Distribution of Bronchial Nonspecific Reactivity in the General Population

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We investigated 654 subjects of a small Lombardy (Italy) town between 15 and 64 years of age who were representative of the general population. By clinical examination, the sample included 535 normal subjects (164 normal smokers, 341 normal nonsmokers, 30 normal subjects with acute upper respiratory illness within 30 days before the challenge), 50 with chronic bronchitis, 26 with asthma, and 43 with allergic rhinitis. Subjects whose FEV₁ was 75 percent or more than the predicted value (654) underwent methacholine bronchial challenge by means of 1 percent metered-dose solution. The test result was considered positive at a drop of more than 15 percent in FEV₁ (compared with buffer). Normal smokers and all of the groups with disease had a significantly different distribution of reactivity compared with normal nonsmokers. The difference between asthmatic and these “normal” subjects was highly significant; nevertheless, a clear cut-off between the two groups does not appear to exist.

Nonspecific bronchial hyperreactivity, besides being a characteristic of asthmatic patients, is sometimes common to those with chronic bronchitis, with allergic rhinitis, and subjects exposed to specific irritant-sensitizing pollutants at work. Although its exact role in the natural history of the disease is not completely known, it is reasonably believed to act as a predisposing factor to the development of COPD. 1, 2

Previously the available data were compared with those from presumably normal populations whose sample sizes were small and not usually representative of the general population. 3

In 1982 an epidemiologic prospective study of COPD was started in the general population of a small Lombardy town in northern Italy (Caronno Pertusella-VA). During the second cross-sectional control, three years later, we included a nonspecific bronchial challenge with methacholine along with routine functional test. The purpose of our study was to evaluate bronchial reactivity in a general population, define a normal range of reactivity, and compare degrees of reactivity with clinical features.

Materials and Methods

Study Population

In this longitudinal study of COPD, a stratified random sample of 916 subjects was selected from the population, between 15 and 65 years of age and resident for more than five years. They were stratified by the age of the head of the household and the number of family members and found to be representative for age and sex of the referent population. 4

Of 916 subjects in the first survey, 654 participated in the second cross-sectional survey (October 1985 to March 1986), and 652 gave their informed consent to be tested with methacholine. Although 28.61 percent of the original sample was lost to follow-up, the sample undergoing methacholine challenge remained statistically representative by sex and age of the referent population.

Study Design

All of the subjects were interviewed by two pulmonary specialists who filled in a detailed health questionnaire of the Italian National Research Council, adapted from the standard NHBLI questionnaire. 5 On the basis of responses to the questionnaire and more detailed histories from clinical evaluations, physicians clinically classified subjects. Diagnosis of bronchial asthma and chronic bronchitis was made according to the criteria set out in the Ciba Guest Symposium in 1959 6 and suggested by the American Thoracic Society. 7

Cross-examinations were employed to classify controversial cases. By clinical examination the sample was found not to be significantly different from the original sample. There were 535 normal subjects (164 normal smokers [NS], 341 normal nonsmokers [NNS], 30 normal subjects with acute upper respiratory illness within 30 days before the challenge [URI]), 50 with chronic bronchitis, 26 with asthma, and 43 with allergic rhinitis. Among 26 asthmatic subjects, five were found to have past asthma (absence of wheezing or breathlessness within the preceding 12 months) and two from asthma only on exposure to the allergen. 8

All of the subjects had to be free from any drug whose effects might alter airway responsiveness. Then, three expiratory maneuvers were performed on a dry spirometer (Vicast 4 Hellige). Those whose FEV₁ was less than 75 percent of the predicted value (from the best of three curves) were subjected to bronchodilation test with salbutamol (200-μg spray); the others whose FEV₁ was more than 75 percent were challenged with methacholine using buffered lypoil methacholine (phosphate buffer, Lofarma). The 1 percent concentration of methacholine was prepared by diluting methacholine in

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distilled water. A metered nebulizer dosimeter, Mefar, delivered the methacholine from a De Vilbiss no. 646 ampule by means of an air compressor (driving pressure, 1.5 kg/cm²). Inhalation time was set on 1.1 s, every inhalation delivering 200 μg of methacholine.

The phosphate buffer was inhaled and five forced expiratory curves were obtained. Then, starting from 200 μg methacholine, increasing cumulative doses (400, 800, 1,600, 3,200, 4,800 and μg) were delivered (with increasing numbers of inhalations). Every inhalation was stopped and considered as positive at a drop of more than 15 percent in FEV₁ (compared to the buffer). Otherwise, it was terminated at the highest cumulative dose of 4,800 μg. The whole test had to be performed within 20 minutes. All the subjects who had positive reactions were given salbutamol spray (200 μg) postchallenge. The protocol was the same for clinically normal and abnormal subjects.

Statistical Analysis

Responsiveness as expressed in log PD 15 FEV₁, has been distributed by 0.25 log dose intervals. All negative tests were assigned to the "more than 8.5 log dose" category (more than 4,800 μg). The lower fifth percentile of log dose on the NNS group was calculated to define the "normal" range.

Since NNS distribution did not fit a Gaussian curve, the nonparametric Kolmogorov-Smirnov test (KS test) and the Spearman correlation (S test) were used to assess, respectively, the group distribution difference and the correlation between bronchial responsiveness and age.³¹⁴ Logistic regression modeling was performed to estimate the relationships between groups, using log dose adjusted for sex and age classification variables.

To accommodate polyomous groups, a nested set of dichotomies was obtained by successive partitions of the ordered groups. Groups were ranked as follows: (1) NNS; (2) NS; (3) subjects with URI; (4) those with allergic rhinitis; (5) chronic bronchitis; (6) asthma. The first was analyzed vs the subsequent groups, the second vs the subsequent groups, and so on.

The dichotomies are constructed so that the likelihood for the polyomous group variable is the product of the likelihoods for the dichotomies.² In addition, separate logistic models fitted with respect to baseline NNS group are also presented. Statistical computing used GLIM.²

RESULTS

Nine abnormal subjects (six with chronic bronchitis and three with asthma) were found to have obstruction (FEV₁/FEV₉ < 0.61 ± 6 percent predicted values) and underwent the bronchodilator test. Only four of them were positive, with an FEV₁ actually 20 percent more than the baseline value 30 minutes after drug administration.

All of the remaining clinically abnormal and normal subjects who underwent methacholine challenge had a baseline FEV₁ within the normal range, and the mean FEV₁ values for all the groups challenged with methacholine were not significantly different. Only two normal subjects refused to undergo methacholine challenge, and 38 (five chronic bronchitis, two with rhinitis, and 31 normal subjects) did not give any satisfactory performance of the FVC maneuvers, and their data were not saved.

Figure 1 shows a bar graph semilogarithmic plot of the distribution of methacholine responsiveness in the whole population and subdivided into various clinical groups. A descriptive analysis of NNS group showed a similar trend of log PD 15 FEV₁ distribution both for females and males up to the penultimate one log dose class. However, statistical analysis of the whole distribution (KS test) shows a significant difference because of an increased female bronchial responsiveness.

The NNS group log PD 15 FEV₁ does not correlate with age (S test). The mean log dose defining the lower fifth percentile was 7.55 (1,900 μg methacholine).

Normal smokers, subjects with URI, and those with allergic rhinitis had a significantly different log PD 15 FEV₁ distribution from the NNS. The difference in the curve between asthmatic and NNS was highly significant (p<0.001). Although there were no normal subjects in the first five classes, where asthmatic subjects are highly represented, a clear cut-off between the two groups does not appear to exist (Fig 2). Subjects with current asthma and subjects with past asthma could not be differentiated on the basis of their distribution of bronchial responsiveness.

There is an overlapping area of NNS and chronic bronchitis (Fig 3), but the chronic bronchitis group was still highly different from NNS (p<0.001). This overlap with NNS was broader than that for asthmatic subjects, but the two disease groups did not significantly differ (0.05 < p < 1). Table 1 shows the number of asthmatic and chronic bronchitis subjects and percentages with positive tests to both feasible cut-offs. At the former
cut-off of 850 µg methacholine (100 percent of specificity), a 52.1 percent sensitivity was observed; at the latter cut-off of 1,900 µg methacholine (95 percent of specificity) a higher sensitivity (78.3 percent) was found.

Results were also obtained via multiple logistic regression. Sex, age (three age classes: <30 years, 30 to 45, and >45), and log PD 15 FEV₁ principal effects were significantly related to group identification, and there was no significant interaction between the previous variables. These results are based on the pooled (total) nested dichotomies (Table 2). However, inspection of the single models revealed that age was an important factor only for the rhinitis compared to the asthma and chronic bronchitis groups, and for the asthmatic vs the chronic bronchitis groups. On the other hand, sex and log PD 15 FEV₁ appear related with all of the nested compared groups. In addition separate binary logit models with baseline NNS group indicated again the strong relationships with log dose level. However, a sex difference appears among normal smokers and chronic bronchitis but not in the other groups, and age was not significant at all (Table 3).

DISCUSSION

Our results confirm, in the NNS group, an increased bronchial reactivity in females which was previously pointed out by Malo and coworkers 4 and Zamel. 15

The absence of correlation between age and bronchial reactivity in the age range of 15 to 65 years is in agreement with the literature. 14,16 Logistic regression modeling did not show any interaction either of age or sex on log PD 15 FEV₁ in ranking subjects. Moreover, it showed that log PD 15 FEV₁, always significantly determines the ranking of NNS subjects. For the effects of smoking habit, URI within one month before the challenge and atopy on responsiveness, conflicting experiences are reported. 17

Our data, both by Kolmogorov-Smirnov test and multiple logistic regressions, confirm that chronic cigarette smoking 18-21 as well as allergic rhinitis 22 significantly affects nonspecific airway responsiveness. The importance of the role of inflammation in inducing bronchial hyperreactivity has been recently assessed both in animal 23,24 and in clinical studies. 25,27 Our survey on the general population confirms such data, focusing on a significantly increased bronchial reactivity in
subjects with URI rather than in NNS.

Log PD 15 FEV$_1$ significantly discriminates chronic bronchitis from asthma. However, the distribution of reactivity in the asthmatic population as compared with the “normals” was found to be continuous, so that it was impossible to establish a clear cut-off point. Our results confirm reports from studies of smaller numbers of cases.\textsuperscript{5,17,28}

We found 21.7 percent of the asthmatic subjects above 1,900 \( \mu \text{g} \) beyond the fifth percentile of the normal range, where the test was considered negative. Two of them were asthmatic with asthma only on exposure to allergen, one was asthmatic with current asthma, and two had past asthma. Therefore, they ought to be defined as asthmatic without constant bronchial hyperreactivity; this implies that asthmatic subjects can have periods when airways are nonhyper-responsive.\textsuperscript{29,30}

Setting the cut-off at 850 \( \mu \text{g} \) (100 percent of specificity), which is approximately the discriminating dose largely accepted for clinical purposes,\textsuperscript{14,29,31} the resulting sensitivity is very low. It must be pointed out that all of our asthmatic subjects challenged by methacholine and therefore with baseline FEV$_1$ values still within the normal range suffered from a clinically mild asthma. That is not the case in hospital units, where the patient spontaneously presents himself because he is symptomatic. Furthermore, we evaluated the case that the most seriously ill subjects might be followed-up by chest units and did not participate in our survey. Our principal aim, however, was to evaluate the specificity of the test, starting from the study of the normal population, because this datum is not available in the literature, at least in the general population.

The latter cut-off, set on the basis of the fifth percentile, is therefore realistic if the bronchial challenge is evaluated according to commonly accepted criteria defining a normal test. The 95 percent of specificity at 1,900 \( \mu \text{g} \) of methacholine is balanced by a much higher sensitivity (78.3 percent). Positive provocative doses for normal subjects within the fifth percentile were then included between 850 and 1,900 \( \mu \text{g} \). A broad area of overlap still remains, where the response to the challenge must be evaluated by means of a more accurate instrumental and clinical approach. Therefore, methacholine challenge represents a reliable diagnostic tool only for those values within 850 \( \mu \text{g} \). We conclude in agreement with Chan-Yeung et al\textsuperscript{12} that the usefulness of measurement of bronchial hyper-responsiveness in the epidemiologic studies in the diagnosis of asthma is limited.

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Table 1—Patients with Asthma and Chronic Bronchitis Who Had Abnormal Reaction to Methacholine Challenge

<table>
<thead>
<tr>
<th>Cut-off Dose, ( \mu \text{g} )</th>
<th>Bronchitis, no. (%)</th>
<th>Asthma, no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>850</td>
<td>(23.07)</td>
<td>(52.10)</td>
</tr>
<tr>
<td>1,900</td>
<td>(41.00)</td>
<td>(78.30)</td>
</tr>
</tbody>
</table>

Table 2—Marginal and Conditional Likelihood Rests for Terms in the Polychotomous Logistic Models

<table>
<thead>
<tr>
<th>Source</th>
<th>Marginal</th>
<th>Conditional</th>
</tr>
</thead>
<tbody>
<tr>
<td>df</td>
<td>Deviance</td>
<td>p</td>
</tr>
<tr>
<td>Sex ( \times ) age ( \times ) PD</td>
<td>10</td>
<td>8.69</td>
</tr>
<tr>
<td>Sex ( \times ) age</td>
<td>10</td>
<td>10.69</td>
</tr>
<tr>
<td>Sex ( \times ) PD</td>
<td>5</td>
<td>1.65</td>
</tr>
<tr>
<td>Age ( \times ) PD</td>
<td>10</td>
<td>4.21</td>
</tr>
<tr>
<td>Sex</td>
<td>5</td>
<td>43.81</td>
</tr>
<tr>
<td>Age</td>
<td>10</td>
<td>20.17</td>
</tr>
<tr>
<td>PD</td>
<td>5</td>
<td>142.30</td>
</tr>
</tbody>
</table>

Table 3—Estimated Odds Ratio

<table>
<thead>
<tr>
<th>Effect</th>
<th>Normal Smokers</th>
<th>Normal With URI</th>
<th>Allergic Rinitis</th>
<th>Chronic Bronchitis</th>
<th>Asthma</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/F</td>
<td>2.98*</td>
<td>1.07</td>
<td>0.70</td>
<td>7.87*</td>
<td>1.99</td>
</tr>
<tr>
<td>30-45 yr/30</td>
<td>1.43</td>
<td>0.49</td>
<td>0.52</td>
<td>5.28</td>
<td>0.67</td>
</tr>
<tr>
<td>&gt;45/30</td>
<td>0.99</td>
<td>0.66</td>
<td>0.60</td>
<td>5.87</td>
<td>0.67</td>
</tr>
<tr>
<td>+ One unit Ln dose</td>
<td>0.44*</td>
<td>0.19*</td>
<td>0.24*</td>
<td>0.10*</td>
<td>0.05*</td>
</tr>
</tbody>
</table>

*p<0.05  
+p<0.01
inhaled methacholine in young asymptomatic smokers. J Appl Physiol 1982; 52:1464-70
19 Dosman JA, Bergstrom K, Clark K, Khaladkar S. Peripheral airways function and nonspecific airways reactivity in cigarette smokers. Chest 1986; 89:45-8