



Application of exhaled nitric oxide (FeNO) in pediatric asthma

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Purpose of review

Fractional concentration of Nitric Oxide in the exhaled air (FeNO) is a moderately good biomarker of type-2 airway inflammation, and its measurement is feasible also in children. The available evidence is still not enough to support the routine use of FeNO to diagnose or manage asthma in every patient in clinical practice. However, its role in identifying asthma with eosinophilic inflammation is of particular interest in the management of severe asthma.

Recent findings

In healthy subjects, FeNO levels increase with age and height, particularly in males, and are also influenced by ethnicity. FeNO measurement can support asthma diagnosis and help in predicting asthma development later in life in young children with recurrent wheezing. FeNO-guided asthma management is effective in reducing asthma exacerbations but may result in a higher daily dose of inhaled corticosteroids. FeNO can also be used as a marker to evaluate adherence to asthma treatment and predict response to different biologicals, especially Omalizumab and Dupilumab.

Summary

This review outlines recent data on the application of FeNO in childhood-onset asthma diagnosis and management, as well as in phenotyping subjects with severe asthma who may benefit from monoclonal antibodies administration.

Keywords

allergy, asthma, biologicals, children, FeNO, Type-2 inflammation

INTRODUCTION

Asthma is one of the most common chronic diseases worldwide, with an estimated 5–10% of affected school-aged children and adolescents [1,2,3^a]. Pediatric asthma is mainly characterized by a personal and/or family history of atopy and the presence of type-2 inflammation markers, including elevated total and specific immunoglobulin E (IgE) or positive skin prick tests and airway eosinophilia [4^a,5]. Conventional lung function testing, such as spirometry, does not show a good correlation with asthma severity and symptom control in children [6^a,7^a].

Therefore, over the last twenty years, several inflammation markers have been studied, mainly in exhaled air, to identify a noninvasive and easy-to-perform test that could further help in asthma management and predict loss of control [8]. To date, the Fractional concentration of Nitric Oxide in the exhaled air (FeNO) is the most studied and reproducible marker. Up to now, a noticeable amount of evidence supports the correlation between FeNO

levels and: (i) eosinophilic airway inflammation (eosinophil counts in blood/sputum/bronchoalveolar lavage), serum eosinophil cationic protein, IgE concentrations, and the number of positive skin-prick tests; (ii) likelihood of oral and inhaled corticosteroids (ICS) responsiveness; (iii) bronchial hyperresponsiveness; (iv) lung function and asthma symptoms, both in children and adults [9,10]. FeNO was brought to the pulmonology scene with the

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KEY POINTS

- FeNO measurement is a noninvasive tool to assess airway inflammation that can be easily performed starting from 5 to 6 years of age; nonstandardized techniques are also available for preschoolers.
- FeNO is a marker for type-2 airway inflammation, which can support the diagnosis of allergic asthma and help in phenotyping preschool children with recurrent wheezing.
- The available evidence does not support the use of FeNO-guided asthma management in routine clinical practice although this strategy can reduce asthma exacerbations in some groups of patients.
- Considering its fast response to oral and inhaled corticosteroids, FeNO can be considered as a tool to monitor treatment adherence.
- FeNO is being increasingly used as a biomarker to personalize treatment with biologicals in severe asthma.

promise of being the optimal thermometer for bronchial inflammation in asthma, and its use is currently spreading in clinical practice due to the growing availability of affordable and portable instruments. However, there is still conflicting evidence to support the routine use of FeNO for asthma diagnosis and management, mainly due to the heterogeneity of design, selection of subjects and outcomes, medication protocols, cut-off values, and lengths of follow-up in the available studies. Moreover, many endogenous and exogenous factors can be confounding factors influencing FeNO levels [11].

This review outlines the most recent findings on the application of FeNO in pediatric asthma, focusing on diagnosis and management, as well as on its role in identifying asthma with type-2 inflammation, providing evidence for more personalized therapeutic approaches.

FeNO MEASUREMENT AND INTERPRETATION IN CHILDREN

Figure 1 summarizes nitric oxide synthesis [12,13] and testing. International guidelines and technical

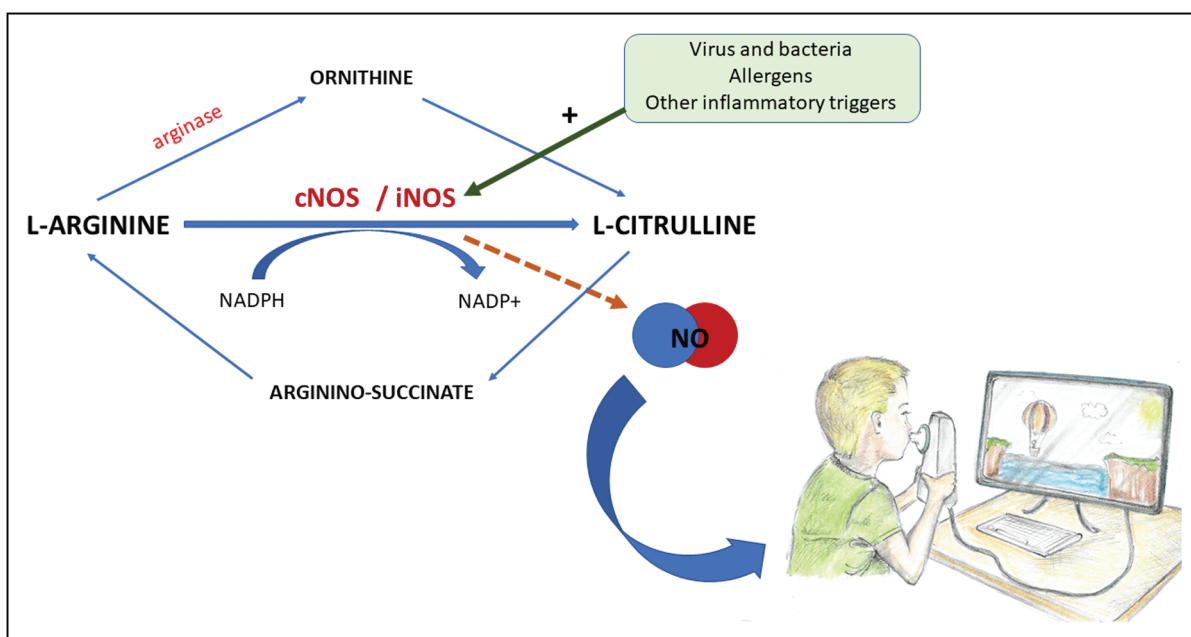


FIGURE 1. Nitric Oxide synthesis and measurement. Nitric oxide is a free radical gas deriving from the oxidation of the amino acid L-arginine to L-citrulline by nitric oxide synthase (NOS), which is involved in many physiological processes, including bronchodilation, vasodilation, neurotransmission, angiogenesis, smooth muscle relaxation, and immune response. Two isoforms of NOS are constitutive (endothelial and neuronal) and one is inducible (iNOS) [12] and all three types of NOS isoforms are expressed in the airways (macrophages, epithelial and endothelial cells, type 2 pneumocytes as well as smooth muscle and other cells) [13]. Pro-inflammatory cytokines such as IL-13 and IL-4 upregulate iNOS, increasing NO release. Exhaled NO is usually measured by chemiluminescence-based analyzers or electrochemical sensor devices. The most used technique is the single-breath online method (details in the text). In childhood, software providing a visual encourager is used to obtain a steady expiratory flow rate of 50 mL/s.

standards are available for FeNO measurement [14,15] and its clinical interpretation [16]. The standardized method for measuring FeNO is the single-breath online method in which the patient must inhale to total lung capacity and then slowly exhale for at least 6–10 s, at a steady flow rate of 50 mL/s and in conditions of velum closure (achieved by using a positive pressure of 5–20 cm H₂O against exhalation) [8,17,18] (Fig. 1). The procedure is non-invasive and can be easily and effectively performed starting from 5 to 6 years of age. According to the American Thoracic Society (ATS) guidelines [16], FeNO levels, expressed in part per billion (ppb), should be interpreted as follows:

- (1) <25 ppb (20 in children aged < 12 years): eosinophilic airway inflammation and responsiveness to corticosteroids unlikely;
- (2) 25–50 ppb (20–35 in children aged < 12 years): values to be considered with caution and to be correlated with clinical context;
- (3) >50 ppb (35 in children aged < 12 years): eosinophilic airway inflammation and responsiveness to corticosteroids are likely.

A reduction of at least > 20% in subjects with FeNO > 50 ppb or > 10 ppb in subjects with FeNO < 50 ppb is suggested as a significant change after the institution of ICS therapy [16].

No standardized method is currently available for FeNO measurement in noncooperating preschool-age children, although possible techniques, such as the offline tidal breathing technique, as well as reference values are also available for this age group [19].

CONFOUNDING FACTORS FOR FeNO LEVELS

FeNO levels may be influenced by several factors that are not currently taken into account by reference values [11] (Table 1).

In childhood, FeNO physiologically increases with age and height. It has been shown that FeNO values increase linearly between 6–14 years of age in girls and 6–16 years of age in boys to then reach a plateau in adulthood [20[■],21]. Garcia *et al.* recently described FeNO trajectories with increasing age in 1791 healthy school-age children (median age 8.4 years at enrolment). In this population, FeNO trajectories were similar in males and females up to the age of 11.5 years, a period coinciding with puberty and early adolescence, after which FeNO levels continued to increase in males, whereas females appeared to plateau [22[■]].

Accumulating evidence shows that also race and ethnicity influence FeNO levels. Healthy Hispanic children have higher FeNO levels than non-

Table 1. Endogenous and exogenous factors affecting FeNO levels

Increased FeNO ↑	Decreased FeNO ↓
Allergic rhinitis	Cystic fibrosis
Atopic dermatitis	Primary ciliary dyskinesia
Allergic Asthma (poor asthma control and/or exacerbations, poor ICS adherence, poor inhaler technique)	Bronchoconstriction
Viral infections	Active and passive smoke
Past asthma in adolescents	Inhaled corticosteroids
Eosinophilic bronchitis	Oral corticosteroids
Exposure to allergens and air pollution	Antileukotrienes
Increasing height	Prematurity
Male gender	Spirometry (transient effect)
Season and environmental pollution	Physical Exercise (transient effect)
Technical factors (high exhalation flow rate, breath holding, ambient NO, mixed oral and nasal NO sampling)	Technical factors (high exhalation flow rate)
Menstrual cycle variations	Menstrual cycle variations
Ethnicity	Ethnicity
Dietary exposures (nitrate rich diet)	Dietary exposures (caffeine, alcohol)

ICS, inhaled corticosteroids; NO, Nitric Oxide.

Hispanic white children [22[■]], whereas healthy black children have higher FeNO levels than both Hispanic and non-Hispanic white children, so it has been suggested to increase the FeNO cut-off point from 35 to 40 ppb for black children [23]. A recent study showed that African American children with nonsevere asthma have higher mean FeNO values at baseline, during an exacerbation, and after a short course of oral corticosteroids (OCS) than non-African American asthmatic children (i.e., white and Hispanics), despite no differences in disease severity, lung function, and ICS treatment doses [24[■]].

Finally, FeNO can decrease during bronchoconstriction and after tobacco smoke exposure [25[■]], although it can increase in specific conditions such as allergic rhinitis, eosinophilic bronchitis, atopic dermatitis, and in adolescents with either a past asthma history [14] or nonasthmatic airway hyper-responsiveness [26[■]].

FeNO USE IN PEDIATRIC ASTHMA DIAGNOSIS

Asthma diagnosis may be challenging in the pediatric population, because respiratory symptoms are

not specific and conventional spirometry testing may not be reliably performed to demonstrate variable airflow limitation, especially in young children.

Since FeNO is a marker for type-2 inflammation, its usefulness in supporting the diagnosis of allergic asthma has long been evaluated, with contrasting results. Since the first studies in the early 1990s demonstrating the presence of endogenous NO in the exhaled air [27,28], many studies have shown that FeNO levels are higher in allergic asthma, both in adults and children [29–31], due to increased activity of NO synthases and iNOS upregulation in the bronchial epithelium caused by type-2 pro-inflammatory cytokines, in particular IL-13 and IL-4 [32,33]. Real-life data from adults with asthma support the validity of current ATS cut-offs to identify and monitor eosinophilic airway inflammation [34]. However, considering all potential confounding factors, FeNO use as a single marker may lead to either over or underestimation of asthma diagnosis [10]. Unsurprisingly, most authors suggest that FeNO levels can better identify a non-eosinophilic asthma phenotype when low, than indicating an eosinophilic phenotype when high [35]. Nevertheless, a recent metaanalysis, including 26 studies with 4518 participants (mainly adults), showed that the overall sensitivity of FeNO in asthma diagnosis was higher than specificity (0.65 (95% CI 0.58–0.72, vs 0.82 (0.76–0.86)) with an area under the curve of 0.80 (0.77–0.85), suggesting that FeNO may be more useful in confirming rather than excluding a diagnosis of asthma [36]. Two further metaanalyses confirmed that diagnostic accuracy of FeNO in the identification of asthma in children is moderate; depending on FeNO cut-offs, the likelihood of having asthma in subjects aged >5 years increases by 2.8–7.0 times given a positive FeNO test result [37,38[¶]]. Some authors suggest combining the use of FeNO with other markers to improve its diagnostic accuracy in children [39]. In a recent study on 275 children with suspected asthma aged 8–16 years, combining FeNO with spirometry, and particularly with FEF25–75%, improved the diagnostic accuracy for childhood asthma (AUC 0.90 (95% CI: 0.86–0.93) [40^{¶¶}].

Taken together, the available evidence does not support the use of FeNO alone in asthma diagnosis in routine clinical practice. Current international guidelines recognize the role of FeNO in identifying type-2 airway inflammation, but recommend its measurement to support (when >35 ppb in children), but not exclude, a diagnosis of asthma in combination with conventional lung function testing and clinical evaluation [3[¶],41[¶],42[¶],43[¶]].

Regarding preschool wheezing, FeNO has been suggested as a potential predictor of asthma

occurrence later in life [44]. FeNO levels are higher in children with recurrent wheezing compared with healthy ones [45], and in preschoolers with persistent wheezing compared with transient wheezing [46]. FeNO levels are higher in preschoolers with recurrent wheezing and positive asthma predictive index [47]. In 166 preschoolers with recurrent wheezing, FeNO measured offline during tidal breathing was associated with an increased risk for asthma at school age [48]. In 306 children with symptoms suggestive of asthma, increased FeNO measured offline at 4 years of age was associated with asthma diagnosis at 8 years [49]. In infants with atopic dermatitis, elevated FeNO measured before the first episode of wheezing was associated with a higher risk of asthma and increased airway reactivity at 5 years of age [50]. White *et al.* recently evaluated the predictive value of FeNO measured using both a flow-controlled method under sedation and an awake tidal-breathing method among 36 children aged 6–24 months with a history of ≥ 3 episodes of physician-diagnosed wheezing treated with bronchodilators or corticosteroids. Sedated single-breath FeNO was significantly higher in children with active asthma at age 6 years than in healthy peers (36.4 vs. 16.9 ppb, $P < 0.0001$), and was also associated with modestly lower lung function. In contrast, awake tidal FeNO was not predictive of active asthma nor lung function at 6 years of age [51^{¶¶}]. Although limited, the available evidence suggests that FeNO in preschool children with recurrent respiratory symptoms may help to assess the risk for future asthma development in addition to clinical evaluation.

FeNO USE IN PEDIATRIC ASTHMA MANAGEMENT

The ultimate goals of asthma treatment are achieving clinical control as well as minimizing the future risk of asthma-related mortality, exacerbations, persistent airflow limitation, and side-effects of treatment [3[¶]]. However, airway inflammation monitoring is not currently included in asthma guidelines, therefore treatment is titrated based on symptoms control [3[¶]].

FeNO may be particularly useful in asthma monitoring since its levels rise before the occurrence of symptoms or when ICS therapy is withdrawn and abruptly fall when OCS [24^{¶¶},52] or ICS are administered [53,54]. However, whether FeNO could represent a reliable marker to monitor eosinophilic airway inflammation, predict exacerbations and guide therapy step up or step down is still controversial, since some previous studies have confirmed its efficacy [55,56], whereas others have not [37,57].

Some metaanalyses addressing data published over the last two decades have shown that pediatric asthma management based on FeNO reduced the likelihood of asthma exacerbations, but has neither significant impact on asthma control, in terms of symptoms, lung function, airway hyperresponsiveness, beta-2 agonist use, nor in final ICS dose when the strategy was compared with traditional guideline-based management [58–60]. Also, some reports have underlined the risk for a tendency to increase ICS dose without significant effects in clinical outcomes when adjusting therapy based on FeNO levels [61,62].

A recent metaanalysis addressing 7 randomized controlled trials (RCTs) on FeNO use to guide asthma treatment in 1112 children (mean age 12.6 years) followed-up for 6–12 months, showed that only a 50% change in FeNO was related to a small increase (11%) in the odds for having uncontrolled asthma in 6 months after baseline, whereas a single FeNO value and absolute change in FeNO over time were unlikely to be clinically useful [63[■]].

In another recent metaanalysis of 23 RCTs examining the effectiveness of FeNO-guided asthma management in 2723 pediatric patients aged 0.6 to 16.5 years with mild to severe asthma, the authors found a reduction in asthma exacerbations and an improvement in FEV₁ in the FeNO group, but at the expense of a significant increase in daily ICS dose (weighted mean difference: 64.17 µg; 95% CI: 53.59–74.75; $P < .00001$) [64[■]].

Regarding the potential use of FeNO to guide ICS dose reduction, a recent analysis by Wang *et al.* on 384 mild-to-moderate asthmatics aged ≥ 12 years, showed that a FeNO ≥ 50 ppb before reducing ICS therapy was associated with a 15% increased risk of exacerbations. Reducing ICS when the estimated exacerbation risk was $< 15\%$ vs $< 10\%$ would result in fewer patients remaining on the same ICS dose, but similar proportions of patients avoiding exacerbations. These findings would suggest considering the gradual reduction of ICS in well-controlled adolescents and adults with mild-to-moderate asthma when FeNO is < 50 ppb [65[■]].

In children aged 6–17 years with uncontrolled asthma, the use of FeNO in addition to Global Initiative for Asthma (GINA) guidelines for titration of ICS therapy resulted in lower daily ICS dose and cost of treatment at 1-year follow-up compared to the use of GINA guidelines only [66[■]]. Further recent metaanalysis data show that FeNO may be useful to guide therapy titration in certain subgroups of asthmatic children, as FeNO-guided treatment is associated with reduced exacerbation risk among children not receiving treatment with leukotriene receptor antagonists, and in nonobese compared to obese children [67[■]].

Lastly, given the response to corticosteroids, FeNO has been studied as a potential marker of low adherence to ICS therapy, which represents one of the most significant factors of poor asthma control in children and adolescents [68,69]. Elevated FeNO levels are associated with nonadherence both in children and adults and this association was found to be greater with FeNO than with lung function or reported symptoms [70,71]. A recent retrospective analysis in 155 asthmatic children aged > 10 years further confirmed these findings by using the percentage of days covered, based on physician prescriptions and pharmacy claims data, over a 2-year period. This study showed that adherence was negatively associated with age and FeNO and no difference in adherence was found between type and number of controllers, status of nasal steroids, atopic comorbidity or predisposition [72[■]].

Taken together, the available evidence is still not enough to support routine FeNO-guided asthma treatment in every patient [3[■]]. Clinical implications of the available studies on FeNO-based asthma management still remain uncertain, due to their heterogeneity in terms of age of the patients, severity of asthma, FeNO cut-offs to adjust treatment, algorithms and definition of exacerbations. FeNO measurement can be considered as an option to support asthma management in asthmatics who are symptomatic despite using ICS [41[■],42[■]]. FeNO efficacy in predicting asthma morbidity is higher when used in combination with other markers, such as blood eosinophils or lung function [73,74]. A crucial point in future studies will be the evaluation of personalized cut-off values based on FeNO personal best in each patient to monitor asthma [16].

FeNO USE IN THE ERA OF BIOLOGICALS

It is increasingly recognized that asthma is a heterogeneous disease, which includes different phenotypes and endotypes. The main endotypes currently recognized are type-2 high and type-2 low asthma, respectively characterized by predominant eosinophilic and neutrophilic airway inflammation [75]. In this context, the advent of monoclonal antibodies (mAb) has dramatically changed the management of severe asthma that represents ~ 2 –5% of childhood asthma [76,77[■]]. Currently, there are a few available biological options for type-2 high severe asthma in pediatric age, namely Omalizumab, Mepolizumab (both from age ≥ 6 years), and Dupilumab (from age ≥ 12 years) [77[■]]. Considering that these biologicals target different components of inflammation and that several others biologicals are in the pipeline, having reliable biomarkers that can help to identify the most

appropriate biological for each case is of paramount importance [78[■]]

FeNO, as a marker of type-2 inflammation, has been shown to be a good predictor of response to Omalizumab (anti-IgE mAb) [79[■]] and Dupilumab (anti-IL4/IL13 mAb) [80], but not to Mepolizumab (anti-IL5 mAb) [81]. The most recent ERS/ATS guidelines on the use of biologicals in severe asthma are largely targeted to adult patients. However, it is recommended using a blood eosinophil cut-off $\geq 260 \mu\text{L}$ and a FeNO cut-off ≥ 19.5 ppb to identify individuals (>12 years) with severe allergic asthma who are more likely to benefit from Omalizumab (low-quality evidence) [77[■]]. The same recommendation is present in the latest GINA guidelines dedicated to severe asthma, which also mention that patients eligible for treatment with Dupilumab should have blood eosinophils $\geq 150/\mu\text{L}$ and FeNO ≥ 25 ppb [82[■]]. FeNO measurement is not required for eligibility for Mepolizumab, which targets an eosinophilic endotype that seems more likely to occur in adult-onset asthma compared to childhood onset-asthma [7[■]].

CONCLUSIONS

After almost 30 years since the first reports of higher levels of FeNO in asthmatics, the usefulness of its measurement in asthma diagnosis and management continues to be controversial especially in children, mainly due to factors influencing its concentrations, as well as the heterogeneity of the available studies. However, FeNO may be considered as a moderately reliable biomarker for type-2 inflammation, rather than a marker for asthma itself, and could be useful in supporting asthma diagnosis and ICS initiation, and also as in monitoring adherence to treatment and predicting asthma exacerbations. FeNO should be used and interpreted with caution, hopefully in the hands of skilled professionals. Future studies are needed to shed more light on those aspects, as well as rethink FeNO cut-off values in children, considering the effect of increasing age and height on developing airways. FeNO is gaining an important role in personalized medicine, for which it could represent a possible guide for a targeted therapeutic approach.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
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