
Exhaled nitric oxide as a predictor of exacerbation in children with moderate-to-severe asthma: a prospective, 5-month study

Anna Lúcia B. Cabral, MD*; William M. Vollmer, PhD†; Rosa M. Barbirotto, MD*; and Milton A. Martins, MD‡

Background: Inhaled corticosteroids (ICSs) are recommended as the first line of treatment in children with moderate-to-severe asthma. Exhaled nitric oxide (ENO) has been proposed as a clinically useful marker of control that might help identify patients in whom ICS dose may be safely reduced.

Objective: To evaluate the ability of ENO to predict future asthma exacerbations in children with moderate-to-severe asthma undergoing ICS tapering.

Methods: This is an observational study with no control group. ENO was measured biweekly for 14 weeks in 32 children with moderate-to-severe asthma who were undergoing ICS tapering. Clinical evaluations and spirometry were performed concomitantly, and families kept daily diaries to record symptoms between visits. We used generalized estimating equations to model the ln (odds) of an asthma exacerbation in the subsequent 2-week interval as a function of ENO level at the start of the interval while adjusting for age, sex, asthma severity, and current medication use.

Results: We were able to successfully lower ICS doses in 10 (56%) of the 18 children with moderate asthma and in 3 (21%) of the 14 children with severe asthma. In 83 of the 187 follow-up clinical evaluations, children were determined to have had an exacerbation during the preceding 2 weeks. ENO levels, whether expressed as a continuous variable or dichotomized, were not associated with future risk for exacerbations in either unadjusted or adjusted models.

Conclusion: ENO was not a useful clinical predictor of future asthma exacerbations for children with moderate-to-severe asthma undergoing ICS tapering.

Ann Allergy Asthma Immunol. 2009;103:206–211.

INTRODUCTION

Although inhaled corticosteroids (ICSs) are the recommended first-line treatment for asthma in children,^{1–3} long-term use can lead to systemic effects,^{4,5} and, therefore, the lowest dose needed to maintain good asthma control should be used. Because of the great variability of the dose required both between children and within individual children, safe methods to guide titration are highly desirable. Once a patient has achieved control over a period, the health care provider can carefully reduce the ICS dosage while retaining good control to help minimize adverse effects. The risks of ICS tapering include worsening of control, which may lead to increased school absenteeism, emergency department visits, and hospital admissions.⁶

According to current guidelines,^{2,3} dose adjustments should be based on asthma symptoms and the results of spirometry. Alternative criteria to improve outcomes have been proposed, including markers of inflammation^{7–18} and hyperresponsiveness.¹⁹ Sputum eosinophils and exhaled nitric oxide (ENO) have been used most often as measurements of inflammation. ENO has received particular interest because such measurements are considerably faster and easier to obtain than sputum eosinophil level or hyperresponsiveness. However, studies examining the ability of ENO to predict future loss of control or exacerbation have yielded inconsistent results.^{13,18,20–22}

The utility of ENO for monitoring children with moderate-to-severe asthma is particularly uncertain. In many of these children, ENO levels have remained persistently high despite appropriate treatment and good asthma control.^{23–27} Also, because of the high variability of ENO levels between and within patients,^{27–30} it is difficult to establish meaningful cut points to guide asthma management. Different cut points have been proposed for adults and children,^{14,16,18,20,31,32} but do these values apply for all levels of asthma severity? Other factors also are known to influence ENO levels, such as atopy, cigarette smoking, and upper respiratory tract infection. These limitations have tempered enthusiasm for using ENO as a monitoring tool and, thus, its role in asthma management remains unproved.

Affiliations: * Division of Pulmonary Diseases, Darcy Vargas Hospital, Sao Paulo, Brazil; † Kaiser Permanente Center for Health Research, Portland, Oregon; ‡ Department of Medicine (LIM-20), School of Medicine, University of Sao Paulo, Sao Paulo, Brazil.

Disclosures: Authors have nothing to disclose.

Funding Sources: This study was supported by the following Brazilian scientific agencies: Fundação de Amparo à Pesquisa do Estado de São Paulo and Conselho Nacional de Desenvolvimento Científico e Tecnológico.

Previous Presentation: Presented in part at the International Meeting of the American Thoracic Society; San Francisco, California; May 22, 2007.

Received for publication December 15, 2008; Received in revised form February 16, 2009; Accepted for publication March 26, 2009.

To better understand the utility of ENO as a tool to safely guide ICS tapering in children with moderate-to-severe asthma, we studied 32 children with moderate-to-severe asthma who were undergoing a period of ICS tapering.

METHODS

Study Population

We examined all 104 children aged 7 to 14 years who met the criteria for moderate-to-severe asthma¹ (based on current history, stability of disease in the previous year, rates of exacerbation, medication needs, and pulmonary function) and, as of the start of the study protocol in October 2004, had been receiving care from us at the outpatient clinic of Hospital Infantil Darcy Vargas, Sao Paulo, Brazil, for at least 12 months. From this group, we excluded 72 children: 34 who had not been regularly taking their ICS medications during the previous 12 months, 18 whose asthma was poorly controlled (explained later) at the time of enrollment into the protocol, and 20 who had a documented respiratory tract infection or an acute asthma exacerbation in the 3 months before the start of the protocol.

For purposes of enrollment, we defined poor control as daytime symptoms more than twice per week, reliever medication use more than twice per week, any nocturnal awakening due to asthma symptoms, limitation of daily activities and exercise, and a forced expiratory volume in 1 second (FEV₁) of less than 60% predicted. Children under our care recorded symptoms and morning peak flow in a diary that they were asked to bring to the hospital at their regular bimonthly visits. At each visit, these diaries were collected, spirometry was performed, and a medical record notation was made as to whether the child's asthma was controlled or not. To enter the study, children had to have had stable asthma for the previous 3 months.

At the time of enrollment, all children had a history of at least 1 positive skin test result or specific IgE response to a panel of common aeroallergens, with the most common response being to dust mites.

A total of 32 children met the eligibility criteria and were invited to participate in the study. All agreed, and both parents and children provided written informed consent. The study protocol was approved by the local research ethics committee.

Study Design

The study protocol took place between October 1, 2004, and March 31, 2005, and was divided into 2 periods: an initial 2-month run-in period and a 14-week tapering period. ENO measurements were taken only during the tapering phase, which spanned the period from December 2004 through March 2005. We chose this time frame (our summer season) to minimize the risk of viral infections, which are known to influence ENO levels.^{27,33} (Sao Paulo has no pollen season because of its subtropical climate.)

Children attended the clinic every 2 weeks throughout the study period, at which times symptom diaries were collected,

asthma control and medication use were checked, and spirometry was performed. All medications were provided free of charge during the study, and children were asked to return their used inhalers before receiving new ones.

Run-in Period

During the initial 2-month run-in period, we adjusted the ICS medications for all participants to equivalent doses of fluticasone administered with a metered-dose inhaler. We also withdrew any long-acting β -agonists the children may have been taking to avoid the potential confounding effect of such medications on our primary outcome (exacerbations). Study staff taught children and parents proper inhalation technique and emphasized the importance of strict adherence to the study protocol. The ICS dose was held constant, and all 32 children remained in good control during this time.

Tapering Phase

During tapering, the dose of ICS was reduced by 25% every 2 weeks as long as the child remained *stable*, which for this phase of the study we defined as having no or mild symptoms, using less than 6 puffs of rescue medication during the last 14 days, and having an FEV₁ equal to or higher than the best FEV₁ recorded during the run-in period. If the patient did not remain stable, the ICS dose was either maintained or increased according to physician discretion; oral corticosteroids were given as needed for exacerbations.

ENO levels were measured a total of 7 times during this phase of the study, including the visit before the ICS withdrawal began. Throughout this time, both the treating physicians and the patients were blinded to the ENO levels.

Study Measurements

Throughout the study, children and their parents used diaries to record daily asthma symptoms, using scores ranging from 0 to 3 (0 indicates none; 1, mild; 2, moderate; and 3, severe). Separate scores were obtained for daytime and nighttime symptoms, including chest tightness, wheeze, cough, and shortness of breath. Children also recorded in the diaries missed school days, the presence of colds or other respiratory tract infections, and morning and evening peak expiratory flow rates. At each clinic visit, these diaries were collected and new diaries were given out.

We performed ENO measurements according to standards recommended by the American Thoracic Society³⁴ using an off-line single-breath exhalation technique before spirometry. The samples were collected in an impermeable bag during the morning. ENO was then measured by chemiluminescence using a fast-responding analyzer (NOA 280; Sievers Instruments Inc, Boulder, Colorado). The analyzer was calibrated with a certified 47-ppb NO source (White Martins, Brazil) and 0 NO filter (Sievers Instruments Inc) before each measurement.

We performed spirometry according to American Thoracic Society standard protocol³⁵ using a spirometer that features a brass pneumotach and combines a portable unit with a computer system (KoKo spirometer; PDS Inc, Ferraris Cardio-

pulmonary Systems Group, Louisville, Colorado). Children were asked to refrain from using their reliever medications for at least 4 hours before testing if possible. The results were interpreted according to the reference equations of Hankinson and coworkers.³⁶

Definition of Primary Outcome

The primary outcome for this analysis was an acute asthma exacerbation occurring between the biweekly clinic visits during tapering. We defined an *exacerbation* as an episode of increased daytime asthma symptoms and concomitant need for reliever use, nocturnal awakening for 3 nights in a row, a morning peak expiratory flow of less than 80% of personal best for 3 consecutive days, or an emergency department or unscheduled visit for worsening asthma. Exacerbations that overlapped adjoining biweekly intervals were considered to have occurred in the first interval.

Statistical Analysis

We used generalized estimating equations to model the ln (odds) of an exacerbation in the subsequent 2-week interval as a function of current ENO levels. We tested both unadjusted models and models that adjusted for the following: (1) exacerbations in the preceding 2-week interval, age, sex, and severity; and (2) these factors plus current medication use. Separate analyses were run for ENO as a continuous variable and for ENO as a binary indicator of risk. For the latter analyses, we used both a cut point of ENO of 22 ppb or greater, as suggested by Zacharasiewicz et al,¹⁸ and a cut point of ENO of 40 ppb or greater (close to the mean value for our population). Models were run using the xtgee procedure in Stata, version 9.2 (Stata Corp, College Station, Texas), with a first-order autoregressive error structure for repeat observations within individuals. Similar results were seen with an exchangeable error structure. We also conducted a receiver operating characteristic (ROC) curve analysis using the Stata roctab procedure.

RESULTS

Of the 32 children who began the study, 27 completed all 7 follow-up visits after the initiation of tapering; the other 5 completed all but the last follow-up visit. All are included in

the analysis. Study participants averaged 10.3 years of age, and 66% were male (Table 1).

At the beginning of tapering, the average FEV₁ was 90.0% of the predicted value and the average ENO level was 38 ppb. As expected, the clinical indicators were worse for the 14 children with severe asthma compared with the 18 with moderate asthma.

We conducted 187 follow-up clinical evaluations; in 83 of these evaluations, children were determined to have had an exacerbation during the preceding 2 weeks according to the criteria previously described. All but 4 of the children reported at least 1 exacerbation. During follow-up, we were able to successfully lower ICS use in 10 (56%) of the children with moderate asthma (with a mean decrease of 35% from the initial dose in these 10 children) and in 3 (21%) of the children with severe asthma (with a mean decrease of 25% from the initial dose in these 3 children).

ENO level, whether treated as a continuous variable or dichotomized into "at-risk" vs "not-at-risk" categories (regardless of the cut point used), was not associated with future risk for asthma exacerbations in any of our regression models (Table 2). The only factor that was associated with subsequent exacerbations in these models was an indicator of baseline severity (severe vs moderate). Even after further adjustment for exacerbations in the immediately preceding 2-week interval (along with continuous ENO, sex, and age), children with severe asthma had a 2.7-fold (95% confidence interval, 1.1–6.6) increased odds of having an exacerbation in the following 2 weeks when compared with children with moderate asthma (data not shown). We also found no evidence of any signal via ROC analysis (Fig 1).

DISCUSSION

Our results showed that ENO measurements were not a predictor of future exacerbation in children with atopic, moderate-to-severe asthma undergoing a period of steroid tapering.

Prior studies addressing the utility of ENO as a predictor of asthma exacerbations have shown conflicting results. Several studies failed to prove that ENO was a good predictor of loss of control or future exacerbation in adult^{9,22,37} patients while

Table 1. Baseline Characteristics of Participants^a

Characteristic	Participants with moderate asthma (n = 18)	Participants with severe asthma (n = 14)	Total (N = 32)
Male, No. (%)	12 (67)	9 (64)	21 (66)
Age, y	9.4 (1.9)	11.4 (2.1)	10.3 (2.2)
FEV ₁ , %	94.2 (16.2)	84.5 (10.3)	90.0 (14.6)
ENO, ppb	29 (13)	50 (20)	38 (20)
PC ₂₀ , mg/mL	0.15 (0.12)	0.33 (0.50)	0.23 (0.35)
Fluticasone dose, µg	667 (149)	893 (128)	766 (179)

Abbreviations: ENO, exhaled nitric oxide; FEV₁, forced expiratory volume in 1 second; PC₂₀, provocative concentration of methacholine producing a 20% decline in FEV₁.

^a Data are given as mean (SD) unless otherwise indicated.

Table 2. Data for Future Exacerbations Associated With ENO Levels

ENO level	Odds ratio (95% confidence interval)	P value (2-sided)
Continuous ^a		
Unadjusted	1.01 (0.99–1.02)	.29
Adjusted (excluding medication use) ^b	1.01 (0.99–1.02)	.53
Adjusted (including medication use) ^c	1.00 (0.99–1.02)	.55
Binary 1 ^d		
Unadjusted	1.12 (0.53–2.38)	.77
Adjusted (excluding medication use) ^b	0.94 (0.43–2.07)	.88
Adjusted (including medication use) ^c	0.90 (0.41–1.98)	.80
Binary 2 ^e		
Unadjusted	1.63 (0.90–2.95)	.11
Adjusted (excluding medication use) ^b	1.49 (0.81–2.74)	.20
Adjusted (including medication use) ^c	1.52 (0.83–2.79)	.18

Abbreviation: ENO, exhaled nitric oxide.

^a Odds ratios are expressed as ratio per 10-ppb increase in ENO.

^b Adjusted for prior exacerbations, disease severity, age, and sex.

^c Adjusted for prior exacerbations, disease severity, age, sex, and changes in medication.

^d At-risk (ENO ≥ 22 ppb) vs not-at-risk (ENO < 22 ppb) category.

^e At-risk (ENO ≥ 40 ppb) vs not-at-risk (ENO < 40 ppb) category.

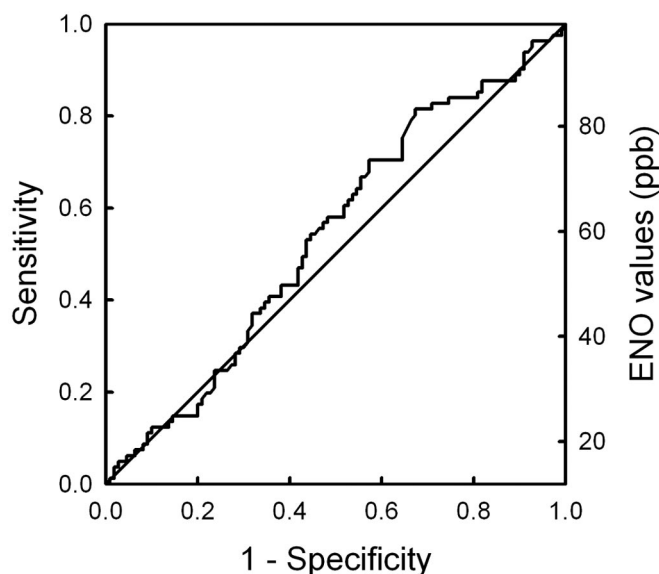


Figure 1. Receiver operating characteristic curve showing the sensitivity and 1-specificity over a range of cutoff points for exhaled nitric oxide (ENO) for predicting future risk of exacerbation.

tapering the ICS dose. More recently, Menzies et al²² evaluated the relationship of ENO levels and exacerbation in primary care, where most patients are routinely treated. They

reported that ENO level was an insensitive method for identifying patients who subsequently had an exacerbation and may, therefore, be of limited utility in primary care.

On the other hand, Jones et al⁷ evaluated the clinical utility of routine monitoring of ENO level for predicting future asthma exacerbations in adults and reported that both single measurements and changes of ENO (10 ppb, or an increase of $>60\%$ over baseline) had positive predictive values of 80% to 90%. Harkins et al,¹³ evaluating adult patients with moderate-to-severe persistent asthma during a routine clinic visits, found significantly higher mean ENO levels in those with an exacerbation.

Few studies have evaluated the utility of ENO as a biomarker of asthma control in children.^{15,18,21,38} Most studies have evaluated heterogeneous groups of children with regard to asthma severity, ICS use, and dose taken, with no consideration of the presence of atopy or seasonality that influence ENO levels.

Zacharasiewicz et al¹⁸ observed 40 children with stable asthma every 8 weeks during steroid tapering and found that an ENO level of 22 ppb or greater was a significant predictor of loss of asthma control. This study, however, has some limitations. It was based on only 15 exacerbations, the period between measurements was quite long, and the potential effects of seasonality, viral infection, and adherence were not clearly addressed.

Fitzpatrick et al²¹ observed 12 children with mild-to-moderate asthma and 16 with severe asthma, nearly all of whom were atopic, for 6 months to identify baseline characteristics associated with subsequent exacerbations. Similar to our results, they found that baseline ENO level was not a predictor of future exacerbations, although several markers of asthma severity ($FEV_1 < 80\%$ predicted, $FEV_1:FVC$ ratio < 80 , and daily nocturnal symptom occurrence) were significant predictors of future exacerbations. They also found significantly lower levels of ENO in children with mild-to-moderate asthma, suggesting that ENO may be a marker of asthma severity. A similar finding had already been reported in the Childhood Asthma Management Program study.³⁹

Recently, Covar et al,⁴⁰ using a regression model to identify factors associated with occurrence of exacerbations in the 48-week Pediatric Asthma Controller Trial Study, reported that in children with mild and moderate asthma available biomarkers were not reliable predictors of exacerbations.

Studies addressing the inclusion of ENO measurements into routine use in outpatients with asthma failed to demonstrate a reduction in subsequent exacerbation rates or an increase in the effectiveness of asthma treatment.^{16,22,31,32} Tailoring the dose of ICSs based on ENO in comparison to clinical symptoms has been performed in different ways, and the results show only modest differences.⁴¹

Recently, Jongste et al⁴² evaluated the contribution of ENO daily monitoring to improve asthma control and found no added value. This study addressed children with mild-to-moderate atopic asthma in a hospital-based selection of patients, whereas our study addressed severe asthma with fre-

quent exacerbations; we also found no benefit in predicting exacerbations while adjusting ICS doses.

Currently, there is no consensus in the literature as to what might be an optimal ENO cut point level to define high risk of exacerbation. Uncertainties exist about what are high and low ENO values. Two studies showed children with persistent high ENO values despite steroid treatment.^{23,24} The response of ENO to ICS varies from patient to patient, and some children have persistent high values despite having their asthma controlled and receiving a high dose of ICS.²³ Both allergy and bronchial hyperresponsiveness drive ENO levels independently of high-dose steroid treatment.²³ Our ROC curve analysis leads us to conclude that our findings would not have changed regardless of the value of ENO chosen.

In clinical practice, the characteristics of the patients vary: ICS doses and asthma severity vary, atopy may or may not be present, and there is a wide range of airway hyperresponsiveness among patients. The ability of ENO to reflect asthma control may vary within these subgroups of patients, and the ability of ENO to reflect asthma control is reduced in patients using high doses of ICSs.³⁸ Atopy is known to raise ENO values independent of lower respiratory tract symptoms.⁴³ Covar et al³⁹ found higher median (first-third quartile) levels of ENO in children with atopic asthma compared with non-atopic children (61.4 [20–115] vs 11.6 [8–25] ppb) and in children with moderate-to-severe asthma compared with mild asthma (83.4 [36–124] vs 34.6 [16–98] ppb). Compared with some other pediatric studies,^{15,18,31} we found higher levels of ENO in our controlled patients (mean, 38 [SD, 20] ppb) than did Zacharasiewicz et al¹⁸ (ENO levels ranged from 8.5 to 33.0 ppb). These differences may possibly be explained by differences among the 2 groups studied, mainly asthma severity and the presence of atopy.

For the reasons previously cited, we limited our population to atopic children with moderate-to-severe asthma, thus purposely reducing its heterogeneity and consequently reducing generalization. However, asthma is a complex collection of biochemical abnormalities superimposed on allergic inflammation⁴⁴ and is not a single disease. Many phenotypes have already been described, and each one may have particular abnormalities. Therefore, ENO levels vary among different asthma phenotypes, making it almost impossible to find a specific marker suitable for all asthmatic patients. Furthermore, this subgroup of patient is the one that will most benefit from ENO as a marker of control because these patients are at higher risk of exacerbation.

We also chose to treat the patients with the same ICS (fluticasone) with no concomitant long-acting β -agonist or antileukotriene use. By controlling these factors, we avoided the concurrent effect of long-acting β -agonist use on our primary outcome (exacerbations). An additional strength of our study is that we did not have to adjust for the influence of pollen or viral infections because of the season in which we chose to conduct the study. None of the published studies previously cited included such a large or relatively uniform

group of asthmatic patients or such a well-controlled medication regimen.

In summary, our results showed that monitoring ENO values is of no value for tapering ICS doses in atopic children with moderate-to-severe asthma. Based on the available evidence, we believe the value of ENO measurements as a predictor of exacerbation appears limited.

ACKNOWLEDGMENTS

We acknowledge the contribution of the American Thoracic Society's Methods in Epidemiologic, Clinical and Operations Research course in the development of this article.

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Requests for reprints should be addressed to:
Milton A. Martins, MD
Departamento de Clínica Médica
Faculdade de Medicina da Universidade de São Paulo
Av. Dr. Arnaldo
455-Sala 1210
01246-903 São Paulo SP, Brazil
E-mail: mmartins@usp.br