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Biomarkers in the Exhaled Breath of Asthmatic Children

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Abstract

Asthma is the most common chronic respiratory disease in childhood. The diagnosis of asthma is based on clinical history, airflow limitation and bronchial reactivity. Asthma treatment focuses on control of the condition; in cases of particular respiratory symptoms, the use of rescue medications, limited daily activity and lung function tests are taken into consideration. However, lung function and symptoms do not always reflect underlying airway inflammation and response to therapy. Consequently, objective parameters of airway inflammation could represent asignificant adjunctive tool for the clinician in tailored management of the disease.

In recent years researchhas focused on biomarkers to identify phenotype, inflammation, pathobiological pathways and to guide the clinician in the diagnosis and personalized management of asthma. More feasible tests in the pediatric population are the collection of exhaled breath condensate (EBC) and the measurement of exhaled nitric oxide (FeNO), which are the most popular tests in practice. Other markers that might predict asthma exacerbation are volatile organic compounds (VOCs) that indicate airway inflammation and the level of asthma control.

Nowadays, the variability and low reproducibility of exhaled biomarkers due to the lack of methodological standardization of pre-collection, collection, post-collection and interpretation of conditions could represent a drawback in clinical practice. Despite these limitations, several biomarkers have been shown to be helpful in distinguishing patients with asthma from healthy children.

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Introduction

There is a 1% to 18% incidence of asthma in people from different countries and it is the most common chronic respiratory disease in childhood. Asthma is a chronic respiratory disease characterized by inflammation of the airways, bronchial hyperresponsiveness, recurrent reversible airway obstruction, deterioration in lung function and respiratory symptoms [1]. The diagnosis of asthma is based on clinical history, limitation of airflow and bronchial reactivity [1]. Treatment focuseson asthma control, particularly of respiratory symptoms, and includes use of rescue medications, patients' reduced daily activity and lung function tests [1]. However, lung function and symptoms do not always reflect possible underlying airway inflammation [2] and response to therapy [3]; therefore, objective parameters of asthma inflammation could be important for the clinician when making a treatment choice.

Currently, diagnostic methods like bronchoalveolar lavage (BAL) and bronchoscopy are the gold standard for assessing airway remodeling and inflammation. These methods, however, are too invasive and have a limited use, especially in pediatric care [2]. For these reasons, in the past years research studies have focused on objective biomarkers to identify phenotype, inflammation, pathobiological pathways and to guide the clinician in the diagnosis and personalized management of the disease [4,5].

An ideal biomarker is easy to collect and measure, inexpensive, noninvasive, feasible in children, and technically simpler when identifying the clinical or treatment response phenotype. The induced sputum technique can be considered a surrogate noninvasive method to evaluate airway inflammation. Nevertheless, this technique may be difficult to apply to pediatric patients and therefore its clinical application is still limited.

Exhaled breath condensate (EBC) and the measurement of exhaled nitric oxide (FeNO) are currently the most frequently used tests in clinical practice [6] and are feasible in the pediatric population. FeNO is an extensively studied marker and its clinical usefulness is supported by guidelines [7]. However, studies regarding the correlation between FeNO and asthma control its efficacy in managing asthma treatment are contradictory [14,15]. FeNO can be helpful to assess asthma control in asthmatic patients and asthmatic patients on treatment. However, its suboptimal sensitivity and specificity may limit its utilization as a single monitoring tool.

On the other hand, although the efficacy and diagnostic roles of inflammatory markers in exhaled breath condensate have been studied, their clinical use is still under debate [16,17]. In diagnosing and monitoring asthma, an approach that involves an ensemble of EBC biomarkers had better accuracy in real-life settings than a single marker. A poor to moderate association of EBC biomarkers with lung function suggests the greater importance of EB Canalysis in the diagnosis of asthma in children.

Other markers that might predict asthma exacerbation are volatile organic compounds (VOCs) that reflect the degree of airway inflammation and asthma control [18]. VOCs in exhaled breath

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showed a potential role in predicting asthma exacerbations in children. Before adopting this in clinical practice, the validity of their capacity to predict asthma exacerbations should be studied in a larger cohort.

Fractional exhaled nitric oxide (FeNO)

In the airways, nitric oxide is mainly produced by two enzymes: constitutive nitric oxide synthase (cNOS) that generates low quantities of NO and epithelial inducible NOS (iNOS) that is induced by various inflammatory cytokines [19]. FeNO has been recognized as a marker of eosinophilic airway inflammation and its measurement has been proposed to assess the level of airway inflammation and the response to anti-inflammatory therapy [20]. FeNO measurement is a noninvasive, repeatable and reproducible method [21]. The gold standard for cooperative children is the single breath on-line method [22]. Other techniques have been evaluated for uncooperative children or in sedated infants [22]. On-line and off-line methods have both been used in uncooperative children without the use of sedatives. Limited experience has been described using single-breath methods in infants. However, these methods have never been validated for clinical purposes and further research is needed to define standardized measurements in this age group. At present, no clear evidence is available regarding the potential clinical application of NO measurements in uncooperative children, particularly regardingits potential application in association with other diagnostic tests, to predict asthma in young children [23].

In an attempt to standardize FeNo measurement procedures, an initial document on FeNO measurement in children was published in 2002 [22], which was jointly revised by ATS/ERS in 2005 [7]. The standardization of techniques made it possible to collect comparable data in different centers for normal subjects and for those with diseases. FeNO levels can be influenced by various factors such as exhalation flow, nasal contamination, ambient air pollution, patient's age, height, gender and race [7]. Furthermore, spirometry or exercise performed before the measurement, diet or exposure to smoke also need to be considered [7].

In children, FeNO increases with age, as reported in the literature [7], and it is recommended that NO analysis should be performed before spirometry because it has been shown that can cause a reduction in transient exhaled NO levels [7]. Patients should also desist from eating and drinking before NO analysis. An increase in FeNO has been found after ingestion of nitrate or nitrate-containing foods [7].Several studies demonstrated that FeNO correlates positively with airway hyper responsiveness, IgE serum levels, bronchodilator response, skin prick tests, asthma symptoms and lung function [24,25].

In allergic asthma, airway inflammation results from the activation of mast cells and a Th2-mediated pro-inflammatory cytokine mechanism that results in the production of IL-4, IL-5, and IL-13 which cause epithelial inducible NO synthase expression that up-regulated via STAT-6, a process which is corticosteroid sensitive, a mechanism of central importance in allergic airway inflammation[26, 27]. Moreover, other studies showed that FeNO levels are correlated with eosinophils in induced sputum, eosinophil infiltration of the airways, blood eosinophilia, serum eosinophilic cation protein and IgE levels in atopic patients [27]. The asthma phenotype characterized by Th2-mediated airway inflammation, eosinophilia, and responsiveness to ICS shows high FeNO values [27].

In addition to clinical history and the lung function test, FeNo is also helpful in identifying patients with asthma eosinophilic phenotype and in predicting asthma exacerbation. The ATS guidelines recommend the use of FeNO to monitor airway inflammation and to guide anti-inflammatory treatment in patients with established asthma [27,28]. High FeNO values, however, are related to allergic rhinitis, eosinophilic bronchitis and allergen or viral exposure; so it is important to keep in mind that not all high FeNO values are linked to eosinophilic asthma. The ATS/ERS document stresses the relevance of a correct interpretation of FeNO values [28]. In children, FeNO values lower than 20 ppb are probably correlated to a lack of response to ICS treatment. On the other hand, FeNO over 35 ppb suggests a response to ICS in support of its role in identifying Th2 airway inflammation responding to ICS treatment [27,29]. It has been widely demonstrated that there is a rapid decrease in FeNO when ICS treatment is started, with a dose dependent mechanism, and a sudden rise when ICS therapy is withdrawn [30]. This trend may be helpful when monitoring patient compliance to therapy [21].

FeNO can also be used in patients in treatment with omalizumab. In fact, some studies showed that FeNO values together with blood eosinophils and BMI can predict response to omalizumab [31]. Experimental data in adults also showed that high FeNO values may indicate a response to treatment with human anti-interleukin-4 receptor monoclonal antibodies that inhibit interleukin-4 and interleukin-13 signaling [32].

Despite the initial enthusiasm for FeNO in the management of asthma in children, the literature is very cautious to support the use of FeNO in addition to standard symptom-based management [33,34], and its utilization is now being reconsidered and is under debate [35]. Therefore, from a practical viewpoint, FeNO may be considered a clinically useful method to identify patients with eosinophilic and Th2-mediated asthma, who are expected to respond to ICS therapy. Furthermore, it may have a practical role in predicting exacerbations and patient compliance to therapy.

Exhaled breath volatile organic compounds

Exhaled breath (EB) volatile organic compounds derive from metabolic fractioning of larger molecules. Airway VOCs originate not only from the upper and lower airways but also from a capillary bed near the alveoli [37]. The measurement of VOCs is a recently proposed method for research and clinical purposes when evaluating respiratory and non-respiratory diseases. The methodical approach to collect VOCs from exhaled breath requires particular attention in order to exclude organic compounds from the ambient air [37].

The collection of airway VOCs may be performed by on-line methods, which allow the technician to directly collect samples via inert tubes inserted into an analyzer, or off-line methods which involve the collection of exhaled air into bags, tubes or syringes. Collection devices need to be made from inert materials such as tedlar bags [38].

After collecting the sample, different techniques can be used to analyze the specific content. Gas chromatography and gas spectrometry (GC-MS) or flame ionization detection (GC-FID) are the most widely used techniques. These methods can distinguish and quantify VOCs at low concentrations but they require highly qualified technicians and expensive apparatus [23]. A new non-selective approach to analyze VOCs in exhaled breath is metabolomic profiling, which can, without

a priori hypothesis, identify and quantify all metabolites in a biological sample. Metabolomic profiles represent the interaction between genetic expression, environmental exposure, microorganisms, medication, nutrition and toxic substances [37,23]. This method allows one to define disease phenotype and is an interesting approach for patient characterization and personalized medication [23]. This approach simultaneously considers a large number of metabolites in a sample and generates metabolite profiles capable of discriminating between different groups of individuals, providing a characterization of all the biochemical processes underway in a given biological system.

More recently, simpler devices with sensor-based techniques such as the electronic nose, colorimetric sensor array and gold nanoparticle sensors have been proposed. They use specific sensors with optical, chemical or electronic properties that can detect and group VOCs in the EB [37]. In recent years, several studies have demonstrated the clinical application of these instruments in pediatric respiratory disease and allergy [39,40]. VOCs in the EB can discriminate patients with asthma from healthy children and atopic from non-atopic children [39,40]. In children, VOCs have also been reported as being capable of predicting asthma exacerbations [18]. VOC collection is also possible in preschool children and their profiles have been shown to be different in children with recurrent wheezing as compared to controls.

Nevertheless, further studies are necessary particularly to evaluate the clinical usefulness of VOC assessment in evaluating asthma severity and monitoring asthma symptoms and response to ICS therapy.

Exhaled breath condensate

Exhaled breath condensate (EBC) is a noninvasive method to evaluate airway inflammation; in it there are analyzing markers and inflammatory mediators that can help to understand asthma pathophysiology. EBC is composed of particles from airway lining fluid collected by the condensation of warm humid breath onto a cold surface in a condensing device. EBC is composed of water vapor, unstable volatiles like CO_2 and H_2O_2, inorganic (O_2,N_2) and organic (CO_2) particles, exogenous and endogenous organic compounds, protein and cytokines [41]. In the respiratory system, H_2O_2 may be released from inflamed cells including neutrophils, macrophages, eosinophils, and epithelial cells. Nitrogen redox forms such as nitrite (NO_2 -) and nitrate (NO_3 -) are present in the epithelial lining fluid of the human respiratory tract.

Concentrations of NO_2 and NO_2+NO_3 were significantly higher in cases of asthma, CF and bronchiectasis compared with healthy controls [41].

EBC collection is typically done using a refrigerated device in compliance withATS/ERS guidelines [42]. It involves 10-15 minutes of tidal breathing during which the airways lining fluid undergoes an aerosolization process and is then condensed in a cooled device (0 to -20°C) [6]. The most frequently evaluated parameters in EBC are pH, exhaled markers of oxidative stress and inflammation.

EBC pH is considered a non-specific marker of airway disease and normative data have been published for children from 0 to 20 years, with a median pH value of 8.0 [43]. Some studies reported that children with stable asthma had a lower pH in EBC than healthy controls and those suffering from severe asthma had a lower pH value than mild asthmatics [44,45]. In addition, ICS naïve asthmatic patients had a lower pH than those ICS treated, and those with acute exacerbation had a higher pH after treatment with budesonide [44,45].

Acidification has also been reported in children with allergic rhinitis and atopic dermatitis [45]. At present, no correlation has been reported with asthma symptoms, lung function, FeNO or airway hyperresponsiveness [46,47].

An important set of potential biomarkers in EBC is related to oxidative stress like H_2O_2, 8-isoprostane, asymmetric dimethylarginine (ADMA), aldehydes and nitrite/nitrate.H_2O_2 in EBC is released from inflamed airways as superoxide anions, an unstable and reactive particle. In the respiratory system, H_2O_2can be released from both inflammatory cells - including neutrophils, macrophages, eosinophils and epithelial cells. The normal level of this molecule in young, non-asthmatic and non-smoking children is 0.09 μmol [42]. H2O2 was found to be higher in asthmatic children during exacerbations and decreased after ICS treatment, which supports the hypothesis that H_2O_2 is a marker of inflammation of the airways [48,49]. However, other studies failed to find a significant difference in H_2O_2 between asthmatics and controls or in its ability to predict exacerbations [50,51].

Asymmetric dimethylarginine (ADMA) is another potential marker of oxidative stress identifiable in EBC by the UPLC-MS/MS technique. It is an analogue of L-arginina that reduces, by inhibiting NOS, the synthesis of NO and increases superoxide. Asthmatic children showed higher values of ADMA than healthy ones with no difference related to ICS treatment [52].

Aldehydes and lipid hydroperoxides derive from the oxidation of the phospholipid membrane and polyunsaturated fatty acid. One study showed high levels of glutathione in the EBC of asthmatic children with exacerbation. That study reported that after 5 days of prednisolone therapy the malondialdehyde level dropped, while glutathione rose [53]. These results suggest that during exacerbations in the airways of asthmatic patients there is an imbalance between oxidative and antioxidant agents.In children with asthma, malondialdehyde levels correlate also with air pollution, lung function and inflammatory markers [54].

8-isoprostane is a product of arachidonic acid and it is also an accurate marker of oxidative stress [23]. Children and adults with asthma present high levels of this marker, especially those with severe asthma or an asthma exacerbation [55]. The concentrations of 8-isoprostane have no correlation with ICS or leukotriene receptor antagonist therapy, lung function or NO [56,57].

Eicosanoids are a large group of markers derived from arachidonic acid that play a role in asthmatic inflammation. The presence of these markers in EBC can be confirmed by specific enzyme immunoassay and radio immunoassays [58].

In children with asthma leukotriene B4 (LTB4), cysteinyl leukotrienes (LTC4, LTD4 and LTE4) are high in EBC compared to healthy subjects [57,58]. The role of cysteinyl leukotrienes (CysLT) in response to ICS therapy is under debate [59-61]. Some authors report a significant reduction of CysLT after a course of oral steroids or 6 months of ICS therapy, whereas others report no changes. A significant reduction of CysLTs has been reported after montelukast [62].

Several other markers of inflammation and oxidative stress such as cytokines and adenosine have been studied. Th2 cytokines are evaluated using the ELISA technique. Some studies showed that the number of Th2 cytokines ishigher and that of Th1 cytokines is lower in the EBC of asthmatic children [63, 64]. IL-4 was higher in asthmatic children, especially in atopic rather than non-atopic children, and it has been proposed as a predictor for asthma diagnosis, whereasit has been suggested that IL-5 is able to predict asthma exacerbations [51]. Children with asthma have also been reported to present with a higher IL-4/INFγ ratio related to Th2 inflammation [64].

Conclusion

In asthma patients, particularly children, noninvasive techniques of sample collection aimed to analyze biomarkers of inflammation of the airways are helpful in assessingthe airway pathophysiology of respiratory diseases. Nowadays, only the use of FeNO has been confirmed as a valid technique in clinical practice as a non-invasive method for assessing eosinophilic inflammation. The standardization of new techniques to collect biomarkers in EB and EBC remains problematic.Variability, low reproducibility in exhaled biomarkers due to a lack of standardization in pre-collection, collection, post-collection and interpretation conditions may represent a drawback in clinical practice. Contrary to FeNo, which is useful and has undergone significant validation, other biomarkers require further research in order to be routinely used in clinical practice.

Competing Interests

The authors declare that they have no competing interests.

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