

Exhaled nitric oxide in the diagnosis and management of childhood asthma

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Abstract: The management of asthma in children and adolescents is currently guided by assessment of clinical symptoms, exacerbation risk and spirometric measure of lung function. The use of biomarkers, an objective measure which indicates normal or pathophysiologic processes and/or the response to a treatment intervention, could greatly enhance the efficacy and safety of current algorithms. Measurement of the fraction of expired nitric oxide in exhaled air (FeNO) has been suggested as a readily determined biomarker that can aid in the diagnosis and management of asthma. FeNO has been used to identify steroid responsive patients, adjust the dose of controller medications, most notably inhaled corticosteroids, and predict relapse during medication taper. In spite of early enthusiasm for the utility of this measure, more recent data suggest a more limited role for FeNO. This review will focus on the use of FeNO in the diagnosis and management of asthma in children and adolescents.

Keywords: exhaled nitric oxide, asthma, children, adolescents

Introduction

An illness that has been recognized since antiquity, asthma is one of the most common chronic diseases of children and adolescents. The accurate diagnosis and optimal management of asthma remains a challenge in spite of numerous advances in the understanding of the pathophysiology and natural history of the disorder. Although much of the diagnosis and management of asthma relies on clinical assessments, the use of objective measures of airway function and, more recently, airway inflammation are playing increasingly important roles. The most recent US and international asthma guidelines [National Asthma Education and Prevention Program: EPR3, 2007; Global Initiative for Asthma, 2008] both stress the importance of monitoring asthma control, which is largely defined on the basis of patient-reported symptoms, disruption of activities of daily living and serious exacerbations. The EPR 3 report further stratifies control into domains of impairment and risk. Impairment refers to the more readily identified symptom and activity domains, while risk refers to future likelihood of severe exacerbations, reduction in lung function and medication adverse effect. The identification of risk is as yet difficult and it is believed that this task might be less onerous if a suitable biomarker that indicated disease activity and/or predicted exacerbations

could be found. Although a number of putative biomarkers have been studied (sputum and peripheral blood eosinophils, airway hyper-responsiveness, serum eosinophil cationic protein, exhaled breath condensate pH or ionic content), the fractional exhaled concentration of nitric oxide (FeNO) has emerged as a measure that might permit more accurate treatment titration in asthma [Reddel *et al.* 2009; Gogate and Katial, 2008; Kharitonov and Barnes, 2006]. The determination of FeNO is relatively easy and several devices approved for clinical use to measure FeNO are now widely available. Although initially quite expensive, some newer eNO analyzers now cost about as much as a high quality office spirometer, and are portable and durable. However, numerous questions remain about the utility of FeNO measures in the diagnosis and management of asthma in children and adults. This review will focus on what is currently known about the utility of FeNO in adjusting treatment in asthmatic children and adolescents.

Biology of NO in the asthmatic airway

Nitric oxide (NO) is a signaling molecule widely distributed in mammalian tissues, including the airway epithelium, vascular and airway smooth muscle and numerous inflammatory cells [Ricciardolo, 2003]. It is synthesized from the

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amino acid arginine by oxidative conversion into the intermediate citrulline and NO. The reaction is catalyzed by nitric oxide synthases (NOS), which occur in several isoforms. A constitutive isoform (cNOS) occurs largely in endothelial (eNOS, NOS3) and neuronal cells (nNOS, NOS1) and an inducible form (iNOS) which is found in airway epithelial cells airway and vascular smooth muscle cells, macrophages, neutrophils, type II alveolar cells, and fibroblasts [Zimmerman and Rothenberg, 2006; Ricciardolo, 2003]. Oxidant exposure in the airways may also increase iNOS via activation of NF- κ B. In the airway, NO functions as a bronchodilator, has antibacterial properties, an anti-proliferative action on fibroblasts, is involved in regulation of ciliary beat frequency and epithelial ion transport [Kharitonov and Barnes, 2006].

The role of NO in the asthmatic airway remains somewhat unclear. The substantial elevation of FeNO in the asthmatic compared with normal airway and reduction with treatment with inhaled steroids suggests an active role in the development or persistence of asthma [Kharitonov *et al.* 1994]. However, elevation of FeNO does not occur in all asthmatics. Moreover, studies using NO synthase inhibitors have not shown reduction in asthmatic symptoms or disease activity [Singh *et al.* 2007]. Consequently, NO may function as a counter-regulatory molecule that is less directly involved in the inception or perpetuation of asthma. Further details on the biology of NO and the technical details of its measurement are reviewed elsewhere [Pijnenberg and De Jongste, 2007; Taylor *et al.* 2006; ATS/ERS, 2005; Ricciardolo, 2003].

Elevation of FeNO seen in allergic asthma is thought to reflect increased iNOS activity in both airway epithelial cells and inflammatory cells in the airways. In most published reports, there is good, direct correlation between clinical markers of eosinophilic airway inflammation (peripheral blood and induced sputum eosinophil counts, allergen sensitivity) and FeNO [Lex *et al.* 2006; Berry *et al.* 2005; Zacharasiewicz *et al.* 2005]. In addition, FeNO also seems to reflect the degree of airway hyperresponsiveness as measured by methacholine challenge test [Taylor, 2006; Smith *et al.* 2004]. Measures of pulmonary function, e.g. FEV₁, bronchodilator response and symptom report typically correlate only weakly with FeNO [Strunk *et al.* 2003; Fitzpatrick *et al.* 2006]. In the cohort of pediatric patients (age 6–17 years) studied in the

severe asthma research program (SARP), both baseline and longitudinal measure of FeNO (over 6 months) was higher in children with severe asthma compared with those with mild-to-moderate disease, even after adjustment for seasonality and IgE levels [Fitzpatrick *et al.* 2006]. Lastly, treatment of asthmatics with inhaled corticosteroids (ICS), potent anti-inflammatory agent 5, typically results in rapid reduction in FeNO, while withdrawal of ICS correlates with a rise in FeNO [Buchvald *et al.* 2003; Kharitonov *et al.* 1996]. As a result of these findings, FeNO has attained status as a potential and clinically useful biomarker or ‘inflammometer’ for the asthmatic airway. Although prominent in children, eosinophilic inflammation is not the primary driver of the airway dysfunction in all asthmatics. Measure of FeNO may not consistently identify non-eosinophilic inflammation. In addition, elevated FeNO is not highly specific for asthma, and is also found in individuals with allergic rhinitis (without asthma), eosinophilic bronchitis, chronic obstructive pulmonary disease (COPD), lung allograft rejection and gastroesophageal reflux disease (GERD) [Taylor *et al.* 2006]. Moreover, studies of the clinical utility of FeNO in the management of asthma have produced conflicting results [Petsky *et al.* 2008; Gelb *et al.* 2006; Taylor, 2006]. Although there are a number of reasons for the inconsistencies among the studies, the type of analyzer used, varying flow rates for measurement and age of the subjects may all play a significant role.

Exhaled nitric oxide has been studied as an adjunct measure for the management of asthma with respect to monitoring disease activity and control, adjustment of treatment, predicting treatment response to inhaled steroids, predicting exacerbations and monitoring adherence to treatment. The initial promise of the measure of FeNO was that it would substantially add to all of the aforementioned elements of disease control and indicate active inflammation in the airways. More recent studies and larger clinical trials suggest that the early promises of FeNO as a global biomarker for asthma may not be fulfilled.

Asthma diagnosis

The diagnosis of asthma has largely rested on a combination of clinical signs and symptoms indicative of airway obstruction (cough, wheeze, shortness of breath) and an objective measure of reversible airflow obstruction and airway hyperresponsiveness. Spirometry, bronchodilator response and inhalation challenge testing using

methacholine or other stimuli are diagnostic tests that can help identify asthma in older children and adults. In groups such as younger children, those with chronic cough as a predominant symptom or recurrent wheeze with normal spirometry, a measured FeNO above certain cutoff levels may be a helpful adjunct for the diagnosis of asthma. If other clinical conditions associated with elevated eNO, such as GERD, allergic rhinitis or other atopic condition, COPD, etc are excluded, an elevated eNO above documented clinical cutoffs (Table 1) can be useful in differentiating asthma from other similar conditions. FeNO has the advantage of being somewhat easier to perform than standard spirometry, making the measure more useful in younger children. However, it is still unlikely that children much younger than 6 or 7 years of age will consistently be able to perform an FeNO measure. Children who have or will develop asthma are also likely to have normal physical examination results and normal pulmonary function testing in between acute episodes, often making clinical assessment of airflow obstruction difficult.

Although there is variability reported in the range of normal values for FeNO, levels between 5–33 ppb (parts per billion) for adults and 5–20 ppb for children have been suggested [Olin *et al.* 2007; Travers *et al.* 2007; Olivieri *et al.* 2006; Buchvald *et al.* 2005]. A number of factors influence the measure of FeNO. Even in normal individuals, recent ingestion of nitrate-containing foods or respiratory infection can result in elevation of FeNO [Dressel *et al.* 2008; ATS/ERS, 2005; Sanders *et al.* 2004]. Males tend to have higher FeNO than females and FeNO tends to rise with age, starting in early childhood [Buchvald *et al.* 2005]. Smoking, recent exercise, bronchodilator use or spirometry can lower FeNO [Dressel *et al.* 2008; Maniscalco *et al.* 2002; Yates *et al.* 2001]. One of the most important contributors to elevated FeNO is the atopic state itself. Non-asthmatic atopic patients with allergic

rhinitis have FeNO levels that are comparable to atopic asthmatics [Buchvald *et al.* 2005; Gratzidou *et al.* 1999]. Non-atopic asthmatics may have FeNO concentration in the same range as normal controls. It is generally accepted that there is not a 'normal range of FeNO' for asthmatic patients. In patients with allergic asthma, FeNO is typically elevated by 50–60% above the upper limit of normal. Likewise, treatment of allergic asthmatics with ICS usually results in a 2–4-fold reduction in FeNO; the effect may be seen within days to weeks of starting therapy [Buchvald *et al.* 2003; Kharitonov *et al.* 2002]. Although FeNO levels may fall with treatment and rise with disease activity, many asthmatics may never achieve FeNO measures within the range for non-asthmatics. A 'best-baseline' FeNO may be a useful measure for the individual patient, with deviation from this level indicating change in disease status. This strategy has appeal, but has not been adequately tested.

The utility of the FeNO in the diagnosis of asthma was examined in a study of 47 patients (age 8–75 years, mean age 41 years) who were referred to a pulmonary center for evaluation of symptoms suggestive of asthma [Smith *et al.* 2004]. Patients completed respiratory questionnaires, performed spirometry and bronchial hyper-responsiveness, sputum eosinophils, PEFr and FeNO were measured. Treatment was instituted and a diagnosis of asthma was assigned if criteria set forth by the American Thoracic Society were met in addition to an objective measure of reversible airflow obstruction. Using a cutpoint of 20 ppb FeNO, both FeNO and induced sputum eosinophil count greater than 3% had a greater sensitivity, specificity and positive and negative predictive value than did baseline lung function tests in identifying asthmatics. Although induced sputum eosinophil percentage was slightly better than FeNO (positive predictive value [PPV] 80 versus 70%), the relative ease of performing FeNO compared with induced sputum, favors the former as a possible diagnostic aid. Other studies in adults and adolescents demonstrated similar results [Berkman *et al.* 2005; Dupont *et al.* 2003; Prasad *et al.* 2006], indicating that distinguishing those with allergy alone from those with allergic asthma is not possible using FeNO.

In a recently published study of 150 children (age 5–18 years, mean 12.5 years) referred to a specialty clinic for evaluation of respiratory

Table 1. Range and interpretation of fractional exhaled concentration of nitric oxide values in adults and children*.

	Low	Normal	High
Adults	<5	5–33	>35
Children 4–10 years	<5	5–20	>20
Children 10–17 years	<5	5–25	>25

*All values are in parts per billion (ppb) and measured at 50 ml/s flow rate.

symptoms suggestive of asthma, FeNO was compared with spirometry and eosinophil counts in induced sputum and spirometry for confirming the diagnosis of asthma [Sivan *et al.* 2009]. The final diagnosis of asthma was made after at least 18 months of treatment by a pediatric pulmonologist (blinded to results of FeNO and sputum eosinophils) and included standard clinical criteria (recurrent episodes of physician-documented wheezing relieved by bronchodilators, a significant response to a bronchodilator determined by spirometry and, when available, results of a bronchoprovocation test). In these children, FeNO values >19 ppb yielded a sensitivity of 80%, specificity of 92%, PPV of 89% and negative predictive value (NPV) of, 86% for the diagnosis of asthma. Percent of eosinophils in induced sputum >2.7% produced almost identical values; however, obtaining induced sputum is far more difficult and time-consuming than obtaining a measure of FeNO. Spirometry (FEV₁ <80%) was far less accurate. In those with FeNO <15 ppb, the false negative rate was <5%, and when FeNO was >23 ppb, the false positive rate was <5%. For those children with symptoms suggestive but not diagnostic of asthma, or in those in whom other diagnostic testing is also not definitive, the rapid and easily performed measure of FeNO may help confirm the diagnosis of asthma and permit institution of appropriate treatment. Those with false negative tests are likely to be among the minority of childhood asthmatics who do not have associated atopy or eosinophilic airway inflammation [Taylor, 2006]. Moreover, in children in whom a symptom report may be more vague (cough, exercise intolerance), a low FeNO may help avoid the misdiagnosis of asthma and use of costly, unnecessary treatments.

Prediction of response to treatment with inhaled steroids

ICS are currently viewed as the most effective treatment for chronic persistent asthma in both children and adults. ICS are the preferred therapy at all stages of treatment for persistent asthma in both the EPR3 [National Asthma Education and Prevention Program, 2007] and GINA guidelines [Global Initiative for Asthma, 2008]. Most, but not all, asthmatics have a significant clinical response to treatment with ICS. Identifying asthmatics who are most likely to benefit from treatment with ICS has heretofore been based in part on trial and error. However, those with eosinophilic airway

inflammation as the predominant driver of asthma are most likely to respond to ICS treatment. Although a number of surrogate measures for predicting response to steroid treatment exist (determination of allergy, magnitude of bronchodilator response, serum ECP levels, eosinophil count in induced sputum), many of the techniques are non-specific, cumbersome to obtain, or available as research measures only [Knuffman *et al.* 2009; Zeiger *et al.* 2006; Anderson and Bramman, 2003; Enright *et al.* 1994].

In both children and adults, the measurement of FeNO can provide a useful index of responsiveness to treatment with ICS. In a study of 60 adults referred to a general pulmonary clinic for evaluation of possible asthma, the utility of FeNO was evaluated in comparison to standard spirometric measures, peak expiratory flow rate (PEFR) variability and bronchial hyper-responsiveness [Smith *et al.* 2005a]. The diagnosis of asthma was made in 27, based on improvement in FEV₁, airway hyper-responsiveness, morning PEFR and clinical response to treatment. A FeNO of >47 ppb had a sensitivity (67%), specificity (78%), positive (47%) and negative predictive value (89%) for improvement of >12% in FEV₁ after treatment with inhaled steroids for 6 weeks. These values were in general superior to use of low baseline FEV₁, degree of PEFR variability, PC20 <8 µg/ml, and degree of baseline bronchodilator reversibility. In a study of pediatric patients, mean age 9.8 ± 2.2 years, a pre-treatment FeNO >25 ppb was predictive of a positive response to treatment with fluticasone (as compared with montelukast) in increasing the number of asthma control days over a 12-month period [Knuffman *et al.* 2009; Zeiger *et al.* 2006]. No other spirometric measure (FEV₁, FEV₁/FVC) was identified as predictive. Since the office measure of FeNO is now quite feasible with the availability of affordable, hand-held eNO monitors, determination of patient responsiveness to ICS treatment could be achieved rapidly and without a prolonged trial of medication use. There are limitations to these studies, however, in that a substantial number of patients with lower FeNO may still respond to ICS as measured by other impairment or risk domains or after longer treatment trials. More work is clearly needed to define subsets of steroid responsive and unresponsive patients based on FeNO.

FeNO as a guide to adjusting treatment of asthma

Current guidelines recommend treatment of asthma to achieve good control, as determined by minimizing impairment (symptoms, activity limitation) and risk (exacerbations, adverse effects and loss of pulmonary function). It is believed that most of these morbidities are driven by airway inflammation and that treating inflammation will improve control. Studies have demonstrated that when asthma treatment is guided by reducing airway hyper-responsiveness or sputum eosinophils (both markers of inflammation), better asthma control is achieved than by using standard symptom- and pulmonary function-based algorithms [Green *et al.* 2002; Sont *et al.* 1999]. However, repeated methacholine challenge testing or collection of induced sputum is time-consuming and expensive. Measurement of FeNO has been proposed as an alternative measure of airway inflammation [Berry *et al.* 2005]. Levels of eNO are elevated in asthmatics who are steroid-naïve and in most patients the levels fall within a week of instituting treatment with ICS [Kharitonov *et al.* 1996]. It should be noted that in some (20–30%) asthmatics who have elevated FeNO prior to treatment with ICS, FeNO remains essentially unchanged following treatment, even with clinical improvement [Pijnenburg *et al.* 2005; Buchvald *et al.* 2003]. As a result it is hypothesized that using the more easily performed serial FeNO measures to adjust treatment would also reflect airway inflammation. Threshold or ‘cutpoint’ FeNO levels could be inserted into an algorithm used to optimize the dose of inhaled corticosteroids to that which decreases airway inflammation, producing good clinical control and minimizing steroid risk. A number of clinical trials have been published in the past few years examining the utility of such repeated measures of eNO in asthma management in children and adolescents. Overall, results have been mixed and more recent larger trials somewhat disappointing. This review will focus on six well-conducted randomized, controlled trials (either single- or double-blinded) reported in the past 4 years (Table 2).

In a study examining the use of FeNO in the treatment of childhood and adolescent asthma, Pijnenburg *et al.* [2005a] randomized 89 children to a group whose treatment was guided by FeNO measures and report of symptoms *versus* a group managed by symptom report (obtained on home diary cards) alone. The study hypothesis stated

that the addition of FeNO to clinical measures would result in use of lower ICS doses and better control. The children were seen every 3 months and followed for 1 year. FeNO was measured at each visit and the ICS dose was increased according to a pre-set algorithm if the FeNO was >30 ppb. ICS dose could be decreased if the FeNO was <30 and symptom score was below a pre-set threshold. Dose adjustment in the control group was based on symptom score alone. The primary endpoint was cumulative steroid dose prescribed over the study period. In this study of allergic asthmatics, use of FeNO to adjust steroid dose did not result in a decrease in cumulative dose, but did result in a reduction in airway hyper-responsiveness as measured by methacholine challenge test. There was a 2.5 doubling dose increase in the FeNO group *versus* a 1.1 doubling dose in the control group with a significant 1.3 doubling dose between-group difference. However, there was no difference between groups in the symptom scores, short acting β -agonist use or symptom free days. FeNO remained stable in the FeNO group, but increased 32% in the control group. These data demonstrated that use of FeNO in allergic asthmatics resulted in an improvement in airway reactivity without significant change in symptoms. Although the ICS dose in the eNO group was not lower than that in the control group, the measured improvement did not come at the expense of an increase in ICS dose. It should be noted that the mean baseline PD₂₀ in the FeNO was somewhat lower than that for the control group; it is possible that some of the effect on airway reactivity was due to regression to the mean.

Smith *et al.* [2005] conducted a randomized, single-blind, placebo-controlled trial in which adolescent and adult asthmatics (age range 12–73 years, mean 44.8) requiring treatment with ICS, were assigned to a management strategy for inhaled steroid adjustment based on standard clinical measures (daytime symptoms, nighttime waking, bronchodilator use, FEV₁ and PEFR variability) or solely with FeNO level. The optimal dose of inhaled fluticasone necessary to maintain asthma clinical control (control group) or a FeNO level <15 ppb (FeNO group) was determined over a period of 2–12 months. Further taper of ICS dose based on symptoms or FeNO was then made for the ensuing year, with visits conducted every 2 months. The primary endpoint in this study was frequency of exacerbations

Table 2. Use of FeNO to manage chronic asthma.

Study	n	Age (years)	Primary outcome	FeNO cutoff	Study duration	Results
Smith 2005	97	12–75	Exacerbation	35 ppb*	1 year [†]	No difference
Pijnenburg 2005	89	6–18	ICS dose	30 ppb	1 year	Lower ICS in FeNO group
Shaw 2007	118	>18	Exacerbation	26 ppb	1 year	No difference in ICS dose Improved AHR
Fritsch 2006	47	12–20	FEV ₁	20 ppb	1 year	No difference in exacerbations Lower final daily dose of ICS in FeNO group
Szeffler 2008	546	12–20	Symptoms days	20 ppb	1 year	No difference in FEV ₁ Higher doses of MEF _{50%} and ICS in FeNO group
De Jongste <i>et al</i> 2009	151	6–18	% symptom-free days	20 ppb 25 ppb >10 years	30 weeks	No difference in symptoms days Fewer prednisone courses No difference in symptom-free days; trend towards fewer exacerbations

ICS, inhaled corticosteroid; ppb, parts per billion.

*Measured at 250 ml/s flow rate; [†]plus run-in lasting 3–12 months.

(defined as minor or major depending on degree of change in symptom score) and the secondary outcome was mean daily dose of ICS. There were no significant differences between the groups in numbers of exacerbations, although there was a non-significant 45.6% reduction in the FeNO group. There was no difference in any other marker of inflammation or lung function between the groups. However, the mean daily dose of fluticasone was less in the FeNO group (370 µg/day *versus* 641 µg/day). The treatment algorithm may have favored a reduction of ICS dose more in the FeNO group than in the control group. Nevertheless, the authors concluded that use of FeNO allowed better titration of ICS dose to control airway inflammation and symptoms responsive to inflammation than did use of symptoms and rescue medication usage. They argue that once inflammation is controlled as measured by FeNO, any residual symptoms are caused by other factors and are unlikely to respond to further increase in ICS dose.

In a larger ($n = 119$) but similar trial, [Shaw *et al.* 2007] adult asthmatics (median age 50 years) who had ICS dose titrated using symptoms and clinical measures [British guidelines, 2003] or FeNO were studied in a single blind, randomized year-long trial. Again, there was no difference in the primary outcome, exacerbations requiring oral corticosteroids or antibiotics between the treatment groups. There was also no difference in PEFR, FEV₁ or Juniper asthma control questionnaire scores [Juniper *et al.* 1999]. Those in

the FeNO group had a higher cumulative dose of ICS over the course of the study (11%, $p = 0.40$), but had a lower daily dose by the end of the study (557 µg/day *versus* >895 µg/day, $p = 0.028$).

Similar results were obtained in a study performed on mild-to-moderate asthmatic children (mean age 12 years); however, in this trial, measure of FeNO was added to standard clinical measures of symptoms, short acting β -agonist (SABA) use and pulmonary function [Fritsch *et al.* 2006]. This trial used a complex treatment algorithm and permitted use of leukotriene receptor antagonist and long acting β -agonist in addition to ICS. Over the course of the 6 month follow-up, there was no significant difference in the primary outcome, FEV₁, or any other measure (symptoms, SABA use, exacerbations). Using a cut point of 22.9 ppb eNO provided the best predictor for exacerbations, with a sensitivity of 80% and specificity of 60%, based on the FeNO measure occurring at the visit prior to the exacerbation. Children in the FeNO group also received higher doses of ICS (316 µg/day *versus* 241 µg/day). A single measure of pulmonary function, the MEF₅₀, was significantly higher in the eNO group compared with controls (83.2 *versus* 68.5% predicted). Of note, this trial did not use FeNO elevation alone as the criterion for increasing the ICS dose, but rather first attempted to improve adherence to the currently prescribed dose. Only when FeNO was elevated in the presence of increased need for albuterol was the ICS dose raised.

A more recently published large, randomized double-blind trial also examined the effect of adding FeNO to measures recommended in the NHLBI EPR 2 guidelines (daytime and nighttime symptoms report, SABA use and FEV₁). This trial was conducted as part of the Inner City Asthma Consortium and randomized 546 adolescents and young adults aged 12–20 years, who had moderate asthma [Szeffler *et al.* 2008]. The participants were all residents of central urban areas and of African-American or Hispanic origin. Following a run-in period during which treatment was optimized based on level of clinical control and pulmonary function, participants were randomized to a control group in which treatment level was adjusted according to symptoms and FEV₁ or an eNO group that added FeNO measure (symptoms, PFT and FeNO). Treatment decisions were made using a computer program with the option for investigator override or adjustment; the treatment steps were based on the EPR 2 guidelines and included ICS, long acting β -agonists (LABA), montelukast and theophylline. The strategy in this trial permitted increasing the treatment level for elevation in the FeNO, but the dose could not be reduced for a low FeNO without a concomitant low level of symptoms. Patients were eligible for a steroid reduction if adherence was at least 50% and symptoms remained well-controlled for at least two consecutive visits. Participants were seen every 6–8 weeks and all study medications were provided. The primary outcome was symptom days. In this trial, most patients in both groups (71–78%) came under good asthma control during the run-in period and remained controlled throughout the ensuing 46 weeks. There was no difference in symptom days between the two groups throughout the study. In addition, there was no difference in LABA use, adherence to treatment, or FeNO between the groups. However, only 35% of participants had a FeNO <20 ppb during the course of the study. Although there were also no differences in the occurrence of emergency visits or hospitalizations for asthma between the two groups, those in the FeNO group had fewer courses of oral steroid prescribed. Moreover, those in the FeNO group received a higher dose of ICS throughout the course of the study and were receiving an average of 118 μ g/day higher dose of fluticasone at the study's end. The marked improvement during the run-in period and the treatment algorithm that did not permit step down in treatment for a lower FeNO without symptom reduction may have influenced the

result in this trial. A *post hoc* analysis suggested that participants who were overweight (BMI >30) and those with multiple positive skin tests for allergy, did benefit from the addition of FeNO monitoring with improved symptom control.

A recently published study examined the utility of daily FeNO monitoring done at home compared with standard care [De Jongste *et al.* 2009]. In this trial, 151 children and adolescents (6–18 years, mean age 11.6 years) were randomized to an asthma management strategy using daily in-home monitoring of FeNO plus recording of symptoms (wheezing, shortness of breath, cough and sleep disturbance) and use of rescue medication, or symptom monitoring alone. The FeNO cutoff for adjusting ICS dose was 20 ppb for children 6–10 years of age and 25 ppb for those over 10 years; a cumulative symptom score of 60 was used. The trial period was 30 weeks, with the primary outcome being symptom-free days occurring over the last 12 weeks of the study. Symptom report was collected using an electronic diary; these data and the FeNO readings were transmitted daily. Useable data were obtained for 86% of FeNO measures and 79% of diary readings. Participants in both groups experienced an improvement in the number of symptom-free days and a reduction in ICS dose. However, there was no significant difference in the symptom-free days or reduction in ICS dose between the groups. There was a trend toward fewer hospitalizations in the FeNO group ($p = 0.10$), but there was no significant difference in the number of exacerbations defined as need for prednisone or emergency visits. Although FEV₁ improved significantly in both groups, there was no between-group difference. The authors concluded that although daily telemonitoring of FeNO and symptoms is feasible in children and adolescents, the addition of FeNO measures did not achieve significant improvement in any important clinical or pulmonary function outcomes.

Although the randomized controlled trials outlined above all have somewhat different designs, eNO cutpoints, treatment algorithms and outcomes, the reported results are quite consistent: the routine use of FeNO as a guide to chronic adjustment of anti-inflammatory treatment in asthma offers little benefit. None of the studies demonstrate a clear improvement in asthma control, significant reduction in exacerbations or a lower ICS burden when FeNO is used, compared, or in addition to standard clinical

measures. Such a result is somewhat disappointing but not totally unexpected. Treatment with ICS is directed at underlying eosinophilic airway inflammation. Since not all airway inflammation in asthma is caused by eosinophils, particularly in severe asthma where neutrophils may be more important, FeNO will not always indicate the presence or degree of inflammation. It is also clear that while the correlation of FeNO with airway eosinophilia is good, it is not ideal. The use of induced sputum eosinophils has consistently performed as well or better than FeNO in studies of asthma control [Zacharasiewicz *et al.* 2005; Jones *et al.* 2001]. Another problem with all of the aforementioned studies is the choice of FeNO cutoff at which treatment is adjusted. All used somewhat different FeNO levels, ranging from 20 ppb to 35 ppb (Table 2). FeNO levels are influenced by a number of factors, including: presence of atopy, age, height, race, smoke exposure, respiratory viral infection and season [Dressel *et al.* 2008; Olin *et al.* 2007; Sanders *et al.* 2004; Maniscalco *et al.* 2002; Yates *et al.* 2001]. Therefore, it is difficult to determine an optimal and accurate eNO level above which treatment should be adjusted in samples of asthmatics that span large age ranges, have variable levels of atopy, differ in race and are sampled at various times of the year. Use of an individual 'best-baseline' FeNO with determination of significant change from baseline cutoff points might be of more use to the individual patient, but such a strategy has not yet been investigated. A recommendation that repeated FeNO measures be used for the routine management of asthma in children and adolescents cannot be made at this time.

Predicting relapse in response to ICS withdrawal

In addition to adjustment of medication in order to maintain asthma control, using FeNO to predict relapse in individuals who have had ICS dose reduced or stopped has also been examined. In a study of 40 children aged 6–18 years (mean age 12.2 years) who were eligible for steroid withdrawal due to clinical disease quiescence for 6 months, FeNO was predictive of relapse [Pijnenburg, 2005b]. In this trial, relapse was defined as need for oral steroids, an increase in SABA use, or diurnal PEFR variability >20%. During this 26-week-long study, patients had FeNO measured at baseline, 2, 4, 12, 24 weeks and spirometry performed at baseline, 12 and 24 weeks. In all, 24% ($n=9$) relapsed; there

was no difference between the groups who relapsed and those who did not in any clinical measure at baseline, including ICS dose, FEV₁, PEFR, FeNO. A FeNO value of 49 ppb had the best performance in identifying those with relapse at 4 weeks (sensitivity 71%, specificity 93%, PPV 71%, NPV 93%). Nevertheless, this was a small study, there was wide variability in FeNO measures and more participants had elevated FeNO who did not relapse than those that did. In a similar study, Zacharasiewicz *et al.* [2005] reported that children and adolescents with a FeNO >22 ppb measured 8 weeks after a steroid taper was indicative of a future exacerbation. However, there were relatively few exacerbations in this trial, as well as wide inter-individual variability in FeNO response. In fact, almost 40% of those with FeNO >22 ppb tolerated a reduction without exacerbation. However, when another measure of inflammation—sputum eosinophils—was examined, all of those with no sputum eosinophilia and who were asymptomatic during the taper, tolerated the reduction without exacerbation.

A recent study by Michils *et al.* [2008] examined the degree of change in FeNO over time and with treatment in an unselected sample of adult asthmatics. Adult asthmatics ($n=341$), mean age 41 ± 16 years, were enrolled from those presenting to an allergy clinic for treatment of persistent asthma. Over a third of the patients were newly diagnosed and steroid naïve; 17 had severe asthma. Patients performed FeNO, spirometry and completed an Asthma Control Questionnaire [Juniper *et al.* 1999]. Follow up measures were available in a subset of patients seen after 3 months of initiating or changing therapy. The degree of change in FeNO from baseline was examined as an indicator of asthma control compared with the Asthma Control Questionnaire (ACQ) which was considered the gold standard. In the group of patients who had suboptimal control at baseline and were treated with low doses of ICS, a 40% reduction in FeNO at follow-up was a reliable predictor of gain of asthma control that persisted for at least 3 months (PPV 80%). Although the change in FeNO in patients with more severe asthma who required high (>1000 mg BDP equivalent/day) doses of ICS was less predictive of change in asthma control, even in these patients, rise in FeNO <15 ppb indicated that a change in asthma control was unlikely. These authors suggest that use of a personalized FeNO profile,

based on degree of change in sequential measures of FeNO in response to treatment, might be of more clinical use than a fixed cutoff for all patients. A similar study has not been done in children or adolescents.

Summary

The use of FeNO as a biomarker for the management of asthma in children and adolescents on face value seems ideal. It is non-invasive, easy to perform even for young children, requires limited operator training and skill and can now be reliably measured using a relatively inexpensive and durable piece of equipment. Are there indeed enough data to support the widespread use of FeNO as an 'inflammometer' for the airways that will guide asthma treatment to a new level of safety and efficacy? Unfortunately, the answer thus far is 'no'. However, what can we say about how to use this measure now and what can we still hope to learn?

First, the measure of FeNO can provide good diagnostic help in identifying children, adolescents and adults with asthma when standard clinical measures are not definitive. For the child with a history of respiratory complaints consistent with but with no physical evidence of airflow obstruction, an elevated FeNO can reliably indicate the diagnosis of asthma with a higher degree of certainty than baseline spirometry. In many instances, FeNO may be easier to obtain (e.g. in younger children) and less costly than bronchodilator responsiveness or inhalation challenge testing. However, distinguishing the patient with atopy alone without asthma is not possible using FeNO. Next, identifying the patient who is likely to benefit from treatment with ICS as opposed to other non-steroidal asthma treatment options (e.g. montelukast) can be aided by elevated FeNO. Moreover, failure of the FeNO to decrease after several weeks of treatment with ICS suggests either poor adherence to or improper use of treatment, or significant underlying allergy and continued allergen exposure, or 'constitutively high' eNO production. In these instances, the clinician would pursue further investigation and discussion with the patient rather than increasing medication.

In what situations might FeNO be of help, but with some degree of caution? FeNO may have some utility as an aid to predicting loss of asthma control or an impending exacerbation during taper or discontinuation of ICS treatment. Stepping down the dose of ICS when asthma

control is maintained for at least 3 months is recommended in current guidelines; can FeNO help predict when this dose reduction will be successful? Substantial reduction in baseline FeNO or a reduction to a near-normal range suggests that the remission on a lower dose of ICS will be sustained at least for a period of months [Michils *et al.* 2008; Pijnenburg *et al.* 2005a; Zacharasiewicz *et al.* 2005]. However, the degree of reported misclassification renders this measure of limited use as a stand alone test for predicting success in tapering steroid dose. In contrast, other data indicate that the absence of eosinophils in induced sputum may be a better measure than FeNO for identifying the asymptomatic patient who will tolerate a lower dose of ICS [Green *et al.* 2002; Jones *et al.* 2001].

Lastly, what of using FeNO to routinely adjust controller medication use in children and adolescents with asthma to achieve good asthma control, prevent exacerbations and use the lowest possible ICS dose? Based on the most recent clinical trials, the answer appears to be 'not necessary and not for everyone'. However, FeNO may be useful when there is discordance reported between patient-reported symptoms and inflammation [Halder *et al.* 2008]. In patients who report few symptoms, but have significant airway eosinophilic inflammation, the use of eNO to titrate ICS dose could lead to fewer exacerbations. Likewise, in patients with high symptom report, but with little airway eosinophilia, such as some obese asthmatics, FeNO measures could lead to ICS dose reduction without adverse effect and to use of other treatments which may be more effective [Szefer *et al.* 2008]. The decision as to when and how to use FeNO in the adjustment of treatment in asthmatics will still need to be based on individual patient characteristics and phenotype. The accurate classification of patient phenotype awaits further research.

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