

The Use of Exhaled Nitric Oxide in the Diagnosis of Asthma in School Children

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Objectives To evaluate the yield of the fractional exhaled nitric oxide (FeNO) in the diagnosis of asthma compared with spirometry and induced sputum cytologic study in school-age children.

Study design Consecutive children referred for evaluation of possible asthma were included. At referral, all children completed FeNO measurement, sputum induction for eosinophil count (eos%) and spirometry. The diagnosis of asthma was performed after 18 months with conventional criteria. Receiver operating curves were used to determine cutoff points for disease status, and accuracy was calculated.

Results A total of 150 children were included: 69 with steroid-naïve asthma, 44 without asthma, and 37 with asthma treated with controllers. FeNO and eos% levels were significantly higher in those with steroid-naïve asthma ($P < .0001$). The area under the receiver operating curve for FeNO and eos% were very high compared with forced expiratory volume in 1 second (0.906, 0.921, 0.606, respectively). The sensitivity, specificity, and positive and negative predictive values for best cutoff points of FeNO (19 parts per billion) were 80%, 92%, 89%, and 86%, respectively, and were similar to eos% (best cutoff = 2.7%): 81%, 92%, 89%, 85%, respectively.

Conclusions FeNO measurement is useful in early diagnosis of pediatric asthma. We suggest considering FeNO measurement in the evaluation of children suspected of having asthma, especially in cases where the diagnosis is not clear. (*J Pediatr* 2009;155:211-6).

Asthma is a chronic inflammatory disorder characterized by the presence of inflammatory cells and the release of inflammatory mediators in the airways. Fractional exhaled nitric oxide (FeNO) is a marker of the airway inflammation. FeNO levels are elevated in asthma, especially when eosinophilic inflammation is present, and predict response to steroid treatment.¹

Conventional measurements for the diagnosis of asthma include symptoms reported by the patient, measurements of airway obstruction by spirometry, reversibility with response to bronchodilators, and assessment of bronchial hyperactivity by provocation tests.² However, self-reporting of symptoms may not always be reliable, especially in children.³ Spirometry and bronchial challenge tests are prone to cooperation problems, decreased compliance, and variable reproducibility. Although airway inflammation may be reflected by the degree of airway obstruction and hyperresponsiveness, their relationship is not a simple one. Children with mild-to-moderate asthma frequently have normal baseline values of forced expiratory volume in 1 second (FEV₁). Hence, tools to measure the status of airway inflammation would be extremely helpful. This may be assessed by measuring the percentage of eosinophils (eos%) in the induced sputum. However, measurement of the fraction of exhaled nitric oxide (FeNO) may be an easier, more practical, and less expensive method for this purpose.

FeNO as a measure of inflammation has been suggested as offering the best combination of disease evaluation and practical “implementation” for improved asthma outcomes.⁴ The place of FeNO measurement in children with asthma is still under debate.⁵ Conflicting results have been reported for FeNO levels in patients with asthma compared with healthy control subjects and its possible diagnostic yield in both adults^{6,7} and children.⁸⁻¹¹ Differences in methodologic factors and patient selection are the main reasons for these inconsistencies.¹² The purpose of this study was to evaluate the yield of the fractional exhaled nitric oxide (FeNO) in the diagnosis of asthma compared with spirometry and induced sputum cytologic study, by analysis of the accuracy of each method in the diagnosis of asthma in school-age children.

AUC	Area under the receiver operating curve
eos%	Percentage of eosinophils
FeNO	Fractional exhaled nitric oxide
FEV ₁	Forced expiratory volume in 1 second
ICS	Inhaled corticosteroid
NPV	Negative predictive value
ppb	Parts per billion
PPV	Positive predictive value
ROC	Receiver operating curve

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Methods

Consecutive children referred for possible asthma to the outpatient pediatric pulmonary clinic of the Dana Children's Hospital at the Tel-Aviv Medical Center were included. Inclusion criteria were (1) nonspecific respiratory symptoms suggestive of asthma for at least 3 months' duration, including cough, wheezing, and shortness of breath with or without trials of treatment with bronchodilators and inhaled corticosteroids; (2) children were cooperative and successfully completed all 3 tests: FeNO measurement, sputum induction, and spirometry; and (3) follow-up at our clinic for at least 1 year. Exclusion criteria included patients with other conditions that could affect FeNO or sputum eosinophil count, including subjects with symptoms of unresolved respiratory tract infection, with systemic clinical manifestations of atopy such as anaphylaxis, angioedema, food allergy, urticaria, or with an underlying systemic or inflammatory disease. Patients with a history of allergic rhinitis or eczema were not excluded because these are common manifestations in atopic asthma. FeNO measurement, sputum induction for analysis, and spirometry were performed in all cases at referral on the same day (at or within 1 week of the first visit). FeNO measurement was performed first followed by spirometry and sputum induction 1 to 2 hours later. All procedures were carried out between 09:00 and 12:00.

Assessment of asthma status was performed by a certified pediatric pulmonologist after at least 18 months of follow-up. Asthma was diagnosed by use of conventional clinical criteria and was based on the patient's history of 2 or more clinical exacerbations of wheezing documented by a physician, dyspnea, or cough relieved by bronchodilators, documented variability in $FEV_1 \geq 15\%$ in response to bronchodilators at any time during the follow-up period (reversibility),¹³ or documented variability in $FEV_1 \geq 15\%$ over time with or without controller medications: inhaled corticosteroids (ICS) or montelukast. Results of provocation tests were included when available. At final diagnosis, the pulmonologist was blinded with regard to the results of the FeNO and eos%. Children in whom asthma did not manifest within 18 months of follow-up were considered as not having asthma. Information collected for all participants included data of FeNO levels, spirometry and induced sputum analysis, and the use ICS or montelukast.

Spirometry was performed with a hand-held spirometer (Micro-lab spirometer ML3500/S, Micro-Medical, Basingstoke, United Kingdom) according to the ATS guidelines.¹⁴ Patients were asked to withhold short-acting bronchodilator for 24 hours. An $FEV_1 \geq 80\%$ of predicted or a ratio of FEV_1 to forced vital capacity (FVC) $\geq 75\%$ were considered to be within normal limits. Bronchodilator response was assessed by measuring change in FEV_1 after inhalation of albuterol 400 μg (4 puffs of 100 μg) delivered by an inhaler via a large-volume plastic spacer.

Sputum induction was performed with 3% saline inhalation for four 5-minute periods. After inhalation, subjects were encouraged to cough and expectorate any sputum. Spu-

tum was separated from saliva to avoid inclusion of squamous cells. Selected sputum (plugs separated from saliva) was processed within 2 hours as previously described,¹⁵ and the total nonsquamous cell count was performed. Differential cell counts were expressed as a percentage of 200 cells, excluding squamous cells. The cutoff for a positive eos% result was defined as $\geq 3\%$.^{6,15}

FeNO was measured online with a chemiluminescence analyzer (Eco Physics CLD88, NO chemiluminescence analyzer; EcoMedics AG, Duernten, Switzerland) and the Denox 88 NO free supplier module (EcoMedics AG) with online recording, during a single breath exhalation, according to the ERS/ATS guidelines and expressed as parts per billion (ppb).^{12,16} Breaths where FeNO was more than 10% off were discarded. The maneuver was repeated with 30 seconds of relaxed breathing between the measurements until 3 reproducible NO values were obtained, and the average was recorded. All children were instructed to avoid the following before the test: eating or drinking for 1 hour, any exercise for 3 hours, exposure to cigarette smoke for 24 hours, medications, alcoholic beverages, and lettuce on the morning of the test. The study was approved by the Hospital and Ministry of Health Ethics (Helsinki) committees.

Data Analysis and Statistics

First, FeNO values were compared with eos% in the induced sputum, regarded as the "gold-standard" for eosinophilic inflammation to assess the accuracy of FeNO as a marker of eosinophilic inflammation in the airways. The 3 variables (FeNO, eos%, and spirometry) were then analyzed compared with the diagnosis of asthma.

Demographic and clinical data were presented as means and standard deviation (SD) for continuous variable. Non-variable tests were also used when the distribution was not normal. Association between the continuous variables (FeNO, eos%, FEV_1) was evaluated by the Pearson and Spearman correlation coefficients. A *P* value $< .05$ was considered statistically significant.

Receiver operating curves (ROC) were used to determine optimal cutoff points for each disease status. Criteria used were: (1) eos% level (≤ 3 vs > 3) for the comparison of FeNO to eos%, and (2) asthma versus non-asthma for the assessment of the accuracy of FeNO, eos%, and spirometry for diagnosing asthma. Several cutoff points were considered for each variable.

For each selected cutoff points that significantly differentiated between patient and normal, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy (%), likelihood ratio of a positive response (sensitivity / [1 - specificity]) and likelihood ratio of a negative response ([1 - sensitivity] / specificity) with confidence interval (CI 95%) were accordingly calculated.¹⁷ The area under the receiver operating characteristics curve (AUC-ROC) was calculated for each diagnostic test with the method described by Hanley and McNeil¹⁸ and Altman and Bland.¹⁹⁻²¹ Statistical analysis was performed by

Table I. Characteristics of patients

Characteristic	Without asthma	Steroid-naïve asthma	Asthma treated with ICS
N	44	69	37
Age (year) mean, range	12.0, 7.0-18.0*	12.6, 5.0-18.0*	12.3, 6.0-18.0*
% male	55*	58*	52*
FEV ₁ (% predicted, mean ± SD)	86.1 ± 17.1 ^{†,‡}	79.3 ± 44.4 [§]	75.0 ± 16.0
FeNO (ppb) (mean ± SD)	12.6 ± 9 ^{,¶}	69 ± 17 [#]	36 ± 57
Eos% (mean ± SD)	1.8 ± 3.9 ^{,**}	18.7 ± 17.1 [#]	15.9 ± 15.9

**P* = not significant among all 3 groups.

[†]*P* = not significant compared with steroid-naïve asthma.

[‡]*P* < .05, no asthma compared with asthma treated with ICS.

[§]*P* < .05, steroid-naïve asthma compared with asthma treated with ICS.

^{||}*P* < .0001, no asthma compared with steroid-naïve asthma.

[¶]*P* = .047, no asthma compared with asthma treated with ICS.

[#]*P* = not significant compared with asthma treated with ICS.

^{**}*P* < .0001, no asthma compared with asthma treated with ICS.

a certified biostatistician using the Statistical Package for the Social Sciences for Windows software, release 14.0 (SPSS, Inc., Chicago, Illinois).

Results

One-hundred fifty children completed all 3 tests and complied with the inclusion and exclusion criteria. All were Caucasian. Six children who were unable to produce adequate sputum were not included in the study. Symptoms for which patients were referred were cough (*n* = 41), dyspnea (*n* = 31), wheezing episodes (*n* = 23), dyspnea on exercise (*n* = 9), cough and dyspnea (*n* = 19), cough and wheeze (*n* = 13), and dyspnea and wheeze (*n* = 14). None of the children were receiving long-acting beta 2 agonists at referral. At the final evaluation 69 patients were diagnosed as having steroid-naïve asthma, in 44 patients the diagnosis of asthma could not be established, and 37 children who were treated with ICS were diagnosed as having asthma. The clinical characteristics are shown in [Table I](#).

The area under the ROC for FeNO versus eos% was 0.886 ([Figure 1](#)). The best cutoff value was 18 ppb, which provided 82% sensitivity, 84% specificity, 89% PPV, and 75% NPV. Thus the accuracy of FeNO for estimating airway eosinophilia was very high. FeNO moderately correlated with eos% (*r* = 0.6, *P* < .005).

The mean FeNO and eos% levels were significantly higher in children with steroid-naïve asthma compared with children without asthma (*P* < .0001, [Table I](#)). With asthma used as the dependent variable, the AUC for FeNO and eos% were very high and were significantly better than for FEV₁ (0.906, 0.921, 0.606, respectively; [Figure 2](#)). The sensitivity, specificity, PPV, and NPV for selected cutoff points of FeNO levels are shown in [Table II](#). The specificity of the measurement of FeNO and eos% for the diagnosis of asthma were maximal at cutoff levels of FeNO >23 ppb and eos% >5%. The sensitivities were, however, maximal at FeNO levels >10 ppb and eos >1%. The best cutoff values, that is, those values that provided

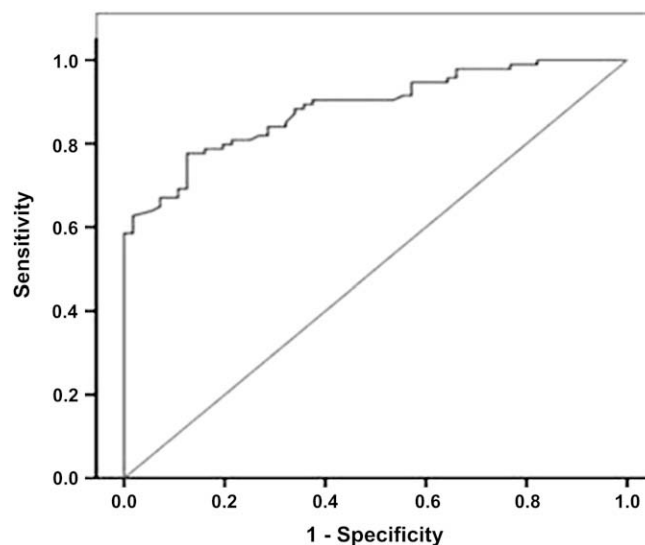


Figure 1. ROC for FeNO versus eosinophil count. The AUC = 0.886.

the highest combination of specificity and sensitivity indicating the best accuracy for the diagnosis of asthma and that had the highest likelihood ratio of a positive response and lowest likelihood ratio of a negative response values were 19 ppb for FeNO and 2.7% for eos%.

Applying both eos% and FeNO for the diagnosis of asthma, that is, that both best cutoffs were met did not significantly increase the accuracy ([Table II](#)) and the AUC (0.920). The application of both a high and low cutoff level, with the best cutoff values that provided maximal diagnosis accuracy for asthma (15 and 20 ppb), improved all accuracy variables ([Table II](#)). However, this resulted with the exclusion of 12/113 (10.6%) cases defining them as inconclusive or “borderline.”

Discussion

This study demonstrates that FeNO in children with asthma is significantly increased when compared with patients with similar symptoms in the absence of asthma. The measurement of FeNO as an inflammatory biomarker in patients with clinical suspicion of asthma provides very similar results to eosinophil count in induced sputum and offers a significantly higher diagnostic yield than the standard approach on the basis of spirometry.

A number of studies reported good sensitivity and specificity for raised FeNO in discriminating between adults and children with and without asthma.²²⁻²⁵ Only a few studies have investigated the diagnostic value of FeNO in an unselected pediatric population and compared it with eos%.

Studies in adults with similar protocols to ours, with follow-up periods from assessment to clinical diagnosis ranging 2 months to 2 years, showed similarly high levels of accuracy (sensitivity, specificity, PPV, and NPV).^{6,23} High accuracy

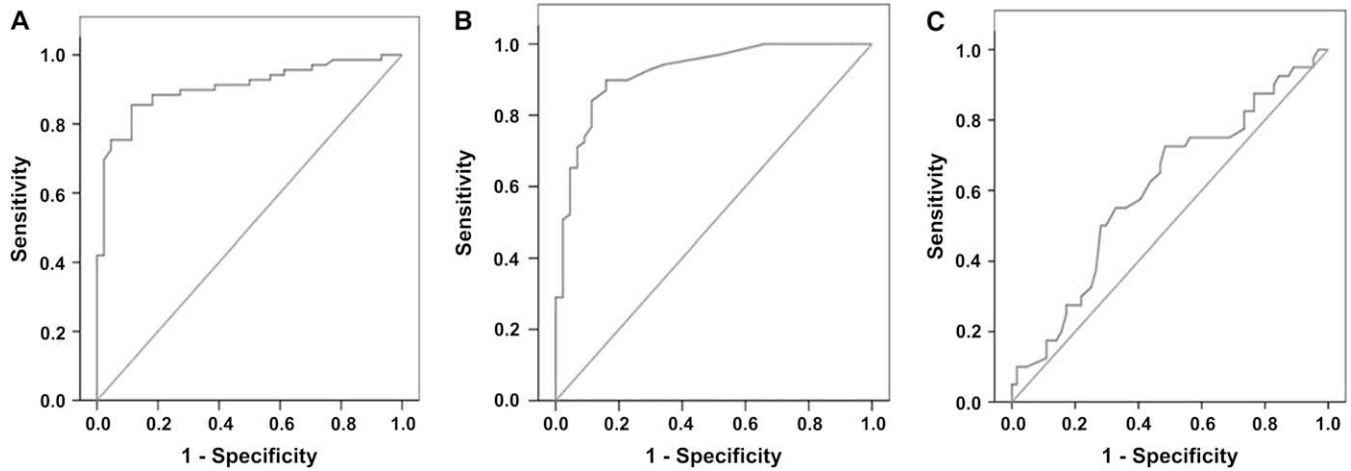


Figure 2. A, ROC for FeNO B, eosinophil count C, and FEV₁ versus the diagnosis of asthma. The AUC are 0.906, 0.921, and 0.606, respectively.

was also observed for FeNO compared with eos%.⁶ A study that compared FeNO with adenosine, methacholine, and exercise provocation tests (but did not include sputum analysis) showed similar accuracy.²⁶ These studies showed significantly better accuracy for FeNO compared with spirometry, for response to bronchodilators, and to treatment with ICS.

Evidence for the overall diagnostic utility of FeNO measurements in children varies. With FeNO > 47 ppb, an epidemiologic survey of 107 schoolchildren showed 47% sensitivity and 93% specificity.²⁷ In a large study of unselected population of 368 schoolchildren, FeNO performed poorly in distinguishing children with asthma from those with non-asthmatic atopia.²⁸ In a selected group of 96 young children with symptoms suggestive of asthma, FeNO discriminated between asthma and healthy control subjects with 86% sensitivity and 92% specificity.²⁹ Another study using a FeNO cutoff value of 25 ppb showed NPV and PPV of 80% and 100%, respectively.⁹ Of note is that these sensitivities and specificities were comparable or even better than those obtained with bronchoprovocation testing. Although one of these studies included sputum analysis,²⁷ comparison of the diagnosis yield of FeNO to sputum eosinophils in children with asthma has been lacking.

It may be argued that the clinical contribution of another technique for the diagnosis of asthma in children is not of major importance because asthma may be diagnosed without difficulty by the primary physician on the basis of typical history, response to therapy, and, when needed, by additional physiological tests; hence, the additional value of FeNO could be seen as limited. Although this statement may hold for many or even most children with clinical symptoms suggesting asthma, it may not be true for a significant amount of children with less specific complaints or who ignore mild-moderate symptoms or in whom result of spirometry may not be abnormal or who do not respond to treatment characteristically. These cases are in need of further evaluation.

FeNO provides a valuable, rapid, easy-to-use, and cost-effective tool that will help to diagnose asthma and initiate therapy early in these children. Bush and Eber³⁰ emphasize that the utility of FeNO may be different in the community compared with a special clinic and that those patients with asthma attending asthma clinics in secondary care are obviously a minority.³⁰ It is this minority that consume more healthcare resources and in whom there is greater scope of benefit for early diagnosis. Combined with the ease of performing the test compared with provocation tests and induced sputum, and the low specificity and sensitivity of spirometry, FeNO may offer the best single and cost-effective test for the diagnosis of asthma in children. Bush and Eber provide a comparison between FeNO, breath condensate, provocation tests for bronchial hyperresponsiveness, and induced sputum with regard to ease to maintain, ease of use, “inflammometry” information, time from test to results, cost and evidence of beneficial outcome, and show the overall advantage of FeNO compared with the other techniques.³⁰

Our finding that the best cutoff threshold of FeNO was 20 ppb is in accordance with previous findings,^{5,6,11,16,31} supporting the validity of our techniques and patient definitions.

Table II. Sensitivity, specificity, PPV, and NPV of FeNO, eos%, and FEV₁ for the diagnosis of asthma

Variable	Cutoff	Sensitivity	Specificity	PPV	NPV
FeNO (best)	19 ppb	86	89	92	80
FeNO	25 ppb	75	89	93	70
FeNO	15 ppb	90	70	83	82
Eos (best)	2.7%	85	89	92	81
FeNO*	>20 or <15 ppb	89	88	92	82
FeNO + Eos (best values)	19 ppb; 3%	87	89	92	81
FEV ₁	80%	52	72	75	48

Only cases that were not receiving ICS during initial evaluation are included (n = 113; no asthma = 44, asthma = 69).

*High and low cutoff levels; N = 101, 65 asthma, 36 no asthma. 12 cases with FeNO levels between 15 and 20 ppb were not included.

The combined criteria for FeNO plus eos% did not significantly improve the diagnostic accuracy. This probably results from the high and similar accuracy of both methods so that none had a significant additional value. This suggests also that overall, both test results were positive in the same patients and also that the 2 techniques failed, that is, were false negative in the same patients. It is possible that the minority of cases with false-negative results present a nonatopic, non-eosinophilic type of asthma. This is in agreement with the 10% rate of children with asthma who primarily have predominance of airway neutrophils.

An overlap between children with and without asthma still exists for FeNO levels. This suggests a limitation of FeNO as a discriminatory test for asthma. However, applying the high and low cutoff levels that improved accuracy left only about 10% of the children out in this "gray zone." The overlap for spirometry variables was much greater.

Symptomatic children with an exhaled NO level not exceeding 15 ppb exhibited a very low chance of having asthma, with a false-negative rate not exceeding 5%. Hence, the data suggest that in these patients, other diseases than asthma should be considered for the reported symptoms. Children with FeNO levels >23 ppb are very likely to have asthma, with a false-positive rate of <5%. In these patients, omitting further diagnostic workup such as challenge testing is justified.

The correlation between FeNO and airway eosinophils is not simple.^{5,30,32} Conflicting results have been reported in adults and children.^{7,10,33,34} Also, in adults, airway eosinophilia characterizes both asthma and eosinophilic bronchitis. The study from the Brompton group showed a 61% concordance between these 2 markers with lower PPV and NPV than ours.¹⁰ However, this study included only 23 children who were under treatment with ICS. They did not include children without asthma.

Atopic, eosinophilic asthma characterizes most of pediatric asthma³⁵ compared with a lower rate in adults. This may not only explain our remarkably better accuracy and yield of FeNO and eosinophil count but also provides evidence and rationale for the clinical use of FeNO for the diagnosis of asthma in children. At present, this test should be reserved for a selected pediatric population of children with respiratory complaints in whom the diagnosis of asthma is not clear despite basic assessment. We suggest that in this population the yield will be at its maximum both as a clinical tool and probably most cost-effective. A significant part of the false-positive cases may have resulted from the inclusion of children with a history of allergic rhinitis and eczema that are associated with increased FeNO levels.³⁶ Nevertheless, we chose not to exclude these cases because these are common manifestations in children with asthma and obviously, excluding children with history of rhinitis or eczema would significantly limit the contribution of this study. Interestingly the false-positive rate was small (< 5%).

This study was not intended to propose FeNO measurements as a guideline for treatment and for the adjustment of therapy, but to investigate the yield of FeNO measurement in the diagnosis of asthma in a large set of children referred to

a tertiary care center. In conclusion, this study provides evidence for the usefulness of FeNO measurement in the early diagnosis or exclusion of asthma in children. We suggest using FeNO measurement in the evaluation of children suspected of having asthma. ■

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