

Exhaled nitric oxide in asthma: From bench to bedside

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With more than 600 publications, exhaled nitric oxide (NO) has been extensively investigated as a noninvasive marker of airway inflammation in a research setting. This clinical rostrum presents a synopsis of the latest research about this novel marker in asthma and suggests how it might move from bench to bedside. Specifically, we review the evidence citing the applicability of exhaled NO in diagnosing asthma, monitoring the response to therapy, evaluating current symptom control, and predicting exacerbations of asthma. These studies support a role for exhaled NO in the evaluation and treatment of asthma in the clinical arena. (*J Allergy Clin Immunol* 2003;111:256-62.)

Key words: Exhaled, nitric oxide, asthma, monitoring

Asthma incidence has been increasing on a global scale and is characterized as a syndrome of chronic airway inflammation (AI), leading to symptoms, variable airflow limitation (AL), and bronchial hyperreactivity (BHR). AL and BHR, in the presence of typical symptoms, are commonly used for diagnosis, whereas the degree of AL is used to follow the course of the disease, including acute exacerbations and response to therapy. However, because more emphasis has been placed on treating AI, there is great interest in monitoring inflammation as the primary pathogenic feature.¹ An "inflammometer" would be helpful if it could assess the degree of AI and its response to therapy, predict exacerbations before AL worsens, and assess adherence to medication. Additionally, perhaps separately targeting and monitoring AI could modulate airway and parenchymal remodeling. Bronchoscopy with bronchoalveolar lavage and biopsy remains the gold standard to assess AI, but invasiveness and cost makes its application impractical and unethical as a routine method.

Currently, several less invasive methods have been evaluated to assess the degree of ongoing inflammation. The peripheral blood eosinophil count has been used as a correlate of ongoing allergic inflammation of the airway, as has urinary leukotriene E4 measurement. However, not all asthmatic individuals are atopic or show increased blood eosinophils, limiting the sensitivity of this method, which also lacks specificity. Induced sputum has

Abbreviations used

AI: Airway inflammation
AL: Airflow limitation
BHR: Bronchial hyperreactivity
EBC: Exhaled breath condensate
FE_{NO}: Fractional concentration of exhaled nitric oxide
LOC: Loss of control
NO: Nitric oxide
NOS: Nitric oxide synthase
PPV: Positive predictive value

received great attention because of its usefulness in analysis of inflammatory cells, particularly eosinophils but also mediators, in the soluble phase.² It is also highly reproducible in experienced hands, and there is a reasonable correlation between sputum eosinophilia and both FEV₁ and methacholine BHR. However, induction is time consuming and not without risk. Skilled preparation and interpretation of sputum differentials are required, and sputum processing takes 1 to 2 hours, meaning that results are not immediately available for physicians to help the decision-making process.

More recently, exhaled breath components, particularly the fractional concentration of exhaled nitric oxide (FE_{NO}) and exhaled breath condensate (EBC), both recently reviewed in the *Journal*,^{3,4} are proposed as means of assessing AI. While an exhaled nitric oxide (NO) test is online and yields immediate results, EBC needs to be collected and processed in the laboratory for the marker of interest. The marker or markers in EBC that will prove optimal for asthma monitoring are unknown. The focus of this clinical rostrum is to examine evidence that could move exhaled NO from bench to bedside.

FROM BENCH . . . Nitric oxide

The saga of NO in biology has unrolled since the 1980s, when NO (or perhaps a rapidly formed metabolite) was found to be an endothelially derived relaxing factor. We now know that NO mediates a broad range of physiologic and pathologic processes (eg, inflammation). This is particularly relevant to AI, which is the primary process involved in asthma.¹

Exhaled NO

Of note is the landmark discovery by Gustafsson et al⁵ in 1991 that NO of endogenous origin can be measured in breath and the subsequent observations by Alving et

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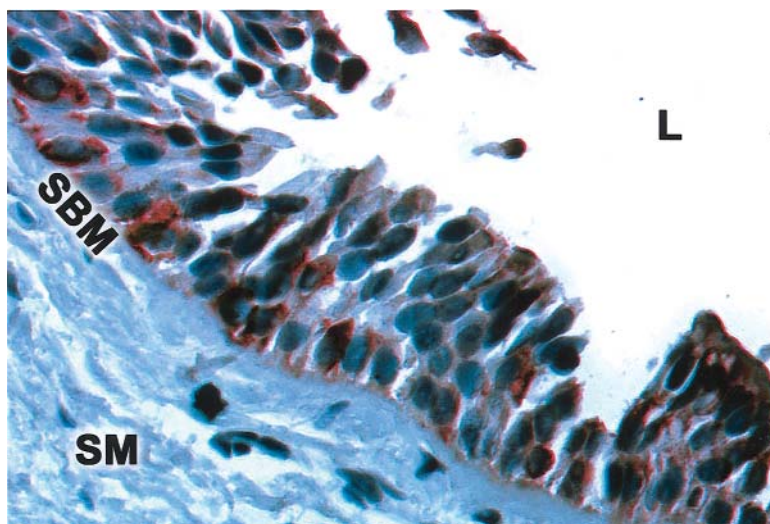


FIG 1. Immunohistochemistry for NOS 1 showing marked expression in an endobronchial biopsy specimen taken from a patient with severe asthma who was receiving chronic oral corticosteroid therapy. *L*, Lumen; *SBM*, subbasement membrane; *SM*, smooth muscle.

al⁶ that levels are high in asthma and by Kharitonov et al⁷ that levels decrease after steroid use. These discoveries have led to intense investigation of the FE_{NO} level in asthma and other lung diseases.

Origin of NO in breath

Once nasal NO is excluded, several studies have established that NO in breath originates in the lower airways⁸ and is synthesized by NO synthases (NOSs),⁹ with the recent suggestion for nonenzymatic sources from reduction of NO metabolites.¹⁰ Constitutive NOSs include neuronal NOS (NOS 1) and endothelial NOS (NOS 3), the names of which derive from their original discovery in the nervous tissue and vascular endothelium, respectively. Constitutive NOS isoforms are steroid resistant and calcium-calmodulin dependent and intermittently produce small packets of NO at picomolar concentrations that diffuse locally and have a physiologic effect. There is indirect evidence that constitutive NOS expression is upregulated in the airways of asthmatic patients.¹¹ Fig 1 shows an endobronchial biopsy specimen from a subject with steroid-dependent asthma in which NOS 1 was highly expressed in epithelial cells. An inducible NOS, NOS 2, might be expressed in a variety of cells (eg, airway epithelium, vascular endothelium, and inflammatory cells) in response to pro-inflammatory cytokines. NOS 2, which produces NO in nanomolar concentrations on a continuous basis, is usually steroid sensitive and is significantly overexpressed in asthma before, but less so after, inhaled steroid therapy.¹²

Standardized measurement

The American Thoracic Society has issued a statement on recommended standardized techniques for measuring FE_{NO} in adults and children.¹³ With online measurement, subjects inhale NO-free gas to total lung capacity and

exhale at a constant flow rate of 50 mL/s because FE_{NO} levels are flow dependent.¹⁴ Exhalation continues until a steady plateau is reached, and repeated exhalations are performed until 3 values are reproducible. A recent task force has dealt with measurement in very young children who cannot maintain a constant flow.¹⁵

Physiologic models of NO output

It would be beneficial to know the relative contribution of the bronchial tree and the lung parenchyma to NO exhalation in asthma and other diseases. This is especially relevant in light of evidence that asthma might affect the distal lung in addition to the major airways. A 2-compartment model (alveoli and airways) has been developed by several investigators to evaluate contributions from each of these areas, and this model explains experimental data.^{11,16-18} Fig 2 presents some important features of this model. The model allows the derivation of flow-independent NO exchange parameters, namely airway wall NO concentration, airway NO diffusion factor, and alveolar NO concentration, which determine the exhaled NO concentration. The relationship between NO output and expiratory flow can be used to derive the relative contributions of the airways and the alveolar compartment to NO output. The exhalation flow determines which compartment contributes more to the total NO output. Alveolar convection of NO predominates at faster exhalation rates (>50 mL/s), whereas airway NO diffusion prevails at slow exhalation rates (<50 mL/s).¹⁶ In asthma most investigators have found that airway NO diffusion is high compared with that seen in healthy subjects, whereas alveolar NO concentration is normal,¹⁹ probably because of avid binding by the pulmonary capillary blood. Airway wall NO concentration, although not significantly increased in patients with asthma,¹¹ decreases after inhaled steroid use by patients with asthma, which is probably related to a

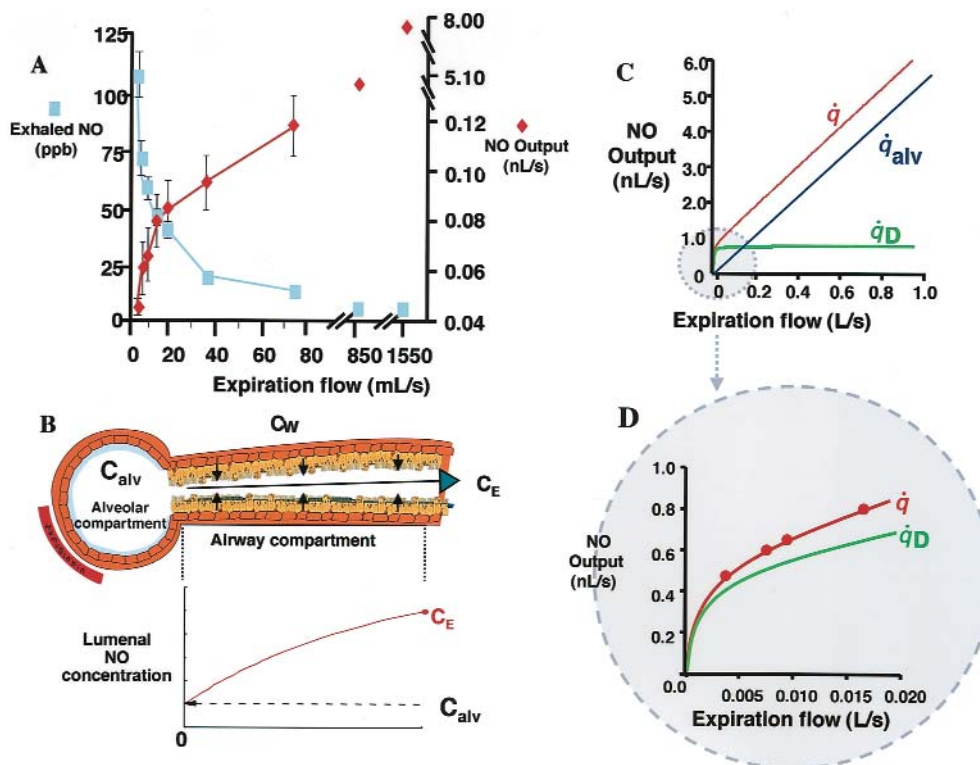


FIG 2. A, The flow dependence of exhaled NO and NO output in 10 healthy subjects taken from the study by Silkoff et al.¹¹ Exhaled NO concentration decreases but NO output increases as expiratory flow increases. B, The 2-compartment model. Alveolar NO concentration (C_{alv}) is low in healthy subjects because of diffusion of NO across the alveolar capillary membrane and avid binding to hemoglobin. NO in the alveolar compartment is convected out of the lungs, and the NO output rate from the alveolar source is directly proportional to the expired flow rate. As alveolar gas ascends the airway, it is conditioned with NO that diffuses from the airway wall to the lumen because of a concentration gradient between the airway wall NO concentration (C_w) and the airway lumen. This diffusion process of NO in the airway is described by an airway diffusion factor for NO (D_{NO}). Airway NO diffusion is expiratory flow dependent because the flow rate affects transit time in the airway limiting the time for NO diffusion. Thus at fast flows, the gas exits fast, and NO conditioning is reduced compared with that at slow flows, which increase transit time and the degree of conditioning. C_E , Expired concentration of NO (equivalent to FE_{NO}) derived from both alveolar convection and airway diffusion. C, Total NO output ($\dot{q}$) is the sum of that derived from airway NO diffusion ($\dot{q}_D$), which increases nonlinearly at low flows and reaches an asymptote at infinite flows, and alveolar NO convection ($\dot{q}_{alv}$), which increases linearly as flow increases. At high flows, the slope of the relationship between NO output and flow is equal to the alveolar NO concentration, and the major proportion of this output derives from alveolar convection ($\dot{q}_{alv}$). D, An enlargement of the low-flow region of Fig 2, C, showing that at low flows, the major proportion of NO output ($\dot{q}$) derives from airway NO diffusion ($\dot{q}_D$).

reduction in NOS 2 expression. Interestingly, airway NO diffusion is high in patients with asthma, even after steroid administration, and appears to be the dominant factor that increases FE_{NO} in asthma.¹¹ The raised airway NO diffusion factor might be related to expression of constitutive NOS isoforms (eg, NOS 1 over a greater airway mucosa area; Fig 1). Furthermore, the maximal airway NO diffusion after steroid use appears to be beneficial in patients with asthma, both reducing reactivity and promoting airway patency.¹¹ The same physiologic models have also been used to evaluate alveolar and airway NO output in patients with interstitial lung disease (eg, scleroderma lung),^{20,21} in whom, in contrast to asthma, alveolar NO convection is increased and correlates negatively with the carbon monoxide diffusion factor, whereas airway NO flux is normal.

Determinants of FE_{NO} in patients with asthma

Assuming a standardized measurement technique, there are several factors that determine an individual's FE_{NO} level, and there is a relatively large interindividual variation in FE_{NO} levels. In children FE_{NO} levels increase with age (perhaps a consequence of having the same fixed expiratory flow in all ages),²² and levels in women are lower.²³ There is an expanding list of diseases that have been associated with both increased FE_{NO} levels (eg, asthma and viral infections) and decreased FE_{NO} levels (eg, cystic fibrosis and ciliary dyskinesia syndromes).

Focusing on asthma, there are several factors that might result in varying levels of FE_{NO} in individuals with similar phenotypic disease. Disease activity is important. FE_{NO}

levels have been shown to increase during the allergy season²⁴ and after allergen challenge²⁵ but to decrease with reduction in allergen exposure.²⁶ Certain anti-inflammatory medications reduce FE_{NO} levels, including corticosteroids⁷ and leukotriene-modifying drugs.²⁷ Individuals with a diagnosis of allergic rhinitis in addition to asthma have significantly higher levels of FE_{NO} than individuals with asthma alone.²⁸ In addition, the presence of allergic rhinitis in and of itself without asthma is associated with increased FE_{NO} levels.²⁹ These individuals might be at risk for the development of asthma in the future.

Genetics play an important role as well. NOS 1 polymorphisms appear to be associated with both asthma symptoms and IgE levels.³⁰ Further work has shown that the number of AAT repeats in intron 20 of this gene correlate with FE_{NO} levels.²⁹ Asthmatic individuals with a higher number of AAT repeats (>12) have significantly lower FE_{NO} levels when compared with asthmatic individuals with fewer repeats. NOS 3 polymorphisms are also associated with atopy and IgE.³⁰ The recent attention to the role of endotoxin in atopic disease will likely prompt further studies in this area and its relationship to NOS induction because LPS is a potent inducer of type 2 NOS. To summarize, there are many factors that create significant variation in FE_{NO} levels between individuals. This will necessitate establishing stable baseline values in individual patients as a basis for comparison.

Relationship of FE_{NO} to AI

There are reports that FE_{NO} levels correlate with induced sputum eosinophil counts.^{31,32} However, if FE_{NO} levels are to be used as an indirect marker of AI, there should be validation against biopsy, but such studies are rare. Payne et al³³ took biopsy specimens and FE_{NO} measurements from 31 children with difficult asthma and 7 children without asthma after a 2-week trial of prednisolone and demonstrated a significant correlation between FE_{NO} levels and mucosal eosinophil inflammation in endobronchial biopsy specimens.³³ They suggest that FE_{NO} levels can be used as a surrogate marker for persistent AI in children with difficult asthma. Van den Toorn et al³⁴ found correlations between FE_{NO} levels and mucosa markers of eosinophil activation, even in subjects whose asthma had remitted. However, Lim et al³⁵ found no relationship between FE_{NO} levels and mucosa eosinophilic indices in patients with mild asthma.

Relationship of FE_{NO} levels to components of asthma

In general, the correlation of FE_{NO} levels with other components of the asthma phenotype are modest. Thus FE_{NO} levels have been reported to correlate with bronchial reactivity to methacholine before, but not after, steroid use.³⁶ In general, FE_{NO} does not correlate with pulmonary function parameters, although the decrease in FE_{NO} levels did correlate with the improvement in pulmonary function in one study.³⁷ The lack of strong correlations between FE_{NO} levels and standard asthma outcomes can be explained by the concept that FE_{NO} is a

distinct component of the asthmatic state; indeed, if there were tight correlations of FE_{NO} levels with, for example, spirometry values, then why measure it?

... TO BEDSIDE

Clinical application

Up to this point, FE_{NO} has been examined as a tool in clinical research. It can be measured in a standardized and reproducible manner and correlates to some degree with AI in biopsy and sputum specimens and with several asthma components. Immediate results can be obtained, and the technology to evaluate this has advanced considerably. In light of these findings, this methodology is poised to advance from the realm of clinical studies and research to its day-to-day use as a clinical tool.

Exhaled NO in diagnosis

The reported correlation of FE_{NO} with BHR suggests that this marker could prove useful as a screening tool for asthma.³⁶ Exhaled NO has proven useful in differentiating chronic cough because of asthma versus cough as a result of other causes. Studies have shown that individuals with chronic cough eventually diagnosed as asthma have significantly increased FE_{NO} levels compared with those with gastroesophageal reflux disease, and in this context FE_{NO} measurement is both sensitive and specific.^{2,38} The usefulness of FE_{NO} in diagnosing asthma has been augmented further by combining FE_{NO} measurement with concomitant measures of BHR.³⁹ Most recently, Deykin et al⁴⁰ reported that receiver operator curves for FE_{NO}, which relate cut-off levels of a test to specificity and sensitivity, indicate that FE_{NO} is a robust discriminator between asthmatic and healthy subjects.⁴⁰ In summary, there is mounting evidence that FE_{NO} measurement might serve as a useful tool in screening for asthma, but further studies are required.

Exhaled NO as an index of response to anti-inflammatory therapy

FE_{NO} levels decrease after anti-inflammatory therapy in asthma but not after bronchodilator use,⁴¹ and FE_{NO} is perhaps one of the fastest responding markers (Fig 3). The agents that have been associated with a decrease in FE_{NO} levels thus far include inhaled and oral corticosteroids and antileukotriene modifiers, as summarized in the section regarding the determinants of FE_{NO} levels. The decrease in FE_{NO} levels after corticosteroid use is rapid, demonstrates a dose response that plateaus at about 500 µg/d beclomethasone, and is highly reproducible when repeated after a short time interval.^{42,43} Exhaled NO has been used more recently in predicting response to therapy in asthmatic individuals treated with corticosteroids.^{44,45} Little et al⁴⁵ reported a positive predictive value (PPV) for an increased FE_{NO} value of 83% for an FEV₁ improvement of greater than 15%, whereas sputum eosinophils had a PPV of 68%.⁴⁵

FE_{NO} and current symptom control

Exhaled NO levels have been followed in the emergency treatment of asthma, where levels were initially

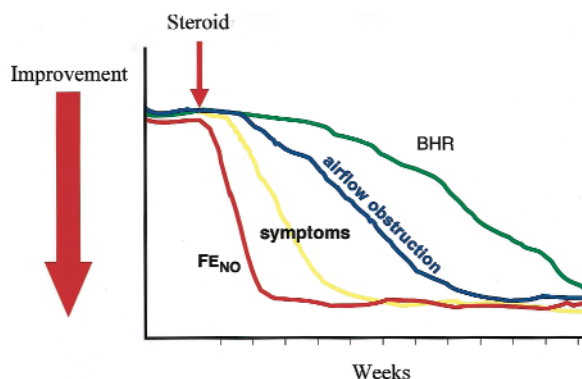


FIG 3. Schematic representation of the relative time courses of response for FE_{NO} measurement and other asthma outcome measures (kindly supplied by Dr Johan De Jongste). Exhaled NO is the quickest marker to respond.

increased and decreased after treatment with glucocorticoids.⁴⁶ This evidence would suggest that FE_{NO} is useful not only in identifying a deterioration in symptoms but can be useful in monitoring the clinical response to therapy.

Although, in the usual case, asthma deterioration is associated with increased symptoms, worsening lung function, and an increased FE_{NO} level, there might be noncoincident responses. In one study by Stirling et al,⁴⁷ inflammation continued to be present, and FE_{NO} levels were increased in individuals with moderate-to-severe persistent asthma, the symptoms of which appeared to be controlled on the basis of spirometry. In the article by Van den Toorn et al,³⁴ continued AI as seen on endobronchial biopsy, increased FE_{NO} levels, and BHR were present in subjects whose asthma was considered to be in clinical remission. Occult AI, as detected by means of FE_{NO} measurement, increased the possibility that remodeling might be occurring despite a lack of symptoms or lung function abnormalities. In the future, identification of silent ongoing inflammation by FE_{NO} levels might indicate that treatment of these individuals is warranted. Thus absent symptoms and normal spirometry results might engender a false sense of security.

Exhaled NO as a predictor of exacerbations

Jatakanon et al⁴⁸ evaluated the utility of FE_{NO} as a marker of increasing AI when patients had their maintenance steroid doses decreased. As peak flows decreased, there was a corresponding increase in FE_{NO} levels, although sputum eosinophil counts were superior to FE_{NO} measurement in predicting exacerbation. In an important study by Jones et al⁴⁹ involving steroid withdrawal, the authors compared FE_{NO} levels with sputum eosinophil counts and BHR to hypertonic saline. Measurement of FE_{NO} at the time of loss of control (LOC), as well as changes in FE_{NO} levels from baseline, had PPVs that ranged from 80% to 90% for diagnosing LOC, which was also accompanied by increased sputum eosinophils and BHR. More importantly, change in FE_{NO} levels from baseline and FE_{NO} levels at the visit before LOC were both good markers of the upcoming LOC.

TABLE I. Advantages and disadvantages of FE_{NO} as a marker

Advantages	Disadvantages
Noninvasive	NO monitors are currently expensive
Reproducible	Technology not widely available
Rapid test with immediate result	Single breath measurement is difficult in the preschool child
Correlates with eosinophilic inflammation and BHR before steroids ³¹	Poor correlation with pulmonary function and BHR after steroids ³⁶
Useful in diagnosis of asthma ⁴⁰	Only measures one component of inflammatory response
Decreases rapidly after anti-inflammatory medications and predicts response	Does not correlate with asthma severity (NHLBI)
Increases during exacerbations	Levels are influenced by several determinants, including atopy and gene polymorphisms ³⁰
High in occult AI ³⁴	The dose response to inhaled corticosteroid is steep and plateaus early ⁴³
Can be used to monitor compliance ⁵⁰	

NHLBI, National Heart, Lung, and Blood Institute.

Correlation with adherence to therapy

FE_{NO} levels increase rapidly after steroid withdrawal, and this could be used to detect nonadherent individuals. Indeed, recent information in a study by Beck-Ripp et al⁵⁰ suggest that FE_{NO} levels correlate well with compliance with study medications. In this study FE_{NO} levels decreased in conjunction with increasing confirmed adherence to anti-inflammatory therapy.

How exhaled NO might be used in the clinic

A suggested way to incorporate this new tool as an adjunct to standard monitoring is as follows. For each patient, FE_{NO} levels would be measured together with spirometry results at each clinic visit. This would allow a stable baseline value to be established for FE_{NO} . New patients with high levels would be expected to show a significant decrease in FE_{NO} levels with effective therapy accompanied by improvement in standard asthma outcomes. Failure of FE_{NO} levels to decrease would alert the clinician to inadequate therapy or poor adherence, which is common with inhaled steroids. A summary of the advantages and disadvantages of FE_{NO} measurement appear in Table I.^{30,31,34,36,40,43,50}

An FE_{NO} level that has increased greater than 20% above the stable baseline should alert the physician to current or upcoming worsening or poor adherence to therapy. Worsening symptoms but a stable FE_{NO} level and spirometry results should raise suspicions for an alternative diagnosis.

CONCLUSION

There is a growing body of evidence to suggest that FE_{NO} measurement might prove to be a useful clinical tool in the evaluation and continued management of asthma. It has been shown to correlate with both indirect and direct markers of AI and provides a useful measure of response to therapies. There is good evidence to suggest that it is a reliable predictor of exacerbations in an asthmatic individual. As this tool becomes more widely available, there is also increasing information, suggesting that FE_{NO} levels correlate well with control of AI and the patient's compliance with medication. Although spirometry and peak flow measurement are the norm for objective measurements of asthmatic control, FE_{NO} promises to broaden the way in which we monitor this disease and to be incorporated into asthma management guidelines. The long-term effect of monitoring exhaled NO on quality of life, health care use, and lung remodeling remains unknown.

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