Analysis of bronchial reactivity in epidemiological studies

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Abstract
The measurement of bronchial reactivity in epidemiological studies has the advantage of quantifying an objective physiological feature of asthma. Bronchial reactivity was developed in a clinical setting and has been conventionally expressed as the dose of agonist producing a 20% fall in FEV₁(PD₂₀). As PD₂₀ can be estimated for less than 20% of subjects in general community surveys with the doses of agonist that are usually given, data from most subjects must be censored. Thus PD₂₀ alone is a poor index of bronchial reactivity for epidemiological studies. Data from 809 aluminium smelter workers were used to evaluate alternative methods of analysing bronchial reactivity. Dose-response relationships were analysed by four methods: (1) PD₂₀ by the conventional method of interpolating the dose on a logarithmic scale between the last two measurements of FEV₁; (2) PD₂₀ (with allowance for extrapolation), estimated by fitting an exponential curve to the dose-response data; (3) the linear regression slope between dose and FEV₁ when significant; (4) the dose-response slope obtained in all subjects as the % change in FEV₁ from baseline in response to total dose. When each of these measures was related to symptoms, diagnosis, and treatment of asthma, all differentiated between "asthmatic" and "non-asthmatic" subjects. The dose-response slope (method 4) had the advantages of simplicity and no censored data, and was shown to be clinically relevant. It is suggested that the dose-response slope should be used for the analysis of bronchial reactivity in epidemiological studies.

Because PD₂₀ can be estimated in only a minority of subjects in community surveys, it is not an ideal measure of bronchial reactivity for epidemiological research. To describe the distribution of bronchial reactivity in a healthy population completely requires a continuously distributed index that summarises the dose-response data for all individuals.

The slope of the dose-response line has been investigated in laboratory studies as an alternative index of bronchial reactivity. These studies have all included substantial numbers of asthmatic subjects, however, and have not considered the value of slope coefficients in epidemiological studies. Empirical evidence favours the use of a linear rather than a logarithmic dose scale. In the present study of aluminium workers two methods for deriving PD₂₀ were compared and alternative indices of bronchial reactivity evaluated.

Methods
The subjects were participants in a longitudinal study of respiratory symptoms and lung function in aluminium smelter workers. After giving informed consent, 809 (96%) of the 843 male employees completed a methacholine challenge, in which they inhaled increasing doses up to 6.14 μmol from hand held glass nebulisers according to a rapid method protocol. FEV₁ was measured from maximal flow-volume loops performed on a water sealed spirometer interfaced with a microcomputer (Gould 2400 system). All subjects wore nose clips and were tested in the sitting position. The test was stopped when FEV₁, had fallen by 20% or the highest dose of histamine had been given. Twelve subjects were not challenged because of impaired baseline lung function (FEV₁ below 65% predicted for age and height); 22 declined to participate. No appreciable side effects were experienced; one subject complained of flushing and a few developed symptoms of hyperventilation. Salbutamol 5 mg was administered by nebuliser when FEV₁ fell by 10% or more, and bronchoconstriction was reversed promptly in all cases. For assessment of reproducibility 727 (90%) of the workers were rechallenged 12 months later, the same protocol being used.

Dose-response relationships were analysed by four methods: 1 PD₂₀ was estimated by the conventional method of interpolating between the last two
measurements on an FEV₁-log dose methacholine plot. 15

2 A Fortran program16 was used to fit the exponential function $FEV₁ = c - \exp (a + b \cdot \text{log dose (µmol methacholine)})$. The parameter $c$ was initially set to post-saline $FEV₁$. Ordinary least squares regression was used to estimate the parameters $a$ and $b$. A new value of $c$ was calculated to minimise the residual sum of squares and the regression repeated. Iteration continued until convergence was achieved or the procedure had been performed 50 times. If the algorithm converged, an “exponential” estimate of PD₂₀ was extrapolated up to 12.28 µmol. Otherwise

“exponential” PD₂₀ was censored at 12.28 µmol.

3 Ordinary least squares regressions were performed on the data for each subject to fit the dose-response line: $FEV₁ = a + b \cdot \text{dose methacholine (µmol)}$. Given a significant linear relationship ($p < 0.05$), the slope parameter ($b$) was considered an index of bronchial reactivity. This parameter showed a severely skewed distribution. As the negative reciprocal of slope ($-1/b$) was still skewed to the right other power and log transformations of this coefficient were explored in an effort to find the most normally distributed index.

4 The dose-response slope was calculated for each subject as % decline in $FEV₁$ from post-saline value/cumulative methacholine dose. 17 This parameter represented a line joining the origin of the the dose-response curve with the final measurement (fig 1). Before it was expressed on a logarithmic scale 1.5%/µmol was added to eliminate negative and zero values.

Descriptive statistics, transformations, correlations, analyses of variance, linear regressions, and associated diagnostics were performed in Minitab. 17 The correlation of a variable with normal scores for all subjects was used to estimate the Shapiro Wilk coefficient. 17 The closer the correlation was to unity the more normal the distribution; the significance of deviations from unity was tested. Correlation between continuous indices was assessed by the Pearson’s product-moment correlation coefficient. 18 Reproducibility of indices was assessed by the intraclass correlation coefficient. 19

Clinical information on any history of asthma, symptoms such as wheeze, and use of bronchodilator medication was obtained from a modified Medical Research Council questionnaire, 20 completed by all subjects before lung function testing. Differences in two means were assessed by Student’s $t$ test and differences in three or more means by analysis of variance. 21 Separation of symptom groups was assessed by the index $D/s$, which was the difference between means divided by the standard deviation for the entire sample. 22

Results

ESTIMATES OF PD₂₀

After the administration of 6-14 µmol methacholine $FEV₁$ increased from baseline in 123 (15%) subjects, showed no change in 21 (3%), a decrease of less than 10% in 509 (63%), and a decrease of 10–20% in 91 (11%) subjects. Only 65 (8%) subjects had a decline in $FEV₁$ of more than 20%. For these 65 cases PD₂₀ was inter-

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**Table 1** Descriptive statistics and tests of normality for different indices of bronchial reactivity (PD₂₀)*

<table>
<thead>
<tr>
<th>Estimate</th>
<th>$n$</th>
<th>Mean (SD)</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Shapiro-Wilk coefficient</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpolated PD₂₀</td>
<td>65</td>
<td>2.8 (1.7)</td>
<td>0.06</td>
<td>6.0</td>
<td>0.984</td>
<td>NS</td>
</tr>
<tr>
<td>Exponential PD₂₀</td>
<td>132</td>
<td>5.6 (3.4)</td>
<td>0.10</td>
<td>12.1</td>
<td>0.981</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Inverse cube root of slope</td>
<td>353</td>
<td>2.69 (0.83)</td>
<td>0.54</td>
<td>5.21</td>
<td>0.997</td>
<td>NS</td>
</tr>
<tr>
<td>Log dose-response slope</td>
<td>809</td>
<td>0.36 (0.29)</td>
<td>-0.73</td>
<td>2.48</td>
<td>0.902</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*See under “Methods” for derivation of the parameters.
polated (see table 1 for descriptive statistics). The distribution of the interpolated PD_{20} (fig 2) did not deviate significantly from a normal distribution.

The exponential function (method 2) successfully extrapolated a PD_{20} value for 132 (16%) subjects (see table 1 for descriptive statistics). The distribution of this estimate of PD_{20} (fig 3) deviated significantly from a normal distribution. This deviation was worsened by log transformation (Shapiro-Wilk coefficient = 0.926). One subject’s value was censored for being too reactive (a greater than 20% drop after the first dose) and 676 “non-reactive” subjects were given the censored value 12.28 μmol.

Complete data for interpolated and exponential estimates of PD_{20} were available for 63 cases. The correlation between the two estimates was 0.94 (p < 0.0001).

SLOPE PARAMETERS

The slope of the line of best fit between FEV_1 and methacholine dose on a linear scale (method 3) provided an index of bronchial reactivity for a much larger proportion of subjects. Linear regressions of FEV_1 against dose yielded a slope coefficient (b) that was significantly negative in 353 (44%) of cases. The most normally distributed transformation proved to be the inverse cube root of the slope (b’’’) (fig 4). The descriptive statistics and normality tests for this index are summarised in table 1.

A dose-response slope could be estimated for all 809 subjects. The distribution of the dose-response slope was severely skewed with two outlying values from extremely reactive individuals. Log transformation yielded a symmetric though significantly non-normally distributed index (fig 5). Descriptive statistics and normality tests for the log dose-response slope are summarised in table 1.

RELATIONSHIPS BETWEEN PD_{20} AND SLOPE PARAMETERS

There was substantial agreement between the ability to obtain a PD_{20} from the exponential algorithm (method 2) and the presence of a significant negative slope (b) to the dose-response line (method 3)—see table 2. Subjects with an exponential PD_{20} below 12.28 μmol are almost entirely within the group with a significant slope. Iterative exponential curve fitting provided an index of bronchial reactivity in six further cases where simple linear regression failed. The mean inverse cube root of the slope of the 126 subjects with both a significant slope and a PD_{20} below 12.28 μmol was significantly less than the mean inverse cube root of the slope for the 227 subjects with a PD_{20} censored to 12.28 μmol (table 3). In other words, subjects with an extrapolated exponential PD_{20} have a steeper dose-response line than those without a PD_{20} value. The correlation between the inverse cube root of the slope and PD_{20} was very strong (r = 0.96, p < 0.0001).

As the dose-response slope is a summary measure of the slope of the the dose-response line, it is not surprising that there is an extremely strong correlation between this measurement and the slope parameter (b) derived from linear regression (r = 0.97, p < 0.0001). The strong negative correlation between log dose-response slope and log PD_{20} (r = 0.98, p < 0.0001). The mean log dose-response slope for the 132 subjects with an exponential PD_{20} below 12.28 μmol was significantly greater than the mean log dose-response slope for the 227 subjects with a significant negative slope but without a PD_{20}, and this in turn was greater than the mean log dose-response slope for the remaining 450 “non-reactive” subjects without a significant negative slope (table 3).

<table>
<thead>
<tr>
<th>PD_{20}</th>
<th>Slope present</th>
<th>Slope absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-censored “exponential”</td>
<td>126</td>
<td>6</td>
</tr>
<tr>
<td>Censored (12.28 μmol)</td>
<td>227</td>
<td>450</td>
</tr>
<tr>
<td>Total</td>
<td>353</td>
<td>480</td>
</tr>
</tbody>
</table>

Figure 3  Distribution of PD_{20} extrapolated up to 12.28 μmol by an exponential algorithm—see text (n = 132).

Figure 4  Distribution of the inverse cube root of the slope from significant dose-response lines by linear regression (n = 353). The quantities are dimensionless.
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Figure 5  Distribution of log transformed dose-response slope—see figure 1 (n = 809). The quantities are dimensionless.

REPRODUCIBILITY
The reproducibility or repeatability of bronchial reactivity indices were assessed by measurement of agreement between indices for the same subjects on rechallenge. The intraclass correlation coefficients are listed for interpolated and exponential PD_{20}, inverse cube root of the slope, and log dose-response slope in table 4. The first three indices could be remeasured for only about 60% of the subjects with non-censored initial values. Adding 1.5%μmol before log transformation failed to eliminate one negative value from the second measurement of dose-response slope. There was a statistically significant agreement between measurements for all indices. The inverse cube root of the slope and log dose-response slope were highly reproducible, exponential PD_{20} was moderately reproducible, and interpolated PD_{20} was less reproducible.

CLINICAL VALIDITY
The clinical validity of the various indices of bronchial reactivity was assessed by comparing subjects who reported features of asthma with those who did not report such features (table 5). Means values of interpolated PD_{20} were significantly lower in subjects with a history of asthma and in those who used salbutamol. Mean values of “exponential” PD_{20} and the inverse cube root of the slope were also significantly lower in subjects who wheezed; mean log dose-response slope was significantly higher in subjects with any of these features. The indices of separation (D/s) indicated that log dose-response slope best separated asthmatic from non-asthmatic individuals; the inverse cube root of the slope was less efficient, and there was greatest overlap for PD_{20}.

Discussion
These results support the use of the dose-response slope for measuring bronchial reactivity in epidemiological studies. In view of the widespread use and clinical understanding of interpolated PD_{20} we could argue that this index should also be reported. Interpolated PD_{20} was of little value in the present study, however, because it applied to only 8% of subjects. Other attempts to reduce censored data, such as the use of exponential PD_{20} and the inverse cube root of the slope, do not appear to have a major role.

The apparent improvement in FEV_{1} with increasing doses of methacholine, seen in 15% of our subjects, has been reported previously. It might be an effect of practice or a consequence of slight stiffening of the central airways with agonist administration moving the equal pressure (choke) point downstream. It is also possible that a decline in FEV_{1} with increasing doses of methacholine could occur from fatigue rather than bronchoconstriction. Subjects had at least two minutes, however, to recover between blows. The reproducibility of bronchial reactivity makes it very unlikely that fatigue is causing a dose related reduction in FEV_{1}.

The usual clinical method of estimating PD_{20} from interpolation between the last two measurements fails to obtain data from most normal subjects. It provided an index of bronchial reactivity for only 8% of this occupational sample. The use of a higher dose of agonist might have provided more subjects with an interpolated PD_{20} as well as information on a plateau response; but as subjects had to return to work after being tested we did not think it appropriate to explore maximal responses to methacholine.

The exponential algorithm yields an estimate for PD_{20} in less than half the number of cases for which simple linear regression yields a significant slope. It has the disadvantage of estimating three parameters from five data points. Extrapolation beyond the dose administered is potentially hazardous for a non-linear function with no estimate of error variance. The results agree well, however, with the conventional interpolated estimate of PD_{20}. The Fortran program operates rapidly and efficiently on a computer.

In summary, the exponential algorithm uses...
Table 5  Comparison of bronchial reactivity indices between subjects with and without features of asthma

<table>
<thead>
<tr>
<th>History of asthma</th>
<th>No history of asthma</th>
<th>Ds</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean interpolated PD_{20}</td>
<td>1.76</td>
<td>3.18</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean exponential PD_{20}</td>
<td>3.84</td>
<td>6.08</td>
<td>0.65</td>
</tr>
<tr>
<td>Mean inverse cube root of the slope</td>
<td>1.91</td>
<td>2.79</td>
<td>1.07</td>
</tr>
<tr>
<td>Mean log dose-response slope</td>
<td>0.964</td>
<td>0.335</td>
<td>1.80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wheeze</th>
<th>No wheeze</th>
<th>Ds</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean interpolated PD_{20}</td>
<td>2.40</td>
<td>3.08</td>
<td>0.39</td>
</tr>
<tr>
<td>Mean exponential PD_{20}</td>
<td>4.10</td>
<td>6.31</td>
<td>0.64</td>
</tr>
<tr>
<td>Mean inverse cube root of the slope</td>
<td>2.26</td>
<td>2.82</td>
<td>0.67</td>
</tr>
<tr>
<td>Mean log dose-response slope</td>
<td>0.556</td>
<td>0.327</td>
<td>0.78</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Salbutamol used</th>
<th>Salbutamol not used</th>
<th>Ds</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean interpolated PD_{20}</td>
<td>1.51</td>
<td>2.99</td>
<td>0.85</td>
</tr>
<tr>
<td>Mean exponential PD_{20}</td>
<td>2.41</td>
<td>5.89</td>
<td>1.02</td>
</tr>
<tr>
<td>Mean inverse cube root of the slope</td>
<td>1.71</td>
<td>2.73</td>
<td>1.24</td>
</tr>
<tr>
<td>Mean log dose-response slope</td>
<td>1.054</td>
<td>0.350</td>
<td>2.39</td>
</tr>
</tbody>
</table>

*Differences in means were assessed by the t test and the index of separation (Ds) was calculated as difference in means/pooled standard deviation.²

In any case, such influence could be considered biologically appropriate as most of the response occurred at higher doses.

The inverse cube root of the slope had the desirable biological and statistical property of being normally distributed, was reproducible, and had clinical validity. The inverse cube root scale is unusual, but not entirely without parallel in respiratory epidemiology. For instance, the latent period for the development of respiratory cancer appears to be related to the inverse cube root of asbestos dose.²⁵ The inverse cube root of the slope is interpreted in the same manner as PD_{20}, in that the larger the value the less reactive the subject. There was excellent agreement between PD_{20} and the cube root of the slope measurements, and subjects lacking an estimate of PD_{20} had significantly greater values for the inverse cube root of the slope.

Subjects with slope alone have measurable reactivity, but of a lesser degree than those who show PD_{20} with both slope and PD_{20}. This was confirmed by the differences in mean log dose-response slope.

The most widely applicable index of bronchial reactivity is the dose-response slope, which could be calculated for all subjects. The single parameter is simple to calculate and agrees well with the slope from linear regression, as has been previously found.¹¹ This model does, however, assume that the first and last measurements of FEV₁ were made without error, which is unlikely, and the absence of any test of statistical significance means that all subjects contribute a dose-response slope and this will include random noise. Subjects showing an increase in FEV₁, from its post-saline baseline to the final dose of methacholine are certainly less reactive than those who show a decrease, but they may differ little from those whose FEV₁ shows little or no change. Thus assignment of a number to their lack of reactivity is hard to justify. Despite the incorporation of data from such subjects log dose-response slope was highly reproducible.

Although the distribution of log dose-response slope was not normal, log transformation allowed bronchial reactivity to be expressed on a familiar logarithmic scale. The increment for eliminating negative and zero values before log transformation requires standardisation. The previously suggested 0.3%/μmol¹¹ would have been insufficient in the present study. Alternatively, untransformed dose-response slope values could be analysed by non-parametric statistical tests, which are sufficiently robust not to be unduly influenced by the severely skewed distribution.

In conclusion, measuring the dose-response slope has the advantages of simplicity, reproducibility, clinical validity, and absence of censored data. Neither exponential PD_{20} nor the inverse cube root of the slope combines these desirable features in a single index. We would recommend using the dose-response slope for analysis of bronchial reactivity in future epidemiological studies.

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