associated with chronic cough and asthma. Identification of lipid-laden macrophages in induced sputum was shown to be a good marker of oropharyngeal reflux and possible gastric aspiration when compared to 24 hour pH monitoring [22]. In 113 patients with persistent cough we found that the percent of eosinophils in IS correlated with the diagnosis of postnasal drip, while a high percentage of lipid-laden macrophages indicated the presence of gastroesophageal reflux disease [23].

Induced sputum in pulmonary tuberculosis and HIV

IS was found to improve the diagnostic yield of patients with infectious diseases, including children with suspected tuberculosis [24], while another study concluded that induction of sputum is costly and had no advantage over routine expectorated sputum [25]. A prospective study compared the diagnostic yield of three induced sputum tests with that of bronchoscopy in subjects with possible active tuberculosis. It was concluded that although the test is cost-effective and obviates bronchoscopy, it may be performed only in respiratory isolation conditions since it carries a high risk of causing tuberculous infection in personnel [26].

IS has also been used to diagnose *Pneumocystis carinii* pneumonia in patients infected with HIV. The performance of polymerase chain reaction for the detection of *P. carinii* from IS samples was found to have high specificity and sensitivity compared to immunofluorescence staining or when PCR was performed in BAL specimens [27]. A more recent report concluded that since 1996, due to the introduction of the highly active antiretroviral therapy, there is a sharp decreased prevalence of

IL = interleukin

HIV = human immunodeficiency virus
PCR = polymerase chain reaction

P. carinii pneumonia and that other pathogens might be responsible for pulmonary diseases in HIV-positive patients. In this context bronchoalveolar lavage should be used as the preferred procedure for diagnosing pulmonary diseases in HIV patients [29].

Induced sputum in cancer

IS was used to investigate and diagnose lung cancer and, later on, was employed as a tool for early detection of primary lung cancer. It was shown that while induced sputum gives better quality specimens and better diagnostic yield in small lesions than spontaneous sputum, very specific molecular screening programs [29] are needed to demonstrate their efficacy in reducing mortality from the disease. Among these molecular markers it was shown that the most effective applications using IS specimens involve specific oncogene activation, tumor suppressor gene deletion, genomic instability and abnormal methylation [30]. Other reports have shown that aromatic hydrocarbon-DNA adducts and 32P-postlabeling of lipophilic DNA adducts can be detected better in IS than in peripheral blood [31].

Induced sputum in children

Sputum induction has been used to study asthma in children since the first description of the technique in 1992. In general, sputum induction in children over 6 years old is safe and has a good success rate (68–100%). The methodology for induction is as previously described for adults, and pretreatment with a beta-2 agonist has been applied in 80% of studies [32]. The procedure should be performed by trained personnel who are experienced in lung function measurement in children and in identification of adverse effects.

A recent study that examined the relationship between airway inflammation and clinical asthma in children [33] demonstrated that the increase in frequency of asthma episodes is accompanied by an increase in sputum eosinophils, sputum eosinophil cationic protein, desquamated bronchial epithelial cells and treatment intensity. In cystic fibrosis as well, a significant inverse correlation was found between FEV1 and polymorphonuclear cell counts, IL-8 and elastase in IS, while airway infection as assessed by bacterial density did not correlate with lung function or indices of inflammation [34].

IS in interstitial lung diseases

Our group was the first to investigate the use of induced sputum in ILD. We compared IS with BAL in the assessment of exposure to hazardous dusts and in the evaluation of pneumoconiotic patients (silica and hard-metal workers) [35]. We found that BAL and IS specimens yielded similar quantitative and qualitative results in terms of the number of particles present in the samples and the chemical analysis of the particles.

Our findings raise the possibility that IS may serve as a future biological monitoring method (as for other toxic materials such as lead and cadmium, and solvents, e.g., toluene, and trichloroethane)

for the periodic physical examinations of healthy workers exposed to hazardous dusts.

As for other interstitial lung diseases of unknown etiology, such as sarcoidosis and idiopathic pulmonary fibrosis, a high percentage of lymphocytes was demonstrated in the sputum from sarcoid patients in both BAL and IS [36]. We analyzed and compared the subpopulations of T lymphocytes in samples obtained by both BAL and IS in 19 patients with pneumoconiosis [36] and in 30 patients with interstitial lung disease [36]. We found that the T cell subpopulations present in the samples recovered by IS correlate well with those recovered by BAL, and that IS can effectively and non-invasively identify CD4+ inflammation in order to distinguish between sarcoidosis and other non-granulomatous interstitial lung diseases.

We also evaluated the value of the IS technique in uveitis and showed that increased CD4/CD8 ratios in induced sputum of patients with uveitis, who also had elevated angiotensin-converting enzyme levels, strongly suggest the presence of ocular sarcoidosis [37].

In another group of diseases, we showed that pulmonary involvement can be demonstrated in patients with Crohn's disease by the accumulation of CD4+ T cells in IS [38]. These findings further support the concept of an uninterrupted recirculation and dynamic balance of lymphocytes in general, and of CD4+ in particular, from mucosa-associated lymphoid tissue with its activated lymphoid follicles to effector sites in the integrated human mucosal immune system.

Conclusions and future directions

Similar to the situation encountered with the introduction of flexible bronchoscopy and BAL in the 1970s, sputum induction was generally considered as a research tool only. The situation has changed and sputum induction is finding its way into clinical practice.

A randomized controlled trial with moderate to severe asthma in adults [16] and in children [33] showed that exacerbations correlate with percent eosinophils in induced sputum and a treatment strategy directed towards normalization of the eosinophilic inflammation in sputum reduced symptoms and hospital admissions [16]. In addition, sputum cell counts are useful in monitoring the anti-inflammatory effect of drugs like theophylline, long-acting beta adrenergic agonists, leukotriene antagonists and newer drugs [19,20]. Moreover, it is a safe and reproducible method for conducting multicenter studies in asthmatic subjects [39].

For lung cancer screening IS is effective in identifying highly diagnostic markers such as oncogene activation, tumor suppressor gene deletion, genomic instability and abnormal methylation. In the field of infectious diseases (i.e., HIV and tuberculosis), further studies are needed to demonstrate its higher diagnostic yield and cost-effectiveness as compared to BAL.

Regarding ILD, IS may have prognostic value for patients with idiopathic pulmonary fibrosis for whom there are clinical contraindications for bronchoscopy or when, for other reasons, tissue samples are not available. This technique is also useful in pneumoconiosis. We propose IS as another first-line test that can

FEV1 - forced expiratory volume in 1 second

be added to those recommended for the initial diagnostic evaluation of patients with suspected sarcoidosis and as another marker of disease activity to those already suggested. Finally, in the field of functional genomics and proteomics, IS may have vast research importance [40].

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