

The clinical significance of exhaled nitric oxide in asthma

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Asthma is an inflammatory disease of the airways, for which many therapeutic options are available. Guidelines for the management of asthma suggest a stepwise approach to pharmacotherapy based on assessment of asthma severity and control. However, the assessment of asthma control presently relies on surrogate measures, such as the frequency of symptoms or the frequency of use of short-acting beta₂-adrenergic agonists. There is no simple, noninvasive technique for the assessment of severity of actual airway inflammation in asthma. The collection and analysis of nitric oxide (NO) levels in exhaled breath has recently become feasible in humans. Based on increased exhaled NO (eNO) levels in patients with asthma, eNO analysis has been proposed as a novel, noninvasive approach to the assessment and monitoring of airway inflammation, and as a basis for adjustments in asthma therapy. In the present paper, the relationship of elevated eNO levels in asthma with inflammatory, physiological and clinical markers of asthma in adults was reviewed. Use of eNO is a promising tool for diagnosing asthma, for monitoring asthma control and for guiding optimal anti-inflammatory asthma therapy. However, because of many unresolved questions, eNO cannot be recommended at present for routine clinical management of adults with asthma.

Key Words: *Airways inflammation; Asthma; Exhaled nitric oxide*

Asthma is an inflammatory disease of the airways that results in respiratory symptoms, bronchial hyper-responsiveness and a variable degree of airflow obstruction. Many effective therapies are available for the treatment of asthma, including bronchodilators for airflow obstruction, as well as anti-inflammatory agents, such as inhaled corticosteroids (ICSs). Guidelines for the management of asthma suggest a stepwise therapeutic approach based on assessment of asthma control. The assessment of asthma control presently relies on surrogate measures, including frequency of symptoms and as-needed use of short-acting beta₂-adrenergic agonists, the degree of airflow obstruction, and the frequency and severity of exacerbations rather than a measure of airway inflammation (1).

ASSESSMENT OF AIRWAY INFLAMMATION IN ASTHMA

Airway inflammation in asthma can be invasively assessed by bronchoalveolar lavage or bronchial biopsy during fibre optic

La signification clinique de l'oxyde nitrique dans l'air expiré des asthmatiques

L'asthme est une maladie inflammatoire des voies aériennes, pour laquelle il existe de nombreuses options thérapeutiques. Les lignes directrices pour la prise en charge de l'asthme proposent une démarche pharmacothérapeutique graduelle fondée sur l'évaluation de la gravité et du contrôle de l'asthme. Cependant, l'évaluation du contrôle de l'asthme dépend de mesures substituts, comme la fréquence des symptômes ou la fréquence d'utilisation d'agonistes bêta₂-adrénergique de courte durée. Il n'existe pas de technique non effractive simple pour évaluer la gravité de l'inflammation des voies aériennes dans l'asthme. Depuis peu, il est devenu possible de recueillir et d'analyser les taux d'oxyde nitrique (ON) dans l'air expiré chez les humains. D'après les taux accrus d'ON expiré (ONe) chez les asthmatiques, on a proposé d'analyser l'ONE à titre de démarche non effractive novatrice de l'évaluation et de la surveillance de l'inflammation des voies aériennes et de fondement pour rajuster la thérapie de l'asthme. Dans le présent article, les auteurs ont examiné le lien entre des taux élevés d'ONE chez les asthmatiques et les marqueurs inflammatoires, physiologiques et cliniques de l'asthme chez les adultes. L'ONE est un outil prometteur pour diagnostiquer l'asthme, pour surveiller le contrôle de l'asthme et pour orienter une thérapie anti-inflammatoire optimale de l'asthme. Cependant, en raison de nombreuses questions toujours sans réponse, il est impossible pour l'instant de recommander l'ONE pour la prise en charge clinique systématique des adultes asthmatiques.

bronchoscopy. Alternatively, the collection and analysis of sputum is a novel, noninvasive approach to the assessment of airway inflammation. However, because of stringent requirements for sample collection and processing, sputum analysis is only available in a few specialized centres. Thus, there is currently no simple, noninvasive, reproducible technique that is widely available for routine clinical use in the monitoring of airway inflammation in asthma.

EXHALED NITRIC OXIDE IN ASTHMA

Since the early 1990s, the measurement of nitric oxide (NO) levels in exhaled gas has been studied as a noninvasive assessment tool in various pulmonary diseases, primarily asthma. Many studies on exhaled NO (eNO) in asthma have focused on children, in whom the assessment of asthma control by symptoms and spirometry is difficult. As well, several recent reviews have focused on the biology of eNO in asthma, technical factors in the measurement of eNO and the use of eNO

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in the management of asthma in children. In the present review, we specifically address the clinical significance of the measurement of eNO levels in diagnosing, assessing and monitoring the response to treatment of adults with asthma, with a focus on practical recommendations for the potential clinical use of eNO as part of a comprehensive asthma clinical monitoring strategy.

Literature search strategy

Studies that reported eNO levels, as well as those that reported the diagnosis, monitoring or treatment of acute and chronic asthma in adult human subjects, were included in the review. Eligible English-language studies were identified by searching MEDLINE (1966 to week 1 of June 2006) and EMBASE (1966 to June 2006) using the search strategy "(exhaled AND nitric oxide) AND asthma". Titles and abstracts were screened to exclude ineligible studies. Included studies were entered into PUBMED and the 'related articles' tool was used to search for other potentially eligible studies. In addition, the bibliographies of included studies and published reviews were also searched. Exclusion criteria included reports in which all subjects were younger than 18 years of age, had another respiratory diagnosis, or in which technical considerations were the focus of the study. Studies were independently reviewed by each author, and disagreements resolved by consensus.

Technical factors in the measurement of eNO

Many technical and biological factors greatly influence the measurement of eNO. For example, eNO levels are significantly reduced after exposure to tobacco smoke and after maximal forced expiratory efforts during spirometry. In contrast, eNO levels are increased during viral upper respiratory tract infection and following ingestion of nitrate-rich foods. Notably, age, sex, body mass index and time of day do not consistently affect eNO measurements. One of the most critical technical factors in eNO analysis is the flow at which patients exhale during exhaled gas collection or NO detection (2), such that increased expiratory flow consistently reduces eNO concentration, but increases total NO output. Rigorous, detailed specifications have been established for the collection and analysis of eNO gas (2). Although attention to technical considerations is essential in the measurement of eNO in humans, we will not specifically address these issues here. Note that the studies reviewed here span from the earliest days of eNO measurement in humans until the present, and thus use differing methodologies and expiratory flow rates. However, within studies, appropriate control groups or serial measurements using the same methodology ensure the biological relevance of findings reported.

Biology of eNO in asthma

Since the early 1990s, it has been recognized that eNO levels are elevated in asthma patients as compared with both normal subjects and nonasthmatic subjects with miscellaneous respiratory complaints (3-6). Although extremely high levels of NO are present in the upper airway, including the nasal passages and paranasal sinuses, increased eNO in asthma is not simply an artifact of upper airway gas contamination of exhaled breath samples. Indeed, an increased presence of NO in the lower airways of asthmatic subjects as compared with control subjects has been directly demonstrated, as assessed by invasive bronchoscopic sampling of lower airway gas (5,6).

The biological basis for increased eNO in asthma is most likely due to increased expression and activity of the cytokine-inducible NO synthase (iNOS) in asthmatic airways (7,8). Increased iNOS protein was detected by immunohistochemistry in bronchial biopsies from 22 of 23 asthmatic patients, versus only two of 20 nonasthmatic subjects (7). Moreover, eNO levels were reduced in nine asthmatic patients following administration of aminoguanidine – a relatively selective inhibitor of iNOS – but remained unchanged in 11 nonasthmatic subjects (9). Elevated eNO levels in asthma may also be derived from neuronal NOS in airway nerves (10), possibly in response to decreased airway sensitivity to the bronchodilator effects of endogenous NO (11). Alternatively, the chemical environment in the airways of asthmatic patients (eg, increased oxidant stress and acidity) may increase NO generation through enhanced metabolism of endogenous NO-containing biomolecules, such as S-nitrosothiols (12).

Use of eNO in the diagnosis of asthma

Based on the consistency of the association between asthma and increased eNO level, a diagnosis of asthma may be facilitated in patients who have a suggestive clinical presentation and an increased eNO level. For example, a threshold eNO concentration of 13 parts per billion (ppb) was 85% sensitive and 80% specific for a diagnosis of asthma in 240 steroid-naïve patients referred for respiratory complaints suggestive of obstructive lung disease (13). Furthermore, an eNO concentration of 16 ppb or greater had 90% specificity and greater than 90% positive predictive value for a diagnosis of asthma. Similarly, a resting eNO level of 12 ppb or greater was highly sensitive for predicting exercise-induced bronchoconstriction in 50 subjects with no history of asthma (14). Low eNO levels also had a negative predictive value of 93% for asthma in 38 patients presenting with chronic cough (15). The high sensitivity and specificity of elevated eNO levels are likely to be significantly lower in the presence of corticosteroid therapy (see below).

Measurement of eNO levels may be as reliable as bronchoprovocation testing for the diagnosis of asthma. Of 85 patients with nonspecific respiratory symptoms and normal lung function followed for two years, 40 were subsequently diagnosed with asthma on clinical and/or physiological criteria (16). A baseline eNO level higher than 7 ppb had 82.5% sensitivity and 88.9% specificity for subsequent asthma diagnosis, yielding an area under the receiver-operating characteristic curve of 0.896, which was similar to methacholine and AMP bronchoprovocation. In summary, although increased eNO may support the diagnosis of asthma in patients with a suggestive clinical presentation, there is currently no consensus on the level of eNO that reliably excludes asthma.

EFFECTS OF AIRWAY INFLAMMATION ON eNO

Asthma is an inflammatory disease, characterized by infiltration of inflammatory cells, especially eosinophils, resulting in airway mucosal edema and epithelial injury. Several studies have assessed the relationship between eNO in asthma and airway inflammation (Table 1). Increased eNO in asthma appears to correlate with increased eosinophils in sputum or peripheral blood (17-20). For example, sputum eosinophil counts and eNO levels were modestly correlated ($r=0.48$; $P=0.003$) in 35 stable, atopic asthmatic patients (17). Similarly, there was a weak correlation ($r=0.31$; $P=0.03$)

TABLE 1
Summary of relationships between exhaled nitric oxide (eNO) levels and inflammatory versus physiological biomarkers in asthma

Study	Biomarker	Correlation with fractional concentration of eNO		Comments
		Strength of rho	P	
Inflammatory markers				
Jatakanon et al, 1998 (17)	Sputum eosinophils	0.48	0.003	Steroid-naïve asthmatic patients
Berlyne et al, 2000 (19)	Sputum eosinophils	0.47	<0.05	Atopic asthmatic patients; effect in steroid-naïve patients only
Deykin et al, 2000 (22)	Sputum eosinophils	No correlation	–	Atopic asthmatic patients; following acute inhalation of LTE4
Lim et al, 1999 (40)	Sputum eosinophils	0.64	<0.05	ICS-treated, atopic asthmatic patients
Jones et al, 2002 (20)	Sputum eosinophils	0.40	0.002	After ICS withdrawal and reinstatement
Crater et al, 1999 (18)	Serum immunoglobulin E	No correlation	–	Acute asthma exacerbation; correlation independent of ICS
Crater et al, 1999 (18)	Blood eosinophils	0.31	0.03	Acute asthma exacerbation; correlation independent of ICS
Lim et al, 2000 (21)	Bronchial biopsy eosinophils	0.14	Not significant	Correlation independent of ICS
Physiological markers				
al-Ali et al, 1998 (24)	FEV ₁	No correlation	Not significant	Steroid-naïve, atopic asthmatic patients
de Gouw et al, 1998 (26)	FEV ₁	Significant correlation	≤0.04	Steroid-naïve, atopic asthmatic patients following histamine, hypertonic saline, AMP bronchoprovocation
Crater et al, 1999 (18)	FEV ₁	–0.29	0.04	Acute asthma exacerbation; correlation independent of previous ICS
Kharitonov et al, 1995 (23)	Late allergic FEV ₁ decline	0.75	<0.01	Steroid-naïve, atopic asthmatic patients
Deykin et al, 1998 (25)	Late allergic FEV ₁ decline	0.80	<0.01	Steroid-naïve, atopic asthmatic patients
al-Ali et al, 1998 (24)	PEFR variability	0.58	0.002	Steroid-naïve, atopic asthmatic patients
Lim et al, 1999 (40)	PEFR variability	0.65	<0.05	Atopic asthmatic patients; effect in steroid-naïve patients only
Sandrini et al, 2003 (44)	PEFR variability	0.46	0.04	Steroid-naïve asthmatic patients; after two weeks of therapy with montelukast
Prieto et al, 2002 (29)	PC ₂₀ AMP	–0.57	0.02	Atopic asthmatic patients during pollen season
	PC ₂₀ methacholine	–0.35	0.14	
al-Ali et al, 1998 (24)	PC ₂₀ histamine	–0.51	0.008	Steroid-naïve, atopic asthmatic patients
Dupont et al, 1998 (30)	PC ₂₀ histamine	–0.65	<0.0001	Steroid-naïve, atopic asthmatic patients; effect lost in ICS-treated patients
Salome et al, 1999 (27)	Dose-response ratio to histamine	0.33	<0.05	Asthmatic and nonasthmatic subjects with airway hyper-responsiveness
Jatakanon et al, 1998 (17)	PC ₂₀ methacholine	–0.64	<0.001	Steroid-naïve asthmatic patients
Lúdvíksdóttir et al, 1999 (28)	PC ₂₀ methacholine	–0.52	0.02	Atopic asthmatic patients
Lim et al, 1999 (40)	PC ₂₀ methacholine	–0.64	<0.05	Steroid-naïve asthmatic patients

FEV₁ Forced expiratory volume in 1 s; ICS Inhaled corticosteroid; LTE4 Leukotriene E4; PC₂₀ Provocative concentration causing a 20% fall in FEV₁; PEFR Peak expiratory flow rate

between eNO and peripheral blood eosinophils in 51 asthmatic patients with acute exacerbations, regardless of previous oral or ICS therapy (18). In contrast, there was no significant correlation between eNO levels and bronchial biopsy eosinophil counts in 32 asthmatic patients, independent of ICS use (21). Similarly, there was no change in eNO levels following acute inhalation of leukotriene E4, despite a significant increase in sputum eosinophils in seven of 16 atopic asthmatic patients (22).

Treatment with ICSs complicates the relationship between sputum eosinophilia and eNO in asthma. For example, in 73 mild to moderate, predominantly atopic asthmatic patients, sputum eosinophils and eNO were significantly correlated

($r=0.47$; $P<0.05$) in steroid-naïve subjects, but not in steroid-treated subjects, in whom eNO levels were significantly lower despite persistent eosinophilia (19). In contrast, following withdrawal of ICSs in 65 asthmatic patients, reinstatement of ICS dose-dependently reduced eNO level, which was significantly correlated after one and eight weeks with reduced sputum eosinophils (20).

Thus, eNO may correlate with some aspects of airway inflammation in asthma, such as eosinophil levels in peripheral blood and sputum. However, the relationship is a complex one, because the time courses of changes in eosinophil counts and eNO levels may differ during responses to acute allergen exposure, and in response to anti-inflammatory ICS therapy.

EFFECTS OF BRONCHOCONSTRICTION ON eNO

Asthma also features a variable degree of airflow obstruction that is due in part to bronchoconstriction. Decreases in forced expiratory volume in 1 s (FEV_1) and the ratio of FEV_1 /forced vital capacity are the most reliable measures of airflow obstruction, but spirometry can be normal in many asthmatic patients. The variability of airflow obstruction can also be assessed through serial monitoring of peak expiratory flow rates (PEFRs). Several studies have addressed the relationships between eNO and parameters of airflow obstruction, including FEV_1 and PEFR variability.

Overall, the relationship between bronchoconstriction and eNO level remains poorly defined (Table 1). For example, a weak inverse correlation was found between FEV_1 and eNO levels ($r=-0.29$; $P=0.04$) in 52 atopic asthmatic patients during an exacerbation (18). Additionally, allergen exposure was associated with an increase in eNO, concomitant with the late allergic response in 25 atopic asthmatic subjects (23). In contrast, FEV_1 and eNO were not correlated in 26 steroid-naïve, atopic asthmatic patients, but eNO levels did correlate significantly with diurnal PEFR variability ($r=0.58$; $P=0.002$) (24).

Several studies have also assessed the effects of induced bronchoconstriction on eNO levels. In 10 steroid-naïve, atopic subjects with thermally-induced asthma, increased eNO levels were recorded during bronchoconstriction induced by either isocapnic, cold-air hyperventilation or specific allergen inhalation (25). Additionally, prechallenge eNO levels were highly correlated with the magnitude of the decrease in FEV_1 during the late-phase allergic response. In contrast to increased eNO in asthmatics following allergen and cold air challenge, inhalation of histamine, hypertonic saline or adenosine was associated with a significant reduction in eNO level in 11 steroid-naïve, atopic asthmatic patients (26).

The conflicting data hinder the use of eNO levels as a marker of bronchoconstriction. As well, the divergent response of eNO levels to various spasmogens suggests differences in the biology of airway NO related to different mechanisms of bronchoconstriction, and possibly different sources of eNO. Moreover, to compare eNO data in different studies, eNO measurements may need to be standardized for airway calibre.

RELATIONSHIP BETWEEN AIRWAY HYPER-RESPONSIVENESS AND eNO

Asthma is characterized pathophysiologically by bronchial hyper-responsiveness or by exaggerated airway smooth muscle contraction and resulting bronchoconstriction in response to nonspecific challenges (eg, exercise) and chemical agents (eg, methacholine).

Several studies have suggested that baseline eNO levels correlate with bronchial responsiveness. For example, there was an inverse correlation between baseline eNO levels and the provocative concentration of histamine causing a 20% fall in FEV_1 (PC_{20} histamine) in 26 steroid-naïve, atopic asthmatic patients ($r=-0.51$; $P=0.008$) (24). Although a weak correlation was also found between eNO levels and histamine responsiveness ($r=0.39$; $P<0.001$) in another study of 306 adults, this result was strongly influenced by the inclusion of 182 normal subjects and only 27 asthmatic patients (27). A significant relationship between eNO levels and methacholine responsiveness was also found in 40 atopic asthmatic patients

(28). There are few data on the relationship between eNO levels and responsiveness to different bronchoconstrictor stimuli, but this is an area of mechanistic interest. For example, in a study of 19 atopic asthmatic patients, eNO levels correlated with PC_{20} AMP ($r=-0.57$; $P=0.02$), but not PC_{20} methacholine ($r=-0.35$; $P=0.14$) (29).

A single study has assessed the effects of corticosteroid treatment on the relationship between eNO and airway hyper-responsiveness (30). A strong correlation between baseline eNO levels and histamine responsiveness in steroid-naïve patients with mild asthma ($r=-0.65$; $P<0.0001$) was subsequently lost following a reduction in bronchial hyper-responsiveness in a steroid-treated subgroup.

Thus, eNO levels appear to correlate significantly with chemical bronchial hyper-responsiveness in some patients with asthma, eg, atopic asthmatics. However, changes in bronchial responsiveness over time, either spontaneous or related to therapy, may not be paralleled by changes in eNO levels. For example, ongoing high-level antigen exposure in nonsensitized asthmatic subjects was associated with more severe bronchial hyper-responsiveness, but not with higher eNO levels (31). The presence and clinical importance of potential differences in the time course of changes in eNO versus airway hyper-responsiveness have not been well studied.

EFFECTS OF ATOPY ON eNO

Atopy, a genetic predisposition to developing immunoglobulin E-mediated responses to common environmental aeroallergens, is the strongest identifiable predisposing factor for developing asthma. In the studies reviewed, atopy was usually defined as the presence of skin-prick positivity (3 mm or larger) to one or more aeroallergens. Atopy in children, in the absence of asthma, is strongly associated with higher eNO levels. However, the relationship between atopy and eNO in adults is weak (19,27,32,33). For example, eNO levels were higher ($P<0.01$) in atopic versus nonatopic young adults without bronchial hyper-responsiveness or asthma (27). Conversely, the presence of atopy, in the absence of clinical or physiological features of asthma, was not associated with elevated eNO levels (19,32). Thus, atopy alone, in the absence of asthma, is not consistently associated with increased eNO levels in adults.

The presence of atopy in asthmatic patients is associated with higher eNO levels (28,34). For example, in 40 asthmatic subjects, eNO levels were higher in the atopic versus nonatopic adult subjects ($P=0.03$) (28). Similarly, eNO levels were higher in 68 atopic versus 19 nonatopic steroid-naïve asthmatic patients ($P<0.001$) (34). In contrast, no relationship was found between total serum immunoglobulin E levels and eNO in 113 subjects, although only 60 subjects had asthma (18).

Ongoing allergen exposure in atopic asthmatics is significantly associated with higher eNO. For example, higher eNO concentrations were found in 26 atopic asthmatic patients with ongoing allergen exposure than in 12 unexposed atopic asthmatic patients (35). Moreover, eNO levels increased significantly during pollen season in 27 sensitized, atopic asthmatic patients (27.2 ppb to 66.1 ppb; $P<0.001$) (36). Interestingly, exposure to an allergen in the absence of specific sensitization does not lead to increased eNO levels. In 43 asthmatic patients sensitized to cat or dog antigen, but not dust mite antigen, ongoing exposure to high levels of dust mite

antigen was associated with more severe bronchial hyper-responsiveness, but not with higher eNO (31).

Thus, although atopy itself does not result in increased eNO levels, iatrogenic or ongoing natural exposure to an allergen in sensitized, atopic asthmatic subjects is associated with higher eNO levels. Differences among allergens remain largely unexplained, but may relate to differences in allergen size, mechanisms of sensitization, or possibly sampling bias.

EFFECTS OF ASTHMA THERAPY ON eNO

There are two aspects to the pharmacological management of asthma: the use of 'controller', anti-inflammatory agents (eg, corticosteroids, leukotriene receptor antagonists [LTRAs]) to reduce airway inflammation and improve control of asthma; and the use of 'reliever', bronchodilator medications (eg, beta₂-agonists) to both prevent and mitigate acute symptoms of asthma. Clinical practice guidelines are available, which provide a stepwise approach to the use of pharmacological therapy based on disease severity (1). Many studies have explored the effects of treatment of asthma with various pharmacological agents on eNO (see below).

Effects of bronchodilators on eNO

A few studies have assessed the acute effects of bronchodilator agents, mainly beta₂-agonists, on eNO levels. For example, inhalation of nebulized salbutamol in 27 subjects significantly improved FEV₁, but had no effect on eNO levels, independent of maintenance ICS therapy (37). Acute inhalation of salmeterol, a long-acting beta₂-agonist, also had no effect on eNO levels in eight of these subjects. Similarly, long-term salmeterol treatment in 14 atopic asthmatic patients had no effect on eNO levels (36). Moreover, salmeterol treatment did not attenuate the significant increase in eNO levels following natural exposure to allergens to which subjects were sensitized. Thus, beta₂-agonist bronchodilators have no apparent effect on eNO levels in asthma.

Effects of corticosteroids on eNO

Inhaled and oral corticosteroids improve symptoms, lung function, airway inflammation, PEFr variability and bronchial responsiveness, as well as reduce the frequency of exacerbations; thus, they are central to chronic asthma therapy. There is extensive evidence that steroid therapy also reduces eNO levels in asthma. For example, many studies have reported lower eNO levels in steroid-treated versus steroid-naïve asthmatics (4,21,30). Moreover, in patients with chronic, stable asthma or acute exacerbations of asthma, initiation of therapy with either oral steroids or ICSs is associated with a significant decline in eNO (6,9,38-41). For example, in a placebo-controlled, double-blinded, crossover study of 11 patients with mild asthma, high-dose inhaled budesonide (1600 µg daily) significantly reduced eNO (6).

The relationship between corticosteroid therapy and reduced eNO in asthma is dose-dependent and reversible. Indeed, following an initial decrease in eNO levels after four weeks of high-dose inhaled fluticasone (1000 µg daily) in 25 atopic asthmatic patients, there was a rebound increase in eNO to pre-fluticasone levels within two weeks after corticosteroid withdrawal (41). Moreover, in 28 steroid-naïve asthmatic patients, the reduction in eNO levels was greater, but the return to baseline eNO levels was more delayed during a one-week washout period following therapy with budesonide 400 µg daily versus 100 µg daily (42).

Thus, anti-inflammatory therapy with either ICSs or systemic corticosteroids is consistently associated with reduced eNO levels in patients with both chronic, stable asthma and acute exacerbations of asthma. In many (but not all) ICS-treated asthmatic patients, eNO levels are in the normal range.

Effects of other asthma therapies on eNO

In some patients with asthma, LTRAs are an important therapeutic option for improved long-term asthma control. A few studies have assessed eNO levels in asthmatic patients treated with LTRAs. For example, in a placebo-controlled, randomized trial of 79 asthmatic patients with poorly controlled asthma despite the use of high doses of ICSs, six weeks of therapy with pranlukast was associated with lower eNO levels and better asthma control (see below) (43). Similarly, in a placebo-controlled, randomized trial of 20 steroid-naïve asthmatic patients, montelukast 10 mg daily reduced eNO (median decrease 1.5 ppb; P=0.005) (44). Thus, LTRA therapy is associated with reduced eNO levels in asthmatics, which appears to be independent of corticosteroid therapy.

Theophylline has long been used in the treatment of asthma, with evidence for both bronchodilator and anti-inflammatory effects. In a double-blind, placebo-controlled trial of theophylline (250 mg twice daily) in 15 mild to moderate, atopic asthmatic patients, there were no changes in eNO despite significant reductions in eosinophils in sputum, bronchoalveolar lavage and bronchial biopsies (45).

RELATIONSHIP BETWEEN ASTHMA CONTROL AND eNO

The natural history of asthma is characterized by episodic loss of control, as manifested by increased symptoms, worsened lung function, increased need for rescue beta₂-agonist use and increased use of health care resources. Currently, there is great clinical and research interest in being able to predict loss of control or detect it before symptoms and lung function worsen. Indeed, asthma is a more severe disease in some individuals, because poor control is associated with chronic symptoms, fixed airflow obstruction, possible airway remodelling, the need for chronic (often high) doses of inhaled and/or systemic corticosteroids, and a greater risk of death.

Repeated measurement of eNO levels has been proposed as a tool to monitor asthma control. However, the relationship between eNO and other markers of asthma control remains unclear. In a cross-sectional study of 100 asthmatic patients treated with inhaled bronchodilators and/or steroids, eNO levels were significantly higher in subjects with evidence of poor control over a two-week period, including frequent symptoms, daily rescue beta₂-agonist use, and a positive bronchodilator response (46). Although eNO levels reflected poor control in the short term, there was a poor relationship between increased eNO levels and chronic severity of asthma. Specifically, there was no correlation of higher eNO levels with fixed airflow obstruction (lower FEV₁ and lower ratio of FEV₁/forced vital capacity), with episodes of respiratory failure or hospital visits, or with a validated asthma severity index.

Monitoring of eNO during changes in therapy

In several studies, changes in eNO following institution or withdrawal of anti-inflammatory therapy have been compared with changes in asthma control. For example, tapering of inhaled budesonide in 14 asthmatic patients led to increases

in eNO and more frequent nocturnal symptoms, but no significant change in daytime symptoms, rescue beta₂-agonist use, FEV₁ or diurnal PEFr variability (47). A subsequent increase in inhaled steroid dose significantly reduced eNO and restored asthma control, as reflected by improved nocturnal symptoms. The decline in eNO levels following institution of steroid therapy may also be associated with improvement in FEV₁ (9) and a reduction in methacholine bronchial responsiveness (39,47). Another study assessed whether changes in eNO levels could predict subsequent loss of asthma control (48). Six weeks after withdrawal of ICS use in 78 patients with mild to moderate asthma, an increase in eNO of more than 60% from baseline, or absolute increase in eNO of 10 ppb or higher, had positive predictive values of 80% to 90% for subsequent loss of asthma control, defined as a 10% fall in PEFr and increased symptoms, nocturnal awakenings, or rescue beta₂-agonist use (48).

Reductions in eNO and PEFr variability were also significantly correlated ($r=0.46$; $P=0.04$) during the second week of montelukast treatment in the study described above (44). In addition, lower eNO levels after six weeks of pranlukast, in 79 asthmatic patients with poorly controlled asthma despite ICS use, were associated with better asthma control, as evidenced by reduced daytime and nocturnal symptoms, less use of rescue beta₂-agonists, improved FEV₁ and higher PEFr (43). Similarly, in 22 patients with mild to moderate asthma, the addition of montelukast (10 mg daily) to either high-dose inhaled fluticasone or fluticasone-salmeterol combination therapy was associated with significant declines in eNO, blood eosinophils and AMP bronchial responsiveness (49).

A single randomized clinical trial has reported the use of eNO measurements to guide reductions in ICS dose in 97 patients with chronic asthma requiring ICS for at least six months (50). During an initial dose optimization phase, ICS dose was reduced if eNO remained less than 15 ppb. During the subsequent 12-month follow-up period, subjects in the eNO group received lower daily ICS doses than subjects in whom ICS doses were adjusted according to conventional guidelines. However, both groups demonstrated similar asthma control, with no differences in exacerbation rate, use of systemic steroids, lung function or sputum eosinophilia. The measurement of eNO levels may thus permit less intense chronic ICS therapy in asthma without a greater risk for loss of control.

Changes in eNO levels in response to therapy may correlate with changes in other clinical, physiological or biological markers of asthma control. The use of sputum eosinophils as a more direct measure of airway inflammation has been promoted in the monitoring of asthma control. The relative clinical utility of sputum analysis versus eNO in monitoring adults with asthma remains poorly defined, but is an area of active investigation.

SUMMARY AND RECOMMENDATIONS

Asthma is consistently associated with increased eNO levels in the absence of anti-inflammatory therapy. Changes in eNO levels in asthma appear to correlate with measures of airway inflammation, but not with the degree of bronchoconstriction or airflow obstruction. Moreover, eNO levels parallel changes in some measures of asthma control in response to modification of anti-inflammatory therapy. These data suggest a potential clinical utility of eNO measurement as a marker of asthma

control. Indeed, changes in eNO levels may be an early marker of loss of control or impending asthma exacerbation.

Clinical approach to using eNO in monitoring of asthma control

Serial measurement of eNO levels is being increasingly used in routine clinical assessment of children with asthma, based on the ease of collection of exhaled gas samples and the increasing availability of sensitive NO analyzers. Routine eNO measurement is also increasingly being promoted and offered as a clinical service in the routine management of asthma in adults. However, the clinical validity of eNO monitoring in adults with asthma is not well established or widely accepted, in large part because of the significant phenotypic heterogeneity of asthma in adults, especially in chronic or severe disease.

The use of eNO in the diagnosis of asthma has been validated, because high eNO levels, and specifically corticosteroid responsiveness, are highly predictive (greater than 90%) of a diagnosis of asthma. However, there is poor consistency among studies on the threshold or cut-off levels, because commonly suggested upper limits of normal eNO levels vary from 25 ppb to 35 ppb, and diagnostically high levels range from 30 ppb to 50 ppb. Moreover, there is no consensus on whether high eNO in asymptomatic individuals should be treated. In contrast, low eNO levels in symptomatic patients (lower than 25 ppb to 30 ppb), in the absence of corticosteroid therapy, effectively rule out significant eosinophilic inflammation and the need for corticosteroid therapy. In this situation, an alternative diagnosis should be considered, including other airway disorders associated with neutrophilic inflammation, such as neutrophilic bronchitis. In the significant grey zone of 25 ppb to 50 ppb, the suggested diagnostic approach is consideration of both eNO and the usual clinicophysiological features of asthma.

In adults with documented asthma, the regular monitoring of eNO may be superior to the current clinicophysiological approach in the assessment of airway inflammation and asthma control. This approach may permit more appropriate and timely adjustment of therapy. Specifically, low eNO levels in a patient treated with ICSs provide evidence for good compliance with therapy, and suggest good control of asthma and airway inflammation; thus, low eNO levels may permit tapering of anti-inflammatory, corticosteroid therapy without risk of exacerbation. The corollary is that routine eNO monitoring, perhaps even daily, may also enhance early detection of asthma exacerbations and the need for more intense or additional therapy. Threshold levels for reduction or intensification of therapy are not well established, but are similar to diagnostic thresholds above. Moreover, rather than absolute levels being uniformly applied to all adults with asthma, individual eNO levels for adjustment of therapy may need to be defined for each patient.

Significant limitations of the studies on the use of eNO in the diagnosis and management of asthma in adults include small numbers of subjects, short-term follow-up and limited recognition of the heterogeneity of asthma in adults. Thus, the use of eNO levels in the routine assessment or follow-up of asthma in adults cannot presently be recommended. Wider acceptance and clinical use of eNO measurements in adults with asthma await more large scale studies to establish significant correlations between eNO levels and important long-term outcomes, such as optimal asthma therapy, including decreased ICS doses and more appropriate use of combination therapy, as well as reduced exacerbations and mortality.

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