Usefulness of bronchial reactivity analysis in the diagnosis of bronchial asthma in patients with bronchial hyperresponsiveness


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Summary We examined the usefulness of some bronchial reactivity indices to identify bronchial asthma in patients with airway hyperresponsiveness.

Eighty-eight consecutive patients with positive response to histamine bronchial challenge (≥20% fall in FEV₁) were included in the study. Dose–response curves were characterised by their sensitivity (PD₂₀) and reactivity. Dose–response slope, continuous index of responsiveness (CIR) and bronchial reactivity index (BRI) with respect to baseline and post-diluent baseline values were determined as reactivity indices. The clinical diagnosis remaining in the case history 2 years after the bronchial challenge was considered the definitive diagnosis.

Asthmatic patients had higher baseline BRI (12.121 ± 0.412 vs. 11.615 ± 0.201; P < 0.001) and post-diluent baseline BRI (12.054 ± 0.368 vs. 11.563 ± 0.531; P = 0.003) than other subjects. Area beneath their receiver operating characteristic (ROC) curve was 82.68% (standard error: 0.77) for the baseline BRI and 81.73 (standard error: 0.76). By multiple logistic regression analysis, baseline BRI was the only independent variable identified as a predictor for diagnosis of bronchial asthma (r = 0.387, P = 0.0007). A cut-off of 11.76 for baseline BRI reached an 87.2% sensitivity and an 80% specificity for bronchial asthma diagnosis.

In conclusion, BRI calculated with respect to baseline FEV₁ should be useful in identifying asthmatic patients among subjects with airway hyperresponsiveness.

Introduction

Airway hyperresponsiveness, defined as an exaggerated airway narrowing upon exposure to a non-specific bronchoconstrictor stimuli, is a functional phenomenon associated with inflammatory disorders of the airways, such as asthma.¹⁻⁶

Airway hyperresponsiveness is usually studied by constructing dose–response curves to pharmacological bronchoconstrictors.⁷ Conventional indices of responsiveness derived from the dose–response curve have been reported using various methods. Hypersensitivity and hyperreactivity specifically refer to a leftward shift and an increase in slope, respectively, of the dose–response curve obtained during inhalation challenge procedures.⁸

Most commonly, the dose–response relationship is described as the dose or concentration of bronchoconstricting agent that causes a specified
decline in airflow or airway conductance, such as PD_{20} FEV_{1}, the dose provoking a 20% decline in FEV_{1}. These measurements are related to asthma severity and provide a useful measure with which to follow the course of individual asthmatic patients.

However, these expressions of responsiveness have an important limitation in diagnostic procedures for asthma. They are particularly suitable for the exclusion of asthma, because of the high sensitivity and high negative predictive value. However, since airway hyperresponsiveness is also described in allergic rhinitis, cystic fibrosis, viral infections, influenza vaccination, congestive heart failure, sarcoidosis, bronchopulmonary dysplasia, hypersensitivity pneumonitis, chronic obstructive pulmonary disease, antigen exposure, outdoor pollution (ozone, NO_{2}, SO_{2}) and healthy subjects, a positive result is not a prerequisite of asthma, and hence, does not rule out other conditions.

An alternative method of expressing the dose–response relationship involves fitting a mathematical model to each individual subject’s data. Simple and more complicated models have been used to describe the shape of the dose–response curve. It has been proposed that the dose–response curve slope could be more useful in the identification of asthmatic patients than the determination of threshold dose or sensitivity.

In the present report, we examine the usefulness of some bronchial reactivity indices in identifying bronchial asthma in patients with airway hyperresponsiveness.

Methods

Subjects

Eighty-eight consecutive patients with positive response to histamine bronchial challenge (≥20% fall in FEV_{1}) were included in the study. Patients were referred to bronchial challenge due to clinical suspicion of asthma. There were 55 women and 33 men, with an age (mean ± standard deviation (SD)) of 38 ± 18 years. Their weight and height were 65 ± 15 kg and 162 ± 19 cm, respectively. Sixteen subjects (18%) were smokers at the time of the study.

Patients stopped use of short- or long-acting beta_{2}-adrenergic bronchodilators for at least 12 or 24 h before the bronchial challenge, respectively. Antihistamine had been withdrawn for at least 1 week before the bronchial challenge. None of the subjects had taken inhaled or oral corticosteroids or leukotriene receptor antagonists in the 3 months before the study. Informed consent was obtained from all subjects.

Pulmonary function

Spirometry was conducted with a MasterScreen Pneumo 4.2 (Jaeger, Würzburg, Germany) according to European Respiratory Society standardisation. The normal values for lung volumes were those proposed by the European Community for Coal and Steel.

A standardised dosimeter technique was used for histamine challenge. Bronchial aerosol provocation system (APS, Jaeger, Würzburg, Germany) with Medic Aid Side Stream nebuliser (Medic-Aid Ltd, Bognor Regis, UK) was used for this procedure. The nebuliser was calibrated to produce an output of 160 mg/ml, with an airflow rate of 100 ml/s. A flow sensor in the expiratory port triggers a solenoid which exposes the nebuliser to compressed air at 138 kPa (20 psi) for about 0.6 s, to give a calibrated output per puff of 9.0 μl. The nebuliser generates heterodisperse droplets with a median aerodynamic mass diameter of 0.5–4 μm.

Each subject was instructed to inhale the aerosols by taking slow deep breaths from functional residual capacity to inspiratory capacity without breathholding. The first aerosol was 0.9% saline followed by doubling doses of histamine diphosphate from 0.03 to 9.4 mmol. A 3-min interval was allowed before each dose increment. FEV_{1} was measured by a MasterScreen Pneumo (Jaeger) 2 min after each dose and the highest of three acceptable measurements within 100 ml was retained to create dose–response curves. The test was discontinued when there was a fall in FEV_{1} of ≥20% compared with the control inhalation (0.9% saline solution) or until the maximal dose was inhaled.

Dose–response curves were plotted for each challenge test as percentage fall in FEV_{1} against the dose of histamine on a log scale and were characterised by their sensitivity (dose of histamine that produced 20% fall in FEV_{1}, PD_{20}) and their slope. When FEV_{1} had fallen by ≥20% from post-diluent baseline value, the challenge was considered positive and PD_{20} was determined by linear extrapolation on a semi-logarithmic scale. Dose–response slope (DRS) was summarised as the expression: per cent decline FEV_{1}/dose, where per cent decline FEV_{1} was defined as the decline in FEV_{1} (from the baseline and post-diluent baseline values) after the final histamine dose was...
administered, and dose was defined as the final cumulative dose administered.\textsuperscript{16} Continuous index of responsiveness (CIR) was determined as the logarithm of the per cent decline from the pre- and post-diluent baseline FEV\textsubscript{1} after the last dose of histamine per unit dose of histamine.\textsuperscript{17} Finally, bronchial reactivity index (BRI) was defined as the log of the per cent decline in FEV\textsubscript{1}/log final histamine dose after adding 10 to eliminate negative values.\textsuperscript{17}

### Clinical diagnosis

A physician blinded to the results from the analysis of airway reactivity indices revised the case history of each patient included in the study. Asthma was defined as a clinical history of intermittent wheeze, cough, chest tightness, or dyspnoea, and documented reversible airflow limitation either spontaneously or with treatment.\textsuperscript{1} Definitive diagnosis was established after a follow-up of 2 years, as the clinical diagnosis remaining in the case history.

### Statistical analysis

Data are expressed as mean±SD. The statistical study was performed using the Statistical Package for the Social Sciences software for Windows Release 8.0 (SPSS Inc, Chicago, IL, USA). The quantitative parameters for groups with and without asthma were compared by \textit{t}-test or Mann–Whitney test. For comparison of qualitative variables, the $\chi^2$ test was applied. $P<0.05$ was considered significant.\textsuperscript{21} Analysis of the individual variables was completed by calculating the areas beneath their receiver operating characteristic (ROC) curves.\textsuperscript{22} Logistic regression was used to assess interaction between the most significant variables with respect to asthma diagnosis.\textsuperscript{23}

### Results

A definitive diagnosis of bronchial asthma was reached in 55 patients (63\%). The 33 remaining subjects were considered to have allergic rhinitis (20 cases), post-infectious airway hyperresponsiveness (11 cases) or absence of recognised respiratory disease after the 2 years of follow-up (two cases).

No significant differences in anthropometric characteristics, smoking habit, baseline FEV\textsubscript{1}, post-diluent fall in FEV\textsubscript{1} or PD\textsubscript{20} were noted between asthmatic patients and non-asthmatic subjects. However, asthmatic patients had higher baseline BRI (12.121±0.412 vs. 11.615±0.201; $P<0.001$) and post-diluent BRI (12.054±0.368 vs. 11.563±0.531; $P=0.003$) than the other subjects (Table 1).

Area beneath ROC curve was 82.68\% (standard error: 0.77) for the baseline BRI and 81.73\% (standard error: 0.76) (Fig. 1). By multiple logistic regression analysis, baseline BRI was the only independent variable identified as a predictor in the diagnosis of bronchial asthma ($r = 0.387$, $P=0.003$).

### Table 1

<table>
<thead>
<tr>
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<th>Asthma group ($n = 55$)</th>
<th>Non-asthma group ($n = 33$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females (%)</td>
<td>63.6</td>
<td>39.4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>39±17</td>
<td>36±19</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161±22</td>
<td>164±11</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>65±13</td>
<td>69±18</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>18.2</td>
<td>18.2</td>
</tr>
<tr>
<td>Atopic subjects (%)</td>
<td>42</td>
<td>48</td>
</tr>
<tr>
<td>Baseline FVC (l)</td>
<td>3.65±1.10</td>
<td>3.75±1.29</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1} (l)</td>
<td>2.99±0.93</td>
<td>3.01±1.04</td>
</tr>
<tr>
<td>Baseline FEV\textsubscript{1}/FVC (%)</td>
<td>81.9±6.7</td>
<td>81.1±12.1</td>
</tr>
<tr>
<td>Post-diluent FEV\textsubscript{1} (l)</td>
<td>2.87±0.97</td>
<td>2.95±0.96</td>
</tr>
<tr>
<td>PD\textsubscript{20} (mmol)</td>
<td>3.31±2.40</td>
<td>2.21±1.98</td>
</tr>
<tr>
<td>Baseline DRS (%/mmol)</td>
<td>106.5±375.4</td>
<td>150.2±550.8</td>
</tr>
<tr>
<td>Post-diluent DRS (%/mmol)</td>
<td>75.4±196.6</td>
<td>84.3±243.4</td>
</tr>
<tr>
<td>Baseline CIR</td>
<td>1.39±0.53</td>
<td>1.40±0.73</td>
</tr>
<tr>
<td>Post-diluent CIR</td>
<td>1.35±0.51</td>
<td>1.30±0.74</td>
</tr>
<tr>
<td>Baseline BRI</td>
<td>12.121±0.412</td>
<td>11.615±0.201\textsuperscript{1}</td>
</tr>
<tr>
<td>Post-diluent BRI</td>
<td>12.054±0.368</td>
<td>11.563±0.531\textsuperscript{1}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Results are mean±SD.

\textsuperscript{1}$P<0.001$ vs. asthma group.
The best cut-off of baseline BRI to discriminate between asthmatic and non-asthmatic subjects was 11.76. This point reached an 87.2% sensitivity and an 80% specificity for diagnosing bronchial asthma (Fig. 2).

Discussion

The main result of our study consists in the identification of BRI as a discriminatory factor between asthmatic and non-asthmatic subjects with airway hyperresponsiveness. In our patients, the BRI calculated with respect to baseline FEV₁ have a notable diagnostic sensitivity and specificity. An important methodological issue needs prior comment. We considered the definitive diagnosis of our patients as that which remained in the case history after a 2-year follow-up. In our opinion, this has two important implications. Diagnosis of each patient was established by a physician outside the study according to standard criteria.1 With this procedure, we tended to avoid bias in subject classification. The objective of the 2-year follow-up was to assess the airway hyperresponsiveness by discriminating between permanent and transitory cases. Moreover, previous studies described that up to 19% of patients with allergic rhinitis and airway hyperresponsiveness develop bronchial asthma in the 2 years following diagnosis.24 Thus, it seems reasonable to follow the clinical evolution of
patients during this period before establishing the definitive diagnosis.

Classical analysis of bronchial challenge curves in terms of PD_{20} sensitivity proposes an important problem. The majority of methacholine or histamine bronchial challenge tests are characterised by a unimodal or continuous distribution of airway sensitivity.\textsuperscript{25} This disposition, without a clear cut-off between studied populations, makes it an unlikely choice for the normal threshold. Therefore, any cut-off chosen will define sensitivity and specificity levels to discriminate between asthmatic and non-asthmatic subjects. This point must be considered arbitrary and variable in relation to population studied and method applied.

Although sensitivity analysis of bronchial challenge has an elevated negative predictive value, its positive predictive value is very dependent on degree of clinical suspicion. For an asthma pre-test probability of 50%, the probability of having asthma when the challenge is positive reaches 86%.\textsuperscript{26} However, after a 2-year follow-up, diagnosis of bronchial asthma was only reported in 39% of asymptomatic subjects with bronchial hyperresponsiveness.\textsuperscript{27} Our patients are in an intermediate situation, because bronchial hyperresponsiveness was associated with bronchial asthma diagnosis in 63%.

Contrasting with recognised limitations of the sensitivity analysis of bronchial challenge dose–response curve, our results show that the reactivity analysis could contribute to better discrimination between asthmatic and non-asthmatic patients. Differences in diagnostic accuracy among the various bronchial reactivity indices analysed should be attributed to their interval characteristics. DRS is a simple expression that summarises each subject’s dose–response relationship by the slope of a line connecting the origin of the dose–response curve with the final point of the curve. Previously, it has been shown that a linear model fits histamine dose–response data better than does a logarithmic model.\textsuperscript{28} Therefore, within the dose range examined, there is a strong linear relationship between dose and per cent decline in FEV\textsubscript{1} for asthmatic subjects.\textsuperscript{16} A previous study\textsuperscript{16} reported that DRS differed markedly between asthmatic and normal subjects. But not one of the nine normal subjects in their study showed airway hyperresponsiveness, determined by a 20% decline in FEV\textsubscript{1}. In contrast, after adding a constant of 10 to eliminate negative numbers, BRI provides a most continuous and relatively normally distributed index of bronchial reactivity. In a study over 522 11-year-old children, reported asthma was associated with increased BRI independent of other factors such as serum IgE levels, symptoms or sex.\textsuperscript{17} Therefore, it is possible that BRI has better discriminatory power between asthmatics and non-asthmatics as a consequence of its better adjustment to normal distribution.

The convenience of analysing bronchial challenge dose–response curve with respect to pre- or post-diluent baseline values is still controversial.\textsuperscript{29,30} From a theoretical point of view, the indices calculated with respect to post-diluent better represent the challenge effect than those calculated with respect to baseline value. The former reflect only the changes induced by the bronchial challenge, whereas the latter are influenced by challenge and diluent nebulisation.\textsuperscript{7} However, in our study, baseline BRI was the only independent variable with diagnostic predictive capacity.

There is evidence that maximum capacity and velocity of shortening in bronchial smooth muscles in patients with asthma are significantly greater than those obtained in healthy subjects.\textsuperscript{31,32} Moreover, it has been demonstrated that the increased extent of shortening in sensitised airway smooth muscles occurs in the early phase of shortening. These findings should explain the greater BRI found in our asthmatic patients. An unresolved question is whether bronchial smooth muscle contractility is altered or if the muscle is made to shorten more simply by increased amounts of agonists released from the mast cells and other cells by the immune reaction. Nevertheless, and due to the phenotypic heterogeneity of bronchial smooth muscle cells, a different cellular composition should also justify the faster shortening of bronchial muscle in patients with bronchial asthma.

Moreover, our results are in accordance with previous results of other studies with different dose–response curve analyses. Prieto et al.\textsuperscript{33} performed a methacholine bronchial challenge in asthmatic patients and in subjects with rhinitis. These authors administered higher doses of methacholine just to achieve a maximal response, characterised by a plateau level of FEV\textsubscript{1}. In their study, fall in FEV\textsubscript{1} in asthmatics was faster than in subjects with rhinitis.\textsuperscript{33}

In conclusion, due to the low positive predictive value of histamine bronchial challenge for diagnosing asthma, reactivity index analysis should contribute to the identification of asthmatic patients among those subjects with airway hyperresponsiveness. Reactivity analysis is neither expensive nor invasive. Its determination is easy and does not require modifying challenge tests. However, it must be considered that the cut-off point obtained in our study could not be applied in other population groups and that its diagnostic accuracy will depend on the prevalence of bronchial asthma in each
group or, in other words, on the degree of clinical suspicion.

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**References**


