

Noninvasive Monitoring of Airway Inflammation in Childhood Asthma

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Abstract

Airway inflammation plays a central role in the pathogenesis of asthma. Accurate assessment of the degree of airway inflammation may allow us to fine-tune the anti-inflammatory treatment. There are many methods of assessing the degree of airway inflammation such as bronchial biopsy and broncho-alveolar lavage. These methods, however, are invasive and can be very difficult to perform in children. Monitoring of induced sputum eosinophils and measurement of bronchial hyperresponsiveness has been found to be useful in adjusting asthma treatment. However, these measurements are not easily performed in the primary care setting. A simple noninvasive measurement of inflammation would be extremely useful to guide individual asthma therapy in the childhood population. Exhaled nitric oxide (eNO) has been found to be measurable in exhaled air and its level has been found to be increased in asthmatic. Use of inhaled corticosteroid can reduce asthma symptoms and eNO. Accurate and reliable tools in measuring eNO are now commercially available. Many research studies in the past few years have confirmed that eNO can be used for the diagnosis, monitoring of control, and guiding treatment of anti-inflammatory therapy. Recent prospective studies have suggested that the addition of eNO monitoring would allow clinicians to use lower dose of inhaled steroid without compromising asthma control. Analyses of exhaled breath condensate (EBC) have been extensively investigated as another possible way to monitor airway inflammation. A variety of markers of inflammation can be measured in the EBC such as cytokines, chemokines, leukotrienes, and hydrogen peroxide. Further research is necessary to standardise the methodology of collection of EBC. Prospective trials are needed to confirm that the additional monitoring of these markers in the EBC may result in better control of asthma and optimal dosing of anti-inflammatory therapy in asthmatics.

Key words Asthma; Child; Monitoring; Noninvasive

Introduction

Airway inflammation is one of the most important components in the pathogenesis of asthma and the cornerstone of treatment of asthma is the use of anti-

inflammatory therapies. The severity of airway inflammation is closely associated with atopy and airway hyperresponsiveness (AHR). Inflammatory cells such as eosinophils and their mediators are consistently identified in asthmatic but not healthy airways, and these cells play a central role in mediating airway remodeling in asthma.¹ Various inflammatory cytokines and chemokines derived from type 2 T-helper (Th2) lymphocytes can also be identified in the asthmatic airways. Traditionally, asthma control is usually monitored by means of assessment of clinical symptoms along with lung function tests. Over the past two decades, there are an increasing numbers of studies evaluating the clinical utility of biomarkers of airway

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Received August 31, 2006

inflammation. The use of bronchoalveolar lavage or biopsy taken during bronchoscopy is rather invasive and these procedures are difficult to perform in young children. Recently, there have been rapid advances in the development of noninvasive techniques to evaluate airway inflammation such as the use of induced sputum, exhaled breath analysis for nitric oxide, and assessment of markers of inflammation in exhaled breath condensate. This paper summarises the recent advance in the development of these noninvasive methods in the clinical management of asthmatic children.

Asthma and Exhaled Nitric Oxide

Among all the methods of assessment of airway inflammation, the measurement of expired gases is the most non-invasive one. Exhaled nitric oxide (eNO) was first demonstrated to be elevated in asthmatics when compared to healthy controls approximately 10 years ago.² The major advantage of eNO measurement is that results can be obtained instantaneously in cooperative subjects, and even in young children. A device to measure eNO is has been approved by the Food and Drug Administration for clinical use.³ Several recent studies have been directed at determining the relation of eNO and other markers of asthmatic inflammation.⁴⁻⁶ Improvement of asthma with the use of inhaled corticosteroid was found to reduce eNO levels in asthmatic patients.⁵ Exhaled NO levels also have been found to correlate with other inflammatory markers such as blood eosinophil count and serum eosinophilic cationic protein.^{5,6} Other recent studies have demonstrated that eNO measurement might be useful for the diagnosis of asthma.^{7,8}

Payne and colleagues assessed the relation between eNO and eosinophilic inflammation in endobronchial biopsies from 31 children with difficult asthma. There was a correlation between eNO and histological 'eosinophil score'.⁹ Using this non-invasive technique, Jones et al reported that eNO was useful in predicting loss of control in mild-to-moderate asthma when corticosteroid was withdrawal. Changes in eNO correlated significantly with asthma symptoms, lung function, sputum eosinophils and degree of AHR.¹⁰ Another study found that eNO significantly correlated with annual rate of asthmatic exacerbation.¹¹

There have been a few studies of reference norms for exhaled nitric oxide in children using standardised methodology in Caucasian children.¹² Among Asian children, there was only one small study (n=215) of healthy Japanese children showing a mean eNO of 25.2 ppb.¹³ We

have performed a reference study aiming at establishing the normal range of eNO in Chinese adolescents.¹⁴ Boys were found to have higher eNO levels than in girls. The mean eNO level in Chinese boys was 17 ppb while it was 10.8 in girls. These levels were higher than those reported in Caucasian children. A subsequent larger study was performed with more than 1000 Chinese children in order to determine the cut-off values to differentiate asthmatics from normal controls.¹⁵ Using a cut-off of 15 ppb for girls, the sensitivity and specificity for differentiating asthma from controls are 86% and 78%; for boys, the sensitivity and specificity are 80% and 79% using a cutoff of 25 ppb. There have also been several recent trials confirming the utility of using eNO measurement in routine practice. Smith et al. performed a clinical trial with 97 asthmatic adults divided into two groups.¹⁶ One group was treated conventionally with adjustment of ICS according to symptoms and lung function while the other group had additional information of regular exhaled nitric oxide measurements. With the additional use of eNO measurements, maintenance doses of inhaled corticosteroids can be significantly reduced without compromising asthma control. Although the rate of exacerbation was lower in the group with eNO measurement, the difference did not reach statistical significance. Another similar study was performed in 85 children reaching similar conclusions.¹⁷ In summary, the measurement of exhaled nitric oxide appears to be a simple and promising tool for noninvasive assessment of airway inflammation. Such measurement may help to obtain the optimal level of control with the lowest possible dosage of anti-inflammatory therapy.

Induced Sputum Analysis

Sputum induction has been widely used as a research tool in the past decade to study the pathophysiology of asthmatic airway inflammation. Subjects were treated with nebulised hypertonic saline which triggers the production of sputum. It can also result in bronchoconstriction indirectly by causing mediator release from inflammatory cells and airway smooth muscle constriction. The most important step is to develop the normal reference ranges of the different cell type before the technique can be applied to clinical use. A recent study in 66 non-smoking volunteers reported that sputum neutrophil and macrophage counts correlated significantly with the age of the subjects, highlighting the need for age matching in studies that measure biomarkers in induced sputum.¹⁸

Green and colleagues have performed a study to evaluate a management strategy that minimises eosinophilic inflammation compared with a standard strategy. In this study of one year duration, they recruited 74 adult patients with moderate to severe asthma. The sputum eosinophil count was 63% lower over 12 months in the sputum management group than in the conventional management group. Furthermore, the frequency of asthma exacerbations and related hospitalisation was lower in the sputum management group when compared with those in the conventional management group.¹⁹ This study suggests the addition of assessment of sputum may improve the care of asthma patients by fine-tuning the dosage of inhaled corticosteroid.

In addition to the measurement of differential cell count, researchers have also studied the various biomarkers in the cell-free supernatant of induced sputum. The biomarkers that have been studied include leukotrienes, lipoxins, isoprostane, nitrite, cytokines, chemokines, and various growth factors. Further research is needed to determine which biomarkers or combinations of biomarkers are useful and practical for clinical monitoring of airway inflammation in asthmatic children. Although the procedure is relatively safe, it is not particularly pleasant for children. In the Childhood Asthma Management Program study,²⁰ 90 of 117 children (77%) were able to provide an adequate sputum sample for analysis, and the procedure resulted in bronchospasm in 9 patients. Higher sputum eosinophils was associated with atopy, higher bronchodilator reversibility, lower FEV₁/FVC ratio, sputum and serum eosinophil cationic protein, more prednisone courses during the treatment period, and greater asthma severity. Depending on the availability of technical support, it may take 2 to 3 hours to process the sputum samples. For measurement of other biomarkers in the supernatant, samples will need to have batch analyses such that the results may not be available for the adjustment of medication.

Biomarkers in Exhaled Breath Condensate

Exhaled breath condensate (EBC) is a rapidly growing field of research in respiratory medicine. The first study identifying surface-active properties of EBC was published in Russia in 1980. EBC 'inflammometer', being totally non-invasive, repeatable and easily to perform, is particularly suitable for children. Molecules up to 65 kDa (e.g. cytokines, leukotrienes) have been identified in EBC.¹⁶ The collection of EBC can either be done using commercial

devices (e.g. EcoScreen, RTube) or by home-made appliances that cool expired air to between -10 to -20°C. The concentrations of biomarkers in condensed exhaled breath thus obtained can then be measured.

We have studied the presence of thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC), two Th2-specific chemokines, as well as the eosinophil-specific eotaxin in EBC from asthmatic and non-allergic children. MDC and eotaxin could be detected in nearly all the EBC samples whereas TARC was present in quantifiable amount only in about one-third of them as measured by commercial enzyme immunoassays. Of the three chemokines studied, MDC was higher in EBC from asthmatic children as compared to non-allergic controls. Children with persistent asthma receiving high-dose inhaled corticosteroids also had lower exhaled MDC concentrations. The levels of MDC and eotaxin in EBC appeared to be quite reproducible.²¹ We have also confirmed that leukotriene B4 levels in EBC are increased in Chinese asthmatic children.²²

The measurement of the level of acidity of EBC in adults and children has also been found to be useful in assessing asthma severity.²³⁻²⁵ Low EBC pH is found in patients with asthmatic exacerbation as well as chronic stable asthma. However, the major problem with the assessment of EBC is standardisation as well as the low concentration of various biomarkers due to the dilution process as they dissolved in the EBC. Many of the commercially available assay kits are not sensitive enough to detect the biomarkers in question. Further development of highly sensitive and reproducible detection systems is necessary to overcome these limitations. In the meantime, EBC remains to be a very useful research tool. Prospective clinical trials are needed to evaluate whether the addition of EBC measurement may improve the clinical control of asthmatic patients.

Conclusions

Over the past decades, there have been rapid advances in the understanding of the inflammatory process in the asthmatic airways. Optimal control of asthma will rely on adequate control of airway inflammation with controller drugs. The measurement of different biomarkers in exhaled breath and induced sputum represent an emerging tool for accurate assessment of airway inflammation and asthma control. Both eNO measurement and sputum analysis have been found to be useful clinically to predict asthmatic

exacerbation. The former has the advantages that it is simple and non-invasive, and the results are obtained instantaneously. Prospective trials have shown that eNO measurement may be helpful to obtain similar control with lower dosage of ICS treatment. The collection of induced sputum is more labour-intensive and may cause bronchospasm in a small percentage of the subjects. The assessment of biomarkers in EBC is a simple and interesting research tool to assess the degree of airway inflammation. Further prospective clinical trails are needed to confirm the clinical utility of EBC measurement in the day-to-day management of asthmatic patients.

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