Diagnostic Value of the Bronchial Provocation Test With Methacholine in Asthma*

A Bayesian Analysis Approach

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The Bayesian analysis was used in this study to investigate the diagnostic value of the bronchial provocation test with methacholine in patients with asthma. The best cutoff value of accumulated concentration of methacholine administered that caused a 20 percent fall in FEV₁, post-saline (PC20) in our sample, determined with a receiver operator characteristic curve, was 15 mg/ml. The interval security of the test was established by a pretest probability between 0.16 and 0.87 and the best test results were obtained when pretest probability was 0.48. The positive final diagnostic gain of the test was maximal at this pretest probability. We conclude that the application of Bayes' theorem, considering the pretest probability of asthma and the sensitivity and specificity of the individual PC20 obtained, increases the accuracy of the bronchial provocation test with methacholine in the diagnosis of asthma. (Chest 1993; 104:149-54)

Traditionally, it has been considered that hyperresponsiveness to inhaled bronchoconstrictor agents is closely associated with bronchial asthma. However, in the last years it also has been clearly pointed out that bronchial hyperreactivity is not a constant feature of this condition and may not be present at all times in a given asthmatic subject. Moreover, hyperreactivity is a frequent finding in other diseases such as allergic rhinitis, chronic bronchitis, and ventricular failure, and even in some normal subjects. Such observations clearly support the concept that bronchial hyperresponsiveness is not synonymous with bronchial asthma. In other words: the presence of bronchial hyperresponsiveness cannot be considered today as a "gold standard" in the diagnosis of asthma.

Nevertheless, the fact that a definitive diagnosis of asthma might not be achieved in this way, does not mean that bronchial provocation tests lack diagnostic value. Physicians usually assign disease probabilities rather than unequivocal diagnosis when interpreting data from clinical symptoms and tests. The disease probability (posttest probability) is so determined considering the test results and the physician's estimate of disease before the test has been done (pretest probability). Thus, when physicians make judgments about the probability of asthma, information about symptoms and bronchial provocation test should be considered. Probabilistic estimation of diseases can be approached by several methods. The most widely used is the application of Bayes' theorem. In the present study, we used the Bayesian analysis to calculate the different posttest probabilities of asthma diagnosis incorporating the prechallenge clinical diagnosis with the response to methacholine.

**METHODS AND MATERIALS**

**Subjects**

Three hundred subjects (130 males and 170 females) recruited from our outpatient clinic or normal volunteers were included in our study. They were 128 healthy individuals, 106 asthmatic subjects (42 atopic and 64 nonatopic individuals), 36 subjects with rhinitis (14 atopic and 22 nonatopic individuals), and 30 subjects with chronic bronchitis.

Asthmatic patients satisfied the American Thoracic Society definition of asthma: all gave a history of episodic dyspnea with wheezing, and all had documented variation in FEV₁ of more than 15 percent, either spontaneously or after use of a bronchodilator. Their mean FEV₁ (percent predicted) value was 98 percent. None had other chest disease and none smoked cigarettes. At the time of the study all of them had had current symptoms within the past 2 weeks. Their symptoms were mild and stable while they were taking aerosol ß₂-adrenergic agonists.

Rhinitis was considered to be present if there was rhinorrhea for 3 months of the year during 2 consecutive years.

Subjects with asthma and rhinitis underwent skin prick tests with a battery of 15 commonly inhaled antigen extracts. Atopy was indicated whenever a patient had one or more immediate positive skin reactions.

Patients with chronic bronchitis had a history of cough and sputum production on most days for as many as 3 months in 2 consecutive years. All of them were smokers. The baseline FEV₁ (percent predicted) was greater than 80 percent in all but five subjects. Nevertheless, the mean FEV₁ values of patients with chronic bronchitis were not significantly different from the other

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NPV = negative provocation test result; PC20 = accumulated concentration of methacholine administered that caused a 20 percent fall in FEV₁, post-saline; PPV = positive provocation test result

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Table 1—Anthropometric Characteristics of Patients and Healthy Individuals*

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>Height, cm</th>
<th>M:F Ratio</th>
<th>FEV₁, % of Predicted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>128</td>
<td>40 ± 1</td>
<td>65 ± 1</td>
<td>1:2</td>
<td>101 ± 7</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>36</td>
<td>35 ± 1</td>
<td>66 ± 1</td>
<td>3:4</td>
<td>102 ± 9</td>
</tr>
<tr>
<td>Chronic</td>
<td>30</td>
<td>55 ± 1</td>
<td>70 ± 1</td>
<td>5:1</td>
<td>88 ± 9</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>106</td>
<td>34 ± 1</td>
<td>68 ± 1</td>
<td>1:1</td>
<td>98 ± 6</td>
</tr>
</tbody>
</table>

*Values are means ± SD.

At the time of inclusion, no subject had suffered an upper respiratory tract infection in the previous 6 weeks. Exposure to sensitizers, use of medications, and smoking were stopped before the study in the terms recommended.15 Informed consent was refused in four cases. A significant response to diluent was observed in ten more cases (all asthmatic subjects). These 14 subjects were excluded.

**Experimental Procedures**

A challenge test with methacholine chloride (Sigma Chemical Company) dilution in buffered saline solution (0.1 to 40 mg/ml) was performed by trained personnel at the same time of the day for all the subjects.

Baseline FEV₁ was recorded with a dry spirometer (Ohio 84) and subjects were excluded if they had a significant response (5 percent fall of baseline FEV₁) to buffered saline solution.

The dilution of methacholine was nebulized with a nebulizer Hudson Up Draft II Neb-U-Mist (output of 0.13 ± 0.002 ml/min) and an electric compressor (Pari Therapiegerät Privat) with an airflow supply of 6 to 8 L/min. The mean aerodynamic mass was 1.8 to 2.3 μg.

The patients inhaled doubling concentrations of methacholine solution through a mouthpiece for 2 min with tidal breathing. The FEV₁ was measured at 30 and 90 s after the end of each inhalation until reproducible results were obtained (within 3 percent). The lowest reproducible FEV₁ was used in calculations. The procedure was continued until the FEV₁, measured from the lowest post-saline to the lowest post-methacholine value had fallen 20 percent or more or until the highest concentration had been administered. The induced bronchoconstriction was reversed by inhalation of salbutamol. Bronchial responsiveness was assessed by measuring the accumulated concentration of methacholine administered that caused a 20 percent fall in FEV₁ post-saline (PC20), expressed as a percentage of the subject's FEV₁. The PC20 value was obtained by a linear interpolation formula of the last two data points.16

**Data Analysis**

For the purposes of this study, individuals were divided into two groups: asthmatic and non-asthmatic subjects.

The ability of the bronchial provocation test for diagnosis of asthma was assessed by calculating the sensitivity, specificity, false-positive rate, and false-negative rate for several possible cutoff values of PC20 (Table 2).

To detect the best cutoff point of PC20 to separate asthmatic and nonasthmatic patients, a receiver operator characteristic curve was graphically constructed by plotting sensitivity against false-positive rate (1-specificity) for each value. The best cutoff point of PC20 corresponds to the value with greatest sensitivity and specificity. With this cutoff value, we determined the posttest probability of asthma after a positive (PPV) or negative (1-NPV) provocation test result and the posttest probability of non-asthma after a PPV for all the possible pretest probabilities according to Bayes' theorem:

\[
\text{PPV: } \frac{(1 - PrT) \times Sp}{(1 - PrT) \times Sp + PrT \times (1 - S)}.
\]

The pretest probability, PrT, is the clinical estimate of asthma, expressed in fractional terms, before the test results are known. The difference between PPV or 1-NPV and pretest probability is the positive or negative final diagnostic gain of the test. In this equation, S is sensitivity and Sp is specificity. Further, PPV represents positive predictive value and 1-NPV negative predictive value.

Finally, we calculated the PPV and 1-NPV for several cutoff points of the metacholine challenge test.

**RESULTS**

Anthropometric data and FEV₁ (percent predicted) of the 300 subjects are presented in Table 1. There were no significant differences between the groups.

Sensitivity, specificity, false-positive rate, and false-negative rate of the test for all the PC20 values are shown in Table 2. For subjects with asthma versus subjects without asthma, as the PC20 cutoff point is increased in a stepwise fashion from 0.50 to 80 mg/ml of accumulated concentration of methacholine, the sensitivity gradually rises toward 100 percent and the specificity falls from 0.99 to 0.53.

The receiver operator characteristic curve for distinguishing asthmatic from nonasthmatic patients is shown in Figure 1. As can be graphically observed, the best cutoff PC20 point to discriminate between them was 15 mg/ml (sensitivity and specificity: 0.84 and 0.86, respectively). Using this PC20 value, the posttest probability of asthma or nonasthma taking into account all the pretest probabilities were determined (Fig 2). The best results of the test (PPV: 0.86; 1-NPV: 0.51).

Table 2—Sensitivity, Specificity, False-Positive Rate and False-Negative Rate of the Bronchial Provocation Test for the Diagnosis of Asthma at Different Accumulated Concentrations of Methacholine

<table>
<thead>
<tr>
<th>PC20, mg/ml</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>False-Positive Rate</th>
<th>False-Negative Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤0.50</td>
<td>0.04</td>
<td>0.99</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>≤1.00</td>
<td>0.27</td>
<td>0.99</td>
<td>0.01</td>
<td>0.73</td>
</tr>
<tr>
<td>≤1.25</td>
<td>0.29</td>
<td>0.99</td>
<td>0.01</td>
<td>0.71</td>
</tr>
<tr>
<td>≤2.50</td>
<td>0.38</td>
<td>0.97</td>
<td>0.03</td>
<td>0.62</td>
</tr>
<tr>
<td>≤5.00</td>
<td>0.58</td>
<td>0.95</td>
<td>0.05</td>
<td>0.42</td>
</tr>
<tr>
<td>≤7.50</td>
<td>0.69</td>
<td>0.93</td>
<td>0.07</td>
<td>0.31</td>
</tr>
<tr>
<td>≤10.00</td>
<td>0.78</td>
<td>0.90</td>
<td>0.10</td>
<td>0.22</td>
</tr>
<tr>
<td>≤12.50</td>
<td>0.82</td>
<td>0.88</td>
<td>0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>≤15.00</td>
<td>0.84</td>
<td>0.86</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>≤17.50</td>
<td>0.86</td>
<td>0.83</td>
<td>0.17</td>
<td>0.14</td>
</tr>
<tr>
<td>≤20.00</td>
<td>0.87</td>
<td>0.81</td>
<td>0.19</td>
<td>0.13</td>
</tr>
<tr>
<td>≤22.50</td>
<td>0.87</td>
<td>0.79</td>
<td>0.21</td>
<td>0.13</td>
</tr>
<tr>
<td>≤25.00</td>
<td>0.87</td>
<td>0.78</td>
<td>0.22</td>
<td>0.09</td>
</tr>
<tr>
<td>≤27.50</td>
<td>0.91</td>
<td>0.78</td>
<td>0.22</td>
<td>0.07</td>
</tr>
<tr>
<td>≤30.00</td>
<td>0.91</td>
<td>0.75</td>
<td>0.25</td>
<td>0.07</td>
</tr>
<tr>
<td>≤35.00</td>
<td>0.93</td>
<td>0.73</td>
<td>0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>≤40.00</td>
<td>0.93</td>
<td>0.70</td>
<td>0.30</td>
<td>0.07</td>
</tr>
<tr>
<td>≤50.00</td>
<td>0.96</td>
<td>0.68</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>≤60.00</td>
<td>0.98</td>
<td>0.59</td>
<td>0.41</td>
<td>0.02</td>
</tr>
<tr>
<td>≤70.00</td>
<td>1.00</td>
<td>0.53</td>
<td>0.47</td>
<td>0.00</td>
</tr>
<tr>
<td>≥80.00</td>
<td>1.00</td>
<td>0.53</td>
<td>0.47</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Bronchial Provocation with Methacholine in Asthma (Perpita et al)
NPV: 0.84) were obtained when pretest probability was 0.48. When pretest probability was higher than 0.48, the PPV of the test increased but it was followed by a decrease in the NPV. Conversely, when the pretest probability was lower than 0.48, the PPV of the test decreased and the NPV increased. The interval security of the test (ie, the pretest probability range when PPV and NPV were greater than 50 percent) was between 0.16 and 0.87.

Posttest probabilities of asthma after a PPV or NPV as well as the maximal positive or negative final gain of the test are depicted in Figure 3. The maximal positive (0.38) and negative (0.42) final gains were achieved when pretest probabilities of asthma were 0.48 and 0.77, respectively.

The curves of PPV and 1-NPV using several cutoff points of PC20 are shown in Figure 4. We could observe that although the PPV of the test increased with lower PC20 values, the 1-NPV of the test also was enhanced.

**DISCUSSION**

Asthma is a common disease for which there is no agreed clinical, physiologic, or epidemiologic definition. Recently, an expert panel on asthma defined it as a lung disease characterized by: reversible airway obstruction, airway inflammation, and increased airway responsiveness to a variety of stimuli. In clinical practice, most physicians diagnose asthma from a history of compatible symptoms and objective measurements of variable airway obstruction. In this way, asthma also has been defined as bronchial hyperresponsiveness to either a bronchoconstrictor or bronchodilator agonist plus symptoms of asthma.

Airway hyperresponsiveness, however, is not a constant feature of asthma. The reported prevalence of bronchial hyperresponsiveness in patients with symptomatic asthma varies between 52 to 100 percent. Conversely, only 47 to 75 percent of subjects with hyperresponsiveness had current asthma. Several reasons have been proposed to explain these discrepancies: one reason is that airway responsiveness is as variable as other clinical features of asthma. It can be episodic, ie, occupational or seasonal allergic asthma, other reasons, such as the lack of specificity of asthmatic symptoms or the continuous distribution of responsiveness in a population, also have been argued. Therefore, asthma frequently is underdiagnosed or overdiagnosed in clinical practice if symptoms and bronchial provocation tests are not accurately interpreted.

The value of bronchial provocation tests in the diagnosis of asthma depends largely on its own interpretation. To differentiate between asthmatic and nonasthmatic subjects, a cutoff value of PC20 has to

**FIGURE 1.** Receiver operator characteristic curve for different cutoff values of PC20.

**FIGURE 2.** Curves for posttest probability of asthma after a PPV (solid line) and posttest probability of nonasthma after a negative test (NPV) (dotted line) using a cutoff value for PC20 of 15 mg/ml.

**FIGURE 3.** Posttest probability of asthma after a PPV or 1-NPV test. G(+), maximal positive diagnostic gain of the test; G(-), maximal negative diagnostic gain of the test.
be determined. An arbitrary cutoff level, generally the value that yields the highest sensitivity, is chosen in most of the studies.\textsuperscript{1,2} In the present study, we use a receiver operator characteristic curve to detect the best cutoff point of PC20 to separate asthmatic from nonasthmatic subjects. It is 15 mg/ml. Using this value, the sensitivity and specificity values of the test were 0.84 and 0.86, respectively.

The sensitivity of the test in our sample is lower than that in other studies.\textsuperscript{1,10} This can be explained by the diverse population considered or the different cutoff value used. The diagnostic usefulness of the test depends on the cutoff point employed. When an arbitrary high cutoff value of PC20 is used, the sensitivity of the test increases but the specificity decreases. The opposite occurs if a low cutoff value of PC20 is chosen. Therefore, only test results applying standardized methods to determine the best cutoff, like the receiver operator characteristic curve analysis, can be compared.

Likewise, the sensitivity of the test is related to the characteristics of asthmatic patients and their current status of asthma symptoms. Airway hyperresponsiveness is greater in patients with recent symptoms than in subjects with past symptoms.\textsuperscript{19,30} It could be argued that the sensitivity of the test in our sample is improved by the selection of subjects with current asthma. However, most of the authors\textsuperscript{33,34} think that the use of hyperresponsiveness as a diagnostic test for asthma in clinical settings only can be helpful when subjects have current symptoms consistent with this.

The inclusion of other nonasthmatic groups with asthma-like symptoms, such as patients with COPD, who might have bronchial hyperresponsiveness also can affect the specificity of the test. The mechanism that causes hyperresponsiveness in patients with COPD is likely secondary to the reduced airway caliber but the different effect of initial airway caliber on the response to bronchial provocation in subjects with asthma or COPD is unclear. While some authors\textsuperscript{35} suggest that in COPD patients there is a clear relationship between FEV\textsubscript{1} and PD20, others\textsuperscript{36} state that this association also can occur in asthmatic subjects. In our study, only five subjects with chronic bronchitis had a baseline FEV\textsubscript{1} (percent predicted) lower than 80 percent, but the mean FEV\textsubscript{1} values of patients with chronic bronchitis were not significantly different for all the other groups. Consequently, we do not think that the exclusion of these five patients would modify significantly the specificity of the test.

If sensitivity and specificity are good determinants of the diagnostic quality of the test, their proper value in epidemiologic or clinical settings can only be established taking into account the prior probability of disease. The pretest probability of asthma should be based on the history, physical examination, and laboratory data of the patient. In our study, we use Bayes' theorem to determine posttest probability of asthma, considering the full range of pretest probabilities and test results. Our findings show that the test could only be considered if prior probability of asthma is established between 0.16 and 0.87. If pretest probability is lower than 0.16, posttest probability of asthma is less than 0.5, being the least worthy probability accepted. This could explain the low diagnostic value of the test reported in epidemiologic
studies where the estimated prevalence of asthma is below 0.15. Likewise, if pretest probability is higher than 0.87, the test result does not significantly increase the prior probability of disease.

Although a numerical estimate of pretest probability was used in our study in order to apply Bayes' theorem, it seems reasonable to think that such estimates are not easily obtained in clinical practice. It is more helpful for the physician to express the different pretest probabilities in words such as "low," "mild," or "high" probability. With this approach, we can say that the test could be useful if it is not performed in patients with lowest or highest probability of asthma.

Using the cutoff value of 15 mg/ml, the best utility of the test is obtained with a pretest probability near to 0.50. The probability of having asthma, when the test result is positive, is 0.86, and the probability of excluding asthma with a negative test result is 0.84. Britton et al. analyzed the results of a study by Cockroft et al., where the prevalence of asthma was 0.51, similar to the best pretest probability in our study. He found that the probability posttest of having or not having asthma was 0.77 and 0.95, respectively. We think that our findings are not consistent with those of Cockroft et al because of the different PC20 values used. The increased sensitivity of the test in their study enhanced the probability of nonasthma after a negative test but decreased the posttest probability of asthma.

Posttest probability of having or not having asthma varies inversely with pretest probability so it is important to make a good clinical estimate before the test has been done.

When the test is positive, we observe that the nearer to 0.5 the prior probability of asthma is, the higher is the posttest probability is of having asthma. On the other hand, if pretest probability of asthma is higher than 0.5, the main application of the test is to exclude asthma diagnosis in those patients whose test results are negative.

The difference between the probability of having asthma before and after the test has been done is the final diagnostic gain of the test, and this represents the contribution of the test to the diagnosis of asthma. As Britton says, "what matters is not so much the absolute predictive value of the test but how much this differs from the probability of disease before the test was performed." The sum of the maximal positive and negative diagnostic gains is the diagnostic content of the test. The maximal positive diagnostic gain of the test in our study (0.39) is achieved when pretest probability is 0.48; above or below this level the positive diagnostic gain decreases. Similarly, the maximal negative diagnostic gain (0.42) is taken when pretest probability is 0.70. Therefore, the diagnostic content of the test is maximal when the prior probability of asthma is between 0.48 and 0.70.

These results show that bronchial provocation tests with methacholine can be considered useful in the diagnosis of asthma if they are properly used.

Recently, Gilbert and Auchincloss, using published data of other authors, have constructed several curves for posttest probability of asthma. They emphasize that changes in the population studied or in the level of the PC20 used as a cutoff value can affect the curves, making the comparisons between different studies inaccurate. Thus, curves for different cutoff values have to be constructed.

We used our data to represent the probability of asthma after a positive or negative test using different cutoff values of PC20 (Fig 4). In this way, posttest probability of asthma is accurately established after the individual PC20 and the clinical estimate of asthma are determined.

From the present work, we conclude that the application of Bayes' theorem, considering the pretest probability of asthma and the sensitivity and specificity of the individual PC20 obtained, enabled us to obtain a good performance of the bronchial provocation test in the diagnosis of asthma.

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