Induced Sputum in Childhood Asthma

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Introduction

Asthma is characterized by variable airway obstruction, bronchial hyper-responsiveness and influx of inflammatory cells, especially eosinophils, into the bronchial mucosa.1 It is a common disease that can cause much morbidity and mortality.2,4 Management decisions in childhood asthma have traditionally been based on assessment of symptoms, results of peak expiratory flow rate (PEFR) or simple spirometry and the frequency of use of rescue medication. However, the abnormal airway physiology is not often present even in cases with severe asthma.5-7 In addition, these measures do not correlate closely to the underlying eosinophilic airway inflammation, which is a predictor of asthmatic exacerbation and precursor of airway remodeling.8 In selected published studies, up to 80% of corticosteroid-naive subjects9-11 and more than 50% of corticosteroid treated subjects12 with concurrent symptoms have sputum eosinophil count outside the normal range. Thus the monitoring of sputum eosinophil may allow better asthma control and provide a useful guide in management. Recent evidence suggests that a treatment strategy directed at normalization of airway eosinophil reduces asthma exacerbations and hospital admissions.9 Current research and reviews on asthma management have also highlighted the potential use of sputum induction as a non-invasive method in assessing paediatric airway inflammation.13-16

Methods for Sputum Induction and Processing

Studies in children indicate that sputum induction can be safely performed in children >6 years of age and the reported success rate is between 68-100%.13 Such variation in success rate could be due to differences in the methods used. Sputum induction in younger children is limited by their poor spirometric technique and low tidal volume, which limits the dose of saline that can be delivered.13 In most study series utilizing sputum induction, the procedure is well tolerated by children and possible side effects reported include cough, bronchospasm, vomiting and anxiety.18-20 The procedure is equally well tolerated by children with severe asthma and those with an acute exacerbation.21,22

Combining hypertonic saline challenge with sputum induction allows the assessment of bronchial hyper-responsiveness and degree of airway inflammation during the same procedure. In adult patients, hypertonic saline challenge has been shown to be sensitive, reliable and it correlates better with serum markers of inflammation than methacholine challenge.25 Whether the same holds true for childhood asthma remains to be seen. Although combined hypertonic saline challenge with sputum induction provides a useful means to compare airway hyper-responsiveness and airway inflammation at the same time, it was reported that its success rate in obtaining adequate sputum allowing for the preparation of good quality cytoslides for differential cell count was lower than sputum induction alone.24

We carry out sputum induction in a dedicated area with negative pressure facilities. Before the start of induction, the patient is thoroughly examined and his temperature checked. We also go through a list of screening questions to ensure that the patient is not suffering from an infectious condition. In our experience, success rate is greatly enhanced by thoroughly explaining the procedure to the subject (Figure 1) and teaching him the proper coughing and expectoration technique. Relatives and parents are asked to wait outside the laboratory to minimize interference. Since inhalation of hypertonic saline may lead to bronchoconstriction, some protocols incorporate pretreatment with beta-agonist (salbutamol) before induction. In our practice a medical personnel is usually present during the process and in addition bronchodilators together with other essential resuscitation equipments are readily available.

Figure 1. The suggested procedure for combined hypertonic saline challenge and sputum induction.
Sputum induction is performed using an inhalation of 4.5% hypertonic saline (HS) through a mouthpiece and large one-way non-rebreathing valve (Hans Rudolph 2700, Hans Rudolph Inc, Kansas City USA) connected to a DeVilbiss ultrasonic nebulizer set at the maximum output. The child is asked to rinse his mouth with water to clear debris and squamous epithelial cells. A nose-clip is worn and baseline forced expiratory volume in 1 second (FEV₁) is measured. Sputum induction is only carried out if the subject’s FEV₁ is at least 65% predicted using local reference values. The child then inhales HS for a period of 30 seconds. Lung function is repeated 1 minute after the inhalation. If no sputum is obtained and lung function remains greater than 80% of the baseline value, the test continues. The child then continues inhalation of HS for periods of 1 min, 2 min, and then three periods of 4 min each. He is encouraged to cough up any sputum after each dose of HS and the sputum sample is collected in a specimen bottle, kept at 4°C, and processed within 4 hours. A record of any side effects experienced by the child undertaking the test and repeat measurements of FEV₁ are made at the end of each elapsed inhalation time period. The study concludes either when the child develops significant clinical bronchospasm during the procedure. Ninety-three subjects (74.5%) were able to produce an adequate sputum sample and we were able to demonstrate that the level of exhaled nitric oxide was a significant predictor for successful induction.²⁸

Clinical Application of Sputum Induction

By assessing the degree of airway inflammation and targeting treatment in relation to response, we could prevent children from being exposed to unnecessarily high dose of inhaled corticosteroid and suffer from its potential side effects. Thus, the greatest clinical application of sputum induction is to study the extent of airway inflammation non-invasively in children as the use of bronchoscopy or biopsies have been very limited due to ethical and safety reasons.²⁹ The use of this technique may

For sputum processing, both selected sputum plug method and entire sputum method have been described.³⁰ The first involves collecting and analyzing the more viscid portions of mucus (plugs) extracted from entire sputum as described by Popov and colleagues³¹ while the latter involves collecting and analyzing the entire sputum, including saliva, as described by Fahy and colleagues.²⁶ The use of dissecting microscope or simply with a pair of forceps have been described in selected sputum plug method, however, no studies had compared the diagnostic yield of the different extraction techniques. Both methods have the same diagnostic value in distinguishing asthmatics from healthy subjects but the selected sputum plug method provides more viable cells for subsequent analysis.³² The selected sputum plug method is the method used by most paediatric centers including ours. The volume of the selected sputum is measured and 0.1% dithiothreitol (DTT) (Sigma Chemicals, Poole, UK) is added to the sputum in a 4:1 ratio in order to break up the disulfide bonds and disperse the cells. The cell suspension is aspirated until homogenized and filtered to remove any remaining debris. Phosphate buffered saline is then added to the cell suspension. The non-squamous cell count and cell viability (with trypan blue) are determined in a haemocytometer. The cell suspension is centrifuged at 400G for 10 min and cytospins are made and stained by May-Gruenwald Giemsa stain. 400 non-squamous cells are counted and the result is expressed as percentage eosinophilia (colour photograph is available in the electronic version).

From October 2003 to December 2004, our unit has carried out sputum induction on 130 asthmatic children with a median age of 11.3 years. Sixty-seven patients (51.5%) were using ICS and the median beclomethasone equivalent dosage was 125 mcg (range 50 mcg-500 mcg, assuming budesonide has equal potency and fluticasone is twice as potent as beclomethasone). Fifty-five patients (42.3%) were using long-acting beta agonists and 8 patients (6%) were taking leukotriene receptor antagonist. All children could perform consistent FEV₁ before the procedure and all had mild/moderate persistent asthmatic with FEV₁ ranged from 70% to 100% predicted. Sore throat was the most common complaint after the procedure, occurring in 20 subjects (15%), followed by chest discomfort, affecting 8 subjects (6%). The procedure was prematurely terminated in three cases, in two because of vomiting and in the remaining one because of the unpleasant taste of 4.5% HS. None of the subjects developed significant clinical bronchospasm during the procedure. Ninety-three subjects (74.5%) were able to produce an adequate sputum sample and we were able to demonstrate that the level of exhaled nitric oxide was a significant predictor for successful induction.²⁸

Figure 2. High quality cytospin slide for differential cell counts. 400 non-squamous cells are counted and the result is expressed as percentage eosinophilia (colour photograph is available in the electronic version).

Figure 3 Differential cell counts: 1, eosinophil; 2, neutrophil; 3, macrophage; 4, epithelial cell (colour photograph is available in the electronic version).
further provide important insight into the pathology and mechanism of asthma and its determinants of severity. Sputum induction also allows the analysis of mediators such as proteins and cytokines that are present in the fluid phase of the sputum sample. Among the various cellular markers found in sputum, sputum eosinophilia is well validated as a marker of airway inflammation. Cai et al. have established the reference range of sputum eosinophil for both normal and asthmatic children, and the upper limit for sputum eosinophil is found to be 2.5% (Table 1). They have also compared the sputum cell counts in normal subjects (NC), controlled asthma (CA) on inhaled corticosteroid, symptomatic asthma (SA) and those with exacerbation of asthma (EA) (Table 2). It was found that eosinophils accounted for a median 0.3% [interquartile range (IQR): 0.1, 0.65] of cells in sputum from healthy children. Sputum eosinophils [4.3% (IQR: 1.5, 14.1) p=0.0005] and epithelial cells [14% (IQR: 6, 19.4) p=0.0005] were significantly higher in children with asthma than in non-asthmatic children. Children whose asthma was well controlled, as well as those with symptoms, had more sputum eosinophils and epithelial cells than the non-asthmatics. Mast cells were found in the sputum of only four of the 42 children with asthma. Sputum eosinophil counts have been found to have good correlation with asthma severity in terms of the degree of airway inflammation and variability in expiratory flows. Moreover, studies have demonstrated that there is a fairly good agreement between the eosinophil counts from sputum, bronchoalveolar lavage (BAL) and bronchial biopsies in asthmatic patients. The percentage eosinophils in sputum was significantly correlated with their percentage in bronchial wash (R²= 0.52, P=0.03) and in BAL (R²=0.55, P=0.02).

Sputum eosinophil counts in asthmatics change substantially in response to different dose and duration of inhaled or oral corticosteroids. In a study by Green et al., they found that higher level of sputum eosinophils correlated with greater airway obstruction and the increase in sputum eosinophils can be considered as a predictor for asthma exacerbations. Apparent stable asthmatics with high eosinophil counts are more likely to develop an exacerbation on reduction of their regular corticosteroid therapy. In their study, they found that the extent of control of eosinophilic airway inflammation and exacerbation was greater in the sputum management group, which targeted at lowering the airway inflammation, than in the British Thoracic Society (BTS) management group. They concluded that a treatment strategy targeted at normalization of sputum eosinophilia would reduce asthma exacerbations and hence a better asthma management. Zacharasiewicz et al. also demonstrated the usefulness of using sputum cell count in the management of childhood asthma. ICS treatment reduction was successful in all children who had no eosinophils in their induced sputum.

Childhood chronic cough is a common and troublesome problem. It is a non-specific symptom that is associated with several unrelated mechanisms and has various causes including asthma. Chronic cough is associated with predominant sputum neutrophilia, but up to 40% of subjects with cough have sputum eosinophil count of more than 3%. The latter has good response to bronchodilator and oral or inhaled corticosteroids. Sputum induction in this scenario is a useful and cost effective tool for diagnosing the group of patients with chronic cough that is going to be responsive to anti-asthma therapy.

**Problems in Sputum Induction for Clinical Use**

Problems of using sputum induction in clinical practice includes: 1) It is a time consuming procedure. 2) Sputum needs to be processed within 2-4 hours after induction and methods for preserving sputum beyond that time limit without compromising validity are still under investigation. 3) The need of laboratory support limits its use as a routine clinical procedure. The European Respiratory Society Task Force has recently provided a review as well as recommendations concerning the induction protocol, safety aspects, processing and analysis of sputum sample, however, many issues concerning the technique itself and the interpretation of results including validation of fluid phase markers and possibilities in automating the procedure.

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**Table 1. Sputum cell count for normal, atopic normal and nonatopic normal children**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Atopic normal</th>
<th>Nonatopic normal</th>
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<tbody>
<tr>
<td>TCC 10⁶ cells / ml</td>
<td>5.14 (1.2-9.08); 1.5 (0.8-3.9)</td>
<td>1.75 (0.89-2.6); 1.0 (0.55-2.15)</td>
<td>8.04 (0.63-15.5); 1.8 (1.05-6)</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>1.57 (0.62-2.52); 0.3 (0-1.05)</td>
<td>2.16 (0.83-3.48); 0.5 (0-2.8)</td>
<td>1.13 (0-2.54); 0 (0-0.6)</td>
</tr>
<tr>
<td>Mast cells %</td>
<td>0.024 (0-0.05); 0 (0-0)</td>
<td>0.03 (0-0.07); 0 (0-0)</td>
<td>0.02 (0-0.06); 0 (0-0)</td>
</tr>
</tbody>
</table>

Data are presented as mean (95% confidence interval); median (interquartile range). TCC: total cell count

**Table 2. Sputum cell counts in normal subjects (NC), controlled asthma (CA) on inhaled corticosteroid, symptomatic asthma (SA) and those with exacerbation of asthma (EA)**

<table>
<thead>
<tr>
<th></th>
<th>NC N = 72</th>
<th>CA N = 15</th>
<th>SA N = 16</th>
<th>EA N = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCC 10⁶ cells / ml</td>
<td>1.5 (0.8, 3.9)</td>
<td>1.9 (1.0, 7.5)</td>
<td>1.7 (0.9, 4.1)</td>
<td>2.5 (1.6, 5.4)</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>0.3 (0, 1.05)*</td>
<td>2.5 (1.5, 0.75)</td>
<td>3.8 (2.4, 15.1)</td>
<td>8.5 (1.5, 20.0)</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>35 (12.0, 88.0)</td>
<td>46.5 (29.5, 58.5)</td>
<td>47.0 (24.8, 57.8)</td>
<td>27.0 (22.5, 42.0)</td>
</tr>
<tr>
<td>Epithelial cell %</td>
<td>1.5 (0.8, 3.0)</td>
<td>10.5 (5.0, 17.5)</td>
<td>11.5 (5.5, 21.3)</td>
<td>18.0 (6.0, 28.0)</td>
</tr>
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</table>

Values are presented as median and interquartile range, or as absolute number or percentage. *p=0.0005, using Kruskal Wallis test. N = 47 for total cell count (TCC) from normal subjects.
are still unclear. In order to incorporate sputum induction as a cost effective tool in our routine clinical practice, the time and manpower needed for processing have to be shortened and this is another major challenge to be resolved.

Sputum induction in its current state is generally considered as a useful adjunct in the management of asthma.

References


