Exhaled nitric oxide to help manage childhood asthma – reality bites

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Abstract
Since exhaled nitric oxide (FeNO) was first demonstrated to be raised in asthmatic patients in the early 1990s there has been a strong interest in its potential role in the diagnosis and management of asthma. This culminated in 2003 when the US Food and Drug Administration (USFDA) cleared the NIOX nitric oxide analyser for clinical application in patients with asthma. The interest in FeNO is based on a number of assumptions; firstly that FeNO is a marker of asthma and asthma control, and, secondly, it reflects eosinophilic airway inflammation. However, the literature remains unconvincing and inconclusive. Furthermore, studies that have management algorithms that include FeNO as a guide to asthma treatment have failed to observe any improvement in asthma control compared to the use of standard asthma guidelines. At present the cost of including FeNO in management guidelines far outweighs any potential benefits.

Keywords
Exhaled nitric oxide, asthma diagnosis, asthma management, eosinophils, atopy
Introduction

Asthma is a chronic inflammatory disorder of the airways (GINA) that is more common in atopic than non-atopic individuals. Exacerbations can be triggered by viruses, allergens and chemical exposures. Exacerbations are characterised by an increase in symptoms, reduction in lung function and increase in bronchodilator use. Treatment is aimed at stabilising asthma, reducing exacerbations and interval symptoms, and minimising the use of bronchodilators. The use of regular inhaled corticosteroids (ICS) is the cornerstone of modern management of asthma. Most clinical guidelines advocate the use of the minimum dose of ICS that allows maintenance of asthma control. However, in children, there are no easy methods of assessing adequacy of control or that predicting loss of control that are better than simply monitoring symptoms. Hence the interest in non-invasive markers of airway inflammation, most notably the fraction of nitric oxide in exhaled breath (FeNO). Momentum is growing for the use of FeNO measurements to guide asthma management and reimbursement for FeNO measurements are now possible in many countries including the USA. There are a number of assumptions that have been made by proponents of the use of FeNO measurements as a clinical tool. They include:

i. FeNO is a marker for asthma
ii. FeNO reflects eosinophilic airway inflammation
iii. FeNO reflects asthma control and can be used to guide asthma management

Comment [SS1]: There is no convincing evidence that regular monitoring of PEF improves control or predicts loss of control.
In this review we will examine the validity of these assumptions and review the limited data from randomised, controlled studies that have included $\text{FeNO}$ measurements in asthma management algorithms.

**Exhaled NO as a marker of asthma**

Increased $\text{FeNO}$ in asthma was first reported in the early 1990s. Since that time studies have consistently demonstrated raised $\text{FeNO}$ in asthmatics but it is now clear that $\text{FeNO}$ is elevated only in atopic asthma. There is also good evidence that $\text{FeNO}$ is raised in atopic non-asthmatics. Despite this atopy is not always considered when determining a diagnostic role of $\text{FeNO}$ in asthma.

A number of studies in adults and children have demonstrated that $\text{FeNO}$ has a high sensitivity and specificity for identifying asthma when comparing with healthy and non-asthmatic symptomatic subjects. Indeed, Smith et al. found that $\text{FeNO}$ performed significantly better than conventional spirometric tests in differentiating between asthma and non-asthma in a group of patients with respiratory symptoms. However, these studies have not adequately accounted for atopy. Despite the high degree of atopy in children without asthma some studies have only compared asthmatics with non-atopic healthy controls, while other studies have either not measured atopy or not included atopy in the analyses.

When atopy is considered, the ability of $\text{FeNO}$ to distinguish between asthma and non-asthma is less clear. For example, in a large community study of schoolchildren Prasad
and colleagues concluded that FeNO was not a useful tool for identifying children with asthma in the community, as increased levels did not discriminate between children with asthmatic or atopic symptoms. In another community study, we observed that FeNO was raised in atopic children with increased airway responsiveness (AR), regardless of the presence of symptoms. We therefore believe that atopy must not be disregarded when considering the validity of FeNO as a diagnostic test for asthma.

**Exhaled NO as a marker of eosinophilic inflammation**

Exhaled NO has been widely accepted as a marker of allergic airway inflammation. Positive correlations have been reported between FeNO and eosinophils measured in sputum, bronchoalveolar lavage (BAL) fluid, blood and bronchial biopsy specimens. However, associations between FeNO and the various markers of eosinophilia are not strong, are inconsistent and significantly affected by the presence of atopy and treatment with anti-inflammatory agents.

**FeNO and airway eosinophilia**

Bronchial biopsy is considered the gold standard for the direct measurement of airway inflammation. The relationship between FeNO and eosinophils in airway biopsies has been investigated in both adults and children. The results of these studies have been conflicting. In adults, for example, Lim et al. found that there was no association between FeNO and mucosal eosinophils in either steroid naïve or steroid treated atopic asthmatics. In contrast, van den Toom reported a significant correlation between FeNO and mucosal eosinophils in steroid naïve atopic asthmatics.
either with current symptoms or in remission. Interestingly, a recent study reported a significant association between biopsy eosinophils and Fe\textsubscript{NO} in subjects with severe asthma but not in those with moderate disease\textsuperscript{25}.

The situation in children is similarly unclear. Payne and colleagues\textsuperscript{28} reported a significant positive association between an eosinophil score in biopsies and Fe\textsubscript{NO} in children with difficult to treat asthma. However, using a similar protocol in a larger group of asthmatic children, the same authors were not able to repeat these findings, although a significant association was evident after removing a single outlier who had high biopsy eosinophils but low Fe\textsubscript{NO}\textsuperscript{29}. Finally, in the most recent study by this group there was no association between biopsy eosinophilia and Fe\textsubscript{NO} in children with moderate to severe persistent asthma\textsuperscript{17}.

The positive relationships between Fe\textsubscript{NO} and eosinophil numbers in sputum and BAL fluid are more consistent\textsuperscript{15-18} but are moderate ($r \leq 0.5$) and have not been observed in all studies\textsuperscript{25,30}.

\textbf{Fe\textsubscript{NO}, atopy and eosinophils}

Atopy is probably the most important single factor affecting the level of Fe\textsubscript{NO}. Not only is atopy associated with increased Fe\textsubscript{NO}\textsuperscript{4-6} but it also has a significant impact on the relationship between Fe\textsubscript{NO} and important physiological characteristics of asthma such as eosinophilia\textsuperscript{6,15,20-22} AR\textsuperscript{6,31,32} and possibly symptoms\textsuperscript{6}. 
The effect of atopy on the relationship between FeNO and eosinophils has been assessed in a small number of studies and there is a consistent finding that a positive correlation between the two variables is only observed in atopic subjects.\textsuperscript{6,15,20-22} Interestingly, Barretto et al.\textsuperscript{20} reported that FeNO was raised in atopic children with high blood eosinophil count but not non-atopic children with a similarly high blood eosinophil count. Therefore, the lack of an association in non-atopics is not due to a limited range of eosinophilia in this group. The role of NO in the allergic airway is yet to be fully determined.

\textit{Impact of anti-inflammatory treatment}

ICS are the mainstay of asthma treatment. They reduce airway inflammation, improve symptoms and lung function, and prevent lung function decline.\textsuperscript{33} Exhaled NO is reduced directly after the introduction of inhaled corticosteroids\textsuperscript{34} and has been proposed as a rapid and sensitive marker of changes in airway inflammation in response to anti-inflammatory treatment.\textsuperscript{35}

The relationship between FeNO and airway eosinophils in treated and untreated asthmatic subjects has been investigated in both cross-sectional\textsuperscript{15,17-19,25} and longitudinal\textsuperscript{26,36,37} studies. In cross-sectional studies, the association between FeNO and sputum eosinophils is stronger in steroid naïve than steroid treated asthmatics.\textsuperscript{15,18,25} However, positive associations between eosinophils in both sputum\textsuperscript{17} and BAL fluid\textsuperscript{17,19} with FeNO have been observed in treated asthmatic children. Interestingly, Lim et al.\textsuperscript{26} found that there was no association between FeNO and airway eosinophils in mild adult asthmatics prior to
a course of inhaled steroids, however, after treatment there was a significant relationship between FeNO and eosinophils measured in BAL fluid.

In clinical trials there is a clear and consistent difference in the response of FeNO and airway eosinophils in response to anti-inflammatory treatment.36,37 For example, Jatakonen et al.36 reported a decrease in both FeNO and sputum eosinophils in asthmatic patients treated with 100, 400 or 1600 μg of budesonide. However, while FeNO was reduced between baseline and 400 μg of drug there seemed to be a plateau in levels with higher doses. On the other hand there was a dose-response reduction in sputum eosinophils across all doses of drug.36 Van Ressen and colleagues37 investigated the changes in FeNO, sputum eosinophils and AR in a group of mild asthmatics during a 4-week trial with inhaled fluticasone dipropionate. Although both eosinophils and FeNO decreased in the study group, there was no significant correlation between these changes.37

Corticosteroids have a direct influence on FeNO by inhibiting the expression of the inducible isoform of nitric oxide synthases (iNOS).38 Two studies have investigated the changes in airway eosinophils and FeNO in subjects given non-steroidal anti-inflammatory treatment.39,40 Lim et al.39 found that, in mild asthmatic adults, there was a decrease in sputum, BAL fluid and mucosal eosinophils but no change in FeNO after treatment with low dose theophylline. Similarly, Razi et al.40 observed a significant decrease in blood eosinophils but no change in FeNO after treating rhinitic children with montelukast. Unlike corticosteroids, these treatments do not have any direct effect on iNOS.
The above studies suggest that there is a dissociation between FeNO and more direct markers of eosinophilic inflammation and that steroid induced reductions in FeNO could, in part, be explained by down-regulation of iNOS, rather than an indirect effect through a reduction in inflammation \textit{per se}.\textsuperscript{15}

\textbf{Asthma management}

Exhaled NO seems to reflect asthma control\textsuperscript{41} and has been suggested as potentially useful for guiding asthma treatment.\textsuperscript{2} Exhaled NO is increased during an asthma exacerbation and falls rapidly with ICS treatment.\textsuperscript{42} In some studies, it has been shown to be able to predict loss of asthma control (LOC).\textsuperscript{43-45} However, this finding remains inconsistent.\textsuperscript{46-48} Repeated measures of FeNO are required to predict an asthma exacerbation.\textsuperscript{43} In the study by Jones et al.\textsuperscript{43} FeNO was comparable to peak flows, FEV1, symptom scores and daily reliever use in predicting LOC, however, unlike these other measures, on-going home monitoring of FeNO is a very expensive option that is not viable at present.

There is a strong interest in using FeNO to help guide asthma treatment based on the premise that FeNO reflects airway inflammation.\textsuperscript{2} To date, there have been four published randomised controlled trials that have assessed the utility of FeNO in asthma management and the data remains unconvincing.\textsuperscript{49-52} None of these studies were able to demonstrate that the use of FeNO was better than current asthma guidelines for controlling asthma exacerbations. However, in the study by Smith et al.\textsuperscript{52} the FeNO group was able to
maintain the same level of asthma control with a significantly lower dose of ICS. Shaw et al.\textsuperscript{51} also found that the daily dose of ICS was lower at the end of the study in the Fe\textsubscript{NO} group, but the cumulative dose over the course of the study was no different between the two groups. In the Austrian study ICS dosage was actually significantly higher in the Fe\textsubscript{NO} compared to control group.\textsuperscript{49}

We believe that the ICS dose outcomes require closer scrutiny as some of the study designs favoured a reduction in ICS dose in the Fe\textsubscript{NO} group. For example, in the study by Smith et al.\textsuperscript{52}, if asthma was controlled and Fe\textsubscript{NO} < 15 ppb the dose of ICS was reduced, but if Fe\textsubscript{NO} was > 15 ppb, the dose stayed the same. Whereas, in the comparison arm, if symptoms were controlled at one visit, subjects had to wait for an additional 2 months before an opportunity to reduce the dose. Similarly, in the study by Pijnenburg et al.\textsuperscript{50} a symptom score > 14 directs management in each arm of the study (“Fe\textsubscript{NO}” and “symptom”). In the Fe\textsubscript{NO} arm, the ICS dose was increased if Fe\textsubscript{NO} exceeded 30 ppb regardless of symptoms. In the “symptom” arm a symptom score > 14 resulted in an increased dose of ICS, whereas in the Fe\textsubscript{NO} arm a score >14 only resulted in an increased dose if Fe\textsubscript{NO} exceeded 30 ppb. Similarly, if the symptom score was < 14, the dose of ICS for a child in the Fe\textsubscript{NO} arm remained the same (Fe\textsubscript{NO} > 30 ppb) or else was reduced (Fe\textsubscript{NO}< 30 ppb) whereas, in the symptom arm the dose was only reduced if a child had a symptom score < 14 for two consecutive visits. Hence, in both of these studies the dose of ICS in the intervention arm is more likely to be reduced for a given level of symptoms.
The use of inappropriate comparators in these studies\textsuperscript{50,52} has led to inferences regarding the use of Fe\textsubscript{NO} and ICS dose that could be misleading. In the study by Smith et al.\textsuperscript{52} the overall reduction in ICS by 42\% in the active Fe\textsubscript{NO} management arm can at least in part be explained by the imbalance in treatment options in the two arms of the study that favour a reduction of ICS in the Fe\textsubscript{NO} arm. In the Pijnenburg study\textsuperscript{50} the final ICS dose is not different between the two groups. Therefore, given that there was an imbalance in treatment options that favoured a reduction of ICS in the Fe\textsubscript{NO} arm, it is not possible to rule out the possibility that individual children in the intervention arm might have received a higher dose of ICS for a given level of symptoms compared to the control arm.

Only two of these studies have been able to demonstrate any significant improvement in outcome measures in the Fe\textsubscript{NO} groups.\textsuperscript{49,50} Pijnenburg and colleagues\textsuperscript{50} observed a significantly greater improvement in AR in the Fe\textsubscript{NO} compared to control group. However, this group had increased AR compared to the control arm at baseline and it is likely that some of the observed improvement was due to a regression to the mean. In the study by Fritsch et al.\textsuperscript{49} there was a significantly greater improvement of mid-expiratory flows in the Fe\textsubscript{NO} group. However, this was achieved at a higher ICS dose and the clinical relevance of the finding is uncertain.

\textit{Is Fe\textsubscript{NO} worth it?}

The observation that each of these four studies failed to demonstrate any improvements in markers of asthma control in patients despite frequent monitoring of Fe\textsubscript{NO} must raise questions regarding the clinical utility of such a strategy. For example, even if the
reduction of ICS dose observed in the study by Smith et al.\textsuperscript{52} was a true indication of what might be achieved in clinical practice by routine measurement of Fe\textsubscript{NO}, what are the likely benefits? One could argue that a reduction in the risk of side-effects particularly in adults where there is a tendency to use higher doses of ICS than in children is desirable. However, this aspect is hard to quantify but it is possible to estimate the relative benefit in terms of a reduction in costs for prescribing of ICS. Although the following is a simplistic approach we believe that it demonstrates how marginal the cost-benefit argument is likely to be. To avoid some of the assumptions about populations one can consider the situation for a single patient. If we assume that a patient formerly requiring ICS 250\textmu g twice daily can be controlled using 125\textmu g twice daily, the cost in Australia for example, of fluticasone dipropionate metered dose inhalers per person would fall from approximately $A474 per year to $A354, a reduction of $A120. Savings would then only accrue if the reimbursement costs for Fe\textsubscript{NO} are below $A120 per year. The latter would depend upon the frequency of measurements and reimbursement level for each test. The study by Smith et al.\textsuperscript{52} measured Fe\textsubscript{NO} monthly, a regime of measurement that would only be feasible in the most severe asthmatics and that would only be cost-neutral if the reimbursement for testing was $A12 or less! Even with a modest reimbursement fee of $A50 a cost-benefit would only accrue if patients were tested no more than twice a year. Since there is no evidence that twice-yearly measurement of Fe\textsubscript{NO} has any clinical benefit such a regime can hardly be supported. Furthermore, even if a marginal cost-benefit regime could be established that is effective in an individual, for any real benefit to be realised a large proportion of patients being treated with ICS would need to have
FeNO measured on a regular basis. The infrastructure requirements to achieve this would be prohibitive for most health services.

In summary, FeNO appears to reflect some physiological and immunological aspects of the atopic airway. However, the relations between airway inflammation and FeNO are inconsistent. Data from randomised clinical trials that use FeNO in an asthma management algorithm do not support the routine use of FeNO in the management of asthma.

References


