Diagnosing Asthma

Comparisons between Exhaled Nitric Oxide Measurements and Conventional Tests

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International guidelines recommend a range of clinical tests to confirm the diagnosis of asthma. These focus largely on identifying variable airflow obstruction and responses to bronchodilator or corticosteroid. More recently, exhaled nitric oxide (F_{ENO}) measurements and induced sputum analysis to assess airway inflammation have been highlighted. However, to date, no systematic comparisons to confirm the diagnostic utility of each of these methods have been performed. To do so, we investigated 47 consecutive patients with symptoms suggestive of asthma, using a comprehensive fixed-sequence series of diagnostic tests. Sensitivities and specificities were obtained for peak flow measurements, spirometry, and changes in these parameters after a trial of steroid. Comparisons were made against F_{ENO} and sputum cell counts. Sensitivities for each of the conventional tests (0–47%) were lower than for F_{ENO} (88%) and sputum eosinophils (86%). Overall, the diagnostic accuracy when using F_{ENO} and sputum eosinophils was significantly greater. Results for conventional tests were not improved, using a trial of steroid. We conclude that F_{ENO} measurements and induced sputum analysis are superior to conventional approaches, with exhaled nitric oxide being most advantageous because the test is quick and easy to perform.

Keywords: asthma; diagnosis; exhaled nitric oxide; induced sputum; lung function

Bronchial asthma is common and in Western populations its prevalence is about 20% in young adults (1). Diagnosis is based on a history of wheeze, shortness of breath, and cough, which are variable in severity and over time (2), but it may not always be straightforward and clinicians frequently wish to validate the diagnosis using objective tests. This is particularly so if maintenance treatment with long-term inhaled corticosteroid therapy is being considered.

International guidelines recommend the measurement of serial peak expiratory flows or spirometry to confirm the diagnosis of asthma (3–5). The response to either inhaled bronchodilator or to a trial of corticosteroid is also included as part of current recommendations. However, these traditional approaches are problematic. First, they are primarily based on demonstrating abnormal airway physiology. Particularly in mild asthma, this is often not present and for this reason the sensitivity of each of these tests is low (6–8). Second, obtaining serial peak flow recordings or repeated spirometry over an interval of 10–14 days (as recommended) requires adequate patient compliance, and it is the experience of many clinicians that this is not easily achieved (7). As an alternative, supportive evidence may be sought using laboratory-based tests for nonspecific bronchial hyperresponsiveness (BHR) (3, 5, 9). BHR is a recognized feature of asthma and is associated with increased vulnerability to exogenous stimuli. But again, the sensitivity of tests such as methacholine challenge is variable (8, 10), and access to testing is not always available.

Updates to current guidelines have highlighted the potential diagnostic role of exhaled nitric oxide (F_{ENO}) measurements and induced sputum analysis (5). F_{ENO} is increased in patients with bronchial asthma (11) and has been shown to distinguish subjects with asthma from those without asthma (11, 12) with a high degree of discriminatory power (13–15). Moreover, F_{ENO} correlates well with airway eosinophilia and with BHR (16–18). Thus F_{ENO} is a potential surrogate measurement of abnormal airway pathology in asthma, and as such may be a more appropriate diagnostic tool than conventional approaches based on abnormal physiology. The test is easily performed in both adults and children (19). A similar rationale applies to the use of induced sputum analysis, but the latter is much more technically demanding, and is not routinely available.

To date, there has been no systematic comparison between F_{ENO} measurements and each of the "routine" tests recommended for diagnosing asthma. In this study, we have compared F_{ENO} measurements and induced sputum cell counts against serial peak flow recordings, spirometry, and airway responses to inhaled bronchodilator and oral steroid, in an unselected population of patients being investigated by their family practitioner. Our principal aim was to evaluate the diagnostic utility of F_{ENO} measurements as a test for asthma. Our data have previously been presented in part as an abstract (20).

METHODS

Subjects

We recruited 47 consecutive patients aged 8–75 years referred by their family practitioner to the Dunedin Hospital (Dunedin, New Zealand) pulmonary function laboratory for investigation of possible bronchial asthma. The laboratory offers spirometry and BHR testing to community-based clinicians as a routine service. No patient had been referred for specialist consultation as such, and there was no prior selection of patients except that each subject was required to have had respiratory symptoms for a minimum of 6 weeks. Subjects were excluded if they had used oral or inhaled corticosteroid in the preceding 4 weeks or if they had had a typical respiratory tract infection in the previous 6 weeks. The study was approved by the Otago Ethics Committee and all subjects gave written informed consent.

Study Design

Patients attended on three separate occasions at 2-week intervals. Short-acting β-agonist and anticholinergic inhalers were permitted during the

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study, but were withheld for a minimum of 6 hours before each study visit. After completing a questionnaire providing details of respiratory symptoms, a fixed sequence of diagnostic investigations was performed at each of the study visits (Table 1). Between Visits 2 and 3, a 14-day trial of oral prednisone (30 mg/day for adults; 0.5 mg/kg per day for children) was given.

At the final visit, the diagnosis of asthma was ascertained on the basis of the following: relevant symptom history (present in all patients), using American Thoracic Society criteria (2), *and* a positive test for BHR *and/or* a positive response to bronchodilator. These were defined as: provocative dose of hypertonic saline resulting in a 15% fall in FEV_1 (PD_{15}) of less than 20 ml (21) and an increase in FEV_1 of 12% or greater from baseline 15 minutes after inhaled albuterol, respectively (6).

Study Procedures

Respiratory symptoms, bronchodilator use, and twice daily peak flows were recorded in a diary card. Data for the 7 days before the trial of oral steroid was used to calculate peak flow variation. Significant variation was defined as an amplitude percent mean of 20% or greater (22). These data were also used as a baseline to calculate the percent increase in mean morning peak flows with steroid. An increase of 15% or greater for both morning peak flows and FEV_1 was considered significant (5).

Exhaled nitric oxide was measured before any forced expiratory maneuvers, according to current guidelines (19). All readings were obtained by technical staff who were blinded as to the clinical status of the patients. Two exhalation flow rates were used (50 and 250 ml/second). For the flow rate of 50 ml/second, F_{ENO} levels were read at the first NO plateau, and for the flow rate of 250 ml/second, they were read at the end-of-exhalation carbon dioxide plateau (23). The correlation between F_{ENO50} and F_{ENO250} was high ($r = 0.99$, $p < 0.0001$). On the basis of these and other data (24), only results for F_{ENO50} are reported. Bronchodilator reversibility was calculated as percent change in FEV_1 15 minutes after inhaling 400 μg of albuterol. An increase of 12% or greater was considered significant (6).

Bronchial hyperresponsiveness to hypertonic saline (4.5%) was measured by means of a modified version of an earlier protocol, from which PD_{15} was established (21). Simultaneously, sputum was collected for cell analysis according to a standardized method (25).

Statistical Analysis

Comparisons between asthmatic and nonasthmatic diagnostic groups were made by Mann–Whitney *U* tests. Sensitivities, specificities, and positive and negative predictive values for the diagnosis of asthma were calculated for each of the diagnostic tests. For FEV_1 the cut point used to define "abnormal" was 80% (6). For the FEV_1/FVC ratio, because an "abnormal" test is less easy to define (6), two cut points were used: 80 and 70%. For sputum eosinophils, the cut point used was 3% (26).

Receiver–operator characteristic (ROC) curves were constructed to compare the diagnostic utility for each test, except for percent change in FEV_1 with bronchodilator and PD_{15}, both of which had been used as diagnostic criteria. The areas under the ROC curves were compared statistically by the method described by Hanley and McNeil (27). Predictive accuracy was defined as the percentage of all test results that correctly identified presence or absence of asthma.

TABLE 1. STUDY PLAN

	Visit 1		Visit 2		
	Initial	2 wk	Initial	2 wk	Visit 3
Clinical asthma assessment	X				
F_{ENO} measurement	X		X		X
Skin allergy test	X				
Spirometry	X		X		X
Bronchodilator reversibility	X				
Hypertonic saline challenge			X		X
Sputum induction			X		
Peak flow measurements		X		X	
Trial of oral prednisone				X	

Definition of abbreviation: F_{ENO} = exhaled nitric oxide.

RESULTS

Fifty-one consecutive patients were enrolled. Four patients withdrew after the first study visit because of the time commitment. The baseline characteristics of the remaining 47 are shown in Table 2. Sputum induction was possible in only 40 of 47 patients.

Seventeen patients (36%) were diagnosed with asthma, 15 having symptoms and evidence of BHR, and a further 2 having symptoms and a positive response to bronchodilator without evidence of BHR. On the basis of Global Initiative for Asthma (GINA) criteria, 1 patient was classified as having severe asthma, 4 had moderately severe asthma, and 12 had mild asthma. Thirty patients (64%) were deemed not to have asthma. The final diagnosis for these patients was as follows: chronic rhinosinusitis (13; 28%); extended postviral respiratory syndrome (8; 17%); gastroesophageal reflux disease (6; 13%); eosinophilic bronchitis (2; 4%); and chronic obstructive pulmonary disease (1; 2%). Thirteen of the 17 patients with asthma (76%) were atopic compared with 13 of 30 (43%) in the nonasthmatic group.

Compared with the nonasthmatic group, the mean FEV_1 (percent predicted) and FEV_1/FVC ratio were significantly lower with asthma (Table 2). F_{ENO} and sputum eosinophils were significantly higher in the asthmatic group (Table 2). The peak flow and spirometric responses to a trial of oral prednisone did not differ significantly between the two groups: mean (SD) morning peak flows increased by 7.0% (9.8) and 2.2% (6.7) and FEV_1 increased by 5.4% (12.6) and 2.1% (4.2) in the asthmatic and nonasthmatic patients, respectively.

The sensitivities, specificities, and positive and negative predictive values for each of the diagnostic tests are shown in Table 3. ROC curves and comparisons of areas under the ROC curves are shown in Figure 1 and Table 4, respectively. Both F_{ENO} and sputum eosinophils provided significantly higher degrees of diagnostic accuracy than did tests based on lung function. For F_{ENO}, the optimum cut point for diagnosing asthma, based on calculating the predictive accuracy for a range of different F_{ENO50} levels, was 20 parts per billion (*see* the online supplement for detailed data).

In contrast, conventional lung function tests (spirometry and peak flow recordings) provided substantially lower degrees of diagnostic accuracy. Measurements taken before and after a trial of oral steroid did not offer an improvement in this overall picture. Given that bronchodilator reversibility and BHR were used to define the diagnosis of asthma, similar comparisons could not be made for these tests. However, significant bronchodilator reversibility was present in only 7 of 17 patients with asthma (41%) (poorly sensitive).

There were significant correlations between F_{ENO50} and sputum eosinophils ($r = 0.67$, $p < 0.001$) and PD_{15} ($r = -0.56$, $p < 0.001$).

DISCUSSION

In this prospective study, we have evaluated a comprehensive range of tests for confirming the diagnosis of asthma and compared those that are recommended in international guidelines with more recent "state-of-the-art" approaches, notably exhaled nitric oxide measurements. Our results show that single measurements of F_{ENO} in patients with undiagnosed chronic respiratory symptoms are strongly predictive of a diagnosis of asthma. On the basis of ROC curve analysis, F_{ENO} was also superior to the majority of conventional tests of lung function, such as peak flow recordings and spirometry, as well as changes in these parameters after a trial of steroid. In addition, we assessed the value of induced sputum analysis, given that it has been shown to reflect the underlying pathology of asthma (25, 28). F_{ENO} measurements were comparable to this technically more demanding approach, but with the advantage of being noninvasive, and quick and easy to perform.

TABLE 2. CHARACTERISTICS OF THE STUDY PARTICIPANTS BY ASTHMA DIAGNOSIS

	Patients with Asthma (*n* = 17)	Subjects without Asthma (*n* = 30)
Mean age, yr	41.6 (range, 9–72)	31.8 (range, 9–64)
Smoking history (mean pack-years)	14 nonsmokers, 3 ex-smokers (11.3)	28 nonsmokers, 2 ex-smokers (12.5)
Sex	8 female (47%), 9 male	19 female (63%), 11 male
FEV_1, L	2.71 (1.16)	3.18 (0.82)
FEV_1, % predicted	90.5 (18.4)	110.0 (13.5)*
FEV_1/FVC ratio, %	77.3 (11.9)	84.9 (6.0)†
Bronchodilator reversibility, %	11.6 (9.6)	4.2 (2.5)*
Peak flow variation, %	8.3 (5.4)	5.5 (2.5)
$F_{E_{NO}}$, ppb (50 ml/second)	52.0 (34.0)	15.7 (12.9)‡
Sputum eosinophils, %	13.8 (10.0)	1.8 (5.0)‡
Sputum neutrophils, %	20.5 (16.7)	35.5 (21.6)†

For definition of abbreviation *see* Table 1.

All values are reported as means (SD) unless otherwise stated. Bronchodilator reversibility is the percent increase in FEV_1 15 minutes after inhalation of albuterol. Peak flow variability is expressed as the amplitude percent mean calculated over 7 days.

* $p < 0.01$, for between-group comparisons.

† $p < 0.05$, for between-group comparisons.

‡ $p < 0.001$, for between-group comparisons.

"Best practice" for confirming the diagnosis of asthma is currently based on assessing abnormal airway physiology, using relatively simple tests, despite a relative dearth of supportive evidence (3–5). Peak flow variation, spirometric values, and responses to bronchodilator have been shown to differ significantly between subjects with asthma and those without asthma (7). For the most part, this was confirmed in the present study (Table 2). However, our results demonstrate that despite significant between-group differences, the diagnostic usefulness of these conventional tests, including the response to a trial of steroid, is limited. They were poorly sensitive and their predictive values were much lower than for $F_{E_{NO}}$ and sputum eosinophils. This is in keeping with the results of two other studies showing that peak flow variation (8) and bronchodilator response (29) are poorly sensitive in diagnosing asthma, particularly in patients who present with mild disease. Thus, although it may be desirable for clinicians to confirm the diagnosis of asthma "on the spot," the overall yield of currently advocated, easily accessible tests is likely to be low.

It might be argued that the thresholds we used to define "abnormal" for peak flow measurements and spirometry were inappropriate in a population with only mild asthma. We examined this issue by altering the threshold of abnormal for both FEV_1 (percent predicted) and FEV_1/FVC ratio. For FEV_1 (percent predicted), no significant improvement in sensitivity was obtained by setting the threshold at 90% (Table 3). Similarly, for the FEV_1/FVC ratio, a cut point of 80% improved the sensitivity to only 47%. This pattern of results strengthens our conclusion that $F_{E_{NO}}$ measurements are of even greater diagnostic value in patients presenting with only mild disease.

The advent of $F_{E_{NO}}$ measurements to evaluate airway inflammation in asthma represents a significant advance in respiratory medicine, but its application in day-to-day clinical practice as a diagnostic tool has not yet been defined. This was recognized

TABLE 3. SENSITIVITY, SPECIFICITY, AND POSITIVE AND NEGATIVE PREDICTIVE VALUES FOR EACH OF THE DIAGNOSTIC TESTS FOR ASTHMA

	Asthma (*n* = 17)		Nonasthma (*n* = 30)		Sensitivity (%)	Specificity (%)	Positive Predictive Value (%)	Negative Predictive Value (%)
	Yes	No	Yes	No				
Bronchodilator reversibility > 12%	7	10	0	30	—	—	—	—
Bronchial hyperresponsiveness < 20 ml	15	2	0	30	—	—	—	—
Peak flow variation > 20%	0	17	0	29*	0	100	NA	70
Peak flow improvement with steroid > 15%	4	13	0	29*	24	100	100	69
FEV_1 < 80% predicted	5	12	0	30	29	100	100	71
FEV_1 < 90% predicted	6	11	2	28	35	93	75	72
FEV_1/FVC ratio < 70%	6	11	0	30	35	100	100	73
FEV_1/FVC ratio < 80%	8	9	6	24	47	80	57	73
FEV_1 improvement with steroid > 15%	2	15	0	29*	12	100	100	66
Sputum eosinophils > 3%	12	2*	3	23*	86	88	80	92
$F_{E_{NO50}}$ > 20 ppb	14	2†	6	22†	88	79	70	92

Figures for bronchodilator reversibility and bronchial hyperresponsiveness to hypertonic saline are not given because both these parameters were used to diagnose asthma.

* Patient unable or unwilling to complete procedure.

† Technical difficulties prevented completion of exhaled nitric oxide ($F_{E_{NO}}$) measurements at 50 ml/second.

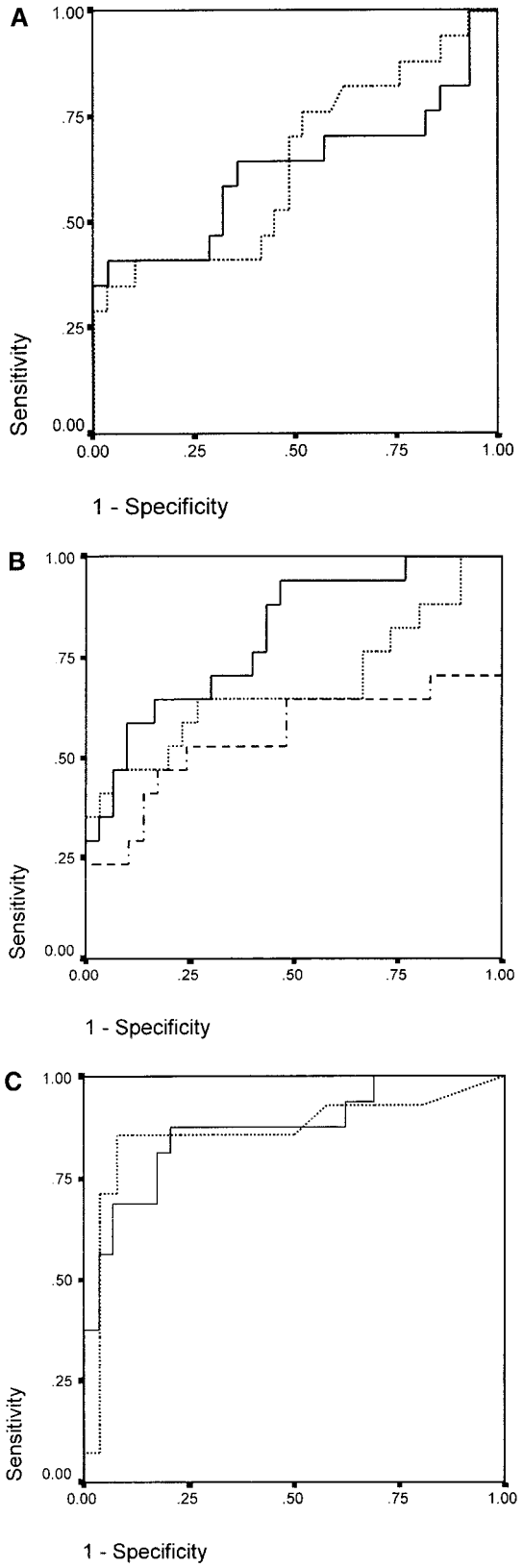

Figure 1. (*A*) Receiver–operator characteristic (ROC) curves for peak expiratory flow variation (amplitude percent mean; *solid line*) and peak expiratory flow steroid response (change in mean morning peak flows between the 7 days before and the final 7 days during the steroid trial; *dotted line*). (*B*) ROC curves for FEV_1% predicted (*solid line*), FEV_1/FVC ratio (*dotted line*), and FEV_1 steroid response (*dashed-dotted line*). (*C*) ROC curves for F_{ENO50} (*solid line*) and sputum eosinophils (*dotted line*).

in the most recent update to GINA guidelines (5). Asthma is an inflammatory disease, for the most part, although not exclusively characterized by eosinophilic airway inflammation, variable airways obstruction, and BHR (30, 31). Given that a significant relationship between F_{ENO} and both sputum eosinophils and BHR has been confirmed in this and previous studies (16, 18), it is therefore much more logical to use F_{ENO} as a surrogate test for airway inflammation than to rely on physiological changes, the measurements of which are variable over time, are often undetectable in mild cases, and correlate only poorly with clinical symptoms. Our results provide evidence in support of this view.

The predictive value of any test is dependent on the "gold standard" used for the diagnosis. The GINA guidelines describe asthma as a "chronic inflammatory disorder characterised by recurrent respiratory symptoms against a background of increased responsiveness to external stimuli giving rise to variable airflow limitation which is reversible either spontaneously or with treatment" (5). On the basis of this description, we elected to define asthma in our study as follows: relevant respiratory symptoms (which were present in all subjects and therefore could not be regarded as discriminatory) plus a positive test for bronchial hyperresponsiveness (BHR) and/or bronchodilator reversibility. A similar approach has been used previously (9, 32). In fact, we performed additional analyses (not reported) using two alternative gold standards. These were "relevant symptoms plus sputum eosinophilia (greater than 3%)" and "relevant symptoms plus one or more of the following: greater than 20% peak flow variability; positive bronchodilator response (spirometry); positive steroid response (peak flows or spirometry); positive test for BHR." By using these alternative definitions even greater accuracy for F_{ENO} as a diagnostic test was obtained. Thus the present results are conservative and have not been influenced by the definition of asthma used.

Of necessity, our particular choice of gold standard also meant that neither bronchodilator response nor BHR could be assessed against F_{ENO} in terms of their diagnostic utility, because both were used to define asthma in the first place. The former is often used to confirm "reversibility." Despite this apparent lack, it is worth noting that even in the asthmatic group, fewer than half (7 of 17) demonstrated reversibility to bronchodilator, indicating low sensitivity for this test. The choice of hypertonic saline rather than another challenge agent was pragmatic in that it permitted simultaneous induced sputum collection. Compared with histamine or methacholine, hypertonic saline appears to be less sensitive but more specific for diagnosing asthma (33, 34). If anything, this will have resulted in a more conservative outcome than if methacholine or histamine had been used.

The present study provides evidence of relevance in a population of patients that is most likely to be encountered in day-to-day clinical practice. By prospectively studying consecutive patients in primary care with hitherto undiagnosed symptoms, and who had relatively mild disease, we have demonstrated that even with low pretest probabilities, F_{ENO} measurements perform well when compared with conventional tests. Our results are consistent with those of three other studies (13–15) in which the diagnostic role of F_{ENO} measurements has been assessed. However, these earlier investigations offer somewhat different and more limited perspectives. In the studies by Dupont and coworkers (13) and Malmberg and coworkers (14), although the diagnostic accuracy for F_{ENO} was similarly high, no direct comparisons with other clinical and laboratory-based tests for asthma were made. Similarly, Deykin and coworkers (15) studied selected rather than unselected patients and focused on the impact of different expiratory flow rates during F_{ENO} measurements on diagnostic accuracy.

In summary, our study confirms the overall superiority of

TABLE 4. MATRIX OF COMPARISONS FOR AREAS UNDER THE CURVE FOR RECEIVER–OPERATOR CHARACTERISTIC CURVES FOR EACH OF THE DIAGNOSTIC TESTS

	AUC	Peak Flow Variation	Peak Flow Steroid Response	FEV_1/FVC Ratio	FEV_1 (*% Predicted*)	FEV_1 Steroid Response	Eosinophils
Peak flow variation	0.626						
Peak flow steroid response	0.640	0.117 (p = 0.453)					
FEV_1/FVC ratio	0.678	0.429 (p = 0.334)	0.303 (p = 0.381)				
FEV_1 (% predicted)	0.804	**1.753 (p = 0.040)**	1.548 (p = 0.061)	1.324 (p = 0.093)			
FEV_1 steroid response	0.554	−0.537 (p = 0.296)	−0.754 (p = 0.225)	−0.926 (p = 0.177)	**−2.118 (p = 0.017)**		
Eosinophils	**0.861**	**2.201 (p = 0.014)**	**2.279 (p = 0.011)**	1.565 (p = 0.059)	0.626 (p = 0.266)	**2.557 (p = 0.005)**	
FE_{NO50}	0.864	**2.086 (p = 0.018)**	**2.332 (p = 0.010)**	1.734 (p = 0.041)	0.786 (p = 0.216)	**2.643 (p = 0.004)**	0.036 (p = 0.486)

Definition of abbreviations: AUC = area under the curve; FE_{NO50} = exhaled nitric oxide at flow rate of 50 ml/second; FEV_1 steroid response = percent change in FEV_1 after trial of steroid.

Column 2: AUC data; a value of 1.0 would occur with a test that is 100% sensitive and 100% specific. Columns 3–8: z scores and p values show significance of differences between each of the diagnostic tests (27).

Figures in boldface represent statistically significant AUC comparisons.

Peak flow steroid response is the percent change in mean morning peak flow after trial of steroid.

FE_{NO} measurements and induced sputum analysis in the diagnosis of asthma compared with conventional tests. FE_{NO} measurements are quick and easy to perform and may be readily incorporated into routine pulmonary function test procedures. This advance offers the possibility that a diagnosis of asthma may be performed more easily and confirmed with much greater confidence than has been possible to date.

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