American Thoracic Society Documents

An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (F$_{E_{NO}}$) for Clinical Applications

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THIS OFFICIAL CLINICAL PRACTICE GUIDELINE OF THE AMERICAN THORACIC SOCIETY (ATS) WAS APPROVED BY THE ATS BOARD OF DIRECTORS, MAY 2011

CONTENTS

Background: Measurement of fractional nitric oxide (NO) concentration in exhaled breath (F$_{E_{NO}}$) is a quantitative, noninvasive, simple, and safe method of measuring airway inflammation that provides a complementary tool to other ways of assessing airways disease, including asthma. While F$_{E_{NO}}$ measurement has been standardized, there is currently no reference guideline for practicing health care providers to guide them in the appropriate use and interpretation of F$_{E_{NO}}$ in clinical practice.
Purpose: To develop evidence-based guidelines for the interpretation of F$_{E_{NO}}$ measurements that incorporate evidence that has accumulated over the past decade.
Methods: We created a multidisciplinary committee with expertise in the clinical care, clinical science, or basic science of airway disease and/or NO. The committee identified important clinical questions, synthesized the evidence, and formulated recommendations. Recommendations were developed using pragmatic systematic reviews of the literature and the GRADE approach.
Results: The evidence related to the use of F$_{E_{NO}}$ measurements is reviewed and clinical practice recommendations are provided.
Conclusions: In the setting of chronic inflammatory airway disease including asthma, conventional tests such as FEV$_1$ reversibility or provocation tests are only indirectly associated with airway inflammation. F$_{E_{NO}}$ offers added advantages for patient care including, but not limited to (*1*) detecting of eosinophilic airway inflammation, (*2*) determining the likelihood of corticosteroid responsiveness, (*3*) monitoring of airway inflammation to determine the potential need for corticosteroid, and (*4*) unmasking of otherwise unsuspected nonadherence to corticosteroid therapy.

Keywords: nitric oxide; asthma; inflammation; airway disease; exhaled breath; clinical application

EXECUTIVE SUMMARY

Nitric oxide (NO) is now recognized as a biological mediator in animals and humans. NO is produced by the human lung and is present in the exhaled breath. It has been implicated in the pathophysiology of lung diseases, including asthma. The measurement of exhaled NO has been standardized for clinical use. Numerous studies have provided evidence regarding the applications of NO measurements in clinical practice, together with the performance characteristics and the strengths and the weaknesses of the test. Based on this evidence, this Clinical Practice Guideline is designed to guide clinicians as to how exhaled NO measurements should be used and interpreted.

EVIDENCE QUALITY AND RECOMMENDATIONS

These recommendations may vary with respect to the particular target population. Where this is the case, this has been included

in the recommendation. If not stated, then the recommendation applies to patients with asthma.

- We recommend the use of $F_{E_{NO}}$ in the diagnosis of eosinophilic airway inflammation (strong recommendation, moderate quality of evidence).

- We recommend the use of $F_{E_{NO}}$ in determining the likelihood of steroid responsiveness in individuals with chronic respiratory symptoms possibly due to airway inflammation (strong recommendation, low quality of evidence).

- We suggest that $F_{E_{NO}}$ may be used to support the diagnosis of asthma in situations in which objective evidence is needed (weak recommendation, moderate quality of evidence).

- We suggest the use of cut points rather than reference values when interpreting $F_{E_{NO}}$ levels (weak recommendation, low quality of evidence).

- We recommend accounting for age as a factor affecting $F_{E_{NO}}$ in children younger than 12 years of age (strong recommendation, high quality of evidence).

- We recommend that low $F_{E_{NO}}$ less than 25 ppb (< 20 ppb in children) be used to indicate that eosinophilic inflammation and responsiveness to corticosteroids are less likely (strong recommendation, moderate quality of evidence).

- We recommend that $F_{E_{NO}}$ greater than 50 ppb (> 35 ppb in children) be used to indicate that eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids are likely (strong recommendation, moderate quality of evidence).

- We recommend that $F_{E_{NO}}$ values between 25 ppb and 50 ppb (20–35 ppb in children) should be interpreted cautiously and with reference to the clinical context. (strong recommendation, low quality of evidence).

- We recommend accounting for persistent and/or high allergen exposure as a factor associated with higher levels of $F_{E_{NO}}$ (strong recommendation, moderate quality of evidence).

- We recommend the use of $F_{E_{NO}}$ in monitoring airway inflammation in patients with asthma (strong recommendation, low quality of evidence).

- We suggest using the following values to determine a significant increase in $F_{E_{NO}}$: greater than 20% for values over 50 ppb or more than 10 ppb for values lower than 50 ppb from one visit to the next (weak recommendation, low quality of evidence).

- We suggest using a reduction of at least 20% in $F_{E_{NO}}$ for values over 50 ppb or more than 10 ppb for values lower than 50 ppb as the cut point to indicate a significant response to antiinflammatory therapy (weak recommendation, low quality of evidence).

Conclusion: Advances in technology and standardization have made $F_{E_{NO}}$ measurement simple, permitting its use as a biomarker that adds a new dimension to the traditional clinical tools in the assessment and management of airways diseases. These guidelines for interpretation of $F_{E_{NO}}$ measurements are meant to enhance their clinical utility, but more work is still needed to better define the use of $F_{E_{NO}}$ in different clinical settings.

INTRODUCTION

NO has long been known as an atmospheric pollutant present in vehicle exhaust emissions and cigarette smoke, and more recently its clinical importance as a biological mediator in animals and humans has been recognized (1, 2). NO is present in virtually all mammalian organ systems and is produced by the human lung. It is present in the exhaled breath of all humans (3). NO is recognized to play key roles in virtually all aspects of lung biology and has been implicated in the pathophysiology of lung diseases, including asthma (4). The functions and effects of NO in the lung/airways reflect its key roles as a vasodilator, bronchodilator, neurotransmitter, and inflammatory mediator (3). Patients with asthma have high levels of NO in their exhaled breath and high levels of inducible nitric oxide synthase (NOS2) enzyme expression in the epithelial cells of their airways, suggesting a role for NO in asthma pathogenesis (5). NO is a highly reactive molecule/free radical and may have oxidant properties directly or in the form of the more noxious peroxynitrite. These properties give NO its bactericidal and cytotoxic effects and may participate in host defense by mediating antimicrobial activity and cytotoxicity for tumor cells (4). The exact pathophysiological role of NO in the airways and lungs is complex (4, 6–8). On the one hand, it may act as a proinflammatory mediator predisposing to the development of airway hyperresponsiveness (AHR) (4, 9). On the other, under physiological conditions NO acts as a weak mediator of smooth muscle relaxation, and protects against AHR (4, 10). In exhaled air, NO appears to originate in the airway epithelium (5, 11–15), as a result of NOS2 up-regulation which occurs with inflammation (5, 12, 13, 16). Thus, exhaled NO may be regarded as an indirect marker for up-regulation of airway inflammation.

The field of exhaled NO measurement has developed remarkably over the last 15 years. The use of chemiluminescence analyzers allowed for the detection of NO in exhaled breath in the early 1990s (17). Patients with asthma were found to have high $F_{E_{NO}}$ in their exhaled breath (18–20) that decreased in response to treatment with corticosteroids (21). This quickly prompted the evaluation of $F_{E_{NO}}$ as a potential noninvasive method to diagnose asthma and monitor the response to antiinflammatory therapy.

Advantages for $F_{E_{NO}}$ include the noninvasive nature of the test, ease of repeat measurements, and the relatively easy use in patients with severe airflow obstruction where other techniques are difficult to perform (22). By providing information about airway inflammation (23, 24), $F_{E_{NO}}$ adds a new dimension to the traditional clinical tools (history, physical exam, and lung function tests).

Before $F_{E_{NO}}$ could become useful as a clinical tool, several issues needed to be addressed (25). In particular, the methods and equipment for measuring $F_{E_{NO}}$ needed to be standardized (26, 27). Large population studies were needed to determine effect of confounding factors and provide the normal range or useful cutoff points of $F_{E_{NO}}$ levels (22, 25). Most of these issues have either already been addressed or are currently under investigation, allowing $F_{E_{NO}}$ measurement to make the transition from research into the clinical arena. Last, but not least, interpretative strategies need to be devised and put in place for the different potential uses and applications (28). The purpose of this document is to address this last requirement.

Wherever possible, the recommendations are based on published material, including abstracts, as referenced, but they are supplemented by nonsystematic observations of experts in the field. The guidelines are provided with the clear understanding that this will be a rapidly evolving area and that periodic updating will be required.

METHODS

Committee Composition, Meetings, and Document Preparation

The project Chair (R.A.D.) assembled a group of international experts in exhaled nitric oxide. Their expertise was in clinical

care, clinical research, or basic science in the area of asthma and/or nitric oxide (five pulmonologists [R.A.D., S.C.E., A.C.O., A.L.P., D.R.T.], an allergist [P.B.B.], two physiologists [C.G.I., J.O.L.], and one pediatric pulmonologist [M.W.L.]). The outline of the Report was proposed by the Chair and modified and agreed upon following input from all Committee members. The Committee was divided into subgroups, each was assigned a specific section, and preliminary drafts were developed. Three face-to-face meetings and nine teleconferences were held. The outline and the drafts were reviewed, and evidence-based recommendations were discussed and finalized by consensus. Committee members disclosed all potential conflicts of interest. All disclosed conflicts of interests were reported to the Chair of the Ethics and Conflict of Interest Committee of the ATS. These were reviewed in detail, and members with perceived conflicts abstained from the discussion of specific questions related to their conflicts of interest. Furthermore, members were reminded to consider their own and other members' potential conflicts of interest during the meetings. The Chair (R.A.D.) integrated the draft sections and composed the entire document into a preliminary document that was circulated among the committee members for further input. The revised document incorporated the comments and input from all Committee members.

Document Structure

This document is structured to provide an evidence-based review of the current state of knowledge regarding the application and interpretation of $F_{E_{NO}}$ measurements in clinical practice. The recommendations regarding interpretive strategy were organized around specific questions according to the GRADE approach to assessing the quality of the evidence (Summary Table E1 in Appendix in online supplement) (29, 30). Relevant section topics and questions were identified by the Committee. Committee members were asked to review the current evidence by independently completing a pragmatic systematic review of the literature using PubMed and OVID. Each Committee member was asked to assess the identified literature relevant to his/her section, and decide about inclusion of individual articles. MEDLINE searches from 1993 to December 2008 were performed by Committee members, with periodic updates during document development and finalization. Searching the literature before 1993 was not done systematically since the discovery of nitric oxide in asthma was first reported in 1993. The search was augmented by searches of Committee member files. The literature search was limited to all relevant studies including randomized controlled trials, cohort studies, case-control studies, and cross-sectional studies published in the English language. Sections that did not yield specific recommendations were written after a thorough review of the available literature in a narrative review format.

Quality of Evidence and Strength of Recommendations

The quality of evidence was determined according to the ATS GRADE criteria (30). For each question, the Committee graded the quality of the evidence available (high, moderate, low, or very low), and made a recommendation for or against. Recommendations were decided by consensus. Recommendations were either "strong" or "weak." The strength of a recommendation reflects the extent to which one can, across the range of patients for whom the recommendation is intended, be confident that desirable effects outweigh undesirable effects (30). Consensus on the recommendations was reached among all the members of the Committee. The strength of a recommendation has important implications for patients, clinicians, and policy makers (30).

Strong recommendation.

- Patients: Most people in this situation would want the recommended course of action and only a small proportion would not
- Clinicians: Most patients should receive the recommended course of action
- Policy makers: The recommendation can be adopted as a policy in most situations

Weak recommendation.

- Patients: The majority of people in this situation would want the recommended course of action, but many would not
- Clinicians: Be more prepared to help patients to make a decision that is consistent with the patient's own values
- Policy makers: There is a need for substantial debate and involvement of stakeholders

Why Should a $F_{E_{NO}}$ Test Be Obtained?

Common reasons for measuring $F_{E_{NO}}$.

- To assist in assessing the etiology of respiratory symptoms
- To help identify the eosinophilic asthma phenotype
- To assess potential response or failure to respond to antiinflammatory agents, notably inhaled corticosteroids (ICS)
- To establish a baseline $F_{E_{NO}}$ during clinical stability for subsequent monitoring of chronic persistent asthma
- To guide changes in doses of antiinflammatory medications: step-down dosing, step-up dosing, or discontinuation of antiinflammatory medications
- To assist in the evaluation of adherence to antiinflammatory medications
- To assess whether airway inflammation is contributing to poor asthma control particularly in the presence of other contributors (e.g., rhinosinusitis, anxiety, gastro-esophageal reflux, obesity, or continued allergen exposure).

Can $F_{E_{NO}}$ Be Used to Diagnose Asthma?

Asthma is a clinical diagnosis and there is no single diagnostic test for the disease. The background pathology of asthma is often but not always due to eosinophilic airway inflammation. The two are not synonymous. This is extremely important in the interpretation of $F_{E_{NO}}$ measurements. It is often claimed that $F_{E_{NO}}$ is a diagnostic test for asthma, but in cases of asthma not due to airway eosinophilia, $F_{E_{NO}}$ may be low. Similarly, the value of exhaled $F_{E_{NO}}$ as a predictor of steroid responsiveness is high even in the absence of induced sputum eosinophils (31).

■ **Recommendations:**

We recommend the use of $F_{E_{NO}}$ in the diagnosis of eosinophilic airway inflammation (strong recommendation, moderate quality of evidence).

We recommend the use of $F_{E_{NO}}$ in determining the likelihood of steroid responsiveness in individuals with chronic respiratory symptoms possibly due to airway inflammation (strong recommendation, low quality of evidence).

We suggest that $F_{E_{NO}}$ may be used to support the diagnosis of asthma in situations in which objective evidence is needed (weak recommendation, moderate quality of evidence).

$F_{E_{NO}}$ is associated with eosinophilic airway inflammation. There are several inflammatory phenotypes in asthma most commonly described as eosinophilic, neutrophilic, mixed, and paucigranulocytic (32). Determination of the subtype may help a physician

decide which therapies to select or stop (33–35). Given the long-established relationship between eosinophilic inflammation and steroid responsiveness in airways disease, the finding that $F_{E_{NO}}$ correlates with eosinophilic inflammation suggests its use as indirect indicator not only of eosinophilic inflammation, but more importantly of the potential for steroid responsiveness (36–42).

There is little evidence directly demonstrating that eosinophilic airway inflammation increases $F_{E_{NO}}$ by increasing NOS2 expression or activity (43). However, eosinophilic airway inflammation may affect $F_{E_{NO}}$ indirectly through NOS2 or via other enzyme pathways. Numerous studies describe the relationship between $F_{E_{NO}}$ and eosinophilic airway inflammation. Eosinophils can be measured in sputum, bronchoalveolar lavage, and biopsies. There are also reports of correlation between $F_{E_{NO}}$ and blood eosinophils (44–46). Warke and coworkers reported that in bronchoalveolar lavage fluid the correlation between eosinophils and $F_{E_{NO}}$ was 0.78 ($P <$ 0.001) (40). Payne and colleagues reported that the correlation between $F_{E_{NO}}$ and eosinophils in bronchial biopsies was 0.54 ($P = 0.03$) (47), but in contrast Lim and coworkers were unable to find a significant correlation in the biopsies (48). In induced sputum, the correlation between $F_{E_{NO}}$ levels and eosinophils ranges from 0.35 (n = 25, $P = 0.09$) (36) to 0.48 (n = 35, $P = 0.003$) (49) to 0.62 (n = 78, $P < 0.001$) (50). In the largest study to date (n = 566), the correlation was of a similar order (0.59, $P <$ 0.001) (39). In this last study, $F_{E_{NO}}$ of 36 ppb (at a flow rate of 50 ml/s) had a sensitivity and specificity for sputum eosinophilia of more than 3% (the cut point deemed by the authors to be clinically significant) of 78% and 72%, respectively. In the study by Shaw and colleagues, a $F_{E_{NO}}$ of less than 26 ppb had a negative predictive value of 85% for sputum eosinophils less than 3% (51). Similarly, Porsbjerg and coworkers have reported that with $F_{E_{NO}}$ less than 27ppb, it is unlikely that sputum eosinophils will be greater than 1% (52). Thus a low $F_{E_{NO}}$ is of value in determining the *absence* of eosinophilic, and, by inference, the likely absence of steroid-responsive airway inflammation.

These limited correlations reflect the fact that whereas sputum eosinophilia is *always* abnormal, exhaled nitric oxide is present even in health with its distribution skewed to the right. It is also necessary to bear in mind that negative and positive predictive values are limited in their generalizablity, given that they depend on the prevalence of the condition in the tested population. Importantly, two studies have shown that the relationship between $F_{E_{NO}}$ levels and airway eosinophilia is independent of the diagnosis of asthma as reported in patients with chronic obstructive pulmonary disease (COPD) (53), and with eosinophilic bronchitis (54). Furthermore, NO and NO metabolites in the airway (e.g., peroxynitrite) alter the REDOX balance in the airways, may cause inflammation, and are in some part steroid sensitive. Thus NO production is to some extent independent of eosinophilic inflammation (4).

$F_{E_{NO}}$ predicts likelihood of corticosteroid responsiveness. Treatment response in asthma is heterogeneous (55). Not all patients respond to corticosteroids and an important reason to use $F_{E_{NO}}$ is to help decide who might benefit from steroid treatment, and who should try other medications (e.g., leukotriene modifiers). $F_{E_{NO}}$ may also be used to determine patients in whom steroid therapy may be safely withdrawn. $F_{E_{NO}}$ has been shown to predict the likelihood of steroid responsiveness more consistently than spirometry, bronchodilator response, peak flow variation, or AHR to methacholine (56–58). The optimum cut point in the study by Smith and coworkers (56), was 47 ppb, with a negative predictive value of 89% for the change in FEV_1 with inhaled steroids. The predictive values were similar for alternative endpoints. Even when patients do not demonstrate sputum eosinophilia, $F_{E_{NO}}$ is highly predictive of steroid response (at a cut

point of 33 ppb) (31). These data are consistent with studies in which high $F_{E_{NO}}$ (> 47 ppb) predicts the likelihood of loss of control when inhaled steroids are reduced or withdrawn in children with a confirmed diagnosis of asthma (59). Conversely, low $F_{E_{NO}}$ (< 22 ppb) predicts the likelihood of successful reduction or withdrawal of inhaled steroids (positive predictive value, 92%) (60). Again, these outcomes may differ somewhat depending on the target population: for the most part these data are derived from patients with mild to moderate asthma. In summary, depending on the prevalence of eosinophilic airway inflammation in the target population, $F_{E_{NO}}$ measurements may provide a signal that is helpful in identifying patients with asthma-like symptoms who are likely to benefit (or not) from corticosteroid treatment.

$F_{E_{NO}}$ can support a diagnosis of asthma. The diagnosis of asthma is well defined, and the background pathology is often but not always due to eosinophilic airway inflammation. Early studies in populations comprising mainly patients with eosinophilic asthma explored the performance characteristics of $F_{E_{NO}}$ as a diagnostic test. The predictive values for $F_{E_{NO}}$ (usually at cut points of > 25 ppb) were shown to be sufficiently robust for it to be used in this context (23, 61, 62). Further, the predictive values for $F_{E_{NO}}$ are higher than for conventional measurements such as peak flows and spirometry (23), and similar to those associated with bronchial challenge tests (62). However, in general, in patients presenting with variable cough, wheeze, and shortness of breath, an increased $F_{E_{NO}}$ provides supportive rather than conclusive evidence for an asthma diagnosis. As stated, the limitations to the diagnostic role of $F_{E_{NO}}$ arise principally because airway inflammation in asthma is heterogeneous and is not always associated with increased $F_{E_{NO}}$ (e.g., neutrophilic airway inflammation). Similarly, in patients who have already been treated with inhaled steroids, the test may be falsely negative. Thus, the importance of $F_{E_{NO}}$ lies in its potential to identify steroid responsiveness, rather than the exact clinical diagnosis. This information is much more clinically relevant because it enables the clinician to bypass an empiric "trial of steroids" or unnecessary long-term corticosteroid treatment.

$F_{E_{NO}}$ may predict AHR. Irrespective of the specific underlying inflammatory signal which $F_{E_{NO}}$ represents, measurements appear to reflect the dynamic interrelationships between the response to allergen or other triggers and evolving eosinophilic airway inflammation/AHR (4, 7, 8, 63). Serial $F_{E_{NO}}$ levels increase progressively in response to allergen exposure and the advent of airway symptoms (63). Because of the practical difficulties involved in measuring AHR, especially in children, it was initially thought that $F_{E_{NO}}$ might be used as a surrogate marker for AHR. The relationship between NO metabolism and AHR in asthma is complex (64). When $F_{E_{NO}}$ was used to predict the presence of AHR, the studies reveal inconsistent relationships and correlations are generally low. The clinical interpretation of $F_{E_{NO}}$ in relation to AHR is even more problematic in subjects who are taking ICS (9, 65) and with long-standing as opposed to recently developed asthma (66). This is demonstrated in studies designed to evaluate pathophysiological relationships in clinical asthma using factor analysis: AHR, airway inflammation, and $F_{E_{NO}}$ belong to different domains (66–68). However, in one study $F_{E_{NO}}$ has been used as a surrogate for AHR testing to support the diagnosis of asthma in children, and the data appear to support its use in this limited context (62).

Is There a Normal $F_{E_{NO}}$ Value?

This section will discuss the normal ranges of $F_{E_{NO}}$. We will also discuss the important clinical cut points and the rationale for selecting these cut points to be used in the interpretation of an
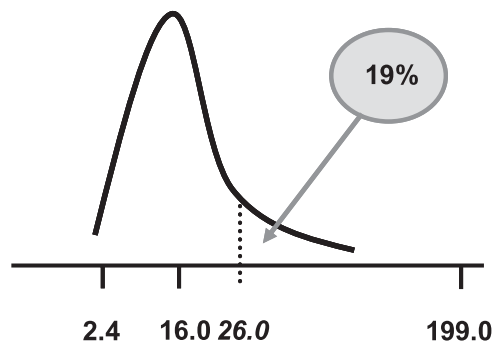
Figure 1. Schematic representation of the distribution of $F_{E_{NO}}$ levels in an unselected population of 2,200 male and female subjects. The median value was 16.0 ppb with a range of 2.4 to 199 ppb. The cut point of 26 ppb is the optimum cut point for significant sputum eosinophilia, indicating that up to 20% of individuals with an $F_{E_{NO}}$ greater than 25 ppb may not necessarily have sputum eosinophilia, and that the clinical context requires to be taken into account. The data used to prepare this composite figure were obtained from Shaw and colleagues (51) and Olin and colleagues (73) after consultation with the authors.

elevated or reduced $F_{E_{NO}}$ value. It is important to choose the appropriate cut point in relation to the clinical setting and question. While this section and the accompanying tables (*see* Tables 3–5) focus on asthma and airway diseases/inflammation, other causes of high and low $F_{E_{NO}}$ levels are listed in the Appendix in the online supplement.

■ Recommendations:

We suggest the use of cut points rather than reference values when interpreting $F_{E_{NO}}$ levels (weak recommendation, low quality of evidence).

We recommend accounting for age as a factor affecting $F_{E_{NO}}$ in children younger than 12 years of age (strong recommendation, high quality of evidence).

Normal Values versus Relevant Cut Points for $F_{E_{NO}}$

This section will discuss the normal ranges of $F_{E_{NO}}$ and what are the important clinical cut points to be used in the interpretation of an elevated or reduced $F_{E_{NO}}$ value. It is unlikely that reference values derived from a "normal" population will be as helpful as cut points in patients with airways disease or respiratory symptoms. The distribution of $F_{E_{NO}}$ in an unselected population is skewed to the right (*see* Figure 1). Even when individuals with atopy or diagnosed asthma are excluded, the upper limit of "normal" ranges from 27 to 57 ppb depending on sex (69). This overlaps with the range of values obtained in
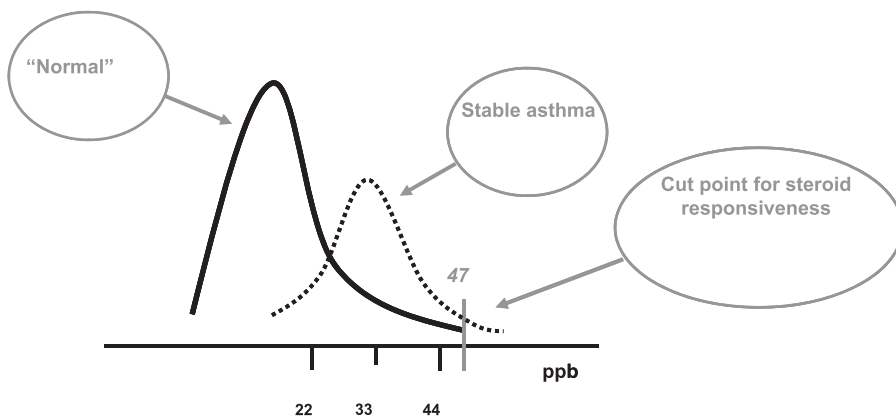
populations with asthma in relation to sputum eosinophilia (*see* Figure 1). In a clinical study, Shaw and colleagues reported that the optimum cut point for a clinically significant $F_{E_{NO}}$ (corresponding to a sputum eosinophil count of $\geqslant 2\%$) was 26 ppb (51). Similarly, studies designed to determine the optimum cut point to diagnose asthma using $F_{E_{NO}}$ have usually pointed to a diagnostic cut point ranging from 20 to 25 ppb (23, 70–72). However, in patients with stable, well-controlled asthma, $F_{E_{NO}}$ values range from 22 to 44 ppb (73). Clearly, there is considerable overlap between mean $F_{E_{NO}}$ levels in healthy and populations with stable asthma. This is illustrated in Figure 2.

Confounding factors that may affect $F_{E_{NO}}$. As discussed in the Appendix in the online supplement, $F_{E_{NO}}$ values can be affected by several factors, including measurement technique, exhalation flow rate, nasal NO contamination, the NO analyzer used (74), age, height, smoking, and antiinflammatory medications. A number of recent publications have reported reference values for $F_{E_{NO}}$ in adults (69, 75–79) (Table 1) and children (76, 80–83). There are important differences between these studies with regard to the size of the examined population, as well as the range of statistical variables that have been included or excluded, limiting their value (76, 77, 80–83). Factors affecting population $F_{E_{NO}}$ levels may be due to one or more variables including genetics, age, sex, atopy, weight and height, current smoking, and diet. The importance of current smoking and atopic status is generally agreed upon (28), but there are inconsistencies between the studies regarding which other factors ought to be accounted for when deriving and applying reference values (Table 1). More detailed information on these biological sources of variability is provided in the Appendix in the online supplement.

Age seems to be important in children (81), but there is less agreement across the studies regarding age in adults, sex, and height. In the largest study to date, Olin and coworkers identified the importance of age and height as factors affecting $F_{E_{NO}}$, but did not find any differences between males and females (69). In contrast, Travers and colleagues (78) and Taylor and coworkers (84) reported consistently higher levels in males. The magnitude of the effect of the patient-related factors alone or in combination is potentially clinically significant. This is demonstrated in Table 2 (data from Reference 69).

Thus, in our present state of knowledge the problems of multiple confounding factors and overlap between normal populations and populations with asthma preclude the routine application of reference values in the clinical setting. The Committee felt that it is more relevant to identify clinically meaningful cut points rather than reference values to interpret $F_{E_{NO}}$ levels as outlined below, keeping in mind that very few of these cut points are well validated. At any one time, however, the most important consideration is whether or not the patient has *current* respiratory symptoms or a prior diagnosis of airways disease; that is, the

Figure 2. An amplification of Figure 1 in which the distribution of $F_{E_{NO}}$ in stable asthma is depicted as a *dotted line.* Taken from Olin and colleagues (73). In that study, the 95% confidence intervals for $F_{E_{NO}}$ in stable asthma was reported to be 22 to 44 ppb. The cut point of 47 ppb is the optimum cut point for steroid responsiveness in patients with nonspecific respiratory symptoms. The other data used to prepare this composite figure were obtained from Smith and colleagues (56) after consultation with the authors.

TABLE 1. STUDIES OF ONLINE FRACTION OF EXHALED NITRIC OXIDE VALUES AT EXHALATION FLOW RATE OF 50 ml/s IN HEALTHY SUBJECTS

Author and Reference	N	Groups for which Reference Values Are Given	"Normal Values" (ppb)	Analyzer
Kharitonov 2003 (75)	59	Mixed population of adults and children	Mean 16.3 ppb, ULN 33.	NIOX (Aerocrine AB, Stockholm, Sweden)
Buchvald 2005 (76)	405	Children aged 4–17 yr Data also available by age stratification	Mean 9.7 ppb, Upper 95% CI: 25.2	NIOX (Aerocrine AB, Stockholm, Sweden)
Olivieri 2006 (77)	204	Male, nonsmoker, nonasthmatic Female, nonsmoker, nonasthmatic (note: atopy not considered)	4.5–20.6 3.6–18.2 (note: values quoted are 5th and 95th centiles)	(CLD88, Ecomedics, Switzerland)
Olin 2007 (69)	3,376	Random population 1,131 never-smoking subjects not reporting any asthma symptom, dry cough or the use of inhaled corticosteroids	See Table 2	NIOX (Aerocrine AB, Stockholm, Sweden)
Travers 2007 (78)	3,500	Male, nonsmoker, nonatopic Male, nonsmoker, atopic Male, smoker, nonatopic Male, smoker, atopic Female, nonsmoker, nonatopic Female, nonsmoker, atopic Female, smoker, nonatopic Female, smoker, atopic	9.5–47.4 11.2–56.5 7.5–38.4 8.8–45.9 7.5–37.4 8.8–44.6 5.9–30.5 6.9–36.4 (note: values quoted are 90% confidence interval)	NIOX (Aerocrine AB, Stockholm, Sweden)
Dressel 2008 (79)	897	Male, nonsmoker, nonatopic, 165 cm Male, nonsmoker, atopic, 165 cm Male, smoker, nonatopic, 165 cm Male, smoker, atopic, 165 cm Female, nonsmoker, nonatopic, 160 cm Female, nonsmoker, atopic, 160 cm Female, smoker, nonatopic, 160 cm Female, smoker, atopic, 160 cm	19.5 29.1 12.2 18.3 15.7 23.5 9.9 14.7	(NOA 280, Sievers, Boulder, CO)

interpretation of $F_{E_{NO}}$ levels should be determined in individual patients with reference to the context in which the measurement is being obtained.

What Are the Clinically Significant Cut Points for $F_{E_{NO}}$?

It is important to choose the appropriate cut point in relation to the clinical setting and question. In this section, we discuss the rationale for selecting these cut points (*see* Tables 3–5). While this section and the accompanying tables focus on asthma and airway diseases/inflammation, other causes of high and low $F_{E_{NO}}$ levels are listed in the Appendix in the online supplement.

■ **Recommendations:**

We recommend that low $F_{E_{NO}}$ (< 25 ppb [< 20 ppb in children]) be used to indicate that eosinophilic inflammation and

responsiveness to corticosteroids are less likely (strong recommendation, moderate quality of evidence).

We recommend that $F_{E_{NO}}$ > 50ppb (> 35 ppb in children) be used to indicate that eosinophilic inflammation and, in symptomatic patients, responsiveness to corticosteroids are likely (strong recommendation, moderate quality of evidence).

We recommend that $F_{E_{NO}}$ values between 25 ppb and 50 ppb (20–35 ppb in children) should be interpreted cautiously with reference to the clinical context (strong recommendation, low quality of evidence).

Low $F_{E_{NO}}$ (< 25 ppb in adults; 20 ppb in children). In a symptomatic adult patient with a $F_{E_{NO}}$ of less than 25 ppb (20 ppb in children), eosinophilic airway inflammation is unlikely. This cut point is based on evidence from a number of sources including the study by Shaw and colleagues (51) and Porsbjerg and coworkers (52), studies investigating the role of $F_{E_{NO}}$ measurements to diagnose asthma (23, 70–72), and studies designed to optimize ICS use (56, 60). The differential diagnosis for symptomatic patients with a low $F_{E_{NO}}$ is given in Table 3. In patients presenting with nonspecific respiratory symptoms, low $F_{E_{NO}}$ suggests alternative diagnoses which are not amenable to an increase in inhaled or oral steroid therapy.

High $F_{E_{NO}}$ (> 50 ppb in adults, 35 ppb in children). High $F_{E_{NO}}$ is likely to indicate significant airway eosinophilia. It is also likely to indicate that a symptomatic patient has steroid-responsive airways inflammation (56, 57, 85, 86). The clinically significant cut point of 50 ppb is based on the results of pragmatic studies. However, this is a general guide and may vary slightly in individual patients. Symptomatic steroid-naïve patients with high $F_{E_{NO}}$ are more likely to exhibit responsiveness to inhaled steroid therapy, irrespective of the diagnostic label (e.g., asthma or nonasthma), with an optimum cut point of 47 ppb (56). In asymptomatic patients with stable asthma, the likelihood of relapse following withdrawal of ICS therapy is greatest in patients whose $F_{E_{NO}}$ increases to above 49 ppb during the 4 weeks after

TABLE 2. FRACTION OF EXHALED NITRIC OXIDE 95% UPPER LIMITS, STRATIFIED FOR SEX AND ATOPY, ACCORDING TO HEIGHT AND AGE AMONG 1,131 HEALTHY LIFELONG NEVER-SMOKING SUBJECTS

Height (cm)	Age 25–49 yr		Age 50–75 yr	
	Women	Men	Women	Men
Subjects without Atopy (n = 845)				
150–159	25	27	34	32
160–169	26	30	36	35
170–179	28	33	39	39
180–189	30	37	41	44
190–199	—	42	—	49
Subjects with Atopy (n = 286)				
150–159	30	58	37	65
160–169	36	63	45	63
170–179	43	54	53	62
180–189	51	50	64	57
190–199	—	50	—	56

Data taken from Reference 69.

TABLE 3. LOW $F_{E_{NO}}$ (< 25 ppb [< 20 ppb IN CHILDREN]): IMPLIES NONEOSINOPHILIC OR NO AIRWAY INFLAMMATION*

Diagnosis
　In a *symptomatic* patient (chronic cough and/or wheeze and/or shortness
　　　of breath for > 6 wk) presenting for the first time, the patient is *unlikely* to
　　　benefit from a trial of inhaled corticosteroid treatment, possible etiologies:
　　Other pulmonary/airway causes:
　　　Rhinosinusitis
　　　Noneosinophilic asthma
　　　Reactive airways dysfunction syndrome
　　　COPD
　　　Bronchiectasis
　　　Cystic fibrosis, primary ciliary dyskinesia
　　　Extended post-viral bronchial hyperresponsiveness syndrome
　　　Vocal cord dysfunction
　　Nonpulmonary/airway causes:
　　　Anxiety-hyperventilation
　　　Gastroesophageal reflux disease
　　　Cardiac disease/pulmonary hypertension/pulmonary embolism
　　Confounding factors:
　　　Smoking
　　　Obesity
Monitoring
　In a *symptomatic* patient with an established diagnosis of asthma, possible
　　　etiologies:
　　Asthma:
　　　Noneosinophilic asthma (probably steroid unresponsive)
　　Additional or alternative diagnosis?
　　　Vocal cord dysfunction
　　　Anxiety-hyperventilation
　　　Bronchiectasis,
　　　Cardiac disease
　　　Rhinosinusitis,
　　　Gastroesophageal reflux disease
　In an *asymptomatic* patient with an established diagnosis of asthma:
　　Implies adequate dosing and good adherence to antiinflammatory therapy
　　Inhaled corticosteroid dose may possibly be reduced (repeat $F_{E_{NO}}$ 4 wk later to
　　　confirm this judgment; if it remains low then relapse is unlikely).

Definition of abbreviations: COPD = chronic obstructive pulmonary disease;
$F_{E_{NO}}$ = fraction of exhaled nitric oxide.
　*The interpretation of $F_{E_{NO}}$ is an adjunct measure to history, physical exam,
and lung function assessment.
　For intermediate $F_{E_{NO}}$ levels (in the range 25–50 ppb [20–35 ppb in children]),
refer to Table 5.

steroid withdrawal (59). The differential diagnosis for high $F_{E_{NO}}$ is shown in Table 4.

Intermediate $F_{E_{NO}}$ (between 25 ppb and 50 ppb in adults; 20–35 ppb in children). The above data indicate that for $F_{E_{NO}}$ values between 25 and 50 ppb, cautious interpretation is required. The weight placed on an $F_{E_{NO}}$ result within this range will depend on whether the test is being used diagnostically in a *symptomatic* steroid-naïve subject, or whether the patient's $F_{E_{NO}}$ has increased or decreased from a previous value by what is deemed to be a clinically significant amount in a patient who is being monitored over time.

■ Recommendation

We recommend accounting for persistent and/or high allergen exposure as a factor associated with higher levels of $F_{E_{NO}}$ (strong recommendation, moderate quality of evidence).

Persistently high $F_{E_{NO}}$ (> 50 ppb in adults, 35 ppb in children). In a patient with ongoing asthma, symptoms may occur despite apparently adequate antiinflammatory treatment (87). In the collective experience of the Committee, a common cause of persistently high $F_{E_{NO}}$ is poor adherence to ICS therapy. Other explanations could be poor inhaled drug delivery or continued exposure to allergen (7, 8).

Continuing or increasing exposure to aeroallergens to which a patient is sensitized may result in a rise in $F_{E_{NO}}$, or the

TABLE 4. HIGH $F_{E_{NO}}$ (> 50 ppb [> 35 ppb IN CHILDREN]) OR RISING $F_{E_{NO}}$ (> 40% CHANGE FROM PREVIOUSLY STABLE LEVELS): IMPLIES UNCONTROLLED OR DETERIORATING EOSINOPHILIC AIRWAY INFLAMMATION*

Diagnosis
　In a *symptomatic* patient (chronic cough and/or wheeze and/or shortness
　　　of breath during past > 6 wk) presenting for the first time, possible
　　　etiologies:
　　Atopic asthma
　　Eosinophilic bronchitis
　　COPD with mixed inflammatory phenotype
　　That the patient is *likely* to benefit from a trial of inhaled corticosteroid
　　　treatment
Monitoring
　In a *symptomatic* patient with an established diagnosis of asthma,
　　　possible etiologies:
　　High persistent allergen exposure
　　Inhaled corticosteroid delivery problems:
　　　Poor adherence
　　　Poor inhaler technique
　　　Proximal drug deposition, with untreated distal airway/alveolar
　　　　inflammation
　　Inadequate inhaled corticosteroid dose:
　　　Likely to respond to increased inhaled corticosteroid dose OR prednisone
　　Rarely: truly steroid resistant asthma (a trial of systemic steroid will
　　　　confirm this: $F_{E_{NO}}$ will remain high
　　Rarely: Churg Strauss syndrome, pulmonary eosinophilia
　In an *asymptomatic* patient:
　　No change in inhaled corticosteroid dosing, but refer to $F_{E_{NO}}$ trend
　　　over time in individual patient
　　Withdrawing inhaled corticosteroid is likely to be followed by relapse
　　An increase in therapy is indicated as some patients are asymptomatic,
　　　but the high $F_{E_{NO}}$ could be a risk factor for an upcoming exacerbation.
　　"High" $F_{E_{NO}}$ may be normal in a certain percent of the population
　　　(Figure 1).

Definition of abbreviation: $F_{E_{NO}}$ = fraction of exhaled nitric oxide.
　*The interpretation of $F_{E_{NO}}$ is an adjunct measure to history, physical exam,
and lung function assessment.
　For intermediate $F_{E_{NO}}$ (levels in the range 25–50 ppb [20–35 ppb in children]),
refer to Table 5.

persistence of an elevated $F_{E_{NO}}$. The magnitude of the effect may be sufficient for $F_{E_{NO}}$ levels to increase beyond the cut point of 50 ppb, and in some patients may occur even in the absence of respiratory symptoms (88–91). More recent evidence suggests that persistent high $F_{E_{NO}}$ in corticosteroid-treated individuals with asthma may also reflect a highly reactive asthma phenotype, and such patients need to be managed with caution (35). However, if the patient is asymptomatic and has a high $F_{E_{NO}}$, then no change in treatment is required. There is a small group of patients whose $F_{E_{NO}}$ remains high despite good asthma control. This probably results from the fact that more than one factor (i.e., not just eosinophilic airway inflammation) is responsible for the elevated $F_{E_{NO}}$. Another explanation may be that the high exhaled NO is derived from constitutive NOS sources which are steroid insensitive. Thus, levels greater than 50 ppb in a well-treated asymptomatic patient may be "normal" for that specific patient.

Can $F_{E_{NO}}$ Be Used to Monitor Airway Inflammation?

The *change* in $F_{E_{NO}}$ value following corticosteroid intervention may be more valid than the absolute $F_{E_{NO}}$ value. The definition of a clinically significant change in $F_{E_{NO}}$, however, remains to be established.

■ Recommendations

We recommend the use of $F_{E_{NO}}$ in monitoring airway inflammation in patients with asthma (strong recommendation, low quality of evidence).

TABLE 5. GENERAL OUTLINE FOR $F_{E_{NO}}$ INTERPRETATION: SYMPTOMS REFER TO COUGH AND/OR WHEEZE AND/OR SHORTNESS OF BREATH*

	$F_{E_{NO}} < 25$ ppb (<20 ppb in children)	$F_{E_{NO}}$ 25–50 ppb (20–35 ppb in children)	$F_{E_{NO}} > 50$ ppb (>35 ppb in children)
Diagnosis			
Symptoms present during past 6+ wk	Eosinophilic airway inflammation unlikely Alternative diagnoses Unlikely to benefit from ICS	Be cautious Evaluate clinical context Monitor change in $F_{E_{NO}}$ over time	Eosinophilic airway inflammation present Likely to benefit from ICS
Monitoring (in Patients with Diagnosed Asthma)			
Symptoms present	Possible alternative diagnoses Unlikely to benefit from increase in ICS	Persistent allergen exposure Inadequate ICS dose Poor adherence Steroid resistance	Persistent allergen exposure Poor adherence or inhaler technique Inadequate ICS dose Risk for exacerbation Steroid resistance
Symptoms absent	Adequate ICS dose Good adherence ICS taper	Adequate ICS dosing Good adherence Monitor change in $F_{E_{NO}}$	ICS withdrawal or dose reduction may result in relapse Poor adherence or inhaler technique

Definition of abbreviations: $F_{E_{NO}}$ = fraction of exhaled nitric oxide; ICS = inhaled corticosteroid.

* The interpretation of $F_{E_{NO}}$ is an adjunct measure to history, physical exam, and lung function assessment. *See* text and Tables 3 and 4 for other details.

We suggest using the following values to determine a significant increase in $F_{E_{NO}}$: greater than 20% for values over 50 ppb or more than 10 ppb for values lower than 50 ppb from one visit to the next (weak recommendation, low quality of evidence).

We suggest using a reduction of at least 20% in $F_{E_{NO}}$ for values over 50 ppb or more than 10 ppb for values lower than 50 ppb as the cut point to indicate a significant response to antiinflammatory therapy (weak recommendation, low quality of evidence).

Monitoring Airway Inflammation in Asthma

Serial measurements obtained when patients' asthma is both stable and unstable allows each patient to act as his/her own control when assessing subsequent measurements and as a result "personal best" can be used (92). The same cut points used in detecting airway inflammation apply when monitoring patients with asthma. In asymptomatic individuals, including patients with well-controlled asthma, low $F_{E_{NO}}$ suggests that ICS dose could be reduced or even that ICS treatment may be withdrawn altogether. In a study of children with stable asthma, withdrawal of ICS did not result in symptom relapse when $F_{E_{NO}}$ remained consistently low (optimum cut point 22 ppb) when measured 2 to 4 weeks after treatment withdrawal (60). In symptomatic patients with low $F_{E_{NO}}$, strategies other than increasing the ICS dose should be pursued. Thus, $F_{E_{NO}}$ values which are either high or low are informative as to the etiology of current symptoms particularly in patients with difficult asthma. Sequential measurements may be important in determining trends. The relatively rapid change in $F_{E_{NO}}$ in response to ICS is thought to add to its utility in monitoring adherence to and response to such therapy (93). However, as a predictor of asthma control, $F_{E_{NO}}$ is no better than more conventional lung function tests (51, 87, 94, 95). The predictive values of a single measurement of $F_{E_{NO}}$ for loss of asthma control are insufficiently sensitive or specific to justify its use for this specific purpose (51, 94, 95).

Minimally Important Differences, and Prognostic Significance of $F_{E_{NO}}$

The within-subject coefficient of variation for $F_{E_{NO}}$ in healthy subjects is approximately 10%, or up to 4 ppb (75, 96). The variation increases to approximately 20% in patients with asthma (75, 96, 97). Since a change of 20% could be due to the variation in the $F_{E_{NO}}$ measurement, the Committee recommends a change of at least 20% to indicate a significant rise or fall in $F_{E_{NO}}$ over time or following an intervention. However, there are very few data that clarify what constitutes a clinically important change in individual patients. In one study, $F_{E_{NO}}$ levels were 50% higher during acute asthma compared with when stability was restored (98). Data obtained from steroid withdrawal studies show that the mean increase in $F_{E_{NO}}$ associated with the advent of loss of control ranges from 16 ppb (99) to 25 ppb (50), the latter representing a 60% increase from baseline. However, the range of the increase in $F_{E_{NO}}$ between stability and loss of control is high (up to 141 ppb) (50). More recently, Michils and colleagues have reported that the transition from good control to poorly controlled asthma is likely to be associated with a rise in $F_{E_{NO}}$ of 40% or greater (100). An acute rise (over 12–24 h) in $F_{E_{NO}}$ may occur after infection or exposure to an allergen to which the patient is sensitized. The magnitude of the rise may be as high as 150 ppb. Ideally, one would wish that a minimally important change in $F_{E_{NO}}$ to a level that is above or below a particular cut point would provide justification for a specific interpretation. Unfortunately, there are insufficient data to recommend this approach. Rather, the current $F_{E_{NO}}$ level, the direction and magnitude of any recent change, and where the measured level sits in relation to the cut points for "high" or "low" values need to be taken into account.

Randomized trials designed to assess whether asthma outcomes are improved using regular $F_{E_{NO}}$ measurements as the basis for adjusting the dose of ICS therapy have failed to show important benefits (51, 87, 95, 101, 102), although in one study ICS dose reduction was facilitated without compromising asthma control (103). Thus in general, $F_{E_{NO}}$ measurements cannot be recommended for this purpose. A recent systematic assessment of published randomized trials of asthma therapy guided by $F_{E_{NO}}$ concluded that the mixed results of these studies (the ASTRAL studies, an acronym for ASthma randomized TReatment ALgorigrm studies) were due to specific design and methodological issues that may have led to incorrect conclusions (104). In his summary, Gibson highlights the following problems: (*1*) the dose–response relationship of the drugs used in relation to the outcomes measured; (*2*) the effects of adherence and nonadherence; (*3*) the algorithms used and their agreement with clinical decision making; (*4*) the selection of $F_{E_{NO}}$ cut points/decision points. Gibson states that future studies would require the use of an additional metric to assess the likelihood that any two algorithms (conventional and biomarker-guided) will give different ICS dosing decisions (104). In a more

recent study, investigators aimed to evaluate the accuracy of baseline $F_{E_{NO}}$ to recognize individuals with difficult-to-treat asthma who have the potential to achieve control with a guideline-based stepwise strategy (105). One hundred two consecutive patients with suboptimal asthma control underwent stepwise increase in the treatment with maximal inhaled corticosteroids for 1 month. Then, those who remained uncontrolled received oral corticosteroids for an additional month. With this approach, 53 patients (52%) gained control. A $F_{E_{NO}}$ cut point greater than or equal to 30 ppb demonstrated a sensitivity of 88% and a specificity of 91% for the identification of responsive individuals with asthma, and a value less than or equal to 30ppb had a negative predictive value for steroid response of 92% (105). Thus, incorporating optimal design features into future $F_{E_{NO}}$ studies should help in obtaining a better estimate of the value of $F_{E_{NO}}$-guided asthma therapy (104). Otherwise, a study is unlikely to detect a positive result in favor of one decision-making algorithm versus the other, even if one truly exists.

How Should a $F_{E_{NO}}$ Measurement Be Interpreted and Reported?

1. Assure proper methodology: follow ATS/ERS guidelines. ATS/ERS guidelines for the measurement of $F_{E_{NO}}$ have been published and are the current standard (26, 27). These guidelines should be followed carefully to obtain accurate and reproducible measurements. These guidelines should be used in conjunction with FDA-approved instructions for the use of specific nitric oxide analyzers. As additional instruments using different technologies to measure $F_{E_{NO}}$ become available, these guidelines as well as the scope of FDA endorsements are likely to change.

2. Determine the reason for the test and the type of subject being tested: does the patient have asthma-like symptoms OR an already established diagnosis of asthma? The interpretation of $F_{E_{NO}}$ begins with whether a patient's symptoms are nonspecific and as yet undiagnosed, or whether they have a confirmed diagnosis of asthma. This upfront distinction between the diagnostic and monitoring uses of $F_{E_{NO}}$ allows for a more appropriate interpretation of the results as outlined in Table 5. Other factors to take into account include whether the subject is a smoker or is on antiinflammatory medications, as well as his/her height and age.

3. Interpretation of $F_{E_{NO}}$ measurement: clinically relevant cut points. The purpose of measuring $F_{E_{NO}}$ is to determine whether the value is within normal limits, high, or low. In addition, when monitoring over time, one must be able to determine when a significant change (increase) has taken place. After correct measurement, and with reference to factors which may be affecting the measurement (e.g., current smoking). Interpretation can be made as follows (*see also* Table 5):

- < 25ppb (< 20ppb in children): eosinophilic inflammation and responsiveness to ICS (post-bronchodilator FEV_1) are unlikely.

- > 50ppb (> 35ppb in children): eosinophilic inflammation is likely; responsiveness to ICS (post-bronchodilator FEV1) is likely.

- Values between 25ppb and 50ppb (20–35ppb in children) must be interpreted cautiously with reference to the clinical context.

- An increase of > 20% *and* more than 25ppb (20ppb in children) may be significant but there are wide inter-individual differences.

- A minimally important decrease of the $F_{E}NO$ value is defined as a difference larger than 20% for values over 50ppb or more than 10ppb for values lower than 50ppb from one visit to the next. A reduction of an elevated $F_{E}NO$ of more than 20% that often occurs 2–6 wk after initiation of anti-inflammatory therapy supports that the treatment was successful in reduction of inflammation.

4. Minimum reporting requirements for $F_{E_{NO}}$. When reporting $F_{E_{NO}}$ results, a minimum information set should be included. This should include but not be limited to: date, time of the day, age, sex, ethnicity, height, smoking status, reason for the test, and prior diagnosis (if known), and whether or not the patient was using inhaled or oral corticosteroids at the time of testing. The format of the reporting should include the device used to make the measurement, the number of measurements made, and the flow rate (currently approved FDA devices use 50 ml/s flow rate). One can choose to include all measurements performed or just the mean value. Results of previous testing (if available) should be included. A listing of the relevant cut point values is usually helpful.

Other Situations in which $F_{E_{NO}}$ May Be Useful

These are emerging areas for the use of $F_{E_{NO}}$ in the clinical setting, but there is not enough literature to provide specific guidelines for their application (106).

COPD. The exact role of exhaled nitric oxide measurements in patients with established COPD remains to be defined. In a significant number of patients, an overlap syndrome comprising features of both asthma and COPD is found (53). The airway inflammatory cell infiltrate may be mixed, including eosinophilic inflammation. Studies show that, at least in the short term, the response to corticosteroids is likely to be greater in patients with COPD who also have sputum eosinophilia (107, 108) or elevated $F_{E_{NO}}$ (109). This raises the possibility that $F_{E_{NO}}$ measurements might be used in predicting steroid responsiveness in COPD. In a small group of 19 patients, Zietkowski and coworkers reported a significant correlation between baseline $F_{E_{NO}}$ and ΔFEV_1 after 2 months with inhaled budesonide 800μg/day (108). de Laurentiis and colleagues (110) reported greater $F_{E_{NO}}$ variability in patients with COPD who subsequently develop exacerbations. More recently baseline $F_{E_{NO}}$ was found to be a predictor for changes in airflow obstruction, but not improvements in functional exercise capacity or health-related quality of life, with corticosteroid therapy (56). There is also some early evidence that a raised $F_{E_{NO}}$ predicts FEV_1 response to ICS in COPD (111, 112).

Pulmonary hypertension. NO is one of the important pathophysiological mediators of pulmonary hypertension (113, 114). It is important to point out, however, that while NO is the most recognized product of NOS, it is not the only one and an activity that is inhibited by NOS inhibition is not necessarily caused by NO (115–119). In the case of pulmonary hypertension for example, NO concentrations 1,000 times higher than those produced by NOS endogenously (normally present in the airways) are required for therapy, and pulmonary hypertension can be treated by nitrogen oxides such as ethyl nitrite that do not produce any nitric oxide at all (119). Thus in this sphere, we use NO to refer to NOS activity, recognizing that NO is a biomarker for NOS activity without always being the effector molecule. In addition to vasodilatation, NO regulates endothelial cell proliferation and angiogenesis, and maintains overall vascular health (121, 122). Interestingly, patients with pulmonary hypertension have low levels of $F_{E_{NO}}$ (123). Although this is a far more complex issue than the simple lack of a vasodilator (124), giving NO therapeutically seems to work well (125). Therapies that target the NO pathway have revolutionized the treatment of this disease, including the widely used phosphodiesterase type 5 (PDE5) inhibitors, which prevent the breakdown of the NO effector molecule 3′,5′-cyclic guanosine monophosphate (cGMP), thus prolonging NO effects on tissues (122). The NO deficiency state in patients with pulmonary hypertension also improves with other therapies that do not directly target the

NO pathway like prostacyclins and endothelin receptor antagonists (125, 126). This seems also to have a prognostic significance, with improved survival in patients who respond to therapy with higher $F_{E_{NO}}$ levels compared with those who do not change their $F_{E_{NO}}$ levels in response to therapy (127). The low $F_{E_{NO}}$ levels in patients with pulmonary hypertension and the improvement with effective therapies suggest that monitoring NO levels over time may be a useful noninvasive marker to evaluate response to or failure of medical therapy in these patients (127).

Cystic fibrosis and nasal NO measurements. Continuous and high production of NO takes place in the human nose and paranasal sinuses (128, 129), and this NO is readily measurable by noninvasive techniques (130). It has been shown that the nasal NO levels are altered in several respiratory disorders—including primary ciliary dyskinesia (PCD) (129), cystic fibrosis (CF) (131, 132), and allergic rhinitis (133, 134), and this has led to the proposal that nasal NO may be clinically useful in diagnosis and monitoring of these diseases. The levels of nasal NO are uniformly extremely low in patients with PCD, and the sensitivity and specificity of the test in this setting is excellent (135–139). The low levels of NO in CF are related to the absence of NOS2 expression in the airway epithelium, which supports the concept of NOS2 contribution to much of the NO detectable in exhaled breath (140–142). There is now abundant evidence that NO levels in CF are affected by a variety of other pathways as well. In addition to NOS2, determinants of exhaled NO in CF include arginase activity (143), superoxide levels (144), S-nitrosothiol metabolism (145), and denitrification pathways/prokaryotic nitrogen oxide metabolism (146, 147). Thus, these various determinants are all important when it comes to clinical interpretation $F_{E_{NO}}$ in CF. As such, response to arginine, response to antioxidants, response to inhaled nitrosothiols, and response to antimicrobial therapy might potentially be monitored in CF, to some extent, by monitoring $F_{E_{NO}}$. Although $F_{E_{NO}}$ is low in PCD, the diagnostic accuracy is considerably greater for a nasal NO test. Therefore this test is attractive for screening for PCD, prior to confirmatory testing (e.g., biopsies with analysis of ciliary structure). In contrast to $F_{E_{NO}}$, a single standardized procedure has not yet been defined for measuring nasal NO. Until this has been agreed upon, nasal NO levels are not yet recommended in routine clinical practice.

In summary, the use of $F_{E_{NO}}$ in COPD and pulmonary hypertension and the use of nasal NO in diagnosis and monitoring of other respiratory disorders (e.g., allergic rhinitis, sinusitis, nasal polyposis, CF) are potentially of interest, but more research is needed before we know how clinically useful these tests can be for these disorders.

Conclusions and Future Directions

Advances in technology and standardization have made $F_{E_{NO}}$ measurements simple, permitting their use as a biomarker in the assessment of inflammatory airways diseases. It is widely acknowledged that asthma is a heterogeneous disease with a variety of underlying pathophysiological abnormalities. $F_{E_{NO}}$ plays a role in identifying these different phenotypes (4, 7, 8, 34, 35, 148). Measurements are easily performed in different settings and may be used in diagnosis and monitoring. Large population studies have identified various confounders that affect $F_{E_{NO}}$ including age, sex, and height, among others. Consistent observations indicate that atopic individuals have higher $F_{E_{NO}}$ levels while smokers tend to have lower $F_{E_{NO}}$ levels (69, 76–78). Reference values have been derived from large population studies, but in practice they have limited application. Rather, evidence-based cut points that are shown to have diagnostic significance

appear to be more relevant. When monitoring individual patients with asthma and assessing their treatment requirements, achieving "personal best" rather than "normal" values is more helpful. In many patients, *changes* in $F_{E_{NO}}$ in relation to a baseline when clinically stable may be even more relevant. $F_{E_{NO}}$ values of themselves do not justify a diagnosis or change in treatment. Rather, they need to be interpreted in relation to the clinical context as discussed in this Guideline. They may be particularly useful in understanding patients with asthma in whom more than one factor is contributing to respiratory symptoms (e.g., obesity, anxiety) and for whom clinical decision making is difficult. Another potential use of $F_{E_{NO}}$ might be during inhalation challenge testing. That is, as with spirometry, giving an allergen inhalation challenge while measuring changes in $F_{E_{NO}}$ before and after the challenge. This may be potentially useful in the assessment of occupational asthma (149, 150).

Although these guidelines for interpretation of $F_{E_{NO}}$ measurements will enhance their clinical utility, we need to continue to investigate how to interpret $F_{E_{NO}}$ measurements in different clinical settings. Inclusion of $F_{E_{NO}}$ as an endpoint in clinical trials would be very helpful in understanding the role of $F_{E_{NO}}$ in monitoring response to therapy (151). Furthermore, $F_{E_{NO}}$ measurement in large population-based studies like the National Health and Nutrition Examination Survey (NHANES) would provide more information on normative values (152). Thus, the guidelines provided here will need to be periodically updated with regard to new developments in this rapidly evolving field.

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