A Comparison of the Validity of Different Diagnostic Tests in Adults With Asthma*

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Study objectives: Diagnosing asthma is not always easy, and there are times when objective tests can be helpful. The extent to which these tests alter the probability of asthma depends on how much more commonly the test result is positive in subjects with asthma compared to healthy subjects and particularly subjects with conditions that are commonly confused with asthma. We set out to compare the sensitivity and specificity of different tests in this setting.

Design: Single-center, cross-sectional, observational study.

Setting: Teaching hospital.

Patients: Twenty-one healthy control subjects, 69 patients with asthma, and 20 subjects referred to the hospital with a diagnosis of asthma who were found to have alternative explanations for their symptoms (ie, pseudoasthma).

Interventions: We measured methacholine airway responsiveness, the maximum within-day peak expiratory flow amplitude mean percentage (derived from twice-daily readings for > 2 weeks), the FEV₁/FVC ratio, the percentage change in FEV₁ 10 min after the administration of 200 μg inhaled albuterol, and the differential eosinophil count in blood and induced sputum. We derived normal ranges (from the 95% upper or lower limit for healthy subjects), sensitivity, and specificity (ie, the percentage of subjects with pseudoasthma who had negative test results).

Results: Most tests were less specific when the reference population was composed of subjects with conditions that can be confused with asthma. Methacholine airway responsiveness and the sputum differential eosinophil count were the most sensitive (91% and 72%, respectively) and specific (90% and 80%, respectively) tests.

Conclusion: We conclude that methacholine airway responsiveness and the sputum differential eosinophil count are the most useful objective tests in patients with mild asthma.

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Key words: asthma; diagnosis; validity

Abbreviations: PEF = peak expiratory flow; A%M = amplitude percent mean; PC20 = provocative concentration of a substance causing a 20% fall in FEV₁

Diagnosing asthma is not always easy, and there are times when objective tests can be helpful. The extent to which these tests alter the probability of asthma depends on the validity of the test, or how much more commonly the test results are positive in subjects with asthma compared to healthy subjects and particularly subjects with conditions that are commonly confused with asthma. Few studies have compared the sensitivity and specificity of different tests in patients with asthma, and none have done so using a reference population of subjects with conditions that are confused with asthma.

The most common strategy that is employed to support a clinical diagnosis of asthma is to demonstrate the presence of an abnormal, short-term, variable airflow obstruction. Spontaneous variable airflow obstruction can be assessed using peak expiratory flow (PEF) monitoring at home,1 or treatment-induced variable airflow obstruction can be assessed in the laboratory by measuring the bronchodilator response to β₂-agonists or the bronchoconstrictor response to short-acting airway smooth muscle spasmogens such as methacholine.2 We have compared the validity of these markers of variable...
airflow obstruction with the FEV₁/FVC ratio and the blood and induced sputum differential eosinophil count in 21 healthy control subjects, 69 patients with asthma, and 20 subjects who were referred with a diagnosis of asthma and who were found to have alternative explanations for their symptoms (i.e., pseudoasthma).

**MATERIALS AND METHODS**

**Subjects**

Patients and control subjects were recruited from among patients attending the Department of Respiratory Medicine, staff, and volunteers. Healthy control subjects had no symptoms suggesting past or current asthma and were nonsmokers. Subjects with asthma had consistent clinical features, were symptomatic at the time of the investigations, had FEV₁ values of > 65% of predicted, and had one or more of the following conditions: a provocative concentration of a substance (methacholine) causing more than a 20% fall in FEV₁ (PC₂₀) of < 8 mg/mL; a > 15% increase in FEV₁ 10 min after receiving 200 μg inhaled albuterol; or a > 20% maximum within-day variability of PEF when measured twice daily for > 14 days. Thirty-seven subjects had mild episodic asthma requiring therapy with β₂-agonists only as required, and 32 subjects had persistent asthma requiring additional regular inhaled corticosteroid treatment (mean daily beclomethasone equivalent dose, 550 μg). No subjects were receiving additional treatment (i.e., therapy with long-acting β₂-agonists or leukotriene receptor antagonists). Pseudoasthma was diagnosed in subjects referred to the hospital with a diagnosis of asthma by a primary-care physician in which the clinical features were considered to be atypical, the symptoms did not deteriorate following the withdrawal of asthma therapy, and symptoms improved following the specific treatment of the underlying condition. The criteria used to make the primary diagnosis and determine the management of subjects with pseudoasthma are shown in Table 1. Subjects were fully characterized and assigned to the appropriate group prior to study entry. Subjects gave full informed consent to participate in the study. The protocol was approved by the Leicestershire Health Authority Ethics Committee.

**Study Design**

This was a single-center, cross-sectional observational study. Subjects attended the study center on 2 days that were separated by at least 14 days. On the first day of attendance, spirometry, allergen skin prick tests, and peripheral blood eosinophil counts were performed, and the subjects were asked to record twice-daily PEF values. On the second day of attendance, a methacholine inhalation test was performed followed by sputum induction after recovery from the test. All laboratory measurements were performed by a blinded observer. Subjects with asthma contin-

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>History</th>
<th>Examination</th>
<th>Investigations</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhinitis</td>
<td>Rhinorrhea, nasal obstruction, sinus pain, sneezing, nasal itch, postnasal drip</td>
<td>Nasal secretions, nasal or pharyngeal mucosal inflammation</td>
<td>Sinus radiograph/CT scan showing mucosal thickening and/or fluid level</td>
<td>Topical budesonide/BDP 100 μg twice daily; in selected cases, topical ipratropium bromide (40 μg twice daily), topical xylometazoline, oral antihistamine</td>
</tr>
<tr>
<td>Gastroesophageal reflux</td>
<td>Heartburn, flatulence, water brash</td>
<td></td>
<td>Barium swallow, endoscopy, and 24-h esophageal manometry and pH in selected cases</td>
<td>Weight reduction, elevation of head of bed, no eating within 2 h of bedtime, acid suppression; prokinetic agent in selected cases</td>
</tr>
<tr>
<td>Chronic bronchitis</td>
<td>Productive morning cough &gt; 3 mo/yr for &gt; 1 year; smoking history</td>
<td>Coarse crackles</td>
<td></td>
<td>Stop smoking</td>
</tr>
<tr>
<td>Postviral cough</td>
<td>Onset following viral upper respiratory tract infection</td>
<td>Normal</td>
<td></td>
<td>Observation</td>
</tr>
<tr>
<td>Hyperventilation syndrome</td>
<td>Sudden dyspnea occurring at rest, associated paresthesias, dizziness</td>
<td>Normal</td>
<td>20 deep breath test</td>
<td>Rebreathing exercises, relaxation, identification of triggering situations</td>
</tr>
<tr>
<td>Obstructive sleep apnea</td>
<td>Daytime somnolence, unrefreshed sleep, snoring</td>
<td>Sleep study</td>
<td>Continuous positive-airway pressure</td>
<td></td>
</tr>
<tr>
<td>Unexplained dry cough</td>
<td>Persistent nonproductive cough</td>
<td>Normal</td>
<td>Antitussives</td>
<td></td>
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</table>

*BDP = beclomethasone dipropionate.*
used to receive their usual treatment throughout the investigation period, although short-acting β₂-agonists were withheld for 6 h before the measurement of airway responsiveness. Subjects with pseudoasthma were studied at least 1 month after stopping all asthma medication.

**Methods**

Spirometry was undertaken using a rolling seal spirometer (Vitalograph; Buckingham, UK), and measurements were recorded as the greater of successive readings within 100 mL. Spirometry was repeated 10 min after the administration of 200 μg inhaled albuterol. Allergen skin sensitivity was measured by skin-prick test reactions to *Dermatophagoides pteronyssinus*, cat fur, grass pollen, and *Aspergillus fumigatus* solutions, compared with those to normal saline solution and histamine controls (Bencard; Nottinghamshire, UK). Atopy was defined as a wheal reaction of > 2 mm more than that for the control. The blood differential eosinophil count was performed using standard hematoxylin techniques. PEF was measured twice daily for 14 days as the best of three blows using a mini-Wright peak flowmeter (Clement Clarke Ltd; London, UK). Airway responsiveness was measured using the tidal breathing method. Sputum was induced using nebulized hypertonic saline solution and was processed using dithiothreitol (Sigma; Poole, UK) as previously described. The differential inflammatory cell count was performed by counting 400 cells after cytospin and staining with Romanowsky stain.

**Analysis**

The maximum PEF amplitude percent mean (A%M) was derived from the maximum within-day variability observed over the 14-day period preceding the second visit as the difference between the highest and lowest PEF values and was expressed as a percentage of the mean PEF. The methacholine PC<sub>20</sub> was calculated by linear interpolation of the log dose-response curve. Spirometric values, the percentage increase in FEV<sub>1</sub> after receiving inhaled salbutamol, and the blood differential eosinophil differential cell count were described as the mean and SEM. The methacholine PC<sub>20</sub> and the sputum differential eosinophil counts were log normally distributed, were log-transformed, and were described as the geometric mean and log SEM. Sputum eosinophil counts of 0% were assigned a value 0.1% to facilitate the log transformation. The upper or lower limits of the normal range that were derived from healthy control subjects were calculated as the mean ± 1.725 × SD. Sensitivity, specificity (with 95% confidence intervals), positive and negative predictive values, accuracy, and likelihood ratios were calculated as suggested by Greenhalgh.

**Results**

Subject details are shown in Table 2. The primary diagnosis among the subjects with pseudoasthma was rhinitis (five subjects), unexplained dry cough (four subjects), gastroesophageal reflux (three subjects), chronic bronchitis and/or mild bronchiectasis (three subjects), hyperventilation syndrome (two subjects), postviral cough (two subjects) and obstructive sleep apnea (one subject). Subjects had received asthma treatment for a median duration of 2 years (range, 0 to 29 years). At the time of referral, most subjects were in British Thoracic Society treatment stages 2 to 4, but three subjects were receiving regular oral prednisolone therapy.

Normal ranges and the distribution of individual values for FEV<sub>1</sub>/FVC percent, PEF A%M, bronchodilator response, methacholine PC<sub>20</sub>, and the sputum and blood eosinophil counts are shown in Table 3 and Fig 1, 2. The sensitivity, specificity, positive and negative predictive values, accuracy, and the likelihood ratios of a positive or negative test result are shown in Table 3. Most tests were less specific when the reference population consisted of subjects with pseudoasthma. The methacholine PC<sub>20</sub> and the sputum eosinophil count were the most sensitive (91% and 72%, respectively) and specific (90% and 80%, respectively) tests (Table 3; Fig 1, 2). None of the subjects with asthma had the combination of normal airway responsiveness and normal sputum eosinophil count, and none of the subjects with pseudoasthma had abnormal results for both of these tests. The sensitivity of the bronchodilator response (37.5% vs 59%, respectively) and the sputum eosinophil count, %† 0.31 (0.12)

**Table 2—Subject Characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Healthy Control</th>
<th>Asthma Patients</th>
<th>Pseudoasthma Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range), yr</td>
<td>35 (19–57)</td>
<td>44 (15–70)</td>
<td>39 (14–76)</td>
</tr>
<tr>
<td>Men, No.</td>
<td>10</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>0</td>
<td>11.5</td>
<td>5</td>
</tr>
<tr>
<td>Atopy, %</td>
<td>14</td>
<td>52</td>
<td>30</td>
</tr>
<tr>
<td>Inhaled steroid use, %</td>
<td>0</td>
<td>46</td>
<td>0</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;, % predicted</td>
<td>103.5 (3.0)</td>
<td>85.0 (1.7)</td>
<td>100.0 (2.3)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC ratio</td>
<td>85.4 (1.1)</td>
<td>73.4 (1.0)</td>
<td>79.6 (1.7)</td>
</tr>
<tr>
<td>Bronchodilator response, %</td>
<td>0.0 (0.4)</td>
<td>3.6 (0.9)</td>
<td>1.3 (0.9)</td>
</tr>
<tr>
<td>PEF A%M</td>
<td>8.7 (1.2)</td>
<td>19.0 (1.1)</td>
<td>15.0 (1.2)</td>
</tr>
<tr>
<td>Methacholine PC&lt;sub&gt;20&lt;/sub&gt;, mg/mL†</td>
<td>&gt; 16</td>
<td>1.5 (0.07)</td>
<td>13.3 (0.04)</td>
</tr>
<tr>
<td>Blood eosinophil count, %†</td>
<td>1.9 (0.6)</td>
<td>4.3 (0.6)</td>
<td>2.0 (0.3)</td>
</tr>
<tr>
<td>Sputum eosinophil count, %†</td>
<td>0.17 (0.08)</td>
<td>3.0 (0.00)</td>
<td>0.31 (0.12)</td>
</tr>
</tbody>
</table>

*Values given as mean (SEM), unless otherwise indicated.
†Values given as geometric mean (log SEM).
ophil count (62.5% vs 81%, respectively) tended to be less in subjects with asthma treated with inhaled corticosteroid therapy, although the differences were not significant. There was no evidence that treatment altered the validity of the other tests.

**Discussion**

This is the first study to derive normal ranges for results and to compare the validity of different tests with reference population that has conditions that are confused with asthma (i.e., pseudoasthma). Our findings indicate that in adults with asthma who have normal or near-normal spirometric values the methacholine challenge (i.e., methacholine PC20) and the sputum differential eosinophil count are the most valid tests and, by implication, are the most clinically useful for discriminating patients with asthma from subjects with pseudoasthma. The acute bronchodilator response to inhaled albuterol and the PEF A%M derived from

*Values given are the mean (range), unless otherwise indicated. BD = beclomethasone dipropionate.*

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Range</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
<th>Positive Predictive Value, %</th>
<th>Negative Predictive Value, %</th>
<th>Accuracy, %</th>
<th>Positive Result</th>
<th>Negative Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1/FVC ratio</td>
<td>&gt; 76.6%</td>
<td>61 (49.5–72.5)</td>
<td>60 (38.5–81.5)</td>
<td>84</td>
<td>31</td>
<td>61</td>
<td>1.5</td>
<td>0.65</td>
</tr>
<tr>
<td>BD response</td>
<td>&lt; 2.9%</td>
<td>49 (37.2–60.8)</td>
<td>70 (49.9–90.1)</td>
<td>85</td>
<td>29</td>
<td>54</td>
<td>1.6</td>
<td>0.73</td>
</tr>
<tr>
<td>PEF A%M</td>
<td>&lt; 21.6%</td>
<td>43 (31.3–54.7)</td>
<td>75 (56–94)</td>
<td>86</td>
<td>28</td>
<td>51</td>
<td>1.7</td>
<td>0.76</td>
</tr>
<tr>
<td>PC20</td>
<td>&gt; 8 mg/mL</td>
<td>91 (84.2–97.8)</td>
<td>90 (76.9–100)</td>
<td>97</td>
<td>75</td>
<td>91</td>
<td>9.1</td>
<td>0.10</td>
</tr>
<tr>
<td>Sputum eosinophil count, %</td>
<td>&lt; 1%</td>
<td>72 (61.4–82.6)</td>
<td>80 (62.5–97.5)</td>
<td>93</td>
<td>46</td>
<td>74</td>
<td>3.6</td>
<td>0.35</td>
</tr>
<tr>
<td>Blood eosinophil count, %</td>
<td>&lt; 6.3%</td>
<td>21 (11.4–30.6)</td>
<td>100</td>
<td>100</td>
<td>27</td>
<td>38</td>
<td>NA</td>
<td>0.79</td>
</tr>
</tbody>
</table>

*Values are the mean (range), unless otherwise indicated.*

**Figure 1.** Individual values for blood and sputum differential eosinophil count (eos) and the provocative concentration of methacholine causing a 20% fall in FEV1 (PC20) in each patient category. Dashed line represents upper/lower limit of normal range.

**Figure 2.** Individual values for FEV1/FVC, the percentage change in FEV1 10 min after the administration of 200 μg inhaled albuterol (beclomethasone dipropionate response), and PEF A%M in each patient category. Dashed line represents upper/lower limit of the normal range.
twice-daily PEF readings were no more valid than the identification of an obstructive spirogram for this purpose.

Making the distinction between asthma and pseudoasthma is an important problem in secondary and tertiary care. A study showed that 160 of 263 subjects referred to a tertiary referral center with suspected asthma received an alternative diagnosis and that many had received prolonged treatment with potentially toxic therapy before the correct diagnosis was reached. Our subjects with pseudoasthma had a range of conditions that was similar to that described in this study, and, thus, they are likely to be representative of a wider population with conditions that are commonly confused with asthma.

As with many studies investigating the validity of diagnostic tests, we studied subjects about whom there was little pretest diagnostic doubt after specialist review, so our findings may not be relevant to the situation in a specialist clinical practice. However, our rank order of validity of the various tests may help clinicians to apportion appropriate weight to the results of tests that might be applied in this situation. A further potential problem is that we studied many patients with asthma who had received treatment, albeit those patients with persistent symptoms. However, there was no evidence that treatment with inhaled corticosteroids differentially influenced results in such a way as to have been responsible for the marked differences in test validity. Furthermore, our approach is most analogous to the situation in everyday clinical practice, in which many clinicians would be uncomfortable about stopping treatment before testing a symptomatic patient.

Our estimates of normal ranges for the FEV₁/FVC percent, PEF A%M, and methacholine PC₂₀ are derived from a small number of healthy subjects but are similar to ranges reported from larger community-based studies. Our estimate of the upper limit of the normal range for the acute bronchodilator response to inhaled albuterol is less than the 7.7% increase in FEV₁ after the administration of isoproterenol reported by Lorber et al, probably because the latter study took no account of the healthy subjects who had a fall in FEV₁ after treatment. Our conclusion that methacholine PC₂₀ is the most sensitive marker of asthma is supported by a number of epidemiologic studies. Higgins et al showed that methacholine airway hyperresponsiveness identifies twice as many subjects with physician-diagnosed asthma than PEF A%M derived from readings made every 2 h for > 7 days, and Siersted et al reported a sensitivities of 69% and 19.2%, respectively, for methacholine airway responsiveness and PEF A%M outside the normal range in a study of adolescents with asthma. The estimates of the sensitivity of methacholine airway responsiveness and PEF variability in these studies are less than those reported by us, probably because the diagnosis of asthma was less precise.

Studies of this kind are hampered by the lack of an accepted “gold standard” for the diagnosis of asthma. We adopted a pragmatic approach based on consistent clinical features and the presence of one or more markers of abnormal short-term variable airflow obstruction. Such an approach is widely recommended and is endorsed by national and international guidelines. Our study could be criticized because we have compared the validity of tests that were included in our definition of asthma. However, our definition did not discriminate among the various markers of short-term variable airflow obstruction and, therefore, is unlikely to have biased our comparisons among these markers.

We studied subjects with mild asthma who had normal or near-normal spirometric values, so our findings might not be applicable to the 20% of subjects with asthma who have significant fixed airflow obstruction. Previous studies have shown that there is considerable overlap between measures of airway responsiveness and PEF variability in subjects with smoking-related COPD and subjects whose fixed airflow obstruction is thought to have an asthmatic basis, suggesting that these markers of short-term variable airflow obstruction are less valid in this group. This is particularly the case with measures of PEF variability. Measuring the bronchodilator response, trials of corticosteroid treatment, or direct assessment of airway inflammation might be a more satisfactory way of clinically categorizing these patients. Our subjects were adults with currently symptomatic asthma, so it may not be possible to generalize our findings to children, in whom the incidence of airway hyperresponsiveness in healthy control subjects is higher, or to subjects with intermittent allergen-related or occupation-related symptoms who may have normal airway responsiveness between attacks.

Our definition of a significant bronchodilator response is less stringent than the 15% improvement in PEF or FEV₁ that is quoted in national and international guidelines. Very few of our patients with asthma had a bronchodilator response of this magnitude, and, even with our less stringent definition, the bronchodilator response was not a particularly sensitive marker of asthma. Our findings are consistent with those of Lorber et al who showed a sensitivity of 37% for an acute bronchodilator response to isoproterenol of 7.7% in subjects with asthma and a baseline FEV₁ of > 72% of predicted. Many clinical trials have used a 15% bronchodilator...
response as a requirement for entry into a study. Our findings and the findings of others suggest that these subjects represent a highly selected subset and that the results of these trials cannot be generalized to a wider population of patients with asthma. This is particularly relevant to some influential trials of long-acting β2-agonists in which the participants were not only highly selected, but were also particularly likely to respond to the study drug.

The validity of the PEF A%M was particularly poor. The PEF is effort-dependent, and monitoring is patient-directed, so there may be more within-subject variability in the results than in spirometric values that are measured by a trained technician. The results of a study using computerized PEF recorders with the capacity to store timed PEF readings has emphasized how inaccurately PEF is recorded. It is possible that the validity of measures of PEF variability might be improved by increasing the frequency of measurements, using different markers of variability, or by incorporating post-bronchodilator readings.

However, community studies have shown that all markers of PEF variability that are derived from PEF readings taken as frequently as every 2 h are substantially less sensitive than methacholine airway hyperresponsiveness at detecting physician-diagnosed asthma. One of the particular problems with our marker of PEF variability was the loss of specificity when the reference population was composed of subjects with pseudoasthma. Many of these subjects had conditions that are associated with upper airway dysfunction and it is possible that false-positive PEF records were due to variable upper airway narrowing, since PEF can be reduced by upper airway obstruction. PEF monitoring can be useful in detecting airway narrowing in response to environmental stimuli, as occurs in occupational asthma, and to guide self-management in subjects who perceive bronchoconstriction poorly. Our findings suggest that PEF monitoring has a limited role in supporting a clinical diagnosis of asthma.

Asthma is associated with eosinophilic airway inflammation and an increased proportion of eosinophils in the sputum. The development of safe, simple, valid, and repeatable methods with which to assess airway inflammation using induced sputum has increased interest in the clinical role of monitoring airway inflammation. We have shown that the result of a sputum eosinophilia test is a valid marker of asthma and is more sensitive than that of a blood eosinophilia test. Similar findings have been reported by Pizzichini et al. Interestingly, the combination of the presence of sputum eosinophilia and/or methacholine airway hyperresponsiveness was a particularly sensitive and specific marker of asthma. Larger studies are required to prospectively test this relationship, but our preliminary observations suggest a clinical role for measuring airway inflammation. The presence of sputum eosinophilia predicts a response to therapy with inhaled and oral corticosteroids in patients with asthma and COPD, so information on airway inflammation also might provide a useful guide to further treatment.

In conclusion, we have shown that the methacholine PC20 is the most sensitive marker of mild asthma and the only one able to discriminate asthma from pseudoasthma reliably. The presence of sputum eosinophilia was the next most valid marker, and the presence of methacholine airway hyperresponsiveness and/or the presence of sputum eosinophilia was particularly sensitive and specific. Our findings suggest that a more invasive approach to supporting the diagnosis of asthma would increase diagnostic accuracy and expedite the search for alternative causes for asthma-like symptoms.

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