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The measurement of exhaled nitric oxide in routine practice

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Key words

Exhaled nitric oxide, asthma, inflammation, diagnosis, control, treatment

Summary

Exhaled nitric oxide (NO) is considered the most easily available clinical test to indirectly assess the level of eosinophilic airway inflammation in asthma, and to predict the efficacy of anti-inflammatory treatment with inhaled corticosteroids (ICS). It is possible to measure the level of exhaled NO using online or offline methods. The most widely used online method employs techniques that enable NO in exhaled air to be measured in a single exhalation, calculating the value at the end-expiratory plateau. Because of the correlation between the level of exhaled NO with the level of eosinophilic inflammation in the airway of asthmatic patients, it has been proposed as a clinical marker in the practice of respiratory and allergy physicians with differing targets. In particular it is considered to be highly effective in the diagnosis of allergic asthma, to be capable of identifying those patients with a higher response probability to inhaled corticosteroids, and to a lesser extent, to be of value in contributing to the management of the disease. The possibility of easily taking measurements of FeNO in an office setting even by relatively young children, and the availability of a portable device, opens a significant perspective for the routine use of FeNO evaluation in daily practice.

Introduction

Though asthma is an inflammatory disease of the airways, requiring regular treatment with inhaled corticosteroids in most cases, control of the disease is mainly based on symptom and lung function measurement, which do not correlate closely to the level of underlying airway inflammation (1,2).
Exhaled nitric oxide is considered the most easily available clinical test to assess the level of eosinophilic airway inflammation in asthma (3,4) indirectly, and to predict the efficacy of anti-inflammatory treatment with inhaled corticosteroids (ICS) (5).
Nitric oxide (NO) is synthesized by different cell types through the enzyme NO synthase. This enzyme is encoded by three different genes in the human genome: nNOS (in neurons), eNOS (in endothelial cells) and iNOS (in macrophages, neutrophils, eosinophils and in epithelial cells). The first two forms are constitutive, the last one inducible, and therefore, the expression of iNOS increases following inflammatory stimulation (6).

Among the variety of biological effects it is involved in, NO mainly relaxes airway smooth muscle, affects ciliary beat frequency and mucus secretion, increases vascular leakage and eosinophil infiltration and is also involved in neurotransmission (7).
In the normal subject, the levels of nitric oxide in the nasopharynx and paranasal sinuses are higher than in the lower airways, this being a defence mechanism that may inhibit the proliferation of bacteria, viruses and parasites in upper airways (8).
In 1991 Gustafsson et al. offered the first description of the presence of NO in exhaled air (9). Levels of exhaled NO have been shown as increased in patients suffering from inflammatory airway diseases, in particular allergic asthma, due to the up-regulation of iNOS (10,11).

Measurement of nitric oxide

In 1997, the European Respiratory Society (ERS) (12), and in 1999, the American Thoracic Society (ATS) (13), defined guidelines for the correct measurement of FeNO, which were updated in a joint document in 2005 (14).
From a practical viewpoint, it is possible to measure the level of exhaled NO using online or offline methods. The most widely used online method employs techniques that enable NO in exhaled air to be measured in a single exhalation, calculating the value at the end-expiratory plateau. This method can be used with cooperating children: the child inhales NO-free air trough a mouthpiece, then exhales for at least six seconds at a constant rate (50 ml/s) through a mouthpiece directly into the analyzer device. For children under age 12, a four second exhalation may be sufficient. It is important to maintain a pressure of between 5 and 20 cm H2O during the exhalation to exclude nasal contamination and to keep flow constant. The test should be repeated twice, with values within 10% of each other (13,15), the final value being the mean. For non-cooperating children, an alternative method has been proposed: the child breathes spontaneously trough a mouthpiece or a facial mask, and the exhalation flow is kept constant manually or adjusted using an automatic control system (16,17).
The offline method is based on collection of exhaled air into a balloon for later analysis. Bodini et al. have evaluated possible differences between samples analyzed at different times, and in different humidity and temperature (18). They concluded that the level of exhaled NO remains stable for nine hours. In the same study, they demonstrated that environmental temperature does not influence the measurement for the first nine hours after collection, but the use of silica gel can alter the results. In current clinical settings, the offline technique is considered obsolete and is no longer recommended.

Exhaled nitric oxide in diagnosis

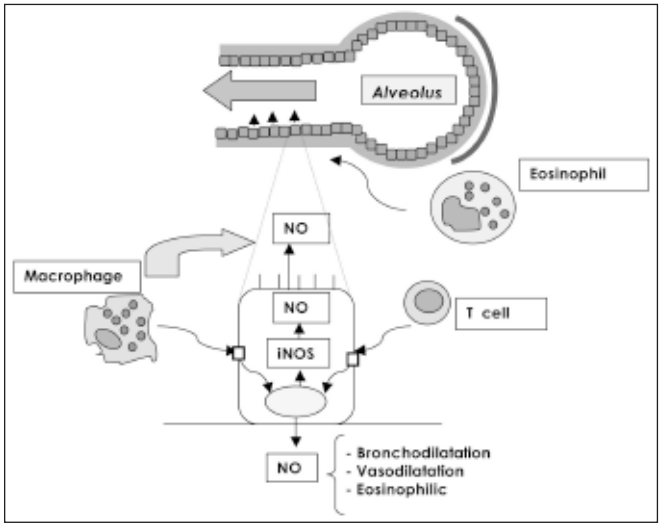
Asthma is an inflammatory disease of the airways characterized by variable clinical symptoms and recurring obstruction of the airways. Traditional diagnostic methods, including lung function, responsiveness of the airways and associated symptoms, often correlate poorly with the underlying level of airway inflammation (19).
According to international guidelines, the diagnosis of asthma should be based on symptoms, peak flow measurement and spirometry including response to bronchodilator, but several studies show that exhaled NO could be a better method for monitoring airway inflammation in clinical practice. In fact, in asthma diagnosis, peak flow measurement and spirometry present low sensibility and may be normal in mild asthmatics.
Smith et al. have shown the superiority of exhaled NO measurements and induced sputum analysis in the diagnosis of asthma compared with conventional tests (20).
In a subsequent analysis of their data, the same authors reported that the combination of FENO (cut-off point 33 ppb) and spirometry (cut-off point for FEV1 of 80% predicted) yielded a sensitivity of 94% and specificity of 93% (21).
Dupont et al. have shown that the concentration of exhaled NO in patients with asthma was significantly higher than in patients with comparable symptoms but without asthma, and in normal subjects (22).
Exhaled NO has also been reported as closely related to asthma and allergy symptoms, whereas spirometric indices, such as percent predicted FEV1, were not (23).
Exhaled NO values are increased in both allergic and non-allergic asthmatic patients, being higher in the first group, without significant correlation to FEV1 (24).
Compared to other techniques, measurement of exhaled NO is easy to implement, reproducible and feasible in young children—it can easily be performed during outpatient visits to follow up asthmatic patients.
One major disadvantage of exhaled NO as a diagnostic test for asthma lies in a number of confounding factors that might influence the level of exhaled NO, like viral infection of the upper airways, which needs to be taken into consideration at the time of each evaluation (10).

The use of exhaled NO to diagnose asthma has been demonstrated as a less expensive alternative to standard diagnostic tests (25).

Exhaled nitric oxide and asthma treatment

In allergic asthma, eosinophils are the main inflammatory cell type, representative of the level of underlying disease at the site of the airway. Accordingly, it is particularly noteworthy that FeNO is significantly correlated to the percentage of eosinophils in samples from induced sputum in patients with allergic asthma (4,26).
Lower FeNO values were observed in subjects for whom bronchial inflammation was not eosinophilic, directing physicians to different diagnoses (neutrophilic asthma, gastroesophageal reflux, chronic obstructive pulmonary disease, etc.) (27).
Additionally, the relationship between eosinophilia and FeNO could also be of interest in patients with difficult asthma, aiming at distinguishing between the eosinophilic and neutrophilic phenotypes (28).
In a clinical setting, the assessment of markers of airway inflammation could have direct implications for the therapeutic approach to asthma patients, particularly children.
During acute asthma exacerbations, exhaled NO is a more perceptive indicator than serum markers, such as eosinophilic cationic protein (ECP) or interleukin-soluble. It would also appear to be a more helpful indicator to assess response to glucocorticoid therapy in young asthma patients (29).
FeNO has been demonstrated to promptly mirror the anti-inflammatory effect of inhaled corticosteroid in asthmatic patients (30) and the rebound of airway inflammation after cessation of therapy (16).
The hypothesis that FeNO can be effective in identifying patients with a higher probability of response to inhaled steroids treatment has been tested further and demonstrated in a study by Smith et al. This study showed that asthmatics with higher levels of FeNO (>47ppb) had a better response in terms of improved symptoms, lung function and airway hyperreactivity compared to those with lower levels of FeNO. (31). Szefler et al. have shown that levels of FeNO were the only indicator capable of identifying children responsive to steroid therapy in a study designed to evaluate the response profiles of fluticasone and montelukast in mild-to-moderate persistent childhood asthma (32).
FeNO measurements have also been reported as practically useful in detecting patients at risk of relapse after withdrawal of inhaled steroid therapy in children with clinical asthma remission (33).
On the basis of the above evidence, since asthma symptoms and lung function measurement correlate poorly with the degree of airway inflammation, biomarkers indicating airway inflammation levels are regarded as potentially useful guides in managing treatment. Because of its simple application in clinical practice, the potential to let FeNO measurement drive decision strategies in the therapy adjustment of asthmatic adults and children has been widely investigated.
In a study by Smith and colleagues, the adjustment of inhaled corticosteroid treatment on the basis of either FeNO, or an algorithm based on conventional guidelines, was compared in 97 patients with asthma (34). That study showed an evident, though not statistically significant, reduction in the number of asthma exacerbations and a 40% lower maintenance dose of inhaled corticosteroid needed to control the asthma, in the group following the FeNO algorithm, concluding that this approach offers a logical alternative to the use of clinical data alone for dose adjustment of inhaled corticosteroids in the management of asthma.
Similar conclusions were reported by Pijneburg et al. in a group of 85 children with atopic asthma in whom treatment with inhaled steroids was guided on the basis of either symptoms or FeNO (35). The authors showed better

Figure 1 – Schematic representation of the source of exhaled NO in the airway. NO synthesized throughout the bronchial tree is harvested by the expiratory flow. The level of NO at the mouth is flow-dependent, with an inversely-related function



control of airway inflammation, lung function and airway hyperreactivity in the group treated according to FeNO levels, though failing to achieve a reduction in the required dose of steroid. They concluded that an algorithm using FeNO for inhaled steroid dose titration every three months for one year was advantageous in comparison to conventional treatment adjustment based on symptoms.

More recently, several studies have failed to show significant advantages when using FeNO as a tool for treatment tailoring in asthmatics compared to conventional approaches based on guidelines (36, 37) or compared to frequent home monitoring of symptoms (38).

In patients with chronic, persistent asthma, corticosteroid treatment can be successfully titrated with the use of FeNO measurements.

In the study by Szefler et al. (36), the authors concluded that addition of fractions of exhaled NO as an indicator of the control of asthma resulted in higher doses of inhaled corticosteroids, without clinically significant improvements in symptomatic asthma control. Nevertheless, the proportion of patients requiring at least one course of oral corticosteroids in the FENO group was 24% lower than the control group. Furthermore, in two important and relatively large subgroups, the primary outcome of the study (maximum number of days with symptoms) was significantly reduced. Thus, patients with a BMI of > 30, representing 28% of all patients, had 0.6 fewer maximum days with symptoms (p=0.0245), and patients with total IgE of > 460 kU/L (33% of patients) had 0.5 fewer maximum symptom days (p=0.0296).

Moreover, Taylor and Bush observed that the FENO management protocol did not allow for a reduction of inhaled corticosteroid dose when FeNO was low in symptomatic patients, which may have affected the conclusions substantially (39).

Gibson has systematically assessed the studies where exhaled nitric oxide has been used to tailor asthma therapy (34-38, 40, 41) and concluded that those studies were disadvantaged by the choice of algorithm decisions being based on healthy subjects rather than on the specific population of asthmatics, with sufficient possibilities for decision-making to enable discernible benefits.

Price et al. have evaluated asthma treatment and management guided by FENO measurement with NIOX MINO instead of symptoms and lung function from an economics perspective (25). They showed that a FENO-based strategy can result in a reduction of annual costs of £341 for patients with mild-to-severe asthma and of £554 for those with moderate-to-severe asthma with similar health benefits.

Conclusions

The body of literature available in the field of nitric oxide measurement in patients with respiratory disease highlights the potential application of this recent marker in the practice of respiratory and allergy physicians with differing targets. In fact, it has been demonstrated as highly effective in the diagnosis of allergic asthma, and capable of identifying those patients with a higher response probability to inhaled corticosteroids, and to a lesser extent, contributing to the management of the disease. The possibility of easily taking measurements of FeNO in an office setting even by relatively young children, and the availability of a portable device, opens a significant perspective for the routine use of FeNO evaluation in daily practice.

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