REVIEW ARTICLE

Assessment of airway inflammation with exhaled NO measurement

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Abstract

Assessing airway inflammation is important for investigating the underlying mechanisms of many lung diseases, including asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, primary ciliary dyskinesia (PCD) and cystic fibrosis. A growing interest has recently directed toward non-invasive methods for the assessment of airway inflammation. Measurement of exhaled nitric oxide in exhaled air is an exciting innovative technique that gives new insights into the pathophysiology of lung disease and asthma in particular, with many potential clinical applications. Careful standardisation of measurement techniques has facilitated the use of this new measurement in paediatric respiratory medicine. Non-invasiveness and instantaneous results potentially make it a suitable instrument for use in children starting from the age of 4, with useful applications both in asthma diagnosis and monitoring. *Hippokratia 2007, 11, (2): 51-62*

Key words: *asthma, exhaled nitric oxide, airway inflammation, non-invasive monitoring, primary ciliary dyskinesia, cystic fibrosis*

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Airway inflammation is centrally important in asthma and other lung diseases. Currently, asthma is viewed as a chronic inflammatory airway disease, and international guidelines place great importance on treating inflammation in this condition[1]. Methods used in everyday clinical practice are not suitable for the assessment of airway inflammation. Symptoms and their perceptions vary widely between individuals and do not accurately reflect the extent of airway inflammation. Lung function measurements are used to monitor disease activity; however, it has been recognized that changes in lung function tests are not closely related to the degree of inflammation, and intensive inflammatory processes may well precede changes in lung function[2, 3].

Conversely, direct assessment of airway inflammation using bronchoscopy cannot be used for monitoring due to its invasive nature. Sputum induction is another way of obtaining cells from the lower airways and it has been demonstrated that its use improves asthma control[4,5]. This sampling method, however, requires inhalation of a hypertonic salt solution, which may provoke bronchoconstriction in asthmatic patients. Furthermore, processing the sputum samples requires ~2 hours work by a well-trained person to obtain reliable readings, and, at the moment, these features limit its wide clinical use.

The need to monitor inflammation in the lungs has led to the exploration of exhaled gases and condensates. Noninvasive monitoring may assist in differential diagnosis of pulmonary diseases, assessment of disease severity and response to treatment. Because these techniques are completely noninvasive, they can be used repeatedly to give information about kinetics, they can be used in patients with severe disease, which has been previously difficult to monitor, and they can be used to monitor disease in children, including infants. This article reviews whether exhaled nitric oxide (eNO) measurement is suitable for routine use in clinical practice.

Markers of airway inflammation in asthma

Invasive markers

In asthma, fiberoptic bronchial biopsies have become the "gold standard" for measuring inflammation in the airway wall, but this is an invasive procedure that is not suitable for routine clinical practice and cannot be repeated often. It is also unsuitable for use in children and patients with severe disease.

Noninvasive markers

A number of markers have been and are being considered as noninvasive markers of airway inflammation. Examples include blood eosinophil counts or serum eosinophil cationic protein (ECP), urinary eicosanoid metabolites, exhaled gasses, mediators in breath condensate or induced sputum (Table 1)[6]. Along these lines,

Table 1. Biomarkers in asthma.

Biomarkers in asthma
Blood/serum
Eosinophils; Eosinophil cationic protein (ECP); EPO; sIL-2R
Urine
9a, 11b-PGF2; LTE4
Exhaled air
Nitric oxide; Carbon monoxide; Hydrocarbons
Exhaled Breath Condensate (EBC)
Hydrogen peroxide; LT metabolites; 8-isoprostane; Nitro tyrosine
Induced sputum
Cell differential; Soluble mediators (ECP, cysLT's)

Definition of abbreviations: EPO: eosinophil peroxidase; sIL-2R: soluble interleukin-2; receptor; LT: leukotriene; cysLT's: cysteinyl leukotriene. (Revised from Reference 6).

the least invasive technique is the collection of exhaled breath samples. Some of them, including nitric oxide (NO) and carbon monoxide (CO), can be measured in the gas phase, while others, such as hydrogen peroxide, adenosine, interleukins and pH, can be determined in the exhaled breath condensate (EBC).

Blood markers

Measurement of blood eosinophil counts and serum ECP levels if correctly performed, are reproducible and consistent [7]. However, blood eosinophil counts have been shown to correlate weakly to eosinophil numbers in biopsies [8] and have poor disease specificity. The correlation of serum ECP with the number of eosinophils in biopsies is variable: although in some studies, a correlation has been reported, this has not been invariably confirmed [8,9]. Serum ECP also lacks disease specificity. Increased levels of ECP can be found in various diseases including cystic fibrosis, whereas conversely, a large degree of overlap exists between normal and asthmatic individuals with varying severity[10,11]. Both eosinophil counts and ECP levels respond to factors known to influence the degree of airway inflammation such as changes in treatment or allergen exposure[12, 13].

Urinary markers

Urinary eosinophil peroxidase (EPX) offers an even less invasive alternative to serum ECP[12,14], especially for children. Another line of investigation is to measure eicosanoid metabolites in urine such as leukotriene (LT) E4 or 9a, 11bPGF2 [15]. These measurements are reliable but require skilled expertise. How precisely they reflect ongoing inflammation in the airways needs to be further evaluated as increased urinary LTE4 levels are not limited to asthma and they do not discriminate between asthma and normal subjects[16].

Exhaled breath condensate (EBC)

Breath analysis is currently a research procedure, but there is increasing evidence that it may have an important place in the diagnosis and management of lung diseases in the future[17]. Exhaled breath condensate is collected by cooling or freezing exhaled air. As the procedure is totally noninvasive and does not influence airway caliber, a major advantage of this technique is that it is extremely well tolerated even by patients with severe airway obstruction and children.

The most common approach is for the subject to breathe via a mouthpiece through a non-rebreathing valve block in which inspiratory and expiratory air is separated. During expiration, the breathing air flows through a condenser, which is cooled to –20 ^{o}C. Exhaled condensate is usually analyzed by gas chromatography and/or extraction specrophotometry, or by immunoassays. The obtained fluid, however, is a complex diluted solution of diverse biomarkers with various chemical stabilities, including a variety of constituents [18]. Due to the complexity of EBC and the fact that it is a much-diluted sample, there are still several unsolved issues surrounding this sampling method. Proposed biomarkers in EBC include adenosine, ammonia, hydrogen peroxide, ions, isoprostanes, prostaglandins, leukotrienes, nitrogen oxides, pH and cytokines. Concentrations of these mediators are influenced by airway diseases, including asthma, Chronic Obstructive respiratory Disease (COPD), Cystic Fibrosis (CF) and bronchiectasis, and are modulated by therapeutic intervention[19]. Differences in condensate chemistry are thought to reflect changes in the airway lining fluid caused by inflammation and oxidative stress. Condensate from asthmatic subjects contains increased levels of leukotriene B4/C4/D4/E4 in addition to several markers of oxidative stress including hydrogen peroxide, nitrotyrosine and 8-isoprostane[20]. Measurement of cytokines has proven less successful to date.

Although the analysis of these various molecules in breath condensate remains to be fully validated, it would seem that they could provide useful information in the disease monitoring. Significant differences in hydrogen peroxide levels were observed between controlled and non-controlled asthma[21], whereas others have shown that 8-isoprostane levels are less sensitive to steroid treatment than eNO or exhaled ethane[22].

Induced sputum

It has been shown that in subjects with obstructive airway disorders, an increased sputum eosinophil percentage has a higher sensitivity and specificity for the diagnosis of asthma than blood eosinophil counts or serum ECP[23]. The degree of sputum eosinophilia was shown to correlate with the clinical severity of the disease, in some studies [24]. In addition, preliminary reports indicate that analysis of induced sputum could help in diagnosing associated conditions such as gastro-esophageal reflux by identifying lipid-laden macrophages [25] or associated left heart failure by screening for haemosiderine-laden macrophages recognized by Prussian-blue staining[26]. The possible role of sputum in diagnosing eosinophilic bronchitis as a cause of nonproductive cough has also been highlighted[27].

An important characteristic of induced sputum is its responsiveness to interventions known to affect the degree of inflammation in asthma. As for eNO, allergen exposure increases the percent eosinophils and metachromatic cells in sputum. This was initially demonstrated following exposure to high doses of allergen given under laboratory conditions to elicit dual asthmatic reactions, which also caused an increase in circulating eosinophil counts[28].

Treatment can also influence sputum eosinophil percentages. Monotherapy with short-acting inhaled b2-agonists has been shown to increase eosinophil counts. However, this effect is not observed when b2-agonists, either short- or long- acting, are given in combination with inhaled steroids[20,29]. Anti-inflammatory asthma treatment decreases sputum eosinophil numbers. This has been illustrated for theophylline[30,31], antileukotrienes[32], but especially for steroids[33-35].

Exhaled air

Carbon monoxide

Other gases that have been measured in exhaled air include carbon monoxide (CO) and hydrocarbons such as ethane and pentane[36,37]. Both are considered to be representative of the level of oxidative stress. Comparison with normal subjects indicates that exhaled CO is increased in non-steroid but not in steroid-treated asthma[38,39].

It is unclear to what extents exhaled CO and NO differ in their steroid sensitivity. The observation that children with persistent asthma, despite treatment with steroids which reduces their NO levels, have significantly higher exhaled CO compared with those with infrequent episodic asthma has led to the proposal that exhaled CO is less steroid-sensitive than eNO [37].

Hydrocarbons

The volatile hydrocarbons ethane and pentane are among the numerous end products of lipid peroxidation of peroxidised polyunsaturated fatty acids that can be measured by gas chromatography from single breath samples. Increased levels have been measured in non-steroid treated asthma. Others have described elevated pentane levels during episodes of acute asthma that returned to normal once the acute asthma subsided[40]. When the hydrocarbon breath test is standardized for clinical use it will likely provide a noninvasive and extremely sensitive instrument for the assessment of oxidative stress status in adults as well as in children in the future.

Exhaled NO

In the last decade there has been an explosion of interest in the measurement of eNO and other volatile substances in exhaled air. Although understanding about the link between NO and airway inflammation remains incomplete, increasing evidence supports the contention that eNO may be considered as being a readout of certain aspects of airway inflammation, particularly in atopic asthma. eNO measurement has characteristics (instantaneous, noninvasive, repeatable, safe) that make it ideally suited for children. The need for developing practical, noninvasive markers to reflect asthmatic airway inflammation is universally recognized[41].

Source of NO in Exhaled Air

Nitric oxide synthases

NO is derived from L-arginine by the enzyme NO synthase (NOS), of which at least three distinct isoforms exist in the human body [42]. (Figure 1, panel A*)*. Two of these enzymes are constitutively expressed and are activated by small rises in intracellular calcium concentration. Neuronal NOS (nNOS, NOS1) is predominantly expressed in neurons and endothelial NOS (eNOS, NOS3) in endothelial cells. A third enzyme that is inducible (iNOS, NOS2), may have a much greater level of activity, is independent of calcium concentration and may be induced by inflammatory cytokines.

Cellular sources of exhaled nitric oxide

Airway epithelial cells may express all NOS isoforms and therefore contribute to NO in the lower respiratory tract[43-46]. In inflammatory diseases, such as asthma, the increase in exhaled NO may reflect, at least in part, induction of NOS2. In adult asthmatic patients there is evidence of increased expression of NOS2 in airway epithelial cells[47]. Pro-inflammatory cytokines, which may at times be involved in asthmatic inflammation, induce the expression of NOS2 in cultured human airway epithelial cells[44,48]. NOS2 may be expressed in other cell types, such as alveolar macrophages, eosinophils and other inflammatory cells[49]. Further evidence that the increase in exhaled NO is derived from increased NOS2 expression is the observation that corticosteroids inhibit induction of NOS2 in epithelial cells[48] and in bronchial biopsies of adult asthmatic patients[49], and that they also reduce exhaled NO concentrations in asthmatic patients[50]. (Figure 1, panel B). Epithelial cell and non-adrenergic,

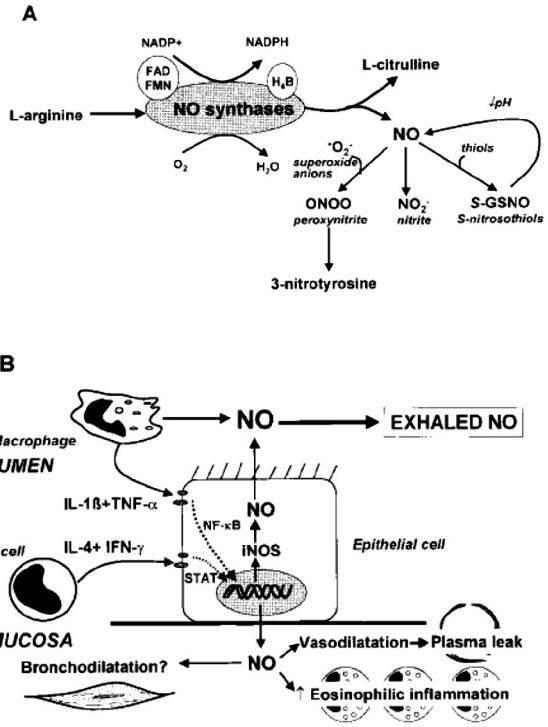

Figure 1. *Synthesis of nitric oxide (NO) and NO-related products (panel A). Sources of NO in exhaled air (panel B). (Revised from Reference 63).*

non-cholinergic NOS1 may also play an important role in determining both the asthmatic phenotype and the expired NO concentration.

Anatomical sites of nitric oxide formation

The levels of NO in the nose and nasopharynx are

much higher than those expired from the mouth, suggesting that upper airways are a major contributor to exhaled NO, at least in normal individuals[51]. However, the lower respiratory tract contributes substantially to exhaled NO. Direct sampling via fibreoptic bronchoscopy in asthmatic patients shows a similar elevation of NO in trachea and main bronchi to that recorded at the mouth, thus indicating that the elevated levels in asthma are derived from the lower airways [52,53].

Measurement of exhaled NO

Guidelines for standardized eNO measurement have been published jointly by ATS and ERS[54,55] and normal reference values with the recommended technique are available for children 4–17 years old[56]. Unfortunately, the application of eNO measurement to infants and young children is limited because the procedure is not well standardized for this age group.

Equipment for direct exhaled NO measurement

There are several commercially available analyzers for exhaled and nasal NO measurements: LR2000 analyzer (Logan Research Ltd, Rochester, UK), NIOX® NO analyzer (Aerocrine, Solna, Sweden), Sievers_ (Ionics Instrument, Boulder, CO, USA), and ECO Physics NO analyzer (ECOPHYSICS, Durnten, Switzerland).

The NO analyzer systems currently used in clinical investigations vary in complexity, but are based on a sensitive chemo-luminescence technique with the required accuracy. Most of the analyzers consist of a sampling system, a computerized NO analyzer with data processing, and user interface. The equipment measures the concentration of NO in sampled air online with high sensitivity in the parts per billion range (ppb), as well as pressure and flow of the sampled air. The software calculates the concentration of NO during a selected time period; displays measured and calculated data on the monitor and saves the information on disk.

The NIOX® NO analyzer has been approved for medical use in the European Union and was also cleared by the US Food and Drug administration for clinical application in patients with asthma in 2003[57].

Single-breath on-line measurement: eNO measurement in school - age children

The single breath online method (SBOL) is the gold standard for eNO measurement in cooperating children (more than 4–5 years old)[58]. Briefly, the child should be comfortably seated and breath quietly for about 5 min to acclimatize. Inspired gas should contain low NO (v5 parts per billion (ppb)). The child inhales to near total lung capacity (TLC) and immediately exhales at a constant flow of 50 mL/sec until an NO plateau of at least 2 seconds can be identified during an exhalation of at least 4 seconds. The expiratory pressure should be maintained between 5–20 cmH2O to close the velum. Repeated exhalations (three that agree within 10% or two within 5%) should be performed with o30-s intervals and mean NO should be recorded[55] (figure 2).

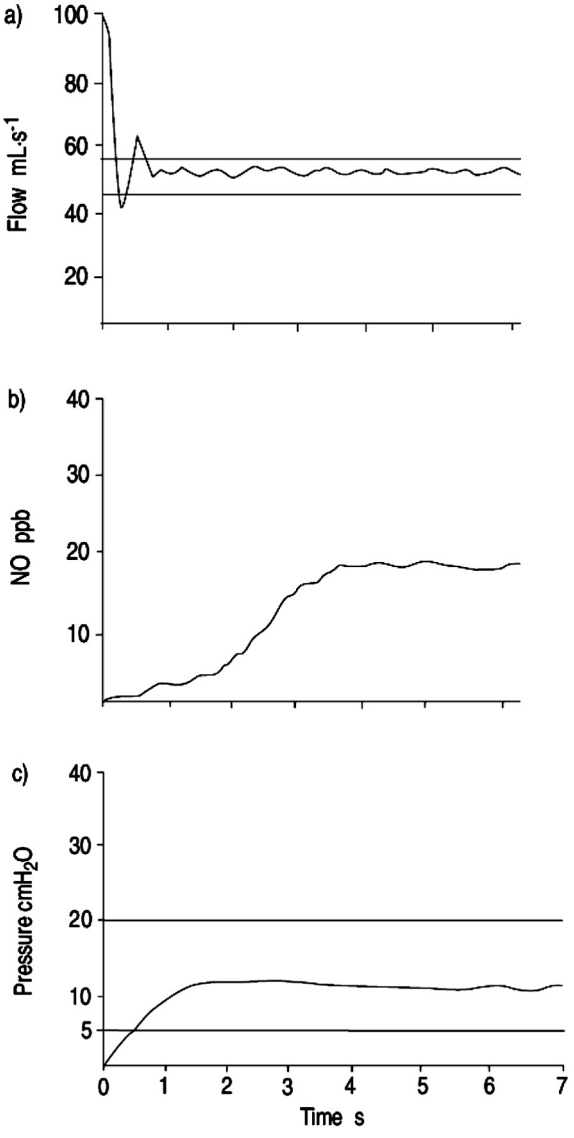

Figure 2. *a) Flow (airflow), b) fractional exhaled nitric oxide, and c) pressure (airway pressure) tracings for a 6-yr-old female using the single-breath on-line method at a flow rate of 50 mL/sec. The exhalation lasted 7sec, a good plateau can be identified and the expiratory pressure is maintained at 11 cmH2O during the manoeuvre. (NO: nitric oxide; ppb: parts per billion). (From Reference 55).*

However, the application of single-breath on-line (SBOL) measurements in preschool children has been reported to be difficult by several investigators [55]. There are several advantages in the SBOL eNO measurement that have made this the "gold standard" technique. These include the following. 1) Maintenance of constant flow. Exhaled NO is flow dependent, low flows result in higher levels and vice versa. The SBOL method keeps flow constant. 2) Exhalation from total lung capacity. SBOL exhalations are performed from (near-) TLC, the most

constant and easily found lung volume. This is important as the degree of lung expansion affects eNO with values measured at the same flow rate, from the functional residual capacity (FRC), being 20% lower than those from TLC. 3) Exclusion of nasal NO. Velum closure is simple and reliable with single-breath exhalations 4) A low constant flow is easily attained. Exhaled NO can also be measured off-line, collecting exhaled air in a reservoir, again with a recommended expiratory flow of 50 ml/sec, but the standardization of this procedure is less precise than for the online method.

eNO measurement in preschool children

On-line eNO measurement during spontaneous breathing has been applied in children aged 2–5 years old [59]. eNO is measured on-line during spontaneous breathing and the exhalation flow is manually adjusted to 50 ml/s by changing the exhalation resistance. The method is not well standardized, however, and still requires passive co-operation in as much as the child needs to breathe slowly and regularly through a mouthpiece.

eNO measurement in infants

There is limited experience with single-breath methods for measuring eNO in infants. eNO can be measured during tidal breathing using a facemask. Hall et al, [60] recently proposed a method for the on-line collection of tidal eNO that enables breath-to-breath monitoring in newborns and infants. A modification of the raised volume rapid thoracoabdominal compression technique (RVRTC) has also been used to measure eNO during a single, slow forced exhalation [61]. With both techniques, it is important to use a two-compartment facemask to avoid contamination by NO from the naso-pharynx. Unfortunately, the latter method requires sedation, specialized equipment and skilled operators.

How to interpret eNO concentrations

In non-smokers, exhaled NO concentration (eNO) between 5–20 ppb can be considered normal. Exhaled NO is significantly higher in asthmatic (24.3±14.8 ppb) than in healthy children (9.9 ±3.4 ppb) [62]. Values >20 ppb are considered as elevated. Elevated eNO is seen in asthma, but other conditions, including viral infection of the airways, bronchiolitis obliterans syndrome and chronic obstructive pulmonary disease (COPD), may also be associated with moderately increased concentrations of exhaled NO [63]. In asthmatic patients, values can increase up to 3–5-fold when compared with normal values.

Markedly increased eNO values are indicative of eosinophilic airway inflammation and predict a good response to steroid treatment. Values <5 ppb are seen in cystic fibrosis (CF) and in patients with PCD.

Factors Affecting Exhaled NO Measurements

Exhaled and nasal NO in healthy subjects is independent of age, sex, and lung function [62]. There is no evidence for significant diurnal variation [64], and exhaled NO measurements are highly reproducible in normal subjects [65, 66].

There are several major factors, which may change NO levels in normal subjects (Table 2). Some routinely used tests can transiently reduce exhaled NO; for example, repeated spirometry [67,68], physical exercise [69], sputum induction [70]. Environmental factors such as NO ozone and chlorine dioxide are known to increase exhaled NO levels [71–73]. Habitual factors such as smoking [74,75], alcohol ingestion [76,77] reduce exhaled NO. Upper respiratory infection significantly increases exhaled NO [78,79] and nasal NO [80].

Table 2. ***Factors affecting exhaled and nasal NO measurements in healthy subjects.***

Increased NO	Decreased NO
Pharmacologic	
Papaverin; Sodium nitroprusside; L–arginine; ACE inhibitors (enalapril)	Oxymetazoline; NOS inhibitors
Physiologic and procedural	
Arginine ingestion, nitrate-enriched food	Repeated spirometry; Acute and transient after forced exhalation; Physical exercise; Menstrual cycle; Sputum induction; Body temperature reduction
Environmental, occupational	
Air pollution (NO, ozone)	Water vapour, CO2; nitrous oxide
Occupational hazards	
Fluoride, dust; Ozone, chlorine dioxide; Rubber latex; Formaldehyde (domestic) exposure; Electromagnetic field generated by cellular phone (nasal NO)	100% inspired O2; Moderate altitude
Habitual	
	Smoking; Alcohol ingestion
Infections	
URTI	

Definition of abbreviations: ACE: angiotensin-converting enzyme; URTI: upper respiratory tract infection. (Revised from Reference 63).

eNO and lung diseases

eNO and atopy

Exhaled NO is elevated in allergic/ atopic adults and children [81]. It is further increased as a result of allergen exposure such as during the late phase response to allergen challenge [82,83], during the grass pollen season [84], or during exposure to indoor allergens [85,86]. Children [81] with atopic asthma have higher levels of exhaled NO than do patients with non-atopic asthma, even without airway hyper responsiveness [87].

eNO and Asthma

Increased levels of exhaled NO have been widely documented in patients with asthma (Figure 3) [88,89]. The increased levels of exhaled NO in asthma have a predominant lower airway origin [90] and are most likely due to activation of NOS2 in airway epithelial and inflammatory cells [91].

Asthma Diagnosis

An elevation of exhaled NO is not specific for asthma, but an increased level may be useful in differentiating asthma from other causes of chronic cough [92]. The diagnostic value of exhaled NO measurements to differentiate between healthy subjects with or without respiratory symptoms and patients with confirmed

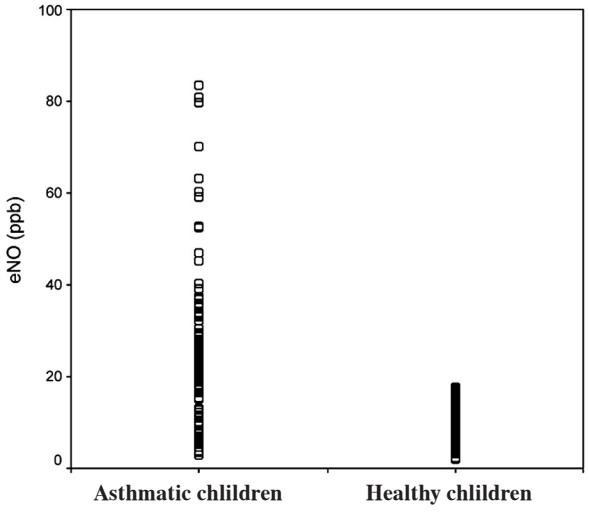

Figure 3. *Scatter gram of exhaled NO in normal and in asthmatic children. eNO was significantly higher in asthmatic (24.3±14.8 ppb) than in healthy children (9.9 ±3.4 ppb); p<0.0001. (From Reference 62).*

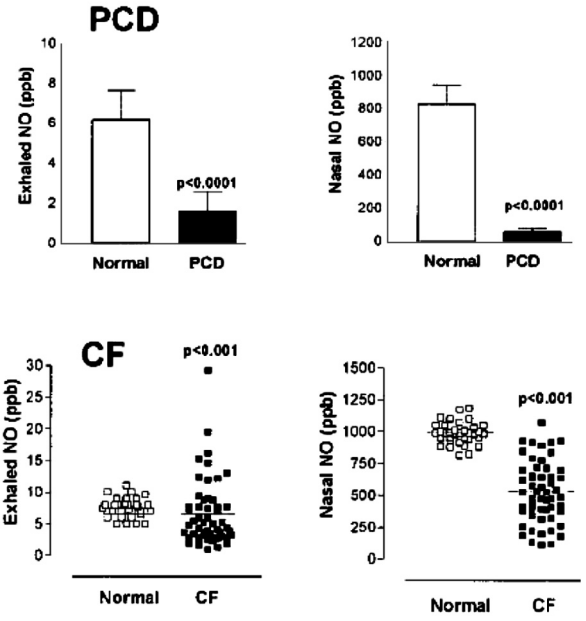

Figure 4. *Exhaled and nasal NO in primary ciliary dyskinesia (PCD) and cystic fibrosis (CF). (From Reference 130 and 135).*

asthma has been recently analyzed by Dupont and colleagues[93] with 90% specificity and 95% positive predictive value when exhaled NO 15 ppb is used as a cut-off for asthma.

Exhaled and nasal NO may be used to identify subjects with atopy, because non-atopic asthmatics have normal exhaled NO[94]. There is a strong association between elevated exhaled and nasal NO and skin prick test scores, total IgE[95], and blood eosinophilia[96] in mild asthma. Elevated nasal NO is also related to the size of skin test reactivity in asymptomatic asthmatic subjects[97]. This may denote "sub clinical" airway inflammation.

Recent studies have demonstrated that exhaled NO is superior to conventional tests, including baseline respiratory function and bronchodilator responsiveness, in identifying patients with probable asthma, both in children and adults[19].

Another potential use of exhaled NO levels in patient management is the prediction of future asthma. An elevated exhaled NO may be found in patients with "sub clinical" forms of asthma (normal lung function, negative bronchodilator tests, and elevated sputum eosinophilic cationic protein concentrations) [98,99]. Elevated levels of NO in patients with "sub clinical asthma" are not in conflict with the specificity of exhaled NO as a marker to diagnose asthma, as lack of current asthma symptoms does not exclude the diagnosis of asthma.

Differential diagnosis

In asthma, levels of eNO can be increased several-fold when compared with healthy subjects [100]; although there is an important overlap between different patients [63,101] (Figure 3). It is important to mention, however, that the typical high increase in eNO is mostly seen in atopic-asthmatic patients, and some studies have shown that normal levels of eNO can be detected in non-atopic asthmatic subjects[102].

The eNO measurement is also useful in the differential diagnosis of patients with dry cough[103]. Patients with chronic cough that is not attributable to asthma have lower NO values, as compared with healthy volunteers and patients with asthma[104], including those with cough due to gastro-oesophageal reflux[105].

Relationship to other markers of asthma

The traditional means of monitoring asthma have limitations. Lung function and PC 20, measurements are not directly related to airway inflammation, have little room for improvement in mild asthma (FEV 1), and are affected by bronchodilators. Both parameters are slow to change and are not able to distinguish the effect of different doses of steroids. There are several areas in which exhaled NO measurements may be advantageous over the traditional means of asthma monitoring: screening for atopy, monitoring the impact of hazardous environmental factors, identification and monitoring of asthma exacerbations, and assessment of the adequacy of anti-inflammatory treatment.

Exhaled NO in patients with asthma is correlated with sputum eosinophils[106-108] and methacholine reactivity[109,110].

Asthma monitoring

It is difficult to monitor the response of different classes of anti-inflammatory drugs in asthma, as there is no single test that can be used to quantify airway in-

flammation. Peripheral blood markers are unlikely to be adequate as the most important mediator and cellular responses occur locally within airways. Eosinophils in induced sputum originate from more proximal rather than small airway[17]. It is clear that different markers of airway inflammation should be considered together to monitor asthma[110].

Exhaled NO has been used to monitor the effect of anti-inflammatory treatment in asthma[111] and asthma exacerbations, both spontaneous and induced by steroid reduction[112,113]. There is a lack of long-term serial studies of exhaled NO, together with other markers of airway inflammation in sputum and exhaled condensate, lung function and symptoms. Exhaled NO behaves as a "rapid response" marker, which is extremely sensitive to steroid treatment, as it may be significantly reduced even after 6 h following a single treatment with a nebulized steroid[114], or within 2 to 3 d after inhaled corticosteroids[111], reaching maximal effect after 2 to 4 wk of treatment[112,115].

Corticosteroids

Systemic corticosteroids have no effect on exhaled NO in normal subjects, but they decease its levels in patients with asthma[116].

Inhaled corticosteroids reduce exhaled NO in asthmatic patients [111] and this effect is dose-related[106]. However, a plateau effect on exhaled NO measured after 6 to 12 h since the last treatment may be seen at a dose of 400 μg budesonide and higher [106]. A gradual reduction in exhaled NO is seen during the first week of regular treatment with maximal effect between 3 wk (105) or 4 wk[106, 115].

The reduction in exhaled NO by anti-inflammatory treatment is explained by the suppression of inflammation, resulting in reduced levels of inflammatory cytokines and, therefore, a decrease in the signal for NO overproduction by iNOS.

Inhaled β2-agonists

Neither short-acting β2-agonists nor long- acting β2-agonists (LABAs) reduce exhaled NO[116]. This is consistent with the fact that they do not have any anti-inflammatory effects in asthma.

Leukotriene antagonists

The leukotriene receptor antagonist pranlukast blocks the increase in exhaled NO when inhaled corticosteroids are withdrawn[117], and montelukast rapidly reduces exhaled NO by 15 to 30% in children with asthma[118]. Antileukotrienes have a moderate effect in patients with asthma and seasonal allergic rhinitis[119].

Disease control and severity

Based on the relationship between changes in clinical measures of asthma control and exhaled NO level, measurement of exhaled NO seems to be a useful marker for monitoring the efficacy of asthma control[57]. This suggestion is further confirmed by the findings of others, showing that eNO predicts asthma relapse in asymptomatic asthmatic patients after the withdrawal of corticosteroids[120].

Treatment with inhaled corticosteroids reduces exhaled NO levels, and therefore exhaled NO cannot be directly related to asthma severity. Exhaled NO levels are almost three times higher in children with recent symptoms than in symptom-free subjects[121], and are further elevated during the asthma attack in both adults[122] and children[123,124].

How to interpret eNO data

In asthma, the use of exhaled NO has been proposed to diagnose asthma, to monitor the response to anti-inflammatory therapy, to evaluate compliance and to predict upcoming asthma exacerbations. Furthermore, it is also proposed that treatment, which is guided by the monitoring of non-invasive markers, such as sputum eosinophils and exhaled NO, could improve overall asthma control.

Elevated eNO values seen in untreated patients confirm the diagnosis of asthma suggestive of ongoing eosinophilic airway inflammation. Successful anti-inflammatory treatment causes a decrease in eNO, which remains low if the patient is stable. If eNO is not decreased, this may be caused by non-compliance or by insufficient dose of corticosteroids. Low eNO values seen in patients stable on anti-inflammatory treatment increase when airway inflammation worsens, for example, due to allergen exposure.

eNO and cystic fibrosis

Exhaled and nasal NO levels are significantly lower in patients with cystic fibrosis (CF) than in normal subjects, despite the intense neutrophilic inflammation in the airways (Figure 4)[125] leading to the release of superoxide anions, which convert NO to nitrate and may result in the formation of peroxynitrite[126]. Increased oxidative stress in CF is likely to be a consequence of this neutrophilic inflammation, malnutrition, and IL-10 deficiency[127, 128]. Although there is a trend toward both exhaled and nasal NO being higher in patients who were not homozygous for the ΔF508 CF transmembrane regulator mutation[129,130], there is no strong association between exhaled NO and disease severity in CF[131] or infection with *Pseudomonas*.

There are several possible reasons for the low levels of NO in patients with CF. First, there is a deficiency of NOS2 in patients with CF[131]. Constitutive expression of NOS2, which has been demonstrated in normal human airway epithelium, and of non-CF mouse is essentially absent in the epithelium of CF airways[132]. Neutrophils enhance expression of NOS2 in normal human bronchial epithelial cells but not in CF epithelial cells[133]. The low expression of NOS2 would account for the low levels of NO in nasal as well as exhaled air.

eNO and primary ciliary diskinesia

Primary ciliary dyskinesia (PCD) presents to general practitioners with symptoms pertinent to a variety of specialists because of the involvement of ciliated epithelium in the upper/lower respiratory tract, ears, eyes and genital tract. There is no easy, reliable screening test for PCD, and thus, the majority of patients remain undiagnosed.

It has been decisively shown that measurement of nasal NO can be used in clinical practice in various specialities to screen suspected patients for PCD, both adults[134] and children[135] (Figure 4). Such low values of exhaled and nasal NO are not seen in any other condition and are therefore of diagnostic value. Measurement of exhaled NO might be used as a screening procedure to detect PCD amongst patients with recurrent chest infections or male infertility due to immotile spermatozoa, and the diagnosis of PCD is then confirmed by the saccharine test, nasal nitric oxide, ciliary beat frequency and electron microscopy[136].

NO plays an important role in bactericidal activity in the lungs, sodium and chloride transport in nasal epithelium, and ciliary beating, so that a lack of endogenous NO production might contribute to the characteristic recurrent chest infections in patients with PCD. Despite the lower levels of exhaled NO in children with PCD, no differences were found in the mean levels of NO metabolites in exhaled breath condensate[137], suggesting that detection of NO in exhaled and nasal breath, but not in the EBC, may be the method of choice in the diagnosis of PCD.

Conclusions

Exhaled nitric oxide has been established as a marker of eosinophilic airway inflammation in asthma and recent studies have included eNO measurement in treatment algorithms. This standardized technique is simple, reproducible and is ready for routine monitoring.

Advances in technology have recently resulted in smaller devices that are easier to use and cheaper. It may be possible to introduce such analyzers in the very near future in family practice and even into patients' homes, so that patients themselves will be able to monitor their own markers and adjust their treatment accordingly.

Exhaled NO may be useful as a diagnostic tool in patients with asthma and in wheezing infants. eNO is better than lung function and bronchodilator responsiveness in identifying preschool children with asthma[138]. Exhaled NO levels can differentiate asthmatic young children from non-asthmatic cases with chronic cough[139]. In children with recurrent wheezing, raised eNO values suggest the presence of eosinophilic airway inflammation and may be useful in identifying patients most likely to respond to inhaled corticosteroid (ICS) therapy and furthermore, high eNO levels have been used to predict the benefits of ICS therapy[140]. Exhaled NO measurement is also useful in asthma follow-up; may predict asthma relapse after discontinuing steroids[141] and can predict the failure of attempts to reduce ICS dosages in children with good symptom control[142].

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