Bronchial responsiveness to inhaled methacholine in epidemiological studies: comparison of different indices

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ABSTRACT: We evaluated the sensitivity and specificity of different indices drawn from the forced expiratory curve in order to detect the best one for evaluating bronchial reactivity in the general population, and particularly, for distinguishing between normal and asthmatic subjects. 654 subjects, between 15 and 64 years of age, who were representative for age and sex of the general population of a small Lombardy town (Italy), were studied. Of the total sample, 448 subjects were clinically normal and asymptomatic, 87 were symptomatic or with acute upper respiratory disease within 30 days before the challenge with methacholine, 43 with allergic rhinitis, 26 asthmatics and 50 chronic bronchitics. All the subjects who had a baseline FEV\textsubscript{1} greater than 85% predicted underwent methacholine challenge. Provocative LnDoses of 6, 10, 15% fall in FEV\textsubscript{1} (LnPD\textsubscript{6}, LnPD\textsubscript{10}, LnPD\textsubscript{15}) and provocative LnDose causing FEV\textsubscript{1} to fall more than 20% below the mean of 5 FEV\textsubscript{1} measurements following buffer inhalation (LnTD) were evaluated. Furthermore we calculated the "Dose-Response slope" (SL) proposed by O'Connor. The cut-off value of each index to define a responder was calculated by discriminant analysis of the response to the challenge in normal and in the asthmatic group. Sensitivity, specificity and predictive value of LnPD\textsubscript{6}, LnTD and SL were 79, 74, 14%; 71, 74, 13% and 46, 97, 48% respectively. LnPD\textsubscript{6} had the best sensitivity (83%) with a high specificity (83%), but, as compared to LnPD\textsubscript{15}, whose sensitivity was 79% and specificity 89%, a lower predictive value (21% vs 27%). Thus LnPD\textsubscript{6} turns out to be the best index to assess bronchial responsiveness in epidemiological studies; with respect to the diagnosis of bronchial asthma however, a low predictivity confirms that the bronchial challenge has only a supplemental role in detecting the disease.

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Recently, studies on bronchial reactivity in the general population began to be published [1-6]. However, the evaluation of sensitivity and specificity of different indices to assess the responsiveness to several bronchial challenge tests has been carried out until now on small samples which were not representative of the general population [7-11]. Indices based on the fall in airway conductance and partial expiratory flow-volume curve measurements after challenge with various increasing doses of bronchoconstrictor agents have been proposed for clinical practice [7-12]. These indices are considered to be more sensitive and independent of the influence of deep inspiration as compared to the more widely employed provocative dose of 20% fall in FEV\textsubscript{1} (PD\textsubscript{20},FEV\textsubscript{1}). However, in epidemiology, only total expiratory curves may be easily obtained and the response to the bronchial challenge is almost always based on the changes in these curves.

The measurement of PD\textsubscript{20},FEV\textsubscript{1} which is widely accepted in clinical practice, raises several problems in epidemiology. In normal individuals, high doses of bronchoconstrictor agents have to be administered, decreasing the safety and compliance of the test [11]; even then many subjects fail to develop a 20% fall in FEV\textsubscript{1} even at very high doses. Several authors [10, 13-15] have proposed the employment of new indices linked to a smaller fall of FEV\textsubscript{1}; these are the histamine or methacholine thresholds, defined as the lowest concentration first producing a fall in FEV\textsubscript{1}, of more than 20% below the mean of four pre-challenge determinations and the provocative doses associated with smaller percentage falls (6%, 10%, 15%) of FEV\textsubscript{1} with respect to the pre-challenge value. Such indices, although reducing the percentage of non-responders, do not completely solve the problem: the distribution curve of the responsiveness to the non-specific bronchial challenge test, in the general population, is still censored at one end of its range, raising difficulties in the statistical evaluation of data.

Furthermore the calculation of the dose required to provoke a given decrement in lung function does not give accurate quantification of bronchial responsiveness.
Indices of Bronchial Responsiveness

It is often completed by the study of the slope calculated in different ways, including all or only some of the points [15]. Recently Neukam and colleagues [16] proposed a new model summarising all the information contained in methacholine dose-response curves simplifying the model in order to apply them to the epidemiological studies.

O’Connor et al. [17] proposed the employment of a simple index represented by the ratio between the percentage decline of FEV1 after the final dose administered and the final cumulative dose: this is a continuous measurement that is not censored at lower levels of bronchial responsiveness.

No epidemiological study conducted on all these indices has yet been published, so that their capability of discriminating between normals and asthmatics is not available. It is also necessary to know their specificity with regard to the general population. A large part of the available data evaluates specificity only with respect to normal subjects, arbitrarily considering hyperreactivity as an exclusive feature of bronchial asthma. But it has been known for a long time that reactivity may be increased in conditions other than asthma, in particular in chronic bronchitis [3-5, 18-20] in cigarette smokers [21, 22], in subjects with recent upper respiratory tract infections [23], and in those with allergic rhinitis [24, 25].

Thus the aim of our study was to define the cut-off values of different indices and to evaluate their sensitivity, specificity and predictive value, in order to detect the best index for distinguishing between normal and asthmatic subjects. We considered clinical diagnosis as the “gold standard” and, on this basis, we evaluated the indices of responsiveness to the challenge.

Methods

Population

In 1982 an epidemiologic prospective study on COPD was started in the general population of a small Lombardy town in Northern Italy (Caronno Pertusella - VA).

A stratified random sample of 916 subjects was selected from the population between 15 and 64 years of age who were resident for more than five years. They were stratified by age of the head of the households and the number of family members. They were found to be representative for age and sex of the reference population [26]. Of 916 subjects in the first survey, 654 participated in the second cross-sectional survey, three years later; although 29% of the original sample was lost, it was still statistically representative, by age and sex, of the reference population [18]. During this second control, we included a bronchial challenge with methacholine along with routine functional tests. All the subjects were interviewed by two pulmonary specialists, who filled in a detailed health questionnaire of the Italian Research Council, adapted from the standard NHLI questionnaire [27]. On the basis of the response to the questionnaire and more accurate history from clinical evaluation, the physicians clinically classified subjects who were suffering from bronchial asthma (A) and chronic bronchitis (CB) according to the criteria set out in the CIBA Guest Symposium [28] and suggested by the American Thoracic Society [29].

Subjects suffering from respiratory symptoms who did not completely meet the criteria for including in group A or CB and those with acute respiratory disease within 30 days before the challenge were considered in another group and classified as “symptomatic” (S). A group of subjects suffering from chronic rhinitis whose allergic etiology was confirmed by positive allergic skin tests (AR) was identified. All the subjects who were not ranked in the above mentioned groups were defined as “normals” (N).

Controversial cases were re-examined in order to achieve an unequivocal diagnosis by means of a more detailed history and physical examination.

By clinical examination the sample was found to be formed by 50 CB, 26 A, 87 S, 43 AR, 448 N, and was not significantly different from the original sample of 916 subjects.

A detailed history about smoking was collected. The number of pack-years smoked was then calculated for each subject. The sample studied and particularly the normal group showed relatively low levels in pack-years [30]; this finding reflecting a considerable effect of the national educational health program concerning smoking habit.

Two normal subjects refused to be tested with methacholine, 38 (31 N, 5 CB, 2 AR) were not capable of performing the bronchial challenge.

On the basis of functional baseline value 9 subjects (6 CB and 3 A) were found to have airway obstruction (FEV1 = 61 ± 6% of the predicted values) and underwent bronchodilation test.

All the remaining clinically abnormal and normal subjects who underwent methacholine challenge had a baseline FEV1 and FEV1/FVC ratio > 85% of predicted [31]. The mean FEV1 values for all the challenged groups were not significantly different.

Methacholine protocol

All the subjects had to abstain from any drug whose effect might alter airway responsiveness and from smoking for at least 12 hours before undergoing the challenge.

Three forced expiratory vital capacity manoeuvres were performed on a dry spirometer Vicatex 4 Hellige (Freiburg im Breisgau, West Germany). The challenge was performed with methacholine using buffered lypo-phile methacholine (phosphate buffer) "Lofarma" (Milan, Italy). The 1% concentration of methacholine was prepared by diluting methacholine in distilled water. A metered nebulizer dosimeter "Mefar" (Brescia, Italy) delivered methacholine from De Vilbiss 646 ampole by means of an air compressor (driving pressure 1.5 kg·cm-2). Inhalation time was set as 1.1 s, and every inhalation delivered 200 μg of methacholine. First the phosphate buffer was inhaled and five forced expiratory curves were obtained. If the largest post-buffer FEV1 did not change by more than 5% as compared to the largest baseline value, increasing cumulative doses of
methacholine (200, 400, 800, 1600, 3200, 4800 µg) were delivered (by increasing the numbers of inhalations from the dosimeter). Every inhalation was performed with a slow submaximal inspiratory manoeuvre, beginning at functional residual capacity. One minute after each dose, three forced expiratory curves were registered and the best FEV₁ was considered. The test was stopped and defined as positive at a drop of more than 15% in FEV₁ compared to the post-buffer value; otherwise it was terminated at the highest cumulative dose of 4800 µg. All the inhalations of methacholine had to be delivered within 20 min in order to obtain a cumulative effect. All the subjects who had positive reactions were given salbutamol spray (200 µg) post challenge.

The protocol was the same for clinically normal and abnormal subjects. Provocative doses of a 6, 10, 15% fall in FEV₁ with respect to the baseline value (PD₆, PD₁₀, PD₁₅) and provocative dose causing FEV₁ to fall 25% below the mean of 5 values of post-buffer FEV₁ (Threshold Dose, TD) were calculated by plotting FEV₁ at each dose on a semilogarithmic scale and joining the points immediately above and below the chosen levels of the percentage fall in FEV₁. All these indices were then transformed to logarithms because of the highly skewed distribution.

All negative tests were arbitrarily assigned to the 8.52 LnDose category (5000 µg).

Furthermore we calculated the "dose response slope" (SL) proposed by O'Conor [17] as the ratio between the percent decline in FEV₁ after the final methacholine dose registered and the final cumulative dose, expressed in µmol; this last dose was that which produced a fall of more than 15% in FEV₁, as compared to the post-buffer value or, in the absence of the above mentioned fall, by the last dose administered.

Statistical analysis

To determine the cut-off value of each index we employed the group of clinically normal subjects and the group of asthmatics. Discriminant analysis was performed in order to determine the boundary values linearly adjusted for the covariates: age, number of pack-years, and baseline respiratory function, expressed as the largest baseline respiratory function, expressed as the largest.

\[
C = M_1 - \sum_{a_j} (X_j - M_j),
\]

where \(M\) and \(\bar{X}\) represent respectively the median and the weighted mean between the mean values of the two groups on the basis of index (1) and covariate (j) and "a's" a site's discriminant coefficients. The above value "C" is a re-scaled cut-off based on Fisher's linear discriminant function. This approach is a "sensible" rule to the discrimination problem; namely it can be made without assuming any particular parametric form for the distribution of the data matrix [32]. Fisher's criterion is intuitively attractive because the linear function is constructed maximising the ratio of the between-groups variance relative to the within-groups variance; moreover its performance is flexible and efficient, comparable to more sophisticated interactive procedures [33]. Thus we might hope this rule would be appropriate in exploratory studies where the gold standard is not exactly definitive.

To evaluate the performance of the boundary values we determined sensitivity, specificity and predictive value [34] for each index, with respect to the whole sample on study, calculated as follows:

Subjects with asthma who are positive to the test
\[
a = \frac{\text{Total number of clinically asthmatic subjects}}{\text{Total number of subjects tested without asthma}} \times 100
\]

Subjects without asthma who are negative to the test
\[
b = \frac{\text{Total number of subjects tested without asthma}}{\text{Total number of subjects with positive test}} \times 100
\]

Subjects with asthma who are positive to the test
\[
c = \frac{\text{Total number of subjects with positive test}}{\text{Total number of clinically asthmatic subjects}} \times 100
\]

Results

The distribution of bronchial responsiveness in the total sample, normal and asthmatic subjects, as evaluated by means of the different indices is represented in fig. 1.
INDICES OF BRONCHIAL RESPONSIVENESS

Table 1. – Mean value and (sd) of each index and the four covariate for normals, asthmatics and the whole sample

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normals</th>
<th>Asthmatics</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>LnPD_{15}</td>
<td>8.35 (0.35)</td>
<td>6.79 (1.05)</td>
<td>8.25 (0.57)</td>
</tr>
<tr>
<td>LnPD_{10}</td>
<td>8.13 (0.61)</td>
<td>8.45 (0.92)</td>
<td>8.02 (0.76)</td>
</tr>
<tr>
<td>LnPD_{6}</td>
<td>7.67 (0.88)</td>
<td>6.06 (0.78)</td>
<td>7.57 (0.96)</td>
</tr>
<tr>
<td>LnTD</td>
<td>7.48 (1.04)</td>
<td>6.16 (0.90)</td>
<td>7.40 (1.09)</td>
</tr>
<tr>
<td>SL</td>
<td>0.56 (0.66)</td>
<td>6.13 (7.68)</td>
<td>0.92 (2.43)</td>
</tr>
</tbody>
</table>

Covariate

| Age       | 39.8 (12.2) | 42.7 (10.8) | 40.0 (12.1) |
| Pack year | 5.18 (10.3) | 0.40 (0.60) | 4.85 (10.0) |
| FEV_{1} ml| 3269 (794)  | 2743 (696)  | 3235 (798)  |
| MMEF ml·s^{-1} | 4079 (1245) | 3151 (910)  | 4019 (1246) |

and their mean values and standard deviation are reported in table 1.

Cut off values computed by discriminant analysis of the response to the challenge in the asthmatic and in the normal group progressively increase from LnPD_{6} up to LnPD_{15}. LnTD is found to have a cut-off value similar to LnPD_{9}.

The frequency of responders with respect to the cut-off value of each index (table 2) is always highest in the asthmatic population: however, a considerable percentage of responders is observed also among subjects with chronic bronchitis and symptomatic subjects, a slightly lower percentage among subjects with allergic rhinitis and a lower one among normal subjects. Sensitivity, specificity and predictive value of the reported cut-off points for each index, with respect to the clinical diagnosis of bronchial asthma on the whole sample on study are reported in table 3. LnPD_{10} showed the highest sensitivity, LnPD_{15} and LnPD_{6} a fairly lower and SL a much lower one. Specificity was very high for SL, followed by LnPD_{15} and LnPD_{6} while for LnPD_{9} and LnTD it was lower. Then LnPD_{6} and LnTD were found to be unemployable because of their low levels of sensitivity, but, above all, specificity and predictivity. SL also appears to be inappropriate, because of its low sensitivity despite a very high specificity and a higher predictive value.

Table 2. – The frequency of responders (% of each group) for the different clinical groups and for the total sample for each index

<table>
<thead>
<tr>
<th>Index</th>
<th>Asthmatics</th>
<th>Chronic bronchitis</th>
<th>Allergic rhinitis</th>
<th>Symptomatics</th>
<th>Normals</th>
<th>Total sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>LnPD_{15}</td>
<td>79.2</td>
<td>44.1</td>
<td>17.2</td>
<td>31</td>
<td>6.3</td>
<td>14.8</td>
</tr>
<tr>
<td>LnPD_{10}</td>
<td>83.3</td>
<td>50.0</td>
<td>31.0</td>
<td>41.4</td>
<td>10.4</td>
<td>20.4</td>
</tr>
<tr>
<td>LnPD_{6}</td>
<td>59.9</td>
<td>55.9</td>
<td>48.3</td>
<td>51.7</td>
<td>19.0</td>
<td>28.3</td>
</tr>
<tr>
<td>LnTD</td>
<td>70.8</td>
<td>47.1</td>
<td>31.0</td>
<td>44.8</td>
<td>22.0</td>
<td>28.1</td>
</tr>
<tr>
<td>Slope</td>
<td>45.8</td>
<td>17.6</td>
<td>0</td>
<td>6.9</td>
<td>1.1</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Table 3. – Sensitivity, specificity and predictive value of each index with respect to the clinical diagnosis of asthma

<table>
<thead>
<tr>
<th>Index</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LnPD_{15}</td>
<td>79.2</td>
<td>88.6</td>
<td>26.8</td>
</tr>
<tr>
<td>LnPD_{10}</td>
<td>83.3</td>
<td>83.3</td>
<td>20.8</td>
</tr>
<tr>
<td>LnPD_{6}</td>
<td>79.2</td>
<td>74.1</td>
<td>14.0</td>
</tr>
<tr>
<td>LnTD</td>
<td>70.8</td>
<td>74.1</td>
<td>12.6</td>
</tr>
<tr>
<td>SL</td>
<td>45.8</td>
<td>97.4</td>
<td>47.8</td>
</tr>
</tbody>
</table>

The choice of the parameter for epidemiological surveys, therefore, by the analysis of our data, falls between LnPD_{10} and LnPD_{6}. LnPD_{6} has a better sensitivity but a lower specificity than LnPD_{15}, resulting in a considerable decrease of predictive value of LnPD_{10} compared to LnPD_{15}.

Discussion

From the end of the 1970s, the concept of bronchial hyperreactivity has attracted the attention of pneumologists. The usual detection in clinical practice of bronchial hyperreactivity in asthmatic subjects and the necessity for an objective measurement to diagnose asthma may have led to overestimation of the diagnostic validity of bronchial challenge tests. Woolcock [35] in her epidemiological studies, bases the diagnosis asthma on the presence of both symptoms and airway hyperresponsiveness to histamine or methacholine. However, in the last years it has been clearly pointed out that bronchial hyperreactivity is not a constant feature of bronchial asthma and may not be present at all times in a given asthmatic subject; for example, it can be present only during the pollen season in some patients with allergic asthma or, in some cases of occupational asthma, it can decrease after removal from exposure [36, 37]. Moreover hyperreactivity is a frequent finding in subjects with chronic bronchitis with or without chronic airflow obstruction, rhinitis, atopy and in cigarette smokers [8]. Such observations clearly support the concept that bronchial hyperreactivity is not synonymous with bronchial asthma.

The question is even more complicated, when the relationship between bronchial challenge test and bronchial hyperreactivity is taken into account, because several tests and, particularly, several indices of measurement have been proposed.

As pointed out by Britton and Tattersfield [8], till now studies on reproducibility, specificity and sensitivity have been carried out only on some of the indices of measurement and on relatively small sized samples, not representative of the general population [7, 9, 11, 14, 38]. The discriminatory values have been set employing groups of normals selected in the laboratory and asthmatic patients of the hospitals units, so that sensitivity and specificity are undoubtedly overestimated: these groups of asthmatics are likely to be more symptomatic
and more reactive than those subjects being studied on field surveys. The employment of such cut-off values runs the risk of being too restrictive. Moreover, the specificity, as evaluated in such a way, is incorrect because of the exclusion of all the other groups who might be affected from bronchial hyperreactivity without being asthmatics. Furthermore, normal subjects selected in laboratory are not usually representative for sex and age of the general population.

On the basis of these assumptions, therefore, we chose to study sensitivity and specificity of some indices of responsiveness to inhaled methacholine in a sample of subjects representative of the general population.

Another problem concerns different diagnostic criteria used to define the study population. Validity of a test is usually expressed in terms of clinical sensitivity and specificity, measured in relation to a "gold standard" for disease. This causes problems when considering asthma, since there is no accepted "gold standard"; we considered specified clinical diagnostic criteria as the "gold standard".

The choice of discriminant analysis yields cut-off values directly from the response value of asthmatics and normal subjects, and allows us to evaluate sensitivity without predetermining specificity.

Another problem is represented by non-responder subjects - even at high doses - who have to be given an arbitrary value for statistical analysis.

This procedure adversely affects the sensitivity of the parameter representing the highest frequency of non-responders (LnPD15). Nevertheless, of the remaining indices LnPD6, best discriminated normals from asthmatics, so that paradoxically this statistical handicap emphasises its validity.

Because of the problem of dealing with non-responders we evaluated the slope of the dose-response curve as proposed by O'Connor [17]. This slope can be criticised for over-simplification; it is calculated only on the last point; however Rucken et al. [39] demonstrated that in studies of subjects with relatively low levels of bronchial reactivity, as may be the case in most population studies, the dose-response curve as expressed by a straight line drawn through origin and last obtained data point will be equivalent to the slope of the individual linear regression of % decrease in FEV1, on mg·ml⁻¹ using all the data points. From our analysis, the employment of this parameter in epidemiology is limited by its low sensitivity, although a very high specificity is present. The mathematical artefact in the calculation of the parameter might lead to a clustering of normals around the lowest values and a considerable scattering of asthmatics in the distribution of the responsiveness. This last one in the total sample was found to be almost similar to that showed by O'Connor [17] in a sample of 465 subjects of the V.A. Normative Aging study. The behaviour of our asthmatics however resulted to be substantially different from the 9 subjects studied by O'Connor. This discrepancy might be explained by the fact that the subjects on study during hospitalisation are surely more symptomatic and then presumably more reactive.

According to the above mentioned points we can state that LnPD6, LnTD, and SL are not useful in epidemiology to discriminate normals from asthmatics.

The sensitivity and specificity of LnPD15 and LnPD6 were relatively similar, LnPD6 having a higher specificity, LnPD15 a higher sensitivity [40], but the resulting predictive value is clearly higher for LnPD6. However predictivity of bronchial asthma of all the above mentioned parameters including LnPD15 is remarkably very low; in addition to sensitivity and specificity the predictive value is influenced by the prevalence rate of disease, in this case relatively low in the general population. Finally LnPD15 turns out to be the best index to assess bronchial responsiveness in epidemiological studies. It is pointed out that its peculiarity of being a highly specific and sensitive index makes it even more valid in clinical practice, where sensitivity is surely much more increased. With respect to the diagnosis of bronchial asthma, a low predictivity confirms however that the bronchial challenge has only the role of instrumental aid in detecting the disease.

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References

supérieure à 2 DS, par comparaison avec la moyenne de 5 VEMS enregistrés après l'inhalation de tampons phosphate. Par ailleurs, nous avons investigué le nouvel indice "pente dose-réponse" (SI) proposé par O'Connor. Les valeurs de "cut-off" de chaque indice pour définir un répondeur, ont été calculées par l'analyse discriminante des réponses à la provocation dans le groupe des sujets normaux et des asthmatiques. La sensibilité, la spécificité et la valeur prédictive de LnPD6, LnTD et SI, ont été respectivement de 79, 74 et 14%; 74 et 13%; et 46, 97 et 48%. LnPD10 manifeste la meilleure sensibilité (83%), avec une haute spécificité (83%); mais, par comparaison avec LnPD15, dont la sensibilité est de 79% et la spécificité de 89%, la valeur prédictive de LnPD10 est plus faible (21% vs 27%). LnPD15 semble donc le meilleur indice pour apprécier la réactivité bronchique dans les études épidémiologiques; toutefois, en ce qui concerne le diagnostic de l'asthme bronchique, une faible valeur prédictive confirme que la provocation bronchique n'a qu'un rôle complémentaire dans la détection de la maladie.

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