

Exhaled Nitric Oxide

An Update



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KEYWORDS

- Exhaled nitric oxide • Allergic airway inflammation • Asthma • Exhaled NO • eNO
- FENO

KEY POINTS

- Fractional concentration of exhaled nitric oxide (FENO) is a noninvasive biomarker of allergic airway inflammation.
- FENO often correlates with eosinophilia, but is driven through different type 2 pathways.
- FENO can be a useful tool to aid in the diagnosis and management of respiratory disease, particularly asthma. However, guidelines on its use remain controversial.

Although originally described as endothelial-derived relaxing factor important in arterial tone, nitric oxide (NO) is now considered an important biomarker for respiratory disease.^{1,2} Studies have confirmed that the fractional concentration of exhaled nitric oxide (FENO) is elevated in the airways of patients who have asthma compared with controls.^{3–6} In addition, the level of FENO is well correlated with the presence and level of inflammation and decreases with glucocorticoid treatment.^{6–8} Because this biomarker is noninvasively and easily collected, it has potential to be used not only as a diagnostic tool but also as a management tool for assessing severity, monitoring response to therapy, and gaining control of asthma symptoms. This article reviews the biology of NO and its role in respiratory disease.

BIOLOGY OF NITRIC OXIDE

NO, a gaseous molecule initially thought to be a deleterious component in environmental fumes, became the molecule of the year in 1992 and was found to be an

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important signaling molecule in various biologic functions. This gas is abundant in the cardiovascular and respiratory systems.

NO and related compounds are generated from various resident and inflammatory cells in the human airway through the oxidation of L-arginine in the presence of a set of enzymes known as nitric oxide synthases (NOSs).^{9,10} This process is both oxygen and NADPH dependent. NOSs are expressed in 3 isoforms that were originally referred to as either constitutive or inducible forms of NOS. The constitutive forms were classified according to their site of expression: neuronal or endothelial. However, these enzymes are now known to be expressed by a wide variety of pulmonary cells and are referred to as NOS1 and NOS3, respectively. The inducible form (iNOS or NOS2) is upregulated by proinflammatory cytokines and stimuli and is likely expressed continuously by human airway epithelial cells under normal conditions.^{11,12}

In the lung, NOS1 is expressed in the NANC fibers and generates NO, which acts as a smooth muscle relaxer.^{13,14} NOS3 has been found in pulmonary endothelium and bronchial and alveolar epithelium.^{15,16} In the bronchial epithelium, NOS3 is thought to function primarily through regulating ciliary beat frequency.^{17–19} Both NOS1 and NOS3 are corticosteroid resistant, and therefore, basal release of NO is not affected by steroids.^{20,21}

NOS2 is a calcium/calmodulin-independent enzyme that is induced by tumor necrosis factor alpha, interferon gamma, interleukin-1 β (IL-1 β), viruses, bacteria, allergens, and environmental pollutants. In the respiratory tract, NOS2 has been reported to be expressed in epithelial cells, endothelium, airway and vascular smooth muscle, fibroblasts, mast cells, and neutrophils.⁹ As opposed to constitutive Nitric Oxide Synthase (cNOS), NOS2 is glucocorticoid sensitive and, although some constitutive expression occurs in the airway epithelium, the level of pulmonary expression can be modulated. Once NOS2 is induced, the production of NO increases within several hours to nanomolar concentrations, levels that are significantly higher than basal levels resulting from cNOS NO production.

The role of NO in the lung includes neurotransmission, vasodilation, bronchial dilation, and immune enhancement. However, NO has a paradoxical role in a disease such as asthma. In low concentrations, animal data support the bronchodilator nature of NO and the NANC-induced bronchodilation effect. However, at higher concentrations, NO seems to promote inflammation. Elevated exhaled NO values seen in disease states are suspected to be a result of overactivity of oxidative pathways, yielding proinflammatory cytokines and subsequent induction of NOS2 activity. The properties displayed by NO gas have allowed it to be measured in exhaled breath and to serve as a diagnostic and management tool for pulmonary disease.

MEASUREMENT AND INTERPRETATION

FENO is measured from a single breath exhalation. Measurement of FENO can be performed with either online techniques, where one exhales directly into an analyzer device and FENO is obtained immediately, or with offline techniques, where one exhales into a collection bag and the contents are later analyzed to determine the FENO. Measurements are more commonly performed online with real-time analyzers that measure NO with electrochemical sensors.²² Online measurements are obtained with a single breath technique whereby a person inhales to total lung capacity and exhales at 50 mL/s (regulated through an inline resistance while providing feedback through a computer graphic interface) for approximately 6 seconds, the time required for the FENO levels to plateau. The maneuver is repeated twice at minimum to assure reproducibility. Exhalation against positive pressure causes the velum to close, which

excludes upper respiratory NO from falsely raising the measurement of FENO.²³ Further information on technique is available in the American Thoracic Society (ATS)/European Respiratory Society (ERS) summary document.²⁴

FENO levels are inversely proportional to the expiratory flow rate. High flow rates result in low FENO and, conversely, low flow rates yield higher FENO levels. Flow dependence is partially explained by the fact that FENO is partly derived from a diffusion-based process in the airways.^{23,25} Because of this flow dependence, the ATS and the ERS adopted a standard for FENO measurements at 50 mL/s.

AIRFLOW MODELING

FENO is thought to provide insight into distal airway inflammation through a noninvasive route. Several investigators have described the sophisticated mathematical modeling, known as the 2-compartment model (alveolar and airway), allowing one to separately derive the relative contribution of the alveolar compartment to the overall NO production.^{24–27} This model allows derivation of flow-independent parameters, such as airway wall NO concentration, airway diffusion factor, and alveolar NO concentration, which collectively determine the total exhaled NO production. The relationship between NO output and expiratory flow rate is used to derive the alveolar component. Applying this model to FENO measurements at multiple flow rates has helped to determine the alveolar and airway contributions. At fast flow rates (>50 mL/s), the alveolar contribution to the total NO value predominates, but at slower flow rates (<50 mL/s), airway diffusion predominates.²⁷

NORMAL VALUES

There is ongoing variability regarding the normal reference range of FENO within adult and pediatric patient populations. Rather than using reference values, the current recommendation for interpreting FENO is to use clinical cut points. The current recommendation is that values <25 ppb (or <20 ppb for pediatrics) indicate a lack of allergic inflammation, whereas values >50 ppb (or >35 ppb for pediatrics) are likely to be consistent with allergic inflammation and responsive to corticosteroid treatment. Values ranging from 25 to 50 ppb (or 20–35 ppb for pediatrics) should be interpreted within the clinical context of each individual patient.²⁸

The data attempting to identify normal levels of FENO are variable, and some study findings indicate different populations should have different cut points. Olin and colleagues²⁹ reported a median value of 15.8 ppb with an interquartile range of 11.9 to 21.4 ppb in 179 nonatopic healthy adults. The atopic asthmatic cohort showed a median value of 29.8 ppb with an interquartile range of 18.2 to 47.8 ppb.²⁹ Buchvald and colleagues³⁰ studied more than 400 children aged 4 to 17 years and found the mean FENO value to be 9.7 ppb. The upper end of the 95% confidence interval (CI) was age dependent, with a value of 15.7 ppb at age 4 and 25.2 ppb for adolescents, suggesting that perhaps there should be age-based FENO normal values.³⁰ A study by Yao and colleagues³¹ also supported the idea of age-based FENO normal values, because they found that FENO increased by an average of 7.4% per year in children. The effect of age on FENO is limited to children, because FENO increases with age until adulthood and then stabilizes.^{32,33}

Another study in pediatric patients demonstrated that in addition to age, race and height were important factors in determining baseline FENO values.³⁴ Olivieri and colleagues³⁵ measured FENO levels in more than 200 non-smoking adults. Men were found to have levels ranging from 2.6 to 28.8 ppb, and women had levels ranging from 1.6 to 21.5 ppb. In another study by Travers and colleagues,³⁶ men consistently

had higher levels of FENO than women. The difference between men and women suggests a possible need to have gender-specific normal values. However, a study by Olin and colleagues³⁷ found that age and height affected FENO, but gender did not. Olin and colleagues³⁸ also published FENO values from a random general population sample and found the median value to be 16 ppb, with the 25th to 75th percentiles being 11 and 22.3 ppb, respectively. Height, age, atopy, report of asthma symptoms in the past month, and reported use of inhaled steroids were positively associated with FENO, whereas current smokers had lower values of FENO. In general, both short-term and chronic smoking cause decreased FENO levels, but FENO levels are usually still elevated in smoking patients who have asthma.^{5,38–40} Gender was not associated with FENO in this study, although other studies have shown FENO to be higher in men than women.^{38,41}

The most recent ATS guidelines on interpreting FENO suggest evaluating changes in an individual's FENO from personal baseline in addition to using generalized cut points. Based on studies evaluating the utility of FENO in guiding dose adjustment of inhaled corticosteroids, an increase or decrease in FENO greater than 20% for levels greater than 50 ppb (or a difference greater than 10 ppb for levels <50 ppb) is significant.²⁸

FACTORS ASSOCIATED WITH ELEVATED NITRIC OXIDE

Allergic and Eosinophilic Inflammation

Allergic and eosinophilic inflammation are central hallmarks of asthma. Studies have confirmed a strong, although not always absolute, correlation between FENO and eosinophilia in induced sputum,^{42,43} bronchoalveolar lavage,⁴⁴ and tissue.^{45–47} Berry and colleagues⁴⁸ demonstrated that an FENO of 36 ppb had a sensitivity of 78% and a specificity of 72% for sputum eosinophils greater than 3%. The meta-analysis of Korevaar and colleagues⁴⁹ found that FENO had a sensitivity of 66% and a specificity of 76% for detecting sputum eosinophils of 3% or greater in adults. Thus, although FENO can be used as a noninvasive surrogate for sputum eosinophils, this may result in several false positives or negatives.⁴⁹ Payne and colleagues⁴⁶ obtained endobronchial biopsies in asthmatic children aged 6 to 17 years treated with prednisolone. Those who had persistent symptoms and a raised eosinophil score showed higher FENO levels.^{46,50} Similarly, in a study of 29 children with asthma and nonasthmatic controls, Warke and colleagues⁴⁴ showed that FENO was positively correlated with BAL eosinophil levels.

Allergic exposures induce T-helper 2 cell (Th2)-mediated inflammation, leading to increased levels of IL-4, IL-5, and IL-13.⁵¹ In addition, type 2 innate lymphoid cells (ILC2) can be stimulated by allergenic or nonallergenic stimuli, such as alarmin cytokines like IL-33, to release IL-5 and IL-13.^{51,52} IL-4 and IL-13 seem to drive FENO, whereas IL-5 drives eosinophilia. Because these cytokines can often increase or decrease at the same time for a given individual, eosinophils and FENO can often increase or decrease in parallel. The correlation is not absolute, however, because eosinophilia and FENO are driven by different cytokines that are produced by various cells, and the relative production of these cytokines will help determine the trajectory of each biomarker.

Recent studies of biologic agents in asthma have helped elucidate the different mechanisms driving allergic and eosinophilic inflammation and their relationship to FENO. In the DREAM study looking at the exacerbation-reducing effect of mepolizumab, a monoclonal antibody to IL-5, subjects were required to meet 1 of 4 criteria that could possibly represent an eosinophilic phenotype: elevated FENO ≥ 50 ppb, blood

eosinophilia ≥ 300 , sputum eosinophilia $\geq 3\%$, or asthma deterioration with a $<25\%$ decrease in Inhaled corticosteroid (ICS) and oral corticosteroid (OCS) dose. The study found that although FENO and blood eosinophils correlated with sputum eosinophils, baseline blood eosinophils were closely associated with a response to mepolizumab, whereas baseline FENO was not.⁵³ A study evaluating the efficacy of lebrikizumab, an anti-IL-13 antibody, for asthma found that treatment with lebrikizumab decreased FENO levels.⁵⁴ Similarly, the use of dupilumab, a monoclonal antibody that inhibits IL-4 and IL-13, appears to decrease FENO. In a pilot study looking at dupilumab's ability to reduce "induced exacerbations" when ICS and Long acting beta-agonist (LABA) were weaned, FENO levels decreased and remained decreased even with a weaning of ICS therapy. There were no significant changes in eosinophil levels in the treatment or placebo group.⁵⁵ A larger, more recent study of dupilumab in adult asthmatics demonstrated a decrease in FENO in both those with elevated eosinophils and those with low or normal eosinophils at baseline.⁵⁶

Although these varied results to the biologics may seem inconsistent initially, understanding the signaling pathway for FENO sheds light on these. Because iNOS is activated by STAT1 and STAT6, upregulation of STAT6 through upregulation of IL-4 and IL-13 would in turn be associated with higher FENO levels. On the other hand, IL-5 upregulates STAT5, which does not have an effect on iNOS or FENO levels. Understanding this pathway further supports the idea that FENO is not a surrogate marker for sputum eosinophils but rather an often parallel marker of airway inflammation.

Glucocorticoid Responsiveness

The correlation of FENO with allergic and eosinophilic inflammation suggests that FENO is also useful to predict steroid responsiveness. In 2003, the US Food and Drug Administration approved the AeroCrine AB (Stockholm, Sweden) FENO monitoring system for clinical application in patients who have asthma. The labeling of this device was restricted to monitoring the response to anti-inflammatory medications, as an adjunct to established clinical and laboratory assessments, in individuals aged 4 to 65.⁵⁷

Little and colleagues⁵⁸ studied the use of FENO as a marker for response to oral glucocorticoids in adults with stable asthma and found that the clinical benefit of oral steroids was greatest in those showing elevated baseline FENO levels. In a study of severe, therapy-resistant pediatric asthma patients, FENO, along with ACT score, forced expiratory volume in 1 second (FEV1), and sputum eosinophils, was examined to predict systemic steroid responsiveness.⁵⁹ Smith and colleagues⁶⁰ found similar results in a study of 52 steroid-naïve adults with chronic respiratory symptoms who received 4 weeks of inhaled fluticasone therapy. The response to ICS as assessed through symptoms and spirometry was greatest in the third of subjects whose FENO was greater than 47 ppb. In the absence of a high FENO, the response to steroids was less likely.⁶⁰ Similarly, Szeftler and colleagues⁶¹ showed that asthmatic children who had high FENO values as well as elevation in other allergic markers (peripheral eosinophils, total immunoglobulin E, and eosinophil cationic protein) were more likely to respond to ICS than those who had lower FENO and other allergic biomarkers. Thus, higher FENO levels appear to predict steroid responsiveness, particularly in allergic asthma.

Similarly, several studies have supported the use of FENO to adjust ICS dose. A study by Pijnenburg and colleagues⁶² evaluated 85 children who had asthma. Using 30 ppb as the threshold above which therapy would be increased, a significant reduction in bronchial hyperresponsiveness was seen in the FENO group, with a concomitant reduction in exacerbations requiring oral steroid use. Katsara and colleagues⁶³

assessed compliance using FENO and a data logger attached to a pressurized meter dose inhaler. This study showed a tendency toward increased FENO with poor compliance. Although these studies do not conclusively prove cause and effect, they suggest the usefulness of FENO as a noninvasive marker in the clinical setting to adjust ICS dose and to monitor patient compliance.

Respiratory Infections

Exhaled NO is increased in association with some viral infections but not with bacterial infections.^{64,65} A study investigating the presence of rhinovirus in nasopharyngeal aspirate from asymptomatic stable asthmatic patients and healthy controls demonstrated rhinovirus prevalence of 4.5% in asthmatics versus 0.9% in controls. The asthmatics who were positive for rhinovirus had higher FENOs than their rhinovirus-negative counterparts, although this did not reach significance.

Unlike infections with rhinovirus, infections with respiratory syncytial virus appear to result in a reduction in NO formation. The difference has been hypothesized to be due to the ability of some viruses to induce iNOS expression. The pathway of this induction is unclear but may be linked to activation of STAT-1 via interferons. In addition, viruses such as rhinovirus and influenza A, are thought to induce ILC2s and IL-33 production, leading to increased production of Th2 cytokines, as discussed earlier, which could account for the increase in FENO.⁶⁶

CLINICAL USE

Asthma

Because FENO has been positively correlated with the presence of airway eosinophilia and allergic inflammation, it may be useful as an asthma diagnostic test to assess underlying inflammation. An official ATS clinical practice guideline published in 2011 based on a committee of FENO experts recommended the use of FENO in diagnosing eosinophilic airway inflammation and in determining steroid responsiveness; however, the 2013 ATS/ERS severe asthma guidelines do not recommend using FENO to guide treatment of severe asthma. Notably, this recommendation is conditional with very low-quality evidence.^{28,67} Similarly, the 2017 Global Initiative for Asthma guidelines do not support the use of FENO for making or excluding a diagnosis of asthma, and they do not recommend using FENO to help decide whether or not to initiate ICS therapy.⁶⁸

Dupont and colleagues⁶⁹ studied steroid-naïve subjects without a diagnosis of asthma who had symptoms suggestive of asthma. Of the 240 people, 160 had asthma based on post-bronchodilator reversibility in FEV1. In this study, FENO levels were found to have a sensitivity and specificity of 85% and 90%, respectively. Schneider and colleagues⁷⁰ used statistical modeling of data from 26 studies to conclude that when the pretest probability is at least 30%, FENO greater than 50 ppb has a positive predictive value of 0.76 (95% CI: 0.29–0.96) for diagnosing asthma. Smith and colleagues⁷¹ compared conventional diagnostic tests for asthma (bronchial hyperreactivity or beta-agonist reversibility) with FENO and induced sputum markers. Of the 47 subjects with symptoms suggesting asthma, 17 were ultimately diagnosed using the conventional diagnostic tests. In these individuals, FENO had a sensitivity and specificity of 88% and 79%, respectively.⁷¹

FENO can also be helpful in diagnosing cough-variant asthma in patients with chronic cough. A meta-analysis of 15 studies by Song and colleagues⁴¹ found that in a population with chronic cough, FENO had a sensitivity of 72% and a specificity of 85% for diagnosing cough-variant asthma, suggesting FENO could be a helpful rule-in test for diagnosing cough-variant asthma. Of note, many of the studies

supporting the use of FENO for diagnosing asthma were conducted in individuals without a prior respiratory diagnosis, which presumably means their disease was mild.

A meta-analysis of 43 studies evaluating the diagnostic accuracy of FENO for asthma concluded that FENO was a more accurate diagnostic tool in children than in adults. The investigators note that in the pediatric population overdiagnosis of asthma is much more common than underdiagnosis.⁷²

Malinovschi and colleagues⁷³ studied FENO as a marker for local inflammation and blood eosinophils as a marker for systemic inflammation to examine their relationship with wheeze, asthma diagnosis, asthma exacerbation, and emergency department (ED) visits. In a population of more than 12,000 children and adults, they found that there was a statistically significant but weak correlation between FENO and blood eosinophils. Both FENO and blood eosinophils correlated positively with asthma, wheeze, and asthma attacks, but only elevated blood eosinophils correlated with ED visits. The investigators hypothesized that the association between exacerbation in patients with severe asthma and eosinophils may be due to rhinovirus-triggered exacerbations, also causing an increase in eosinophils through the ILC2 pathway. FENO and blood eosinophils were found to have an additive value in predicting asthma and wheeze. In another study of FENO and asthma control, Malinovschi and colleagues^{73,74} investigated the utility of using FENO and blood eosinophilia to predict bronchial hyperreactivity and uncontrolled asthma in asthmatics aged 10 to 35. They found that using both FENO and blood eosinophils predicted poor asthma control better than using blood eosinophils or FENO alone.

To correlate FENO values and asthma control, Jatakanon and colleagues⁷⁵ used a steroid reduction model. They evaluated 15 asthma patients twice weekly over 8 weeks after inhaled steroid reduction. Elevated FENO levels significantly correlated with decreased airway function, decreased FEV₁, and increased frequency of beta-agonist use. In another study, asthma control was assessed in 30 patients based on conventional measures of symptoms and lung function. FENO correlated with both asthma control and severity level.⁷⁶

Several studies have evaluated the use of FENO to adjust treatment as well as to assess asthma control. Meyts and colleagues⁷⁷ conducted an office-based evaluation of asthma control in 73 children aged 5 to 18 years. Three levels of control (good, acceptable, and insufficient) were determined based on frequency of beta-agonist use, day and nighttime symptoms, and spirometry. The subjects determined to have insufficiently controlled disease had the highest median levels of FENO (median 28 ppb).⁷⁷

Similar to other studies,^{78,79} a prospective trial by Smith and colleagues⁸⁰ evaluated the usefulness of repeat measurements of FENO to guide therapy as compared with a guideline-based management strategy. The FENO group experienced a 46% reduction in the number of exacerbations, but based on a predefined cutoff for analysis, this reduction did not reach statistical significance. However, a statistically significant reduction did occur in mean ICS use over 12 months for the group managed using FENO measurements.⁸⁰ In a study of asthma in pregnancy, an FENO-guided treatment algorithm resulted in fewer exacerbations than symptom-guided treatment.⁸¹ Powell and colleagues⁸¹ demonstrated that using FENO to guide ICS dose, in addition to using symptoms to guide LABA use, reduced asthma exacerbations when compared with medication dosing guided by symptoms alone. In addition, the FENO group required significantly less Short acting beta-agonist (SABA) use, and the mean daily ICS dose of those in the FENO-guided group was less than the symptom-guided group.

In contrast, Szefer and colleagues⁸² demonstrated that adjusting treatment based on FENO levels in an inner-city asthmatic pediatric population resulted in higher doses of inhaled corticosteroids compared with management based on symptoms alone. Moreover, there is a small fraction of asthmatic patients in whom FENO fails to improve despite treatment with corticosteroids.⁸³ In atopic children with asthma, persistently elevated FENO may be due to ongoing allergen exposure and related to the level of allergic sensitization.⁸⁴ The negative results in Szefer's study may have been due to a combination of ongoing allergen exposure in the inner-city cohort as well as to the fact that their asthma and FENO levels were optimized before randomization, leading to difficulty detecting a difference when using symptoms as an outcome measure. Despite these findings, most studies to date support the concept that using a measure of inflammation, such as FENO, to help guide therapy may lead to more effective disease management, gauged by minimizing exacerbations and optimizing medication use.

Chronic Obstructive Pulmonary Disease

Because of its usefulness in diagnosing and assessing inflammation in asthma, FENO as a tool for diagnosing and monitoring chronic obstructive pulmonary disease (COPD) was investigated. FENO has not been found to be elevated in stable COPD,^{85,86} likely due to the prevalence of cigarette smoking and predominance of neutrophilic inflammation seen in these patients.⁸⁶ A small but detectable difference is seen in patients who have COPD and are current smokers compared with COPD patients who are ex-smokers or those who have never smoked.⁸⁷ Obtaining an FENO level may have a role in patients who have unstable COPD and those experiencing COPD exacerbations. These patients were found to have elevated FENO levels when compared with patients who had stable COPD (both smokers and ex-smokers).⁸⁸ There may also be a role for FENO in assessing patients with asthma-COPD overlap.

SUMMARY

Before the use of FENO, direct assessment of airway inflammation was limited to invasive techniques. Continued understanding and correlations of FENO measurements will likely continue to complement conventional diagnostic and assessment tools for inflammatory lung disease. Because the technique is noninvasive, it has gained popularity and is becoming a more common tool used not only for initial diagnosis but also for routine assessment of disease severity, response to treatment, and compliance. Furthermore, monitoring of FENO can help characterize various asthma phenotypes within the asthma syndrome and guide the appropriate use of inhaled corticosteroids as well as potentially biologics. Although a precise normal has yet to be defined, strong data exist for high positive and negative predictive values for diagnosing and treating asthma at both ends of the spectrum of FENO values. High FENO values (>50 ppb) suggest poor control, the presence of persistent inflammation, and a need for increased anti-inflammatory treatment. Low values (<25 ppb) suggest low levels of inflammation and may allow for anti-inflammatory treatment withdrawal. Currently, FENO serves as a complementary tool in managing patients who have asthma and other inflammatory diseases in addition to conventional measures of symptom control and response to therapy.

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