

FeNO as biomarker for asthma phenotyping and management

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ABSTRACT

The current review aims to revisit literature on exhaled nitric oxide (FeNO) in asthma phenotyping and management to clarify the utility of this test in clinical practice. It is increasingly evident that multiple profiles characterize asthma as a complex disease for which is necessary to find tools able to discriminate among these phenotypes to achieve the best therapeutic strategy for all asthmatic patients. Current findings indicate that FeNO, a noninvasive and easy-to-obtain biomarker, can be considered a useful tool in predicting asthma developing and exacerbation, in identifying specific asthma phenotypes, in improving asthma diagnosis and management in a selected population, and in monitoring efficacy of standard corticosteroid and biologic therapy. Based on this evidence, FeNO might become an appropriate tool for physicians to better define specific asthma phenotypes and to better deal with asthma worsening.

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Exhaled nitric oxide (FeNO) has ever been considered an airway eosinophilic inflammatory marker mainly related to allergic inflammation, but recent literature focused on its complementary role for differentiating asthma phenotypes. This review will discuss the most important issues where FeNO measurement may be clinically relevant.

FeNO AND CHILDHOOD ASTHMA PHENOTYPES

Three phenotypes of wheezing can be assigned to children: 1) the transient early wheezing, characterized by at least one lower respiratory tract illness with wheezing during the first three years of life but no wheezing at six years of age; 2) the nonatopic wheezing, characterized by respiratory tract infection and wheezing during the first three years of life and with wheezing at six years of age; 3) the atopic persistent wheezing, characterized by wheezing in more than half of all cases before age three and associated with atopy and wheezing at 6 years.^{1,2} Recent studies highlighted a predictive role for FeNO in children developing asthma (Fig. 1). In infants with recurrent respiratory symptoms, high FeNO was associated with maternal asthma history and augmented bronchial hyperreactivity (BHR), suggesting that FeNO may have implications in clinical practice for these infants.³ In a perspective study with

preschool children, FeNO was increased in those children with later asthma (at school age) compared with children not developing asthma, indicating that elevated FeNO in infancy is associated with increased risk for school-age asthma.⁴ In asthma-free children, Bastain *et al.* showed that the risk of new-onset asthma in the highest FeNO quartile was more than two-fold compared with the lowest quartile, reinforcing the concept of FeNO as a strong risk factor of new-onset asthma.⁵ In atopic adolescent asthmatics in clinical remission, elevated eosinophilic bronchial counts positively correlated with FeNO, suggesting that it is able to reveal subclinical airway inflammation, which may determine the risk of an asthma relapse later.⁶ Wheezing phenotypes are matter of discussion concerning childhood asthma, and two recent studies explored the role of FeNO in identifying specific phenotypes related to high FeNO. In a longitudinal latent class analysis study, five wheezing phenotypes were identified by different onset age in relation to FeNO, measured at four and eight years: FeNO at four years was higher in intermediate onset and persistent wheeze in comparison with never and transient wheeze, and FeNO at eight years was higher in persistent phenotypes of wheeze (including intermediate onset, persistent, and late onset wheeze) compared with never and transient wheeze but only among children with allergic sensitization at eight years, suggesting that the pathophysiology of wheeze phenotypes differs between atopic and nonatopic children.⁷ In another study in preschool children, FeNO was higher in persistent wheezers (particularly late onset wheezers) than transient wheezers and nonwheezers, and among persistent and late-onset wheezers, FeNO was higher in those with atopy and BHR compared with nonatopic and non-BHR, indicating that FeNO is a more appropriate marker of wheez-

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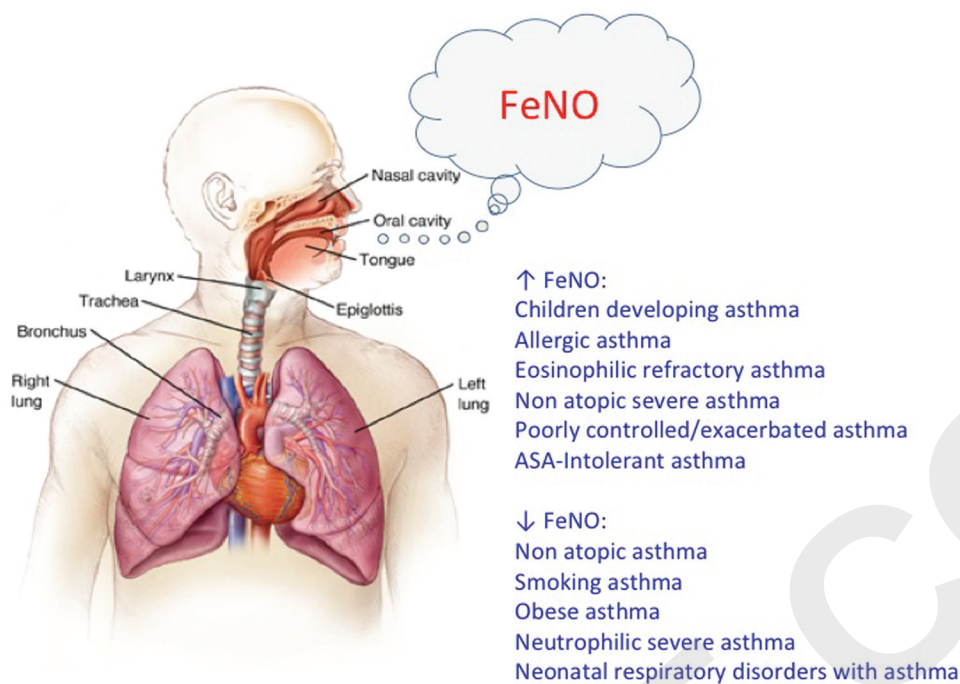


Figure 1. FeNO levels in different asthma phenotypes. ↑ = FeNO more than 26 ppb; ↓ = FeNO less than 15 ppb.

ing phenotypes in preschool children than spirometry.⁸ Another factor affecting FeNO in allergic asthma is related to the lung development: a lower FeNO was present in atopic asthmatic children who had neonatal respiratory distress syndrome (Fig. 1) potentially associated with factors responsible for down-regulation of NOS activity.⁹ This hypothesis is in agreement with Baraldi's study who demonstrated a lower FeNO in children with bronchopulmonary dysplasia than in control subjects.¹⁰

FeNO AND ADULT ASTHMA PHENOTYPES

Multiple different approaches have been used to identify adult asthma phenotypes, including clinical characteristics (*e.g.*, age at onset), factors that trigger asthma (*e.g.*, aspirin), and pathophysiological characteristics (*e.g.*, eosinophilic inflammation).¹¹ Five distinct clusters of asthma phenotypes were recently identified: the early-onset mild atopic asthma, the early-onset mild to moderate atopic asthma, the late-onset nonatopic asthma, the severe atopic asthma, and the severe asthma with fixed airflow.¹²

Other cluster analyses identified different phenotypes with high FeNO (including the early onset atopic and the inflammation predominant) and with low FeNO (such as obese nonallergic and adult-onset asthma) (Fig. 1).¹³ In late-onset asthmatics, obesity is associated with a decreased L-arginine/asymmetric dimethylarginine ratio, explaining why FeNO is negatively related to body mass index in obese late-onset asthmatics with lowered L-arginine/asymmetric dimethylarginine ratio. These subjects showed increased

respiratory symptoms, less allergic inflammation, reduced asthma quality of life, and lower lung volume, suggesting that obesity is a factor of asthma worsening related to low FeNO.^{14,15}

A direct association of FeNO with asthma severity was never confirmed,¹⁶ but in severe refractory asthmatics, FeNO was higher in subjects with eosinophil phenotype (Fig. 1) compared with subjects with negative eosinophil phenotype.¹⁷ Lower FeNO in severe than in mild asthmatics was associated with neutrophilic inflammation (Fig. 1), suggesting that neutrophilic-dependent oxidative stress in severe asthma is able to convert NO in nitrative radicals.^{18,19}

A complex relationship between arachidonic acid metabolites and NO synthesis was described in asthma. After lysine-aspirin (L-ASA) inhalation, FeNO increased significantly only in aspirin-inducible asthmatics, whereas no change was observed in aspirin-tolerant asthmatics and controls. Sputum eosinophils significantly enhanced after L-ASA inhalation only in aspirin-inducible asthmatics, and a significant correlation between the increase of both sputum eosinophils and FeNO was shown after ASA challenge. Thus, FeNO can be used as indicator of eosinophilic airway inflammation during ASA exposure in ASA-inducible asthma (Fig. 1).²⁰

In a distinct phenotype of severe adult-onset asthma (mostly nonatopic, 66%), high FeNO and sputum eosinophils are factors potentially associated with asthma severity, suggesting that adult-onset severe asthma is a phenotype characterized mainly by a nonatopic status and a persistent eosinophilic airway inflammation re-

lated to FeNO (Fig. 1).²¹ Severe asthma was also associated with the highest levels of inducible nitric oxide synthase (iNOS) expression and the index of iNOS mRNA to arginase2 mRNA in epithelial cells (by bronchial brushing) compared with mild-moderate asthmatics and control group.²² In addition, iNOS mRNA/protein expression in bronchial epithelial cells strongly predicted FeNO values, in particular in severe asthmatics.

In the severe asthma research study, asthmatics with high FeNO had more airway reactivity, sputum eosinophils, atopy, and hyperinflation but reduced perception of their symptoms.¹⁶ Among severe asthmatics, high FeNO identified those patients with the strongest airflow obstruction and hyperinflation, frequently needing emergency care. These data suggest that FeNO provides an autonomous classification of asthma severity and in severe asthmatics recognizes the worrisome asthma phenotype.

FeNO is mainly produced in the conducting airways, although a small quantity comes directly from the alveoli.²³ FeNO at 50 mL/s predominantly reflects large airway NO flux, but the single constant expiratory flow rate is not able to discriminate the individual contributions of large airways NO flux versus small airway/alveolar production sites. To interpret the different components that intervene in exhaled NO generation, a lung two compartments (alveolar and bronchial) model was proposed.²⁴ Using this model, central airway NO flux and small airway/alveolar NO concentration can be estimated by measuring exhaled NO at multiple expiratory flow rates and plotting NO output versus expiratory flow rates. The slope of the linear regression line between NO output versus varying expiratory flow reflects small airway/alveolar NO concentration, whereas the y intercept reflects large airway NO flux.²⁵ Airway wall concentration of NO and maximum flux of NO in the airways reflect the tissue production rate of NO, and they can be modified by corticosteroids. The airway wall diffusing capacity of NO depends on diverse physical and anatomic determinants of the airways and can be modified by structural and physiologic changes, which are characteristics of airway remodeling.²³ The alveolar concentration of NO (CalvNO) represents the degree of small airway inflammation, and the persistence of high CalvNO in patients treated with inhaled corticosteroids (ICSs) could reflect the incapacity of these drugs to reach distal airways.²³ In a recent study, CalvNO, corrected for NO axial back-diffusion from proximal to peripheral airway/alveoli,²⁵ was negatively correlated with forced expiratory flow₂₅₋₇₅ in patients with stable asthma, suggesting that CalvNO, in respect to FeNO, is a more specific marker of peripheral airway obstruction along with peripheral airways/alveolar inflammation in stable asthma.²⁶ In mild untreated asthmatics,

the alveolar component of FeNO is associated with the lack of asthma control, determined by the Asthma Control Test, supporting the notion that abnormalities of peripheral airways are involved in the mildest forms of asthma.²⁷

FeNO AND ASTHMA DIAGNOSIS

Asthma is a clinical diagnosis, and there is no single diagnostic test for the disease because of its heterogeneity. Present ATS guidelines have underlined a potential diagnostic role of FeNO in asthma with airway eosinophilia because of the strong recommendations and high degree of discriminatory power in this context.²⁸ The use of clinical cut-off points rather than predictive equations when interpreting FeNO values is supported by ATS guidelines.^{28,29} Alternatively, the normal range for a patient can be identified by reaching the "personal best" FeNO level via a course of oral corticosteroids.³⁰

FeNO can be useful to distinguish asthma from other disorders. FeNO measurement in patients with undiagnosed chronic respiratory symptoms showed a predictive power of asthma diagnosis higher than spirometry.³¹ FeNO specificity and sensitivity for asthma diagnosis, when associated with spirometry and BHR, become 94% and 93%, respectively.³¹ The predictive values for FeNO (cut point >25 ppb) were sufficiently robust for eosinophilic asthma diagnosis. In patients presenting asthma-like symptoms, increased FeNO provides helpful evidence for asthma diagnosis.^{31,32} However, the limitation to FeNO diagnostic role in asthma arises principally from airway inflammation heterogeneity and lack of association with increased FeNO in some phenotypes (*e.g.*, neutrophilic airway inflammation). The test may result falsely negative also in patients who have already been treated with inhaled or oral steroid. Thus, FeNO is very important to recognize steroid responsiveness in asthma. The recent FeNO clinical practice guidelines indicate that the cut-off of 26 ppb is the optimum cut point for significant sputum eosinophilia.²⁸

In subjects without asthma diagnosis with a FeNO more than 50 ppb (>35 ppb in children) significant eosinophilic airway inflammation and responsiveness to ICSs are expected (Fig. 2). In subjects without asthma diagnosis, FeNO less than 25 ppb (<20 ppb in children) suggests that eosinophilic airway inflammation and ICS responsiveness are unlikely, suggesting possible alternative diagnosis. On the other hand, during monitoring of subjects with diagnosed asthma, the presence of symptoms with FeNO more than 50 ppb in adults (>35 ppb in children) may indicate: inadequate ICS doses, high allergen exposure, or risk for exacerbation. In subjects with diagnosed asthma, FeNO less than 25 ppb in

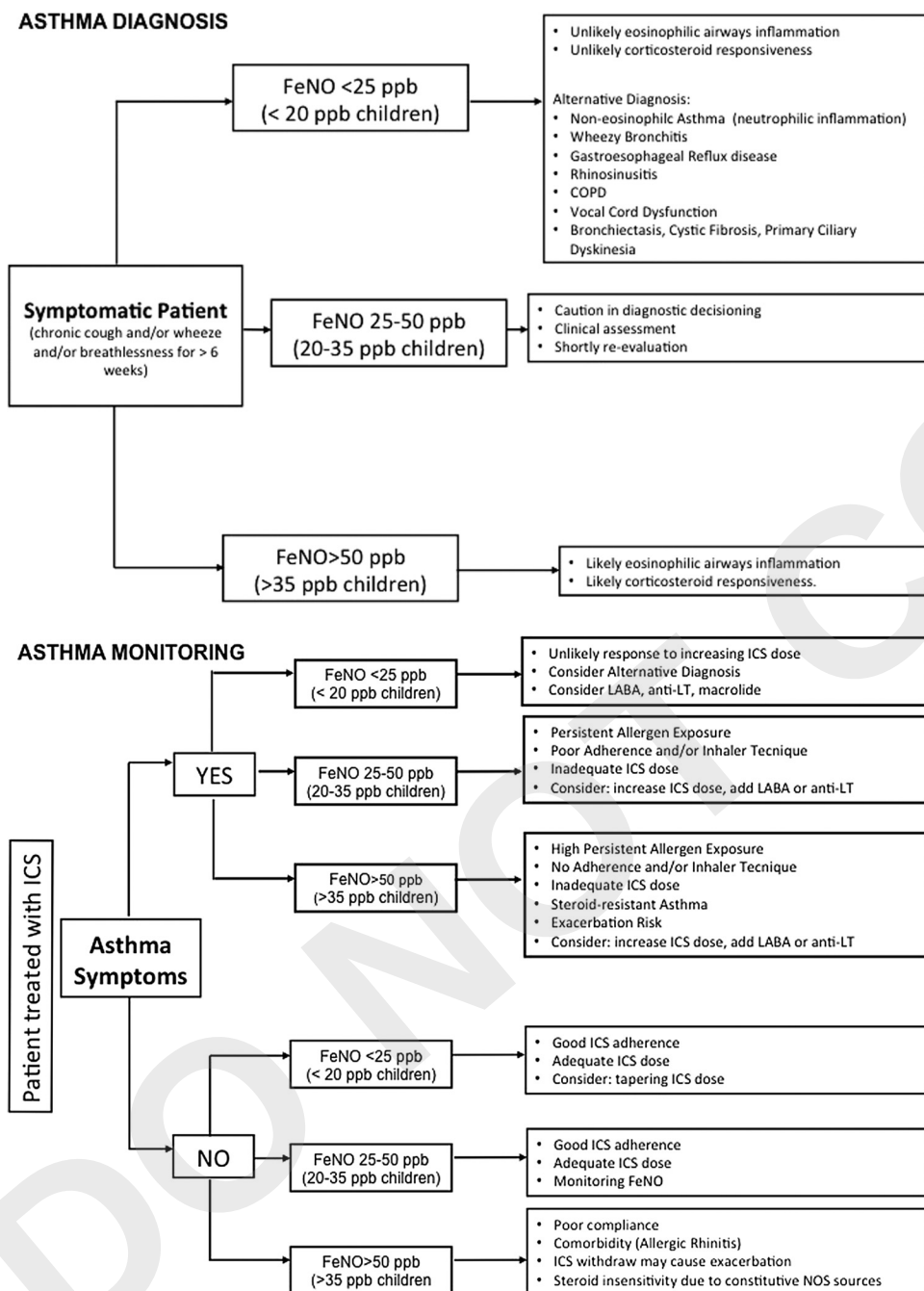


Figure 2. Flow chart concerning the application of FeNO measurement in the diagnosis of asthma (such as at the first visit) extrapolated from reference 28.

Figure 3. Flow chart concerning the application of FeNO measurement in the monitoring of patients with asthma (such as at the follow-up visits) extrapolated from reference 28.

adults (<20 ppb in children) may indicate no benefit from increasing ICS (Fig. 3). In monitored subjects with diagnosed asthma without symptoms, if FeNO levels are more than 50 ppb in adults (>35 ppb in children), ICS dose reduction may result in relapse, whereas in patients with diagnosed asthma, the absence of symptoms with FeNO less than 25 ppb in adults (<20 ppb in children) indicates that ICS dose could be reduced or even withdrawn.

A general practice study evaluated patients with lower airways complaints for a potential asthma diagnosis based on FeNO.³³ FeNO cut-off value of 46 ppb

reached a sensitivity of 32%, a specificity of 93%, and a positive predictive value of 80% to diagnose asthma. Based on study data, asthma diagnosis could be excluded with FeNO values less than or equal to 12 ppb, whereas a FeNO more than 46 ppb could be diagnostic of asthma. Alternatively, the normal range for a single patient is to reach the personal best FeNO level after a course of oral corticosteroids.³⁰ Finally, a recent report showed that asthma could be determined at FeNO more than 31 ppb (positive predictive value 82%) and rejected at FeNO less than or equal to 12 ppb (negative predictive value 81%) in pneumologists' routine work-

up.³⁴ FeNO accuracy increased when patients with neutrophilic inflammation were omitted from analysis, indicating that the diagnostic accuracy of FeNO was pleasing in the whole study population and remarkably increased when inflammatory patterns were considered.

Moreover, an increased FeNO provides supportive rather than conclusive evidence for asthma diagnosis in patients presenting symptoms of shortness of breath, cough, and/or wheeze. In conclusion, FeNO per se has a good specificity but a relatively low sensitivity for asthma diagnosing, limiting the use of elevated FeNO as a diagnostic testing.

FeNO AND ASTHMA CONTROL

The Jones's longitudinal study firstly showed FeNO utility to predict and diagnose poorly controlled asthma with respect to other common markers of airway inflammation and clinical parameters.³⁵ FeNO was able to forecast loss of control with a positive predictive value between 80% and 90%. Globally, over time changes in FeNO had higher positive predictive value, sensitivity, and specificity both for predicting and diagnosing loss of control than any single other measurement. These data showed that absolute FeNO value of more than or equal to 15 ppb or 60% over baseline is a useful inception for ongoing airway inflammation detection and positively predicts breakthrough symptoms arrival.

Several studies showed an increase of FeNO in asthma, further heightening when asthma control declines or when exacerbation occurs (Fig. 1).³⁶ During corticosteroid therapy, FeNO changes come before symptoms, FEV₁ and sputum eosinophilia improvement. Thus, FeNO can be considered a sensitive predictor of loss of asthma control.³⁷ FeNO is the quickest marker of response to corticosteroid therapy rapidly decreasing (in terms of weeks) after treatment as compared with other markers predicting response to therapy.³⁸ A recent study concluded that FeNO value more than 300% of predicted identifies subjects at risk of excessive use of rescue medication and needing oral corticosteroids within one year.³⁹

FeNO AND ASTHMA MANAGEMENT

A precise evaluation and monitoring of inflammation in asthma is clinically relevant to optimize the management of such patients, because symptoms of asthma, including wheeze and dyspnoea, are nonspecific and may not necessarily reflect the intrinsic inflammatory process. FeNO predicts the probability of CS responsiveness more consistently than spirometry, bronchodilator response, or BHR.⁴⁰ In stable asthmatics, both FeNO and sputum eosinophilia predicted a positive therapeutic response to oral prednisolone.⁴¹ A

study on patients with suspected asthma showed that a baseline FeNO of at least 47 was able to identify a positive steroid response in terms of improved lung function.⁴² A study in nonallergic asthma reported a FeNO cut-off of 33 ppb predicting a therapeutic response to ICS.⁴³

On the contrary, randomized trials, investigating asthma control using regular FeNO measurements to adjust ICS therapy, have failed to show important benefits,^{44–46} but in one study a reduction of ICS dose was facilitated without compromising asthma control.⁴⁷ This study for the first time provided proof-of-concept that FeNO could be used to guide asthma treatment.⁴⁷ A recent meta-analysis on the efficacy of adapting asthma interventions based on FeNO showed that the advantages of employing a FeNO strategy were not statistically different for asthma exacerbations, but the FeNO strategy allowed a reduction in the final daily ICS dose in adults.⁴⁶

Children with difficult asthma poorly respond to ICS therapy: these patients often have low FeNO levels. In a study, difficult asthma children remained uncontrolled after dispensing maximum doses of inhaled fluticasone for one month, followed by a course of oral corticosteroid for an additional month.⁴⁸ Baseline FeNO was a relevant marker for predicting therapeutic response: a FeNO value more than 30 ppb showed a 87% sensitivity and a 91% specificity for identification of controlled patients. A multivariate analysis also demonstrated that only FeNO (odds ratio 47.7) independently correlated with the achievement of control. A recent meta-analysis was carried out to estimate the FeNO clinical value in pediatric asthma management, and the results suggested that the use of FeNO to tailor the dose of ICSs in children cannot be recommended for routine clinical practice, because there is a danger of excessive ICS doses in children without significant changes in clinical outcomes.⁴⁹ Another study in children with moderate-severe asthma showed that ICS dose was approximately the same between FeNO-driven and standard asthma management group, thus showing a good risk/benefit ratio.^{50,51}

Moreover, FeNO-based asthma management strategy significantly reduced exacerbation rate, indicating that FeNO-guided asthma management is superior to management by clinical parameters alone.⁵¹

A 12-month study in mild to moderate asthmatics showed a reduction of ICS dose and a progressive symptoms score improvement associated with FeNO and sputum eosinophilia decrease, suggesting that titrating antiinflammatory therapy in relation to both FeNO and sputum eosinophils in adult stable asthmatics generates a fine long-term clinical control.⁵²

Powell *et al.* showed that FeNO can be used in targeted rather than general asthmatic population: 203 pregnant asthmatic women, some of whom without

receiving ICS at study entry, were monitored.⁵³ Patients with asthma symptoms and low FeNO (<16 ppb) received long acting bronchodilator agents and the minimum dose ICS. Patients with symptoms and high FeNO (>29 ppb) received higher doses of ICS by step-wise titration. Exacerbations were significantly reduced from 0.62/pregnant patient to 0.29 in the FeNO group. At term, the number of patients using ICS was superior in FeNO group (77%) compared with control group (46%), but the mean ICS dose was significantly lower in FeNO group, suggesting that FeNO should be selectively used in a specific targeted asthmatic population.

Several studies in both adult and children with allergic severe asthma and high FeNO showed the decrease of FeNO after treatment with omalizumab, suggesting a role for it as predictive marker of efficacy during therapy with an anti-IgE antibody.^{54–56}

Calhoun *et al.* compared three different approaches: physician-based, biomarker-based (using FeNO), and symptoms-based management in adult mild to moderate asthmatics.⁵⁷ Among the three groups, no difference was observed in time to treatment failure. This study may have underpowered to identify a difference in a population of well-controlled asthmatics, and the results in these patients may not predict treatment response in partially/poor-controlled asthmatics.⁵⁷

Finally, FeNO is not generally recommended for asthma management, but a systematic assessment of published randomized trials of asthma therapy led by FeNO concluded that the composite results of these studies (ASTRAL studies: ASthma Treatment ALgorithm) were due to methodological issues that may have conducted to incorrect conclusions.⁵⁸ Therefore, to potentially achieve optimal asthma therapy by using FeNO, new studies, following ASTRAL issues, should be specifically designed.

CONCLUSIONS

FeNO is an easy-to-obtain and noninvasive biomarker for defining and confirming airway allergic/eosinophilic inflammation in patients with asthma. Recent publications^{59,60} highlighted the advantages of routinely FeNO use, when added to traditional assessment tools, in terms of clinical (improved diagnostic accuracy and better tailored therapeutic regimens) and direct/indirect cost-saving implications. The main disadvantage of FeNO technology is expensiveness, which makes it not widely available. Several nondisease-related factors like rhinitis, obesity, and smoke can affect FeNO and should be taken into account when interpreting FeNO. Asthma is a multifaceted disease with a plethora of phenotypes. Therefore, it is not possible to advocate the use of a single biomarker to monitor asthma and determine different responses

to treatments. At present, FeNO is the best noninvasive biomarker to inform us about corticosteroid-sensitive airway inflammation, and it is clinically relevant in different aspects of asthma, including risk of asthma developing, identification of various severe asthma phenotypes, prediction of biologic therapy efficacy, support of asthma diagnosis, and amelioration of asthma management in selected asthmatics. A limited number of studies compared FeNO-based asthma management algorithms with standard clinically based algorithms in targeted populations. Therefore, formal recommendation about the routine FeNO assessment cannot be made. We sustain the idea that FeNO measurement, in combination with clinical evaluation, lung function test, and sputum analysis, may guide physicians in the management of different asthma phenotypes.

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