

Pediatric biomarkers in asthma: exhaled nitric oxide, sputum eosinophils and leukotriene E4

Shaila Gogate and Rohit Katial

National Jewish Medical and Research Center, Denver, Colorado, USA

Correspondence to Rohit Katial, MD, Associate Professor of Medicine, Director, Allergy and Immunology Training Program, and Director, Weinberg Clinical Research Unit, National Jewish Medical and Research Center, 1400 Jackson St, Denver, CO 80206, USA
Tel: +1 303 270 2498; e-mail: katialr@njc.org

Current Opinion in Allergy and Clinical Immunology 2008, 8:154–157

Purpose of review

Markers of disease status that provide a numerical measure of disease activity, biomarkers, have come into more routine use in medicine. This is evidenced by troponin and brain natriuretic peptide when measuring cardiac function or glomerular filtration rate in relation to kidney function. Similar markers to assess inflammation in the asthmatic lung have emerged as possible tools to guide treatment. Three biomarkers, fractional exhaled nitric oxide, sputum eosinophils and leukotriene E4 in the urine and exhaled breath condensate, have been heavily investigated.

Recent findings

Recent literature indicates that exhaled nitric oxide, sputum eosinophils and leukotriene E4 in the urine, and exhaled breath condensate could serve as good markers of inflammation in the asthmatic airway. These markers, when combined with conventional measures of lung function – forced expiratory flow in 1 s, peak flow or methacholine challenge – will be of benefit in improving asthma control in the pediatric population.

Summary

Exhaled nitric oxide and urinary leukotriene E4 are relatively easy to attain in the clinical setting. Sputum eosinophils are an excellent tool for assessing inflammation, however sputum induction can be challenging for a young child. Despite small limitations, all three biomarkers are potentially valuable when used in conjunction with conventional methods for airway control.

Keywords

biomarkers, eosinophils, exhaled nitric oxide, pediatric asthma

Curr Opin Allergy Clin Immunol 8:154–157
© 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins
1528-4050

Introduction

The recent update of the National Asthma Education and Prevention Program (NAEPP) guidelines highlights the need to assess asthma control, however, this is especially challenging in the pediatric population. Conventional methods for determining control (spirometry, peak flow and clinical symptoms) have limitations in a young child. Although asthma is an inflammatory disease of the airway, we currently do not have a direct measure of airway inflammation but rather the above-mentioned tests are used as surrogate markers for inflammation in the asthmatic airway. More recently, noninvasive markers of airway inflammation, such as fractional exhaled nitric oxide, induced sputum eosinophils and urinary leukotriene E4 (LTE4) have been studied as potential tools for assessing airway inflammation. The goal of this review is to discuss the correlation of these markers with clinical symptom control and the conventional tests used to assess control.

Fractional exhaled nitric oxide

Nitric oxide is a gaseous signaling molecule that is present in the respiratory system. It functions as a signaling

molecule both in healthy and disease states. Nitric oxide is produced by nitric oxide synthase (NOS); three isoforms of the enzyme have been identified. Recent evidence supports upregulation of the NOS-2 isoform in the asthmatic airway, leading to increased production of nitric oxide from epithelial cells and alveolar macrophages [1]. After synthesis, nitric oxide diffuses into the airway lumen that is essentially nitric oxide free, thus leading to increased levels in the exhaled air of children and adults with asthma.

Nitric oxide can be measured using a chemoluminescence analyzer. The child inhales to total lung capacity (TLC) and exhales at a constant flow rate, 50 ml/s, until a plateau of greater than 2 s is reached [2]. The 2005 American Thoracic Society guidelines [3] suggest the use of a mouthpiece that excludes nasal nitric oxide by breathing against a resistor since the upper airway, particularly the paranasal sinuses, can generate a high concentration of nitric oxide. Handheld devices for measuring nitric oxide have recently been studied. A recent study by Menzies *et al.* [4•] demonstrated good correlation between the handheld device and the

nonportable chemoluminescence analyzer. Only one device for measuring exhaled nitric oxide (eNO), however, is currently approved by the US Food and Drug Administration (FDA), the Aerocrine Niox (Solna, Sweden).

One large study consisting of 522 school age children [5] has helped to establish the range of normal values of eNO. A value between 15 and 25 ppb is considered normal for children between the ages of 4 and 17. Buchvald *et al.* measured eNO in children from ages 4 to 17. They reported upper normal limits of eNO of 15 ppb in young children and 25 ppb in adolescents. Their study showed an increase in eNO by 1 ppb/year with age. Sixteen patients had eNO values of greater than 34.9 ppb; 88% of these children were older than 10 and had symptoms of rhinitis.

A number of studies have demonstrated the increased production of nitric oxide in the asthmatic airway and improvement of that value with use of inhaled corticosteroids. One of the earliest studies was by Byrnes and colleagues [6]; they examined nitric oxide levels in children with and without asthma. They measured eNO from 39 healthy children, 15 children with asthma on bronchodilator therapy alone and 16 children on regular use of inhaled corticosteroids. They found a statistical difference in eNO levels between the healthy controls and children with asthma not on a corticosteroid. The eNO was 49.6 ppb (SD 37.4) in normal children, while children with asthma not on a corticosteroid had a level of 126 ppb (SD 77.1). The eNO of children with asthma on a corticosteroid was similar to that of the normal controls, 48.7 ppb (SD 43.3). They concluded the concentration of eNO was similar between the healthy control and asthma group on corticosteroids, while it was statistically higher in the group with asthma not on a corticosteroid. A similar study performed by Kharitonov *et al.* [7] a few years earlier had similar findings. In a retrospective analysis, Panickar *et al.* [8] examined the effect on eNO after a single dose of intramuscular triamcinolone. One single injection of triamcinolone reduced eNO values and improved symptom scores for a period of 4 weeks.

eNO has also been correlated with asthma severity and asthma control days. Fitzpatrick *et al.* [9^{••}] used noninvasive markers of airway function and inflammation to assess asthma severity. Twenty-eight out of 75 children were followed for a period of 6 months after baseline characterization with methacholine challenge, eNO and lung function testing. The children with severe asthma had significantly higher eNO and aeroallergen sensitization. A recently published study by Miragalia *et al.* [10] investigated nitric oxide levels in the exhaled air of children on different combinations of formoterol, monte-

lukast and budesonide. Their study showed a decrease in eNO on combination therapy with montelukast and budesonide. In a study of school age children, however, Zeiger *et al.* [11^{••}] showed eNO improved significantly on fluticasone. Also eNO was a good predictor of asthma control days and differentiating asthma control days resulting from fluticasone versus montelukast.

Strunk *et al.* [12] examined the correlation of eNO to inflammatory markers in children with persistent asthma. The study enrolled nearly 150 children aged 6–18 with mild to moderate persistent asthma who were taking albuterol as needed. eNO along with spirometry pre- and postbronchodilator, peripheral blood eosinophil count, serum eosinophilic cationic protein, total serum immunoglobulin E and urinary leukotrienes were measured at the initial visit. A repeat measurement of eNO was taken 1 week after the initial visit. In addition, a methacholine challenge and aeroallergen skin prick testing was performed. eNO correlated with peripheral blood eosinophils, serum eosinophil cationic protein and the number of positive aeroallergen skin test. It only weakly correlated with the forced expiratory volume in 1 s (FEV₁)/forced vital capacity (FVC) ratio, bronchodilator response and methacholine PC20. Additionally, eNO did not correlate with clinical characteristics, such as age, symptoms and skin test reactivity or peak flow measurement.

eNO has shown to be correlated with eosinophilic inflammation as well. Silkoff *et al.* [13] sought to examine the relationship of persistent eosinophilia with eNO in the adult population. They measured eNO and then performed endobronchial biopsy and bronchoalveolar lavage on 24 patients with severe persistent asthma, 15 individuals with moderate–mild asthma and 17 healthy controls. The endobronchial biopsy samples were assessed for eosinophilic infiltration (EOS+). In patients with severe persistent asthma, eNO correlated with tissue eosinophilia with a correlation coefficient of 0.54 and a significant *P* value of 0.007. A similar study by Smith *et al.* [14] assessed eosinophilic inflammation in relation to eNO and steroid responsiveness. They enrolled 52 patients with undiagnosed respiratory symptoms, age range 12–70, in a single blind, fixed sequence, placebo-controlled trial of inhaled fluticasone for 4 weeks. They concluded eNO of greater than 47 ppb was associated with steroid responsiveness in patients with undiagnosed respiratory symptoms. A study by Pijnenburg *et al.* [15] specifically addressed this point. They enrolled 85 children with atopic asthma. The inhaled corticosteroid dose was titrated by either symptoms alone or symptoms in combination with fractional eNO level. The cohort monitored with eNO and symptoms did not show higher use of steroids and improved in airway hyperresponsiveness and inflammation compared with the group monitored with symptoms alone.

In summary, ENO is a good noninvasive marker for eosinophilic inflammation. When considered with more conventional measures of airway control such as FEV₁, it can be a powerful tool for improving asthma control and treatment.

Induced sputum eosinophil count

Induced sputum, though more invasive than eNO, can be easily attained in an appropriate setting from children with asthma. Induced sputum is usually attained by inhalation of hypertonic saline for no longer than 12–15 min. At 2–3 min intervals the child is asked to produce sputum, which is then collected. Close monitoring for FEV₁ and peak flow needs to be undertaken since inhalation of hypertonic saline can cause a decrease in pulmonary function.

Sputum eosinophils are a good marker for asthma control and their decrease in the sputum correlates well with control. Gibson *et al.* [16] recruited 146 children with asthma and 37 healthy controls for sputum induction. The frequency of asthma episodes increased during a 12-month period; the increase was mirrored in the sputum eosinophils and the eosinophil cationic protein. Zacharasiewicz *et al.* [17] evaluated the usefulness of sputum eosinophils and eNO in initiating step-down therapy in children with well controlled asthma. Dose reduction was successful in all children with zero sputum eosinophils at the time of entry to the study. Increased eNO and number of eosinophils in sputum were significant predictors of medication reduction failure.

The Denver branch of the Childhood Asthma Management Program (CAMP) [18] evaluated the use of sputum induction in asthma. Sputum induction, eNO, circulating eosinophils counts, and eosinophilic cationic protein were measured in 90 children after discontinuation of treatment. The corticosteroid group had lower percentage sputum eosinophils than the placebo group. Higher values of sputum percentage eosinophils were associated with worsening asthma control. They found that a greater percentage of sputum eosinophils was associated with higher bronchodilator reversibility, lower FEV₁/FVC ratio, higher eNO levels, circulating eosinophils, sputum and serum eosinophil cationic protein, more prednisone courses during the treatment period, and greater asthma severity.

In summary, sputum eosinophils is a helpful marker for asthma control. An increase in sputum eosinophils is a marker for a possible asthma exacerbation. Sputum induction can fairly easily be attained in an adult, however, it can be challenging for a young child, therefore an adequate sample may be difficult to attain.

Leukotriene E4

LTE4 can also be considered a biomarker for asthma. LTE4, along with LTC4 and LTD4, are considered cysteinyl leukotrienes. They are products of arachidonic acid metabolism by the cyclooxygenase 1/2 pathways. LTE4 is the stable end product of this pathway and can be measured. A number of studies have examined the level of LTE4 in exhaled breath condensate and urine in children with asthma [19,20,21].

Shibata *et al.* [19] evaluated the clinical utility of measuring LTE4 from the exhaled breath condensate in children with mild persistent asthma. They measured the level of LTE4 in 37 children with mild asthma and six healthy controls. The asthma group was further subdivided into three groups according to time of last wheezing episode. They found the level of LTE4 was significantly elevated in the asthma group versus the healthy controls. Further, the level of LTE4 increased between wheezing episodes. Children who were wheezing at the time of LTE4 measurement had higher values than the healthy controls and children who had not wheezed for more than 6 months.

Rabinovitch *et al.* [20^{*}] assessed the relationship between daily urinary LTE4 production and lung function in children treated with an inhaled corticosteroid and long-acting β agonist. This study found that an increase in urinary LTE4 correlated with decreases in FEV₁. Rabinovitch *et al.* also showed leukotriene receptor agonist protected against daily decreases in FEV₁. The effect of leukotriene receptor antagonist on LTE4 production has also been investigated by Montuschi *et al.* [21]. They studied the effect of montelukast on exhaled LTE4 in atopic children with and without asthma. The groups consisted of 17 and 16 children in each group, respectively. The level of LTE4 was higher in the group with asthma; the LTE4 levels also decreased in this group with the addition of montelukast. Atopic children without asthma did not have a change in their production of LTE4 upon the addition of montelukast.

In summary, emerging data indicate that cysteinyl leukotrienes may play an important role in the assessment of asthma control in children. Additionally, measurement of LTE4 in the urine and in exhaled breath condensate may be appropriate.

Conclusion

Asthma control assessment can be very challenging in children. The goal of asthma management is to maximize therapy until symptoms are controlled, thereafter treatment should be decreased according to symptoms. Conventional markers, FEV₁, peak flow variability and clinical symptoms assessment help in step-down therapy

by serving as surrogate markers of airway inflammation. Inflammatory biomarkers, eNO, sputum eosinophils and LTE₄, however, can be of additional value to conventional measurements by giving a more direct measure of airway inflammation. Therefore, biomarkers can be used as adjuncts to conventional tests in managing treatment of children with asthma.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 203–204).

- 1 Fischer A, Folkerts G, Pierangelo G, Groneberg D. Mediators of asthma: nitric oxide. *Pulm Pharmacol Ther* 2002; 15:73–81.
 - 2 Zacharasiewicz A. Fractional exhaled nitric oxide and induced sputum. *Paediatr Respir Rev* 2007; 8:94–96.
 - 3 American Thoracic Society/European Respiratory Society. ATS/ERS recommendations for standardized procedures for the inline and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide. *Am J Respir Crit Care Med* 2005; 171:912–930.
 - 4 Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: comparison with the 'gold standard' technique. *Chest* 2007; 131:410–414.
- This study showed similarity in values of eNO between currently accepted Niox and portable machines.
- 5 Buchvald F, Baraldi E, Carraro S, *et al.* Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J Allergy Clin Immunol* 2005; 115:1130–1136.
 - 6 Byrnes CA, Dinarevic S, Shinebourne EA, *et al.* Exhaled nitric oxide measurements in normal and asthmatic children. *Pediatr Pulmonol* 1997; 24:312–318.
 - 7 Kharitonov SA, Yates DH, Chung KF, Barnes PJ. Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. *Eur Respir J* 1996; 9:196–201.
 - 8 Panickar JR, Bhatnagar N, Grigg J. Exhaled nitric oxide after a single dose of intramuscular triamcinolone in children with difficult to control asthma. *Pediatr Pulmonol* 2007; 42:573–578.
 - 9 Fitzpatrick AM, Gaston BM, Erzurum SC, Teague WG, National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma research Program. Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. *J Allergy Clin Immunol* 2006; 118:1218–1225.
- This study elucidated high eNO values in children with severe asthma.
- 10 Miraglia del Giudice M, Piacentini GL, Capasso M, *et al.* Formoterol, montelukast, and budesonide in asthmatic children: effect on lung function and exhaled nitric oxide. *Respir Med* 2007; 101:1809–1813.
 - 11 Zeiger RS, Szefer SJ, Phillips BR, *et al.* Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute. Response profiles to fluticasone and montelukast in mild-to-moderate persistent childhood asthma. *J Allergy Clin Immunol* 2006; 117:45–52.
- This study showed the effectiveness of fluticasone in lowering eNO values. It also showed eNO was a predictor of asthma control days.
- 12 Strunk RC, Szefer SJ, Phillips BR, *et al.* Childhood Asthma Research and Education Network of the National Heart, Lung, and Blood Institute. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003; 112:883–892.
 - 13 Silkoff PE, Lent AM, Busacker AA, *et al.* Exhaled nitric oxide identifies the persistent eosinophilic phenotype in severe refractory asthma. *J Allergy Clin Immunol* 2005; 116:1249–1255.
 - 14 Smith AD, Cowan JO, Brassett KP, *et al.* Exhaled nitric oxide: a predictor of steroid response. *Am J Respir Crit Care Med* 2005; 172:453–459.
 - 15 Pijnenburg MW, Bakker EM, Hop WC, De Jongste JC. Titrating steroids on exhaled nitric oxide in children with asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2005; 172:831–836.
 - 16 Gibson PG, Simpson JL, Hankin R, *et al.* Relationship between induced sputum eosinophils and the clinical pattern of childhood asthma. *Thorax* 2003; 58:116–121.
 - 17 Zacharasiewicz A, Wilson N, Lex C, *et al.* Clinical use of noninvasive measurements of airway inflammation in steroid reduction in children. *Am J Respir Crit Care Med* 2005; 171:1077–1082.
 - 18 Covar RA, Spahn JD, Martin RJ, *et al.* Safety and application of induced sputum analysis in childhood asthma. *J Allergy Clin Immunol* 2004; 114:575–582.
 - 19 Shibata A, Katsunuma T, Tomikawa M, *et al.* Increased leukotriene E₄ in the exhaled breath condensate of children with mild asthma. *Chest* 2006; 130:1718–1722.
 - 20 Rabinovitch N, Zhang L, Gelfand EW. Urine leukotriene E₄ levels are associated with decreased pulmonary function in children with persistent airway obstruction. *J Allergy Clin Immunol* 2006; 118:635–640.
- This study demonstrates the potential benefit of urinary leukotrienes in the management and diagnosis of asthma.
- 21 Montuschi P, Mondino C, Koch P, *et al.* Effects of a leukotriene receptor antagonist on exhaled leukotriene E₄ and prostanoids in children with asthma. *J Allergy Clin Immunol* 2006; 118:347–353.