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EXHALED NITRIC OXIDE IN THE DIAGNOSIS AND MANAGEMENT OF ASTHMA:

CLINICAL IMPLICATIONS

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Abstract

Exhaled nitric oxide (eNO) utilized as an aid to the diagnosis and management of lung disease is receiving attention from pulmonary researchers and clinicians alike because it offers a noninvasive means to directly monitor airway inflammation. Research evidence suggests that eNO levels significantly increase in individuals with asthma prior to diagnosis, decrease with inhaled corticosteroid administration, and correlate with the number of eosinophils in induced sputum. These observations have been used to support an association between eNO levels and airway inflammation. This review presents an update on current opportunities regarding use of eNO in patient care, and more specifically, on its potential usage for asthma diagnosis and monitoring. The review will also discuss factors that may complicate use of eNO as a diagnostic tool, including changes in disease severity, symptom response, and technical measurement issues. Regardless of the rapid, convenient, and noninvasive nature of this test, additional well-designed, long-term longitudinal studies are necessary to fully evaluate the clinical utility of eNO in asthma management.

Keywords

Exhaled Gas Analysis; Nitric Oxide; Lung Inflammation; Asthma

INTRODUCTION

In the last several years, there has been a great deal of interest in the analysis of breath constituents for purposes of monitoring oxidative stress and inflammation in the lungs. Nitric oxide (NO) has garnered the most attention. NO is truly a “multipurpose” molecule as it mediates physiologic processes in the majority of organs. For example, NO is thought to be an essential part of diverse physiologic processes, such as smooth muscle relaxation, platelet inhibition, neurotransmission, apoptosis, and immune regulation.¹ In the lung alone,

endogenous NO influences outcomes in airway disease, pulmonary hypertension, lung injury, and infection.

NO Synthesis

Nitric oxide has distinct chemical properties largely as a result of the unpaired electron it possesses. NO is a highly reactive molecule known as a free radical or reactive nitrogen species. Nitric oxide synthase (NOS) catalyzes the generation of nitric oxide via the enzymatic conversion of the amino acid arginine to citrulline. Three NOS isoforms have been identified, endothelial NOS (eNOS), neural NOS (nNOS), and induced NOS (iNOS), and they differ in (among other things) tissue distribution and activity.¹ Of the three, iNOS is of most interest because it is highly inducible by a variety of inflammatory cytokines, produces large quantities of NO, and is responsible for most increased exhaled NO (eNO) levels in the disease state of asthma.² The two constitutive isoforms (nNOS and eNOS) require influx of calcium (Ca^{2+}) and calmodulin for activation.³ In contrast, iNOS is ready for physiologic activity immediately after translation due to a pre-existing bond between Ca^{2+} and calmodulin.⁴ The isoform of nNOS, released from nerve cells,⁴ is known to be up-regulated by heat, electrical activation,⁵ light, ischemic injury,⁶ and sex hormones, such as estradiol and testosterone.⁷ Alternatively, nNOS is downregulated by pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α).^{5,8} The isoform of eNOS is released by endothelial cells and is up-regulated by shear stress and proliferation of tissue,^{9,10} and down-regulated by endotoxin or cytokines.⁴ In the respiratory system, when proinflammatory cytokines or oxidants activate nuclear factor κB (NF- κB),¹¹ iNOS is expressed in various cells: bronchial epithelial cells,¹²⁻¹⁴ alveolar macrophages,^{12,15,16} nasal vascular epithelial cells and nasal ciliated epithelial cells.¹⁷ Most importantly, it should be recognized that iNOS has a dual nature - it involves cell protection as well as cell damage.¹⁸⁻²⁰

Physiologic Actions of NO

In the respiratory system, NO produces regulative, protective, and deleterious effects.¹⁸⁻²⁰ As a regulator, NO controls tracheobronchial circulation,²¹⁻²³ suppresses airway plasma exudation^{24,25} and stimulates mucociliary clearance,²⁶⁻²⁸ a primary airway defense mechanism. As a cytoprotective agent, NO is known to attenuate toxicity mediated from reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), alkyl hydroperoxides, and superoxide.^{20,29} The up-regulation of iNOS activity increases NO production and creates a toxic environment for viruses, bacteria, fungi, parasites, e.g., herpes simplex virus, mycobacterium tuberculosis.³⁰ This activity of iNOS in airway epithelium is important for airway host defense. Although NO itself is not inherently cytotoxic, depending on intracellular redox milieu, NO can react as a cytotoxic or cytoprotective agent. Reaction of NO with oxygen or redox metal complexes produces NO and NO-derived chemical species thereby creating its deleterious effects. Peroxynitrite (ONOO^-), one of powerful oxidant formed by the reaction between NO and O_2^- , inhibits enzyme function, causes damage in DNA, induces lipid peroxidation, and increases cellular susceptibility to radiation, toxic metals, and alkylating agents.^{20,31}

Clinical Relevance

Traditionally, direct measurement of inflammation associated with lung disease has not been a part of routine clinical practice. Instead, indirect measures are commonly used, e.g., symptom reports, spirometry, and sputum analysis. Direct measures are available but all are invasive and therefore impractical for frequent use, e.g., transbronchial biopsy and bronchoalveolar lavage. The search for a direct, noninvasive method to monitor airway inflammation led to the exploration of the potential utility of exhaled gases and condensates as a means to accomplish this goal. An emerging body of research suggests that noninvasive monitoring of NO may

assist in differential diagnosis, assessment of disease severity, and response to treatment.³² Until recently, monitoring of eNO has been a research procedure. Mounting evidence of the utility eNO analysis led to US Food and Drug Administration clearance of an eNO system for clinical application in patients with asthma in 2003³³ and in 2005 to American Thoracic Society and European Respiratory Society recommendations for standardized measurement techniques.³⁴

This review will focus on several topics that address the efficacy of eNO for the diagnosis and management of asthma, the first disease to be associated with altered NO levels.¹ Selected articles were identified and reviewed from a Medline search using the key terms Exhaled Gas Analysis, Nitric Oxide, Lung Inflammation, and Asthma. Since the first asthma/NO paper in 1993,³⁵ hundred of studies have attempted to discern the possible role of NO in asthma as a monitor of disease status. This review will critique and summarize selected studies in this field to identify: expected changes in eNO in normals versus treated and untreated asthmatics; the potential utility of eNO as a diagnostic test for asthma and the potential contributions of this technique to asthma management.

ATOPY AND ALLERGIC INFLAMMATION

The association between eNO and airway inflammation is supported by observations that NO levels are increased in subjects with asthma,³⁵⁻³⁷ decreased in subjects receiving inhaled corticosteroids,^{36,38} and correlated with the number of eosinophils (differential count) in induced sputum.³⁹ In 1999, Salome and colleagues⁴⁰ examined the distribution and reproducibility of eNO levels in 306 young adults and, in an innovative application, attempted to determine if there was evidence of elevated NO in adults who reported wheezing but had normal spirometric values and normal airway responsiveness. The sample was divided into two groups on the basis of airway hyperresponsiveness (AHR), defined as dose of histamine that provoked a 20% fall in FEV₁ equal or less than 3.9 μ mol histamine or a positive response to bronchodilators. The correlation between eNO and dose-response ratio remained significant in the hyperresponsive group ($r=0.33$, $p<0.05$), but not in the normoresponsive group ($r=0.12$). Atopic adults without AHR had eNO levels significantly higher than nonatopic adults without AHR, both in the wheeze group ($p<0.01$) and in the normal group ($p<0.01$). Salome et al.⁴⁰ concluded that eNO was greater in atopic than in nonatopic adults, suggesting that mild airway inflammation is present in atopic subjects. In line with the suggestion that eNO may be a noninvasive marker of airway inflammation,³⁸ Smith and coworkers⁴¹ found eNO to be significantly higher ($p<0.001$) in steroid-free adults with asthma when compared to normals and significant correlations between eNO and sputum eosinophils ($r=0.67$, $p<0.001$).

Among others supporting this thinking,⁴²⁻⁴⁴ Malmberg and colleagues⁴⁵ compared 62 healthy children with 21 children having probable asthma treated only with beta-2 agonists and reported significantly higher levels of eNO in the latter group ($p<0.0001$). In addition to the recognition that eNO is elevated in subjects with asthma compared to normal individuals, NO levels have been shown to vary with disease activity and in response to anti-inflammatory therapy.^{38, 46-48} In another study with very practical clinical implications, Chatkin and coworkers have demonstrated that, in a population of adults with chronic cough, eNO discriminated well between those with and those without asthma.⁴⁹

Elevated levels of eNO have been validated against invasive measurements of inflammation by bronchoscopy^{50,51} and induced sputum,⁵² and a significant correlation has been found between eNO and iNOS positive granules in sputum eosinophils.⁵³ Nonetheless, when subjects were using inhaled corticosteroids, findings from other studies have been inconsistent regarding the correlation between eNO and differential count sputum eosinophils.⁵⁴⁻⁵⁶ Jones and colleagues⁴⁴ avoided this potentially confounding effect by temporarily withdrawing

maintenance inhaled corticosteroid therapy in adult subjects. Measured longitudinally, the changes in eNO correlated significantly not only with changes in the other markers of airway inflammation but also with measurements of airway caliber and symptoms. It is notable, however, that in the first study to systematically examine the relationship between eNO and sputum eosinophil (differential) count in a large heterogeneous population of adults with asthma (n=566), investigators found a significant positive association between these two markers of airway inflammation in 56% of subjects on inhaled steroids.⁵⁷

In summary, the evidence discussed in this section suggests that eNO may have potential utility as a means of monitoring airway inflammation in individuals with asthma. However, in addition to the potential confounding effects of corticosteroids, a number of other variables such as age, diet, cigarette smoking, caffeine and alcohol consumption may influence eNO levels also. In a subsequent section, these potential confounders will be discussed in some detail within the context of the usefulness and utility of eNO for guiding therapy.

DIAGNOSTIC UTILITY OF eNO

For a number of years, guidelines for the management of asthma have emphasized the importance of maintaining disease control by preventing chronic symptoms and recurrent exacerbations.⁵⁸ This strategy minimizes the need for rescue medications (such as short acting beta-agonists) and promotes maintenance of normal pulmonary function and activity levels. As asthma care has become more sophisticated, it has become possible to maintain symptom control over a wide range of disease severity. Of note, asthma control and asthma severity are conceptually separate, therefore should not be mistaken for the same entity. For example, many patients with mild asthma have poorly controlled symptoms, robust reversibility of airflow obstruction, and require frequent rescue medications for any number of reasons. Conversely, a patient with moderately severe asthma may be receiving appropriate controller therapy, have few symptoms or exacerbations with minimal reversibility of airflow obstruction.

Jakakanon and colleagues⁵⁹ completed one of the few longitudinal studies examining changes in eNO. Exhaled NO levels were examined for 8 weeks in 15 adults with a previous history of asthma who had been treated for at least 3 months with inhaled corticosteroids. Seven of these subjects developed mild exacerbations during the 8 weeks of the study, while the other 8 subjects did not. Exhaled NO levels were not significantly different at baseline. However, significant correlations were found between the change in eNO and the changes in FEV₁ (r = -0.70, p = 0.004) and the amount of rescue inhaler use (r = 0.56, p = 0.028) in data pooled from both groups. While these results suggest limitations in utilizing eNO as an accurate marker of control of inflammation in asthmatic subjects using inhaled corticosteroids, they may also suggest that NO is sensitive to the inhibitory effect of corticosteroids.

Severity versus Loss of Control

Exhaled NO levels have been found by some to reflect asthma control better than asthma severity.⁶⁰ For example, Stirling and coworkers⁶¹ found higher levels of eNO in adults with greater asthma severity regardless of corticosteroid use, and Kharitonov and colleagues⁶⁰ demonstrated that a reduction in the dose of inhaled corticosteroids increases eNO even without a significant change in peak flow variability or spirometry. There is also evidence to suggest that an increase in eNO may be seen before any significant deterioration in airway hyperresponsiveness, sputum eosinophils, or lung function during an asthma exacerbation induced by steroid reduction.^{59,62} This is consistent with the findings of Jatakanon and coworkers who were unable to demonstrate significant correlations between eNO and other markers of airway inflammation in patients with asthma who had been treated with inhaled corticosteroids.⁶³ However, some have argued that eNO may be more sensitive to inhibition by corticosteroid therapy than other markers of inflammation. For instance, even 2 weeks after

discontinuation of inhaled corticosteroids in adults with mild to moderate asthma, one investigative group found that eNO was not a significant predictor of subsequent asthma control, whereas sputum eosinophils were.⁶⁴

Exhaled NO levels are typically elevated in mild asthma, but almost normal in stable moderate asthma adequately treated with corticosteroids.³⁶ In some, but not all studies, eNO levels were further elevated in patients with severe⁶¹ and uncontrolled asthma.⁵⁸ However, although eNO has demonstrated usefulness in research studies as a marker for monitoring airway inflammation and thus asthma control, the lack of consistency in findings suggests caution. Only through additional well-designed, longitudinal studies can the clinical usefulness of this technique be convincingly elucidated.⁶⁵

UTILITY OF eNO IN GUIDING THERAPY

Jones and colleagues⁴⁴ conducted an 11-week study with 78 adults (having predominately atopic asthma) to evaluate the predictive and diagnostic value of eNO in assessing loss of control in asthma following steroid withdrawal. The majority (77.9%) developed loss of control, according to predetermined study criteria, within 6 weeks after the cessation of steroid treatment. The median time to loss of control was 17 days, and fall in the mean morning peak expiratory flow rate and asthma symptoms that were disagreeable or distressing were the most frequent criteria of loss of control. Exhaled NO was associated with a positive predictive value of between 80 and 90% for predicting and diagnosing loss of control. The outcomes also reflect poor sensitivity but good specificity for eNO measurements. Nevertheless, values for eNO compared favorably to the more elaborate techniques of sputum induction and hypertonic saline challenge to assess change in asthma control.⁴⁴

Though the above findings provide support for the use of eNO measurements as a tool for assessing airway inflammation and long-term asthma control, many patients with asthma are prescribed maintenance inhaled corticosteroid therapy. Thus, clinical application of the findings is problematic in steroid-dependent patients. Nevertheless, it should be remembered that other studies (of shorter duration) offer support for the usefulness of eNO in the presence of inhaled corticosteroids. In yet another (longitudinal) study to offer support for the usefulness of eNO in the presence of inhaled corticosteroids, Baraldi and coworkers⁶⁶ examined eNO levels in children with mild asthma before, during, and after the pollen season. The investigators suggest eNO is a useful longitudinal assessment tool for asthma because, even though greater than one-third of the children were taking inhaled corticosteroids, a rise in eNO levels (but without FEV₁ change) was seen during the pollen season consistent with increased airway inflammation induced by allergen exposure. This is consistent with findings of no strong link between eNO, FEV₁ and symptoms in mild steroid-naïve asthma measured under stable conditions.^{35,36} However, as a pediatric study, the results may not generalize directly to an adult population.

These results do, however, support those from a study by Dupont and coworkers⁶⁷ that measured eNO in steroid-treated and untreated adults with mild asthma. Findings suggest that eNO reflects airway hyperresponsiveness in adults with mild asthma who have not already been treated with inhaled steroids. It is notable in this study⁶⁷ that those with asthma treated with inhaled corticosteroids had eNO levels comparable to levels in control subjects, although airway hyperresponsiveness *could still be demonstrated* (Figures 1 & 2).

While eNO levels have been recognized to be elevated in steroid-naïve asthmatic subjects and decreased in asthmatic subjects with corticosteroid treatment,⁴⁷ it is less clear whether eNO can reflect changes in airway inflammation in subjects who remain symptomatic despite corticosteroid treatment. Jatakanon and colleagues⁶³ speculated that there may be differences in the mechanism of airway inflammation between asthmatic adults who display varying

degrees of treatment efficacy on corticosteroids. Accordingly, they designed a study to compare eNO levels in adults with uncontrolled asthma despite continuous corticosteroid therapy. The subjects included patients with mild asthma receiving occasional inhaled β_2 agonist treatment (n=23), moderate asthma requiring medium doses of inhaled corticosteroids (n=16), and severe asthma despite high dose corticosteroid treatment (n=16). The results suggested that eNO levels were highest in those with mild asthma using occasional short-acting B_2 agonists (Figure 3).⁶³ Significant differences in eNO were found between normal adults and those with mild asthma ($p<0.001$), mild vs. moderate asthma ($p<0.05$), and normal subjects vs. severe asthmatics ($p<0.001$). However, no significant difference in eNO was noted between those with moderate and severe asthma.

As previously noted, current thinking posits an association between eNO and airway inflammation, supported by the observations that NO levels are increased in subjects with asthma,^{35,36} decreased in subjects receiving inhaled corticosteroids,^{36,38} and significantly correlated with the number of eosinophils in induced sputum.³⁹ As a marker of airway inflammation, eNO levels might conceivably correlate with indicators of disease control in asthmatic patients. In order to clarify this potential relationship, Sippel and colleagues⁵⁸ analyzed a cross-sectional survey of 100 adults with asthma. Over half reported using inhaled corticosteroids during the previous year. Those who reported asthma symptoms during the prior 2 weeks had significantly higher eNO levels than those without symptoms, irrespective of whether there was adjustment for inhaled corticosteroid use. However, those who reported asthma symptoms during the past 1 or 6 months did not have significantly higher eNO levels compared with those without symptoms. The use of daily rescue medications also correlated positively with eNO, as did a positive bronchodilator response.

The above findings clearly seem to challenge the clinical utility of eNO as means of monitoring airway inflammation once corticosteroid treatment has been started. The controversy surrounding this issue is nicely illustrated by two recent longitudinal randomized controlled trials. Smith and colleagues⁶⁸ assigned 97 adults with asthma who were regularly receiving treatment with inhaled corticosteroids to have their corticosteroid dose adjusted by either an algorithm based on conventional guidelines or by eNO measurements. The subjects were followed for 12 months (after the optimal dose of inhaled steroid was determined) for the outcomes of asthma exacerbation frequency and mean daily dose of inhaled corticosteroid. Investigators determined that with eNO measurements at 4 to 8 week intervals for 12 months, a significantly lower maintenance dose of inhaled corticosteroid was needed for asthma control compared to conventional guidelines. In fact, a 40% reduction in required inhaled corticosteroid dose, without compromised exacerbation rates, was demonstrated in the group receiving eNO measurements.

However, in the largest study of its kind to date, Shaw and colleagues⁶⁹ tested the hypothesis that titrating inhaled corticosteroid using eNO results in fewer severe asthma exacerbations compared to traditional management. One hundred eighteen adults with asthma were randomly assigned to management based on either eNO measurements or British Thoracic Society guidelines, and followed monthly for four months and then every two months for the next eight months. Results showed that compared with traditional asthma management, the use of eNO measurements to guide treatment decisions did not result in a decreased exacerbation frequency or in a lower maintenance dose of inhaled corticosteroid. While subjects in the eNO group were, at the conclusion of the study, receiving a significantly lower dose of inhaled corticosteroid, use of inhaled corticosteroid over the entire 12 months of the study did not differ between groups.

Several potential confounders need to be considered when interpreting eNO values. Cigarette smoking is known to decrease eNO levels, possibly because NO in cigarettes down-regulates

NOS production.⁷⁰ An alcohol and caffeine consumption-related influence on eNO has been reported, but whether such effects are consistent in both healthy individuals and patients with lung disease (e.g. asthma) is not yet clear.⁷¹ Although the effect of diet on eNO levels is not readily apparent, there may be some influence from food supplements containing L-arginine, or nitrate.⁷²⁻⁷⁴ Therefore, it is recommended that patients refrain from eating, drinking caffeinated beverages, or smoking for one hour before measurements are made.⁷⁵ The possible effect of ambient NO on eNO is still a matter of some controversy,⁷⁶⁻⁸¹ and in addition, the influence of body mass index, gender, and age on eNO is not yet clear.^{82,83}

In summary, it seems reasonable that future studies examining eNO in asthmatic patients should not only be longitudinal in nature, but should also consider utilizing methods capable of reliably measuring all the pertinent variables: symptoms, lung function, airway inflammation, and hyperresponsiveness. Current data suggests asthma deterioration may start to occur while one or more of these factors remain within normal limits. Complicating analysis and interpretation of any NO measurements are a number of potential confounders.

ONLINE VERSUS OFFLINE MEASUREMENTS

Expiratory flow rate has been demonstrated to have a most dramatic influence on measured eNO values - with eNO declining with increasing expiratory flow rate.^{84,85} Collection techniques (offline reservoir bag or online) that use slow expiratory flow rates produce higher NO value differences between normals and individuals with asthma,⁸⁶⁻⁸⁸ suggesting that low-flow collection methods allow for greater discrimination.^{87,89} However, low-flow collection maneuvers may be uncomfortable.⁹⁰ Standard offline measurements are considered a second choice because of they do not permit real-time monitoring of exhalation (in order to be able to exclude technically poor maneuvers) and risk contamination through leaks in the collection bag.⁹¹ Offline collection techniques do have the benefit of facilitating remote collection with convenient centralized use of a single analyzer.

Deykin and colleagues⁸⁴ conducted a systematic investigation of the effects of online and offline collection technique and flow rates to determine the ability of eNO to discriminate between adults with and without asthma. The study involved offline measurement of eNO at several expiratory flow rates with the goal of differentiating those with asthma (n=34) from healthy subjects (n=28) and comparing these findings to online measurements made at similar flow rates in a subgroup of the subjects. The subjects were using no asthma medications except for short-acting bronchodilators withheld for at least 8 hours before testing. No systemic or inhaled corticosteroids were used within 8 weeks of enrollment. All 62 subjects performed offline eNO collections at five expiratory flow rates (50, 100, 200, 350, and 500 ml/s). Individuals with asthma had higher eNO values as compared with healthy subjects at each flow rate, and offline eNO values fell with increasing flow rate (Figure 4). A subgroup of 18 adults with asthma and 17 healthy subjects performed online eNO collection at four expiratory flow rates (42, 108, 210, and 250 ml/s). As in the case of offline measurements, subjects with asthma had higher mean eNO values at each flow rate compared to healthy subjects, and online eNO values fell with increasing expiratory flow rate (Figure 4).

If a prevalence rate of asthma in the general population of 0.05 is used, extrapolation of the findings of Deykin and colleagues⁸⁴ indicates that eNO as a test for asthma would demonstrate a positive predictive value of approximately 12% with a negative predictive value of 97 to 98%. These findings provide support for the assertion that eNO is most useful as an *exclusionary* test for asthma. Taken as a whole, these findings may be very important to consider given commercial marketing of eNO measurement as a method for clinical monitoring of patients with asthma. Based on these results, investigators and clinicians may choose the eNO flow rate that is most comfortable and convenient for the population under study -

provided the same flow rate is used for repeated measurements within the same individual and for legitimate comparisons between individuals.⁸⁴

In summary, measurement of eNO via offline or online methodology is rapid, convenient, and noninvasive. While either method is potentially attractive as a monitoring approach, the preferred technique for any given diagnostic or management scenario will likely remain online measurement in order to allow for real-time monitoring of exhalation and to reduce the risk of contamination through leaks in an offline collection bag.

MULTIPLE FLOW RATE eNO MEASUREMENT

As previously discussed, eNO concentration is known to be dependent on exhalation flow rate. This is a characteristic of NO that differs from other exhaled gas constituents, such as carbon monoxide.⁸⁷ Gelb and colleagues⁹² used 100 ml/sec as the lowest flow-rates because of difficulty in collecting reproducible data at 50ml/sec in asthmatic adults with FEV₁% less than 80% predicted.⁹² However, in the studies using much lower flow rates,^{93,94} 100% of subjects with average FEV₁% less than 70% predicted were able to tolerate the protocol. Intraclass Correlation Coefficient was 0.99.⁹⁴ To further demonstrate this unique characteristic, NO exchange models have been developed by several researchers.⁹⁵⁻⁹⁸ The most widely used technique is the two-compartment model designed to provide measures of NO levels in alveoli (C_ANO, ppb) and flux of NO from the airway wall (J_{AW}NO, nl/sec).⁹⁸ For detailed methodological information on this procedure, the reader is referred to the review by George and colleagues.⁹⁹ This technique provides information about inflammation that involves both airways and alveoli and how the contribution of eNO from these different compartments can be analyzed separately.¹⁰⁰ Several studies have used this technique in asthmatic adults^{92-94, 101-103} to test the feasibility of performing multiple flow-rates eNO measurement in asthmatic adults with various levels of severity and compare C_ANO and J_{AW}NO among groups by types of treatment and disease severity. In all selected studies, no difficulty was reported obtaining flow rates that matched the desired rates, suggesting that patients can easily master this technique and produce reproducible values. When comparing results of studies, difference in equipment should always be considered.

Studies reported to date suggest that monitoring C_ANO by multiple-flow rate eNO measurement may provide the following benefits: 1) diagnosis and monitoring of peripheral airway inflammation in individuals having asthmatic symptoms with normal lung function,¹⁰² which may provide a means to guard against under-treatment; 2) evaluation of peripheral airway inflammation in nocturnal asthma;¹⁰¹ and 3) measurement of distal lung inflammation in refractory asthma which is difficult to control with inhaled steroids.^{93,94,103}

The concentration of alveolar NO may provide a useful initial estimate of the presence of peripheral airway inflammation in nocturnal asthma when a direct measurement is not available. In the study by Lehtimäki and coworkers,¹⁰¹ C_ANO was higher in asthmatic adults with nocturnal symptoms than in patients without these symptoms and controls.¹⁰¹ J_{AW}NO was higher in all asthmatic patients regardless of nocturnal symptoms. This finding indicates that nocturnal asthma also involves peripheral airways, in addition to underlying central airway inflammation. The suggestion of inflammation of this extent in nocturnal asthma is supported by the analysis of serum inflammatory markers.¹⁰¹ In this study, serum eosinophil protein levels in a nocturnal asthma group were significantly higher than in asthma patients with no nocturnal symptoms. In addition to high serum eosinophil protein, patients with nocturnal asthma had significantly higher levels of other markers, such as eosinophilic cationic protein, myeloperoxidase, and interleukin-6, compared with patients without nocturnal symptoms.

In three recent studies, higher C_ANO levels were consistently reported in severe, or refractory, asthma patients who cannot get benefit from the treatment with inhaled corticosteroids.^{93,94,}

¹⁰³ In the initial study by Berry and coworkers,⁹³ no significant difference in sputum eosinophil counts or J_{AW}NO was evident between the mild-to-moderate (FEV₁ pred 94.2 ± 7.4) and refractory asthma group (FEV₁ pred 62 ± 21.1). However, C_ANO was significantly higher in the refractory asthma group compared to the mild-to-moderate group. When changes in C_ANO were compared before and after either oral or inhaled corticosteroid treatment in refractory asthma patients, C_ANO was significantly decreased by oral steroids, whereas no change occurred with inhaled corticosteroid use. In addition, the study by van Veen and colleagues¹⁰³ also reported high C_ANO in severe asthma patients compared with mild-to-moderate asthma. However, while the study by Berry and colleagues⁹³ reported decreased C_ANO after oral steroid therapy, C_ANO in severe asthma patients who were already on oral steroids was still higher than severe asthma patients with no oral steroids. Similar results were reported from the study by Brindicci and coworkers, which estimated C_ANO in 58 asthmatic adults with different severity.⁹⁴ Here, C_ANO was significantly increased in moderate asthma patients during exacerbations and in severe asthma patients on either inhaled corticosteroids or oral steroids. However, due to study limitations such as small sample size and of the use of different expiratory flow rates and equipment, it is difficult and perhaps somewhat misleading to compare results. Nevertheless, both authors suggest that additive information from C_ANO may have a potential for 1) estimating peripheral lung inflammation and 2) thereby guiding the dosages of systemic anti-inflammatory therapies.^{94,103}

SUMMARY

Despite the fact that exhaled breath analysis of NO has until very recently been strictly a research practice, mounting evidence of the potential utility of eNO for the diagnosis and monitoring of lung disease is bringing it ever closer to being a practical clinical procedure that can be used to assist in differential diagnosis, assessment of disease severity, and response to treatment in patients with asthma.³² Observations that eNO levels are increased in subjects with asthma,^{35,36} decreased in subjects receiving inhaled corticosteroids,^{36,38} and significantly correlated with the number of differential count eosinophils in induced sputum³⁹ have been used to support an association between eNO and airway inflammation. Nevertheless, a synthesis of prior studies suggests many challenges in using eNO for diagnosis and management of asthma. Interpretation of eNO results can be complicated in patients who remain symptomatic despite corticosteroid treatment as well as by variations in the intensity of any given individual's disease activity. There is also a lingering question of whether eNO maintains its clinical utility as an accurate inflammatory marker for monitoring the control of airway inflammation once corticosteroid treatment has been started, suggesting that eNO may be more sensitive to inhibition by corticosteroid therapy than other indices of inflammation such as eosinophil levels in induced sputum.

The interpretation of eNO results in any given population is complicated by several factors. Flow rate, exhaled breath assay method, and use of inhaled NO-free air prior to the exhalation maneuver can and often does vary between studies. The extent to which these factors, as well as demographic characteristics and inter-individual variation influences the reported NO values is difficult to assess. Accordingly, care should be exercised before attempting to generalize study findings to a larger population.

Measurement of eNO via offline or online techniques is rapid, convenient, and noninvasive and therefore attractive as a monitoring approach. Evidence suggests eNO levels are highly reproducible among subjects with and without asthma.¹⁰⁴ Exhaled NO has also been convincingly demonstrated its usefulness as a marker for monitoring airway inflammation in patients who are not receiving steroids. Only through additional well-designed, long-term studies can the clinical usefulness of this technique be more comprehensively understood.

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Figure 1. Relationship between exhaled NO (ppb) and PC₂₀ histamine (mg/ml) in steroid-naïve patients with mild asthma ($r = -0.65$; $p < 0.0001$).
Note: From Dupont et al.⁶⁷ Reprinted with permission.

Figure 2.

Relationship between exhaled NO (ppb) and PC₂₀ histamine (mg/ml) in steroid-treated patients with mild asthma ($r = -0.18$, $p = \text{NS}$).

Note: From Dupont et al.⁶⁷ Reprinted with permission.

- Normal
- ▽ Mild asthma receiving occasional inhaled b2-agonist treatment
- ◆ Moderate asthma requiring medium dose of inhaled corticosteroids to maintain control
- Severe asthma despite high dose corticosteroid treatment

Figure 3. Exhaled NO in mild, moderate, and severe asthma. * $p < 0.05$; *** $p < 0.001$.
Note: From Jatakanon et al.⁶³ Reprinted with permission.

Figure 4.

Effect of flow rate and collection techniques on measured exhaled NO in healthy subjects ($n = 34$) and subjects with asthma ($n = 28$). In all subjects, exhaled NO fell with an increasing flow rate. Exhaled NO was lower at each measured flow in comparison to the next lowest rate ($p < 0.001$ for all comparisons with the exception of online 210 vs. 250 ml/s, $p = 0.58$ and 0.27 for healthy subjects and subjects with asthma, respectively). At each rate and with both collection techniques, exhaled NO was higher in subjects with asthma as compared with healthy subjects ($p < 0.001$).

Note: From Deykin et al.⁸⁴ Reprinted with permission.