Paediatric Respiratory Reviews 16 (2015) 205–212

Contents lists available at ScienceDirect

Paediatric Respiratory Reviews

Mini-symposium: Biomarkers and Phenotype

Childhood asthma biomarkers: present knowledge and future steps

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EDUCATIONAL AIMS

The reader will be better able to:

- List the various sources of biomarkers and their advantages and disadvantages
- Understand the use and limitations of exhaled fractional excretion of nitric oxide in asthma management
- Recognize the variety of different biomarker classes available from exhaled breath condensates from asthmatic children

ARTICLE INFO

Keywords:
Asthma
Children
Biomarkers
Exhaled breath condensate

SUMMARY

Asthma represents the most common chronic respiratory disease of childhood. Its current standard diagnosis relies on patient history of symptoms and confirmed expiratory airflow limitation. Nevertheless, the spectrum of asthma in clinical presentation is broad, and both symptoms and lung function may not always reflect the underlying airway inflammation, which can be determined by different pathogenetic mechanisms. For these reasons, the identification of objective biomarkers of asthma, which may guide diagnosis, phenotyping, management and treatment is of great clinical utility and might have a role in the development of personalized therapy. The availability of non-invasive methods to study and monitor disease inflammation is of relevance especially in childhood asthma. In this sense, a promising role might be played by the measurement of exhaled biomarkers, such as exhaled nitric oxide (FE_{NO}) and molecules in exhaled breath condensate (EBC). Furthermore, recent studies have shown encouraging results with the application of the novel metabolomic approach to the study of exhaled biomarkers. In this paper the existing knowledge in the field of asthma biomarkers, with a special focus on exhaled biomarkers, will be highlighted.

INTRODUCTION

Asthma affects from 1% to 18% of the population in different countries [1], and is the most common chronic respiratory disease of childhood. This disorder is characterized by airway inflammation, bronchial hyperresponsiveness and recurrent episodes of reversible airway obstruction [2]. Current standard clinical diagnostics rely on a patient's history of symptoms, confirmed expiratory airflow limitation and bronchial reactivity of variable severity [1,3]. The spectrum of asthma in terms of its clinical presentations is broad, however, and the pathogenic mechanisms involved may differ considerably, making this disorder a collection of heterogeneous disease subtypes [2,4]. What is more, symptoms and lung function measurements may not always reflect the underlying airway inflammation [2], and response to therapy varies across the asthma spectrum [4]. For all these reasons, research has focused in recent decades on finding objective asthma biomarkers capable of orienting the diagnosis, phenotyping, management and treatment of this disorder. It would be of great

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http://dx.doi.org/10.1016/j.prrv.2015.05.001
1526-0542/

clinical utility to identify quantifiable biomarkers that can shed light on the pathobiological pathways involved in a given asthma patient and provide information on how adequately the disorder is controlled. Such biomarkers might also have a role in the development of personalized treatments.

Currently, tissue-specific diagnostic methods such as bronchoalveolar lavage (BAL) and bronchoscopy with bronchial biopsy are considered the gold standard for assessing airway inflammation and remodeling in asthma. However, the invasiveness of these procedures limits their use in daily clinical practice [2]. Sputum induction is also considered too invasive and technically complex, especially in children under eight years old [2,5]. When working with children, it becomes particularly important to devise non-invasive methods for studying and monitoring asthma-related inflammation.

Peripheral blood/serum collection is easier to achieve and only minimally invasive. Most of the studies assessing serum/blood biomarkers have focused on markers of eosinophilic or Th2 inflammation [6]. For instance, eosinophilia in the blood correlates with bronchial hyperresponsiveness and asthma-related inflammation, but this finding is scarcely specific because it can be found in cases of allergy, autoimmune disease, and also with parasitic infection. On the other hand, it seems that variations in eosinophil activation state represented by integrins and other eosinophil surface proteins may be associated with asthma activity [7]. Another emerging Th2-high serum biomarker is the extracellular matrix protein periostin, which may have a part to play in defining asthma phenotypes and predicting response to therapy. Despite the promising results, however, further investigations are needed before this molecule can be used in clinical practice [8].

Urine is possibly the least invasive bio-fluid that can be collected for the purpose of measuring biomarkers, and this makes it highly suitable for assessing asthma in young children [9]. Urinary leukotriene E4 (uLTE4) assay has been validated as a method for assessing total body cysteinyl leukotriene production [5]. Recent studies have also reported that this metabolite may be a marker of environmental exposure to tobacco smoke and ambient air pollution, a predictor of asthma exacerbation risk related to tobacco smoke, and also a marker of response to leukotriene receptor antagonists [10]. Despite these findings, uLTE4 does not appear to have gained widespread acceptance in clinical practice [4].

Among the non-invasive methods for assessing asthmatic inflammation, there is a special place for the measurement of exhaled biomarkers, such as exhaled nitric oxide (FE_{NO}) and molecules in exhaled breath condensate (EBC) [11]. Fractional exhaled nitric oxide seems to correlate with the eosinophilic asthma phenotype, but also with the degree of atopy [12]. Studies on the usefulness of this biomarker in orienting asthma therapy in adults and children have produced mixed results [13]. As for exhaled breath condensate, it can be used to study the pathological processes underway in the lung because its composition is believed to mirror that of the respiratory fluid film [14]. EBC contains volatile and non-volatile compounds, some of which have been measured and proposed as markers of inflammation or oxidative stress, both of which are pathogenic mechanisms involved in asthmatic patients [11].

More recently, a role has emerged for the novel metabolomic approach to the study of exhaled asthma biomarkers [2,15]. This untargeted method has the potential to identify the metabolic fingerprint characterizing a given condition.

The purpose of this paper is to summarize the present knowledge in the field of asthma biomarkers, with a special focus on exhaled biomarkers, drawing attention to key strengths and limitations of each approach and trying to suggest the directions that future research might take.

EXHALED NITRIC OXIDE (FE_{NO})

The presence of gaseous nitric oxide in exhaled human breath was first reported in 1993 [16]. Four years later, it was found to be higher than normal in children with asthma [17]. As a result, the early 2000s saw a considerable number of publications exploring the relationship between the fractional concentration of exhaled nitric oxide (FE_{NO}) and asthma. In the respiratory system, nitric oxide is produced mainly by two enzymes: constitutive nitric oxide synthase (NOS), which constantly generates low concentrations of NO and inducible NOS (iNOS), the expression of which is induced by various inflammatory cytokines [18].

FE_{NO} measurement is non-invasive, rapid, repeatable and reproducible [19]. It can be performed using different techniques, including chemiluminescence, electrochemical detection, and laser spectroscopy [20]. Online and offline methods are available for collecting and analyzing FE_{NO}, but only the single breath online method (SBOL) has been fully standardized and it is now the gold standard for FE_{NO} measurement in children who are able to cooperate [21]. Several techniques have been developed for measuring exhaled NO even in young or uncooperative children, as well as in sedated infants [21–23]. A first statement by the European Respiratory and American Thoracic Societies on FE_{NO} measurements in children (paying special attention to preschool children) appeared in 2002 [21], and the standard method for measuring NO in exhaled air was last revised by the ATS/ERS in 2005 [24]. Several factors can influence FE_{NO} levels, such as flow rate, nasal contamination and ambient air [24]. Other confounders unrelated to any disease that need to be taken into account when measuring exhaled NO are age, height, gender, race, spirometry or exercise before testing, diet and exposure to smoke [18,19].

In pediatric asthma, several potential applications of FE_{NO} have been explored. These range from predicting asthma in pre-school children with respiratory symptoms to its possible use for asthma management.

Measuring FE_{NO} in pre-school children

Respiratory symptoms are extremely common in pre-school children, and the differential diagnosis is broad [19]. Studies undertaken in young children have reported that FE_{NO} may be useful in discriminating asthmatic cases from healthy controls and from non-asthmatic children with chronic cough [25], since atopic wheezers have higher FE_{NO} levels than non-atopic ones [26]. Higher than normal FE_{NO} levels proving sensitivity to steroid treatment have been found in children with recurrent wheezing [27,28]. A limitation of such studies conducted in young children relates to their having measured FE_{NO} using off-line techniques, which have yet to be well standardized [21]. Further research is needed to develop standardized FE_{NO} measurements for this age group, which could be useful – in association with other diagnostic tests - in predicting asthma in pre-school children.

FE_{NO} in asthma diagnosis and inhaled corticosteroids (ICS) responsiveness

In allergic asthma (the most common form of pediatric asthma), airway inflammation is due to the production of cytokines and IL-4, IL-5 and IL-13 by mast cells and antigen-specific type 2 T-helper cells. In particular, IL-4 and IL-13 induce an upregulated epithelial iNOS expression. Exhaled NO directly reflects Th2-mediated pro-inflammatory cytokine mechanisms [20,29].

In several studies, FE_{NO} levels were shown to correlate with sputum eosinophils, blood eosinophilia, serum eosinophilic cationic protein, and IgE levels [29]. High exhaled NO levels are consequently considered a marker of a common asthma endotype