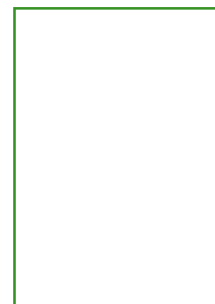




Biomarkers for Asthma: Are They Useful Clinical Tools?

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Introduction

Airway inflammation is a key component in asthma, and the former is closely associated with atopy and airway hyperresponsiveness (AHR). Eosinophils and their mediators are consistently identified in asthmatic but not healthy lungs, and these cells play an important role in mediating airway remodelling in asthma.¹ The asthmatic airway is also characterised by a predominance of cytokines and chemokines derived from type 2 T-helper (Th2) lymphocytes. Asthma control is conventionally monitored by means of clinical symptoms and lung function tests, particularly FEV₁ and FEV₁/FVC. There is growing interest in determining the role of biomarkers in asthma care. Initial studies characterising asthma-related airway inflammation have relied upon the relatively invasive technique of bronchoalveolar lavage taken during bronchoscopy. Various emerging techniques are also available to evaluate airway inflammation (e.g. induced sputum, exhaled breath analysis for nitric oxide and other molecules). This article summarises the usefulness and pitfalls of these latter methods in the clinical management of asthmatic patients.

Asthma and exhaled nitric oxide

Studies in adults have shown that improved asthma control can be attained by use of AHR to methacholine² to modulate asthma therapy rather than waiting for clinical symptoms to appear or pulmonary functions to deteriorate. Nevertheless, bronchial provocation is contraindicated in asthmatics with significant airflow limitation because this procedure may induce severe bronchospasm. In the recent decade, the application of biomarkers to assessment of asthma management has become more widespread. Exhaled nitric oxide (eNO), being totally non-invasive, is the most widely used method in assessing airway inflammation. Results can be obtained instantaneously in cooperative subjects, and even in young children. A device to measure eNO is now Food and Drug Administration approved for use.³ Clinicians are questioning the applicability of measuring eNO in clinical practice, as well as the interpretation of results.

Nitric oxide is produced from arginine by nitric oxide synthases³, and eNO is significantly increased in asthma.^{3,4} Exhaled NO measurement was found to be superior to conventional tests (e.g. bronchial provocation, spirometry, bronchodilator responsiveness, peak expiratory flow rate measurement) in diagnosing asthma.⁵ 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'.⁶ 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 withdrawn. Changes in eNO

correlated significantly with asthma symptoms, lung function, sputum eosinophils and degree of AHR.⁷ Another study found that eNO significantly correlated with annual rate of asthmatic exacerbation.⁸

One important finding to note is that eNO in our local Chinese children were found to be significantly higher than the values measured in age-matched Caucasian children living in Hong Kong.⁹ Because of similar environmental influences, this finding may be explained by differences in the genetic regulation of NO production. However, there is still limited data on the reference values of eNO in Chinese subjects. Clinicians should thus be cautious when interpreting eNO results in our local population. At present, it would be more useful and practical to apply this technique as a monitoring tool in the serial assessment of airway inflammation in asthmatic patients.

Induced sputum analysis

Sputum induction is increasingly used as a research technique and as a clinical tool. Hypertonic saline is delivered using a nebuliser, with subjects pretreated with β_2 -agonist to minimise bronchospasm.^{10,11} This technique can also be used to measure AHR. Hypertonic saline results in bronchoconstriction indirectly by causing mediator release from inflammatory cells, which then activate airway smooth muscle. As with other biological measurements, it would be crucial to establish normal ranges of differential cell count in induced sputum before this technique can be applied clinically with confidence. 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.¹²

Treatment decisions in asthma are conventionally based on assessments of symptoms and spirometry, which do not relate closely to underlying eosinophilic airway inflammation. Green and colleagues assessed whether a management strategy that minimises eosinophilic inflammation reduces asthma exacerbations compared with a standard strategy. Among 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. The former group of patients had significantly fewer severe asthma exacerbations and related hospitalisation than did patients in the conventional management group.¹³

Apart from measuring differential cell count, a number of studies suggested significant association between asthma and biomarkers in the cell-free supernatant of induced sputum (Table 1). It is appealing that sputum analysis is a useful tool





in assessing asthma control. Birring et al elaborated on this issue by comparing airway inflammation in patients with chronic cough and asthma. The concentrations of histamine, cysteinyl-leukotrienes, prostanoids, and interleukin-8 were measured in induced sputum supernatants. Sputum histamine concentrations were significantly higher in patients with idiopathic chronic cough and cough variant asthma/eosinophilic bronchitis than in normal subjects, whereas sputum prostanoids were significantly higher in all categories of chronic cough. The findings cast doubt on the clinical application of sputum analysis in asthma diagnosis because there might be similarities in the mechanism of cough in a diverse range of conditions.¹⁴

Table 1 Biomarkers in induced sputum and EBC that are reported to be associated with asthma

Biomarker	Induced sputum	EBC
Differential cell count	✓	×
Acidity (pH)	×	✓
Ammonia	×	✓
Leukotrienes (e.g. LTB ₄ , cysteinyl-LTs)	✓	✓
Lipoxins (e.g. LXA ₄ , 15-epi-LXA ₄)	✓	×
Oxidative stress (e.g. hydrogen peroxide, 8-isoprostane, aldehydes)	✓	✓
Anti-oxidant status (e.g. glutathione)	✓	✓
Nitrite / nitrate / nitrotyrosine	✓	✓
Adenosine	×	✓
Cytokines (e.g. IL-4, IL-6, IL-8, IL-13, IL-18)	✓	✓
Chemokines (e.g. eotaxin, TARC)	✓	×
Chemokines (e.g. MDC)	×	✓
Vascular endothelial growth factor	✓	×
Platelet-derived growth factor	×	✓
Matrix metalloproteinase-9	✓	×
Plasma proteins (e.g. albumin, fibrinogen)	✓	×
Eosinophil cationic protein	✓	×
Soluble E-cadherin	✓	×
Temperature	×	✓

IL, interleukin; LT, leukotriene; LX, lipoxin; MDC, macrophage-derived chemokine; TARC, thymus and activation-regulated chemokine.

✓ : Association reported; ×: Association either nil or not reported.

The safety issue of sputum induction in paediatric asthma has recently been addressed in the Childhood Asthma Management Programme study.¹⁵ Ninety of 117 children provided an adequate sputum sample for analysis, and sputum induction resulted in bronchospasm in 9 patients. These subjects had more severe disease. Higher sputum eosinophils was associated with atopy, higher bronchodilator reversibility, lower FEV₁/FVC ratio, higher eNO, circulating eosinophils, sputum and serum eosinophil cationic protein, more prednisone courses during the treatment period, and greater asthma severity.

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.

Recently, my research group found that plasma levels of thymus and activation-regulated chemokine (TARC), a Th2-specific chemokine, were increased in children with chronic stable asthma.¹⁷ Among children with asthmatic exacerbation, plasma TARC and macrophage-derived chemokine (MDC) levels also correlated with their peak expiratory flow rates at hospitalisation.¹⁸ Subsequently, our group studied the presence of these two Th2 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.¹⁹ Besides, my group has recently reported leukotriene B₄ levels in EBC to be increased in local children with asthma.⁴

Table 1 provides an overview of the EBC biomarkers reported to be associated with asthma. In contrast to the inflammatory mediators, one can readily and instantaneously measure the acidity of EBC in adults²⁰ and children²¹ using small online pH meters. Although highly reproducible, EBC pH is not a direct measure of acidity in the airway lining fluid but rather an integrated signal representing excessive production and volatilisation of acid from one or more locations in the airway. Low EBC pH is found in patients with asthmatic exacerbation as well as chronic stable asthma.

Despite these attractions and the apparent simplicity of EBC collection, there are at present many methodological problems associated with this emerging, non-invasive method. Comparison and interpretation of data on this rapidly growing field is hampered mainly by the lack of standardisation on collection methods and conditions, unavailability of specific high-sensitivity detection assays and poor reliability of biomarker measurement. More sensitive and reproducible detection systems are required to overcome these limitations. Overall, EBC remains at present to be a research tool for asthma. Clinical trials should be performed in the near future to investigate the efficacy of treatment-decision algorithms that based on changes in EBC biomarkers on the clinical outcomes of patients with asthma.

Conclusions

Biomarkers in exhaled breath and induced sputum represent an emerging approach in assessing airway inflammation and asthma control. Both eNO measurement and sputum analysis are found to be useful clinically to predict asthmatic exacerbation. The former has the advantages that it is totally non-invasive, and patients require little effort to generate the results instantaneously. Thus, eNO measurement can be considered as a clinically useful tool in asthma management. Sputum induction and processing, on the other hand, is relatively labour-intensive, and this technique may also induce bronchospasm. The quantification of biomarkers in EBC is a novel monitoring tool in respiratory and inflammatory research. More than a dozen biomarkers are reported in EBC from asthmatic patients. However, none of these has been shown to be able to reflect clinical asthma control or severity at present.





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