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Induced sputum in childhood asthma

兒童哮喘的誘導痰液

Asthma is characterised by variable degrees of airway obstruction, airway hyper-responsiveness, and chronic airway inflammation. Current guidelines emphasise that inhaled corticosteroid treatment is the mainstay of asthma therapy because it targets the underlying airway inflammation. It is prudent to use the lowest possible dose of inhaled corticosteroid compatible with good asthma control. In clinical practice, the use of or the reduction of inhaled corticosteroid dosage is based on symptoms and lung function, both of which have been shown to have a poor correlation with airway inflammation. The use of induced sputum as a marker of airway inflammation improves asthma monitoring and optimises treatment in adults. This review discusses the technique of sputum induction, its clinical application, and our experience of its use in asthmatic children.

哮喘包括不同程度的氣道阻塞、氣道反應過敏，以及慢性氣道炎症。皮質類固醇吸劑用於防治最根本的慢性氣道炎症，是目前主要的哮喘療法。謹慎的處理方法是盡可能以最少量的皮質類固醇吸劑以有效控制病情。臨床方面，使用或減低使用皮質類固醇吸劑，要視乎病人的症狀和肺功能，但兩者跟氣道炎症的相關性不大。以誘導痰液來識別成年患者是否出現氣道炎症，能更有效監察哮喘的病情並提升治療效果。本文回顧誘導痰液的技術、其臨床應用，以及分享把技術用於哮喘患兒的經驗。

Introduction

Asthma is characterised 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 symptom assessment, results of peak expiratory flow rate or simple spirometry, and the frequency of use of rescue medication. However, abnormal airway physiology is not often present, even in cases of severe asthma.[5-7] In addition, these measures do not correlate closely with the underlying eosinophilic airway inflammation that is a predictor of asthmatic exacerbation and precursor of airway remodelling.[8] In selected published studies, up to 80% of corticosteroid-naive subjects[9-11] and more than 50% of corticosteroid-treated subjects[12] with concurrent symptoms had a sputum eosinophil count outside the normal range. Thus the monitoring of sputum eosinophil counts may allow better asthma control and provide a useful guide to management. Recent evidence suggests that a treatment strategy directed at normalisation of the airway eosinophil count reduces asthma exacerbations and hospital admissions.[9] Current research and reviews of asthma management have also highlighted the potential use of sputum induction as a non-invasive method of assessing airway inflammation.[13] This review examines the methodology of sputum induction in children, the clinical application of the technique, and its associated problems.

Key words:
Asthma;
Child;
Eosinophils;
Sputum

關鍵詞：
哮喘；
兒童；
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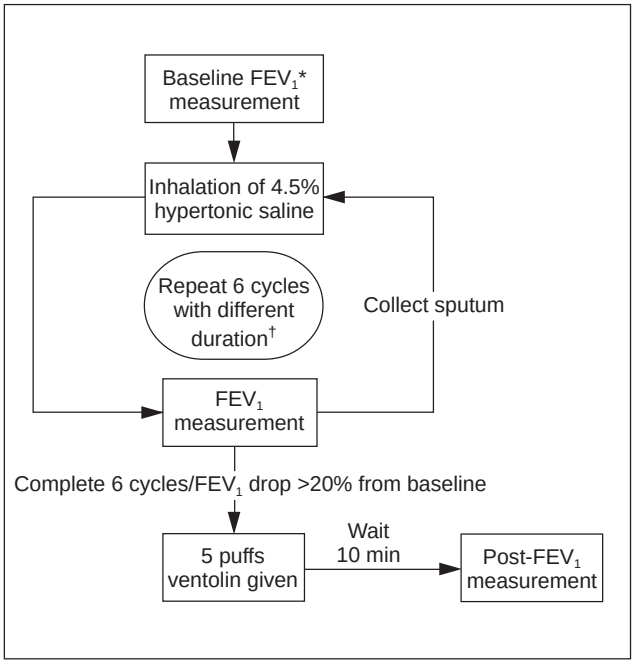
Methods for sputum induction and processing

Studies in children indicate that sputum induction can be safely performed over the age of 6 years with reported success rates between 68% and 100%.[13] Such variable success may be due to differences in the methods used. Sputum induction in younger children is limited by their poor spirometric technique and low tidal volume. This limits the dose of saline that can be delivered.[14] In most study series utilising sputum induction, the procedure was well tolerated by children. Possible side-effects included cough, bronchospasm, vomiting, and anxiety.[15-17] The procedure is equally well tolerated by children with severe asthma and those with an acute exacerbation.[18]

Combining hypertonic saline (HS) challenge with sputum induction allows simultaneous assessment of bronchial hyper-responsiveness and degree of airway inflammation. In adult patients, HS challenge is sensitive and reliable, and correlates better with serum markers of inflammation than methacholine challenge.[19] It is unknown if the same holds true for childhood asthma. The reported success rate of the combined technique in obtaining adequate sputum for the preparation of good-quality cytospin slides for differential cell counts is lower than that achieved for sputum induction alone.[20]

We carried out sputum induction in a dedicated area with negative pressure facilities. Prior to induction, the patient was thoroughly examined and the body temperature was measured. A list of screening questions was also checked to ensure that the patient was not suffering from any infection. In our experience, success was greatly enhanced by thoroughly explaining the procedure (Fig 1) and teaching the child the correct coughing and expectoration technique. Relatives and parents were asked to wait outside the laboratory to minimise interference. Because inhalation of HS may lead to bronchoconstriction, some protocols incorporated pretreatment with a beta-agonist (salbutamol). In our practice, a doctor was usually present during the process and bronchodilators together with other essential resuscitation equipments were readily available.

Sputum induction was performed following inhalation of 4.5% HS through a mouthpiece and a large one-way non-rebreathing valve (Hans Rudolph 2700; Hans Rudolph Inc, Kansas City, United States) connected to a DeVilbiss ultrasonic nebuliser set at the maximum output. The child was asked to rinse



* FEV_1 forced expiratory volume in 1 second
† Duration: 30 s, 1 min, 2 min, 4 min, 4 min, 4 min

Fig 1. The suggested procedure for combined hypertonic saline challenge and sputum induction

his mouth with water to clear debris and squamous epithelial cells. A nose-clip was worn and baseline forced expiratory volume in 1 second (FEV_1) measured. Sputum induction was performed provided FEV_1 was at least 65% predicted using local reference values. The child inhaled HS for a period of 30 seconds and lung function testing was repeated 1 minute later. If no sputum was obtained and lung function remained greater than 80% of the baseline value, the test continued. The child then continued inhalation of HS for periods of 1 minute, 2 minutes, and then three periods of 4 minutes each. He/she was encouraged to cough up any sputum after each dose of HS. A sample of sputum was collected in a specimen bottle, kept at 4°C, and processed within 2 hours. A record of any side-effects experienced by the child undertaking the test and repeated measurements of FEV_1 were made at the end of each elapsed inhalation time period. The study concluded when the child developed troublesome symptoms, when lung function dropped below 80% of the baseline value, or if the child could not be persuaded to complete the whole inhalation procedure. If the child had a greater than 20% drop in FEV_1, 0.5 mg of salbutamol was administered using a metered dose inhaler with a spacer, and recovery monitored. The subject was allowed to leave only when FEV_1 had returned to the baseline value. The procedure was stopped if no sputum was obtained after 20 minutes of inhalation despite stable FEV_1.

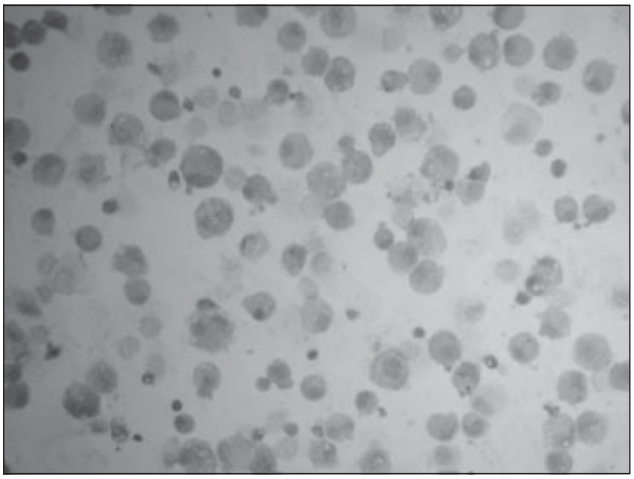


Fig 2. High-quality cytospin slide for differential cell counts
400 Non-squamous cells are counted and the result is expressed as percentage of eosinophils

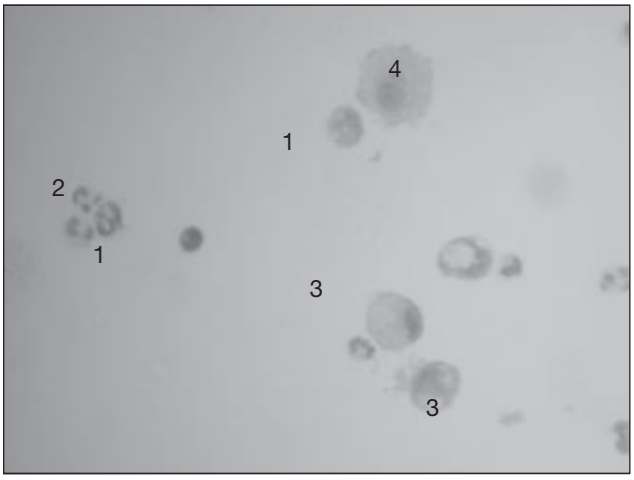


Fig 3. Differential cell counts
1: eosinophil; 2: neutrophil; 3: macrophage; 4: epithelial cell

The selected sputum plug method and entire sputum method have been described for sputum processing.[13] The first involves collecting and analysing the more viscid portions of mucus (plugs) extracted from the entire sputum as described by Popov et al.[21] The second involves collecting and analysing the entire sputum, including saliva, as described by Fahy et al.[22] The use of a dissecting microscope or simply a pair of forceps has been described in the selected sputum plug method. Nonetheless, no studies have 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.[23,24] It is also the method used by most paediatric centres including ours. The volume of the selected sputum is measured and 0.1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) added to the sputum in a 4:1 ratio to break up the disulphide bonds and disperse the cells. The cell suspension is aspirated until homogenised 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 400 g for 10 minutes and cytospins made and stained by May-Griunwald Giemsa stain. Four hundred non-squamous cells are counted: an adequate sample is defined as less than 50% squamous cells (Figs 2, 3). The eosinophil count is then expressed as a percentage of the total cell count.

From December 2003 to April 2004, the authors' unit performed sputum induction in 60 asthmatic children aged from 7 to 17 years with a mean age of 11.9 years. Ten were corticosteroid naive. The range of Becotide equivalent dosage of inhaled corticosteroids being used by the subjects was 50 μg to 1 mg. None of the subjects used a long-acting beta-agonist or leukotriene antagonist. All children could perform consistent FEV_1 before the procedure and all had mild or moderate persistent asthma with an FEV_1 range of 70% to 100% predicted. Of 60 children, 59 completed the procedure and one failed to finish the procedure because of the 'unpleasant taste of the HS aerosol'. Adequate sputum sample was obtained in 45 (75%) children and none had to terminate the procedure because of side-effects. These results are similar to those reported in the literature and reiterate the feasibility and safety of this procedure in children with asthma.

Clinical application of sputum induction

By assessing the degree of airway inflammation and targeting treatment in relation to response, it is possible to avoid exposing children to unnecessarily high doses of inhaled corticosteroids and the associated side-effects. Thus, the greatest clinical application of sputum induction is to non-invasively study the extent of airway inflammation in children: the use of bronchoscopy or biopsies is limited due to ethical and safety reasons.[25-29] The use of this technique may further provide important insight into the pathology and mechanism of asthma and determinants of severity.[25] Sputum induction also allows the analysis of mediators including proteins and cytokines that are present in the fluid phase of the sputum sample.[22,26] Among the various cellular markers found in sputum,

Table 1. Sputum cell counts* for normal, atopic normal, and non-atopic normal children[30]

	Normal	Atopic normal	Non-atopic normal
Total cell count ($x10^6$ /mL)	5.14 (1.20-9.08); 1.5 (0.8-3.9)	1.75 (0.89-2.60); 1.00 (0.55-2.15)	8.04 (0.63-15.5); 1.80 (1.05-6.00)
Eosinophils (%)	1.57 (0.62-2.52); 0.30 (0-1.05)	2.16 (0.83-3.48); 0.5 (0-2.8)	1.13 (0-2.54); 0 (0-0.6)
Mast cells (%)	0.024 (0-0.050); 0 (0-0)	0.03 (0-0.07); 0 (0-0)	0.02 (0-0.06); 0 (0-0)

* Data are presented as mean (95% confidence interval); median (interquartile range)

Table 2. Sputum cell counts* in normal subjects (NC), controlled asthma (CA) on inhaled corticosteroids, symptomatic asthma (SA), and those with exacerbation of asthma (EA)[30]

	NC, n=72	CA, n=15	SA, n=16	EA, n=11
Total cell count ($x10^6$ /mL)	1.5 (0.8-3.9)†	1.9 (1.0-7.5)	1.7 (0.9-4.1)	2.5 (1.6-5.4)
Eosinophils (%)	0.30 (0-1.05)‡	2.5 (1.5-0.75)	3.8 (2.4-15.1)	8.5 (1.5-20.0)
Neutrophils (%)	35.0 (12.0-88.0)	46.5 (29.5-58.5)	47.0 (24.8-57.8)	27.0 (22.5-42.0)
Epithelial cell (%)	1.5 (0.8-3.0)	10.5 (5.0-17.5)	11.5 (5.5-21.3)	18.0 (6.0-28.0)

* Data are presented as median (interquartile range)
† n=47
‡ P=0.0005, using Kruskal-Wallis test

sputum eosinophilia is well validated as a marker of airway inflammation.[13,26] Cai et al[30] have established the reference range of sputum eosinophil for both normal and asthmatic children: the upper limit of normal for sputum eosinophils is 2.5% (Table 1). They have also compared the sputum cell counts in normal subjects, controlled asthma on inhaled corticosteroids, symptomatic asthma, and those with exacerbation of asthma (Table 2). Eosinophils accounted for a median of 0.30% (interquartile range [IQR], 0-1.05%) of cells in sputum from healthy children. Sputum eosinophils (median, 4.3%; IQR, 1.5-14.1%; P=0.0005) and epithelial cell counts (median, 14%; IQR, 6.0-19.4%; P=0.0005) were significantly higher in children with asthma than that 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.[30] Sputum eosinophil counts correlate well with asthma severity in terms of the degree of airway inflammation and variability in expiratory flows.[11,13,31,32] In addition, studies have demonstrated fairly good agreement between eosinophil counts from sputum, bronchoalveolar lavage, and bronchial biopsies in asthmatic patients. The percentage of eosinophils in sputum was significant when correlated with their percentage in bronchial biopsy sample (R_S=0.52, P=0.03) and in bronchoalveolar lavage (R_S=0.55, P=0.02).[33]

Sputum eosinophil counts in asthmatics change substantially in response to different doses and duration of inhaled or oral corticosteroids.[31] In a study by Green et al,[9] a higher level of sputum eosinophils correlated with greater airway obstruction. The increased sputum eosinophils were considered a predictor of asthma exacerbations. Apparent stable asthmatics with high eosinophil counts were more likely to develop an exacerbation on reduction of their regular corticosteroid therapy. The extent of control of eosinophilic airway inflammation and exacerbation was greater in the sputum management group, which aimed to lower airway inflammation, than in the British Thoracic Society management group. The authors concluded that a treatment strategy targeted at normalisation of sputum eosinophilia would reduce asthma exacerbations and improve asthma management.[9] A similar study has not been carried out in children but the same theoretical rationale should apply.

Childhood chronic cough is a common and troublesome problem. It is a non-specific symptom 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 a sputum eosinophil count of greater than 3%.[34,35] The latter responds well to a bronchodilator and oral or inhaled corticosteroids.[36-38] Sputum induction in this scenario is a useful and cost-effective tool for diagnosing children with chronic cough who will respond to anti-asthma therapy.

Problems in sputum induction for clinical use

There are three main problems associated with the use of sputum induction in clinical practice. First, it is a time-consuming procedure. Second, sputum needs to be processed within 2 hours of induction. Methods of preserving sputum beyond this time without compromising validity are still under investigation. Third, the need for laboratory support limits its use as a routine clinical procedure. The European Respiratory Society Task Force provided a review as well as recommendations concerning the induction protocol, safety aspects, processing, and analysis of sputum samples.[39] Nonetheless, many issues concerning the technique itself and the interpretation of results including validation of fluid phase markers and possibilities in automating the procedure remain unclear. In order to incorporate sputum induction as a cost-effective tool in routine clinical practice, the time and manpower needed for processing have to be shortened. This is another major challenge. Sputum induction in its current state is generally considered a research tool only, but it is rapidly becoming recognised as a useful adjunct to the management of asthma.

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