

FRACTURED EXHALED NITRIC OXIDE AND BIOLOGIC THERAPIES FOR PAEDIATRIC ASTHMA

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Abstract

Bronchial asthma is the most frequently diagnosed chronic respiratory disease in children. Treatment approaches should aim to achieve the disease control, reduce limiting symptoms, and improve the quality of life. Routine treatment of patients with asthma relies on assessment of symptoms and spirometry results. These diagnostic and therapeutic strategies do not consider the level of inflammation in the airways as a fundamental pathognomonic feature of the disease. The use of biomarkers is increasing in the context of efforts to better understand individual asthma pathways (asthma endotyping), with the potential for personalized treatment with innovative biologics. Elevated levels of exhaled nitric oxide (FENO) represent an indirect marker of T2 inflammation in airways. FENO is one of the few biomarkers that have been applied in routine clinical practice. High levels predict a good therapeutic response to treatment with corticosteroids and selected biologics (Omalizumab, Dupilumab, Mepolizumab, Tezepelumab), or an increased risk of asthma exacerbation. The aim of this review is to evaluate the advantages, disadvantages, and potential applications of this test in relation to new treatment options using biologics for asthma.

Keywords: asthma, biologics for asthma, biomarkers, children, exhaled nitric oxide, T2 type inflammation

INTRODUCTION

Bronchial asthma (AB) is the most common chronic respiratory disease in children (1). The prevalence of asthma is constantly increasing, resulting in an increased economic impact on its diagnosis and treatment, particularly in developed countries. According to an epidemiological analysis by the US Center for Disease Control and Prevention, the prevalence of asthma in the United States in 2017 was 7.9%, with a higher prevalence in children (8.4%) than in adults (7.7%) (2).

The main pathognomonic entity of asthma is chronic inflammation of the lower airways associated with reversible bronchial hyper-responsiveness and structural changes in the tracheobronchial tree (3). Recurrent bronchial obstruction is linked with various clinical symptoms, including anamnestically reported dyspnea, wheezing, chest tightness, limitation of physical activity, and chronic cough. It is a heterogeneous clinical syndrome with a specific etiopathogenesis and variable clinical manifestations. Considering this, great efforts have been made to understand the individual pathophysiological processes to classify this disease into unique endotypes and phenotypes (4). Asthma treatment focuses on monitoring the activity and severity of the disease (5).

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The treatment of an asthmatic patient that relies solely on the control of reported symptoms may not be successful over a long period. Achieving control over the limiting symptoms does not reflect the risk of predicting future asthma flares (6). In young children, the diagnosis of asthma is particularly challenging. The diagnostic approach in children is often complicated by inability to perform some tests used in older individuals (7, 8). Common methods for asthma diagnosis (questionnaire methods, peak expiratory flow, spirometry, and bronchial challenge tests) do not assess the level of airway inflammation (9, 10).

BRONCHIAL ASTHMA AND EOSINOPHILIC INFLAMMATION

AB is a multifactorial disease with heterogenous patterns of airway inflammation. A combination of heterogeneous extrinsic and intrinsic factors determines the development and persistence of chronic inflammation of the bronchial wall in genetically predisposed individuals. In the new era of evidence-based personalized medicine, efforts are being made to identify measurable pathomechanisms that target a specific group of affected patients (11). This approach enables access to targeted, effective, and innovative treatment. By blocking specific causal pathways, we can achieve better disease control, reduce limiting symptoms, and decrease the use of conventional therapy (12). Finally, attention should be paid to the health of individual patients and their significant improvements in quality of life.

The T2 endotype of asthma is predominant in childhood, in contrast to adults. This endotype is characterized by inflammation with a predominance of eosinophils in an allergic or non-allergic immune microenvironment (13). Considering this, we do not have to prove laboratory sensitization to inhalant allergens in a proportion of pediatric patients. The Th2 subpopulation of lymphocytes and natural lymphoid cell type 2 (ILC2) are crucial for the pathogenesis of the T2 endotype (14). Damage to the airway epithelium due to exogenous factors (allergens, viruses, and pollutants) leads to a loss of functional and structural integrity of the bronchial epithelial barrier (15). The activation of innate and adaptive immune mechanisms causes to the production of epithelial cytokines called alarmins (IL-25, IL-33, and TSLP-thymic stromal lymphopoietin). TSLP is a cytokine produced by the epithelial cells in the skin, gut, lungs, and thymus. It is a member of the interleukin-7 (IL-7) family of cytokines. TSLP binds to a receptor called TSLP receptor (TSLPR), which is a heterodimer of the IL-7 receptor alpha chain. TSLP and other members of the alarmin group directly activate Th2 and ILC2 cells to produce a characteristic cytokine spectrum (IL-4, IL-5, and IL-13) with pleiotropic functions (16, 17). This results in typical functional and anatomical changes in the affected bronchi, such as goblet cell hyperplasia, smooth muscle hypertrophy, neovascularization, mucosal tissue fibrosis, and bronchial hyper-responsiveness (18). IL-13 is the main effector cytokine of T2 inflammation (Fig.1).

Eosinophils are essential components of the inflammatory processes. They are granulocytes that originate in the bone marrow from a myeloid precursor. They also play an important role in maintaining the internal environment of the body within physiological limits (19). For example, resident eosinophils are involved in the regulation of glucose metabolism and adipose tissue (20). Secretory granules located in the cytoplasm of these cells contain many cytotoxic mediators and vasoactive substances that participate in defense against viral and parasitic pathogens (21–23). In contrast, pro-inflammatory eosinophils are responsible for a variety of eosinophil-derived diseases that can affect almost any organ system. Eosinophils help activate bronchial fibroblasts to produce pro-fibrotic factors by secreting various mediators. Therefore, they participate in bronchial remodelling and the thinning of the basement membrane of the epithelial layer. Furthermore, synthesized pro-inflammatory mediators promote bronchial smooth muscle contractility and inhibit relaxant stimuli (24, 25). The IL-5 produced by immunocompetent cells is crucial for the growth, maturation, and migration of the pro-inflammatory phenotype of eosinophils to the site of the inflammation (26). Inflammation-activated epithelial cells also synthesize special chemokines called class

1 (CCL11) and class 2 (CCL24) eotaxins. Eotaxins act on the endocrine system of eosinophils after binding to a specific receptor on their cell surface (predominantly through CCR3). The initiation of connected molecular interactions triggers signalling pathways that result in their migration to the site of the inflammation. Therefore, eotaxins regulate the bone marrow as potent chemoattractants (27,28). The main pathways that result in the development of T2 type asthma are shown in Fig. 2.

Patients with the T2 endpoint often demonstrate a good therapeutic response to inhaled corticosteroids and a better response to bronchodilator therapy with beta-adrenergic receptor agonists (13). They also show a favourable response to properly selected monoclonal antibodies that block cytokines from T2 inflammation. Elevated peripheral blood eosinophils, increased number of eosinophils in bronchoalveolar lavage fluid, and total IgE antibodies are markers of this asthma endpoint (29). FENO belongs to this category. Typically, during endotyping and phenotyping of asthma, the focus is on the number of eosinophils in the peripheral blood, induced sputum, or bronchoalveolar lavage fluid. The paediatric population faces significant limitations in the collection of biological materials owing to their specificities. In exceptional cases, induced sputum is examined. It requires active cooperation (troublesome in children) and the inhalation of a hypertonic solution (risk of bronchospasm). The evaluation of bronchoalveolar lavage requires flexible invasive bronchoscopy associated with general anaesthesia. These limitations render these tests suitable for more serious and complicated diagnostic cases. Peripheral blood eosinophil counts are widely available as a part of differential blood cell counts. Interestingly, peripheral eosinophilia does not correlate with the number of eosinophils in the tissues (30).

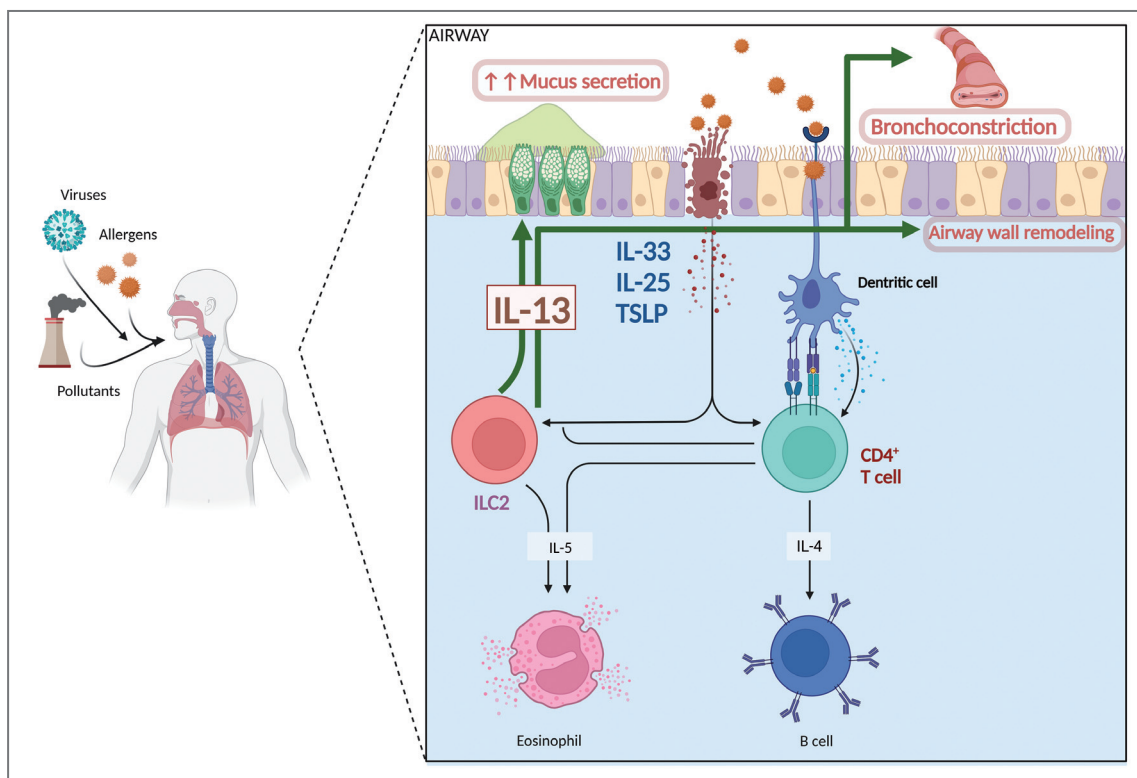


Fig. 1 IL-13 as a central effector cytokine of the T2-mediated inflammatory process, created in Biorender.com, **abbreviations:** IL-25: interleukin 25, IL-33: interleukin 33, TSLP: thymic stromal lymphopoietin, ILC2: natural lymphoid cell type 2

However, clinical cases of children with difficult-to-treat AB after treatment with systemic corticosteroids showed a more significant correlation with decreased FENO values than the number of eosinophil histological samples from the bronchial mucosa (31). Both FENO and eosinophils are closely associated with T2-type inflammation in patients with asthma. However, its production is regulated by different inflammatory cascades (IL-5: eosinophils versus IL-13: FENO)(32). Therefore, it is important to precisely define their specific roles in the etiopathogenesis of AB as independent, interrelated, and complementary biomarkers in the holistic concept of the disease.

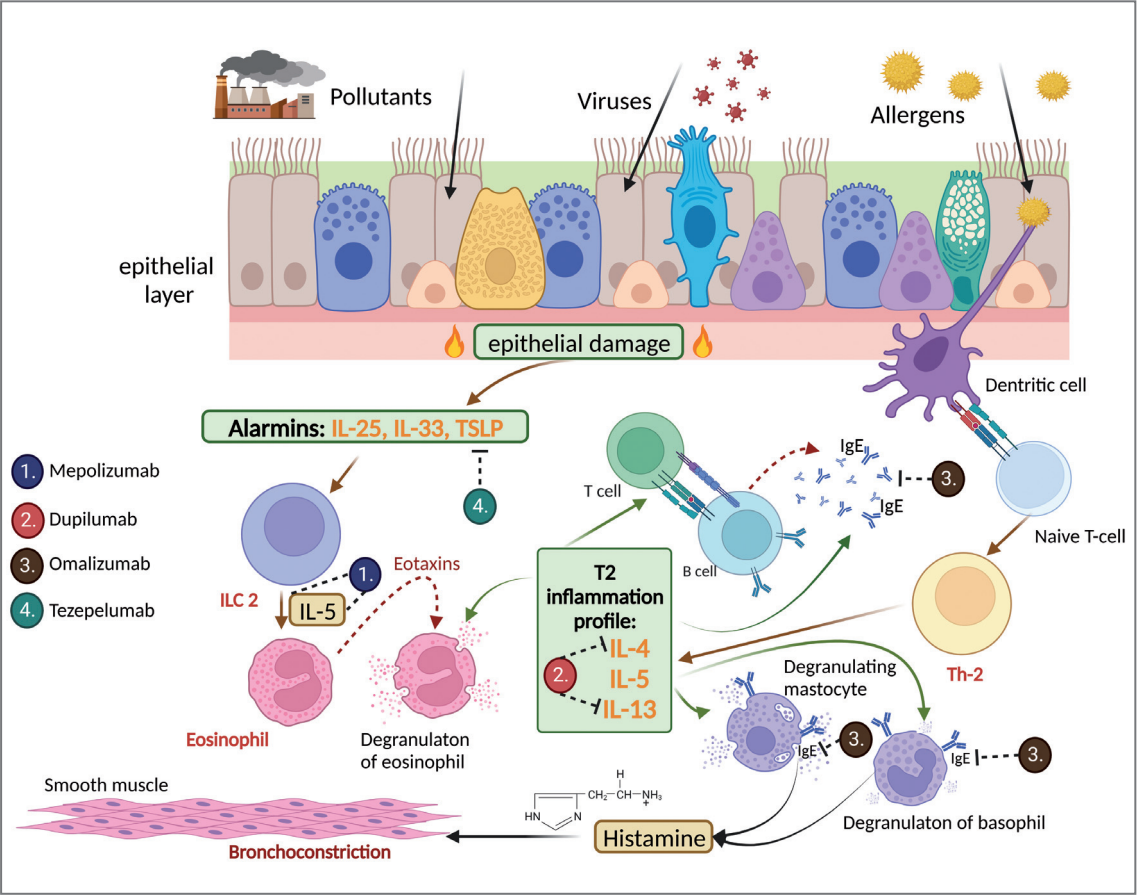


Fig. 2 Main pathways resulting in development of T2 type asthma and selected biologicals related to FENO, source: own elaboration, created in Biorender.com, **abbreviations:** IL-25: interleukin 25, IL-33: interleukin 33, TSLP: thymic stromal lymphopoietin, ILC2: natural lymphoid cell type 2, Th2: Th2 lymphocytes.

VARIABILITY OF FENO

FENO levels depend on various determinants and should be interpreted in the context of anamnestic data and clinical presentations. Factors with a possible impact on the measured values include atopy, height, age, sex, race, upper or lower respiratory tract infection, nitrate consumption in food, caffeine, alcohol, and active or passive smoking (Tab. 1) (33). Paradoxically, smoking and bronchoconstriction contribute to a decrease in these values.

Obesity is a growing global health problem and is negatively associated with asthma control. Dysregulation of metabolic and neuroendocrine processes and interactions in adipose tissue enhances inflammation in the body. Surprisingly, lower FENO values were confirmed despite significant sputum eosinophilia in obese patients (34). Exhaled NO undergoes diurnal changes simultaneously. Anderson et al. showed that the results of morning measurements were significantly higher than those of night measurements (35). While systemic and inhaled corticosteroids decrease FENO levels in children, antihistamines increase them (36). T2 inflammation-related diagnoses (atopic dermatitis, allergic rhinitis, and eosinophilic bronchitis) contribute to increased exhaled nitric oxide levels. Exercise is also an important factor. Clinical studies have demonstrated the effect of intense physical activity on higher FENO levels in less active children and adolescents (37). Finally, age should be considered when interpreting the results. The increase in age and height in specific age groups of the paediatric population determines the variation in FENO. A linear increase in the values was observed in girls aged 6–14 years. A similar linear relationship was also described for the opposite sex in the age group of 6–16 years. Both sexes reach a plateau of maximum concentration during adolescence, which persists into adulthood (38,39). Ethnicity and race also predict different basal levels of FENO in healthy and asthmatic children. In the US, Hispanic and African American children had higher mean FENO levels than Caucasian children (40).

Some studies have suggested the use of FENO measurements for the detection of cough-variant asthma and eosinophilic bronchitis in adult patients with chronic cough. Considering the many determinants of the variability of the FENO value, the importance of correctly assessing the results in correlation with selected anamnestic data has increased (41). The authors of this article investigated the possible association between FENO levels and cough reflex sensitivity in childhood asthma. There was no correlation between the elevated values of the parameters studied and the increase in the rate of cough reactivity in the asthmatic group or the control group of healthy probands (42).

The ability to investigate preschool-aged children is also clinically valuable. Based on the availability of devices using electrochemical analysis techniques, children above the age of five have a success rate of approximately 70% (43). In our clinical experience, most children over 8 years of age can handle this test.

Table 1 Various factors affecting FENO levels

FENO levels increase with:	FENO levels decrease with:
Allergic rhinitis	Early allergic reactions
Atopic dermatitis	Bronchoconstriction
White race	Corticosteroids (oral and/or inhaled)
Intake of a nitrate-rich diet	Passive and active smoking
Acute infection	Obesity
Morning (diurnal variation)	Reduced age in children
Male sex	
Caffeine	
Alcohol	

FENO AND SELECTION OF BIOLOGICS

Severe refractory asthma (subphenotypes of asthma that do not respond to current standard therapy) is a serious health problem. Pediatric asthma is often more treatable than adult asthma with adequate adherence to patient treatment. Severe asthma can also occur in children. Innovative biologics are available in cases where the disease control cannot be achieved despite the exhaustion of standard therapies. These drugs belong to a group of licenced monoclonal antibodies that block the T2 signalling pathway of inflammation. They act by disrupting associated T2 cytokines and IgE antibodies. Selective biomarkers can be used to identify suitable treatment responders. Omalizumab was the first approved monoclonal antibody for the treatment of asthma with a history of more than 20 years. This is an IgE-blocking IgG1 antibody. The mechanism of action involves interrupting the binding of IgE antibodies to coupled receptors on the cell surface. Omalizumab is indicated for add-on treatment of children aged six years of age. The role of FENO in selecting right patients and predicting a good therapeutic response remains unclear (44). A post-hoc analysis examining the effect of Omalizumab treatment on FENO levels yielded interesting conclusions. The study group of pediatric patients (>12 years) with higher FENO levels (>19.5 ppb) had a more significant reduction in exacerbations after 48 weeks of treatment (53% vs. 16%) compared to the group with lower FENO levels (45).

Eosinophils are therapeutic targets for the treatment of asthma. Since IL-5 is the major cytokine involved in eosinophil migration, maturation, and activation, the IgG1 monoclonal antibody Mepolizumab was specifically designed to target this cytokine. Treatment can also be initiated in children with severe eosinophilic asthma up to the age of 6 years. The outcomes of Mepolizumab treatment showed clinical evidence of efficacy in a real sample of paediatric patients with severe asthma when administered in routine care settings in Slovakia (46). The results of realized studies do not support the relevance of FENO as a biomarker of therapeutic success in patients treated with Mepolizumab (29,47). On the contrary, one study partially confounds the conclusions of previous clinical observations. A post-hoc analysis of the phase 2b DREAM trial data showed that adult patients treated with Mepolizumab with higher FENO (>25 ppb) and higher eosinophil (300 cells/ μ L) showed more significant reduction in exacerbations than the group with lower FENO (<25 ppb) and elevated eosinophils (62% vs. 34%) (48).

NO production is associated with the IL-13 signalling pathway. Dupilumab belongs to the family of biologics available for the treatment of AB. Dupilumab inhibits IL-4R signalling induced by both IL-4 and IL-13, and down-regulates T2 inflammation in a different allergic disorder (49). This dual mechanism inhibits the action of both pro-inflammatory cytokines in patients with T2-positive asthma. Dupilumab is indicated for patients aged 6 years and older with allergic and non-allergic eosinophilic asthma with FENO > 25 ppb, who depend on oral corticosteroids to control their symptoms, regardless of the blood eosinophil count (50). The role of FENO as a predictor of a good therapeutic response was confirmed in the Liberty Asthma Quest trial (NCT02414854). Probands aged 12 years and older with mean FENO values >25 ppb had a significantly reduced number of exacerbations (approximately 50%) compared with patients with FENO values <25 ppb (51). Furthermore, another study confirmed that a consistent decrease in baseline FENO values in adolescents and adults was associated with the chronic administration of anti-IL-4R. This suggests the potential of FENO as a pharmacodynamic biomarker for Dupilumab treatment (52).

Recently, Tezepelumab has been approved for the treatment of severe asthma. It targets one of the known epithelial cytokines, TSLP, which plays an important role in AB pathogenesis. The European Medicines Agency (EMA) has approved it for the treatment of adolescents aged ≥ 12 years and adults with severe asthma, regardless of phenotype, and without other indication limitations (positive biomarkers). Tezepelumab reduced eosinophil counts, FENO, and IgE in a clinical efficacy study of participating probands, suggesting its multi-suppressive action on individual inflammatory pathways. Similar to Dupilumab, patients

with higher FENO levels have significantly reduced disease flares (53). While innovative biologics are very effective in treating asthma, they do have some risks. These risks include an increased number of acquired infections, allergic and autoimmune reactions and in rare cases serious side effects (54).

Studies using biologics performed so far have focused on the usefulness of this biomarker in predicting therapeutic responses. Significantly less research has been devoted to assessing the significance of the dynamics of FENO values associated with treatment with these monoclonal antibodies. Indeed, the subject of future studies in this field may be its prognostic relevance to changes in biologics in the patient's therapeutic regime (55).

CONCLUSIONS

AB is a complex, multifactorial disease. Similar to other allergic disorders, the number of new cases diagnosed in children is expected to increase continuously in the future. In clinical practice, the diagnosis and care of pediatric patients with asthma is predominantly based on the evaluation of symptoms. However, this approach has some limitations. In many cases, this can lead to misdiagnosis. Therefore, methodologies that aim to assess the functional status of the airways, including bronchial hyper-responsiveness, have a unique place in the diagnostic algorithm of AB. However, these tests do not directly assess the severity of airway inflammation, which is the principal pathognomonic feature of AB.

FENO is a non-invasive, easy-to-implement, repetitive measurable, and economically rentable biomarker that accurately reflects the level of T2 inflammation in the bronchi. IL-13 production and activity in the airway epithelia affect the dynamic variability of FENO levels. This represents another additional tool to improve diagnosis, especially in paediatric patients with AB. FENO does not replace commonly used diagnostic options but complements them when appropriately indicated and interpreted. The application of this tool may be particularly beneficial in more severe and complicated cases of asthma. Knowledge of the relationship between decreasing FENO values in the context of the biological effects of inhaled and systemic corticosteroids suggests its role as a predictor of the possible therapeutic response and indirectly the assessment of adherence to treatment. Additionally, it may be helpful to stratify patients according to the future risk of the disease exacerbation and a decrease in spirometric

Table 2 Review article highlights

Highlights
➤ FENO as asthma biomarker reflects the level of T2 eosinophil inflammation in the bronchial tree. ➤ FENO is a non-invasive, easy-to-implement, repetitive measurable, and cost-effective biomarker. ➤ FENO can be measured by exhalation into an analyser in young children aged 5 years. ➤ Nitric oxide production in asthma inflamed airways is associated with the IL-13 signalling pathway. ➤ FENO levels depend on various determinants and should be interpreted in the context of the patient's history and clinical symptoms. ➤ FENO is helpful to recognise responders on corticosteroids and to follow the response and adherence to treatment. ➤ FENO can be useful biomarker to predict therapeutic response and efficacy in reducing the risk of future asthma exacerbations in candidates for biologic therapy with Omalizumab, Mepolizumab, Dupilumab and Tezepelumab.

parameters. Other potential use of FENO is to predict which children who have repeated episodes of wheezing are likely to be diagnosed with asthma in the future. New findings have revealed the importance of the FENO in the context of potential for positive therapeutic response and monitoring of treatment with selected biologics (Omalizumab, Mepolizumab, Dupilumab, Tezepelumab).

The implementation of more well-designed studies with sufficient evidence will help to better understand the importance and targeted implementation of FENO in cases of severe asthma treated with biologics.

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