Analysis of bronchial reactivity in epidemiological studies

Michael J Abramson, Nicholas A Saunders, Michael J Hensley

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 FEV\(_1\) (PD\(_{20}\)). As PD\(_{20}\) 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 PD\(_{20}\) 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) PD\(_{20}\) by the conventional method of interpolating the dose on a logarithmic scale between the last two measurements of FEV\(_1\); (2) PD\(_{20}\) (with allowance for extrapolation), estimated by fitting an exponential curve to the dose-response data; (3) the linear regression slope between dose and FEV\(_1\), when significant; (4) the dose–response slope obtained in all subjects as the % change in FEV\(_1\) 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 PD\(_{20}\) 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 PD\(_{20}\) 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. FEV\(_1\) was measured from maximal flow-volume loops performed to American Thoracic Society standards 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 FEV\(_1\) had fallen by 20% or the highest dose of histamine had been given. Twelve subjects were not challenged because of impaired baseline lung function (FEV\(_1\) 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 FEV\(_1\) 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. PD\(_{20}\) was estimated by the conventional method of interpolating between the last two
measurements on an FEV\textsubscript{1}-log dose methacholine plot.\textsuperscript{15}  
2 A Fortran program\textsuperscript{16} was used to fit the exponential function \( FEV_1 = c - \exp (a + b \times \text{log dose (\text{\textmu}mol methacholine)}) \). The parameter \( c \) was initially set to post-saline \( FEV_1 \). 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 PD\textsubscript{20} was extrapolated up to 12.28 \textmu mol. Otherwise

![Figure 1](image1.png)  
**Figure 1** Dose-response slope (DRS) representing a line joining the baseline with the final measurement of \( FEV_1 \). Dose-response slope is calculated as % change in \( FEV_1/\text{total dose methacholine (\textmu mol)} \).

3 Ordinary least squares regressions were performed on the data for each subject to fit the dose-response line: \( FEV_1 = a + b \times \text{dose methacholine (\textmu mol)} \). Given a significant linear relationship (\( p < 0.05 \)), the slope parameter (\( b \)) was considered as 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 \( FEV_1 \), from post-saline value/cumulative methacholine dose.\textsuperscript{21} This parameter represented a line joining the origin of the dose-response curve with the final measurement (fig 1). Before it was expressed on a logarithmic scale 1.5%/\textmu mol was added to eliminate negative and zero values.

Descriptive statistics, transformations, correlations, analyses of variance, linear regressions, and associated diagnostics were performed in Minitab.\textsuperscript{17} The correlation of a variable with normal scores for all subjects was used to estimate the Shapiro Wilk coefficient.\textsuperscript{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.\textsuperscript{18} Reproducibility of indices was assessed by the intra-class correlation coefficient.\textsuperscript{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,\textsuperscript{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.\textsuperscript{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.\textsuperscript{22}

**Results**

**ESTIMATES OF PD\textsubscript{20}**

After the administration of 6–14 \textmu mol methacholine \( FEV_1 \), 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 \( FEV_1 \), of more than 20%. For these 65 cases PD\textsubscript{20} was inter-

![Figure 2](image2.png)  
**Figure 2** Distribution of PD\textsubscript{20} interpolated in the usual manner between the last two measurements of \( FEV_1 \) on a log dose scale (\( n = 65 \)).

**Table 1** Descriptive statistics and tests of normality for different indices of bronchial reactivity (PD\textsubscript{20})*

<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 PD\textsubscript{20}</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 PD\textsubscript{20}</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.
bulol (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 mmol.

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 mmol 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 invasive cube root of the slope of the 126 subjects with both a significant slope and a PD_{20} below 12.28 mmol was significantly less than the mean invasive cube root of the slope for the 227 subjects with a PD_{20} censored to 12.28 mmol (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 mmol 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 2. Cross tabulation between non-censored and censored "exponential" PD_{20} values and the presence or absence of a significant negative slope coefficient on linear regression of dose on FEV_{1} data

<table>
<thead>
<tr>
<th>PD_{20}</th>
<th>Slope present</th>
<th>Slope absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-censored &quot;exponential&quot;</td>
<td>126</td>
<td>6</td>
</tr>
<tr>
<td>Censored (12.28 mmol)</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 mmol 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.
Analysis of bronchial reactivity in epidemiological studies

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 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}.

Table 3 Comparison of mean inverse cube root of the slope and log dose-response slope in patients according to their reactivity categories (exponential PD_{20} < 12.28 μmol, significant negative slope on linear regression but censored PD_{20} and non-reactive according to both indices)

<table>
<thead>
<tr>
<th>PD_{20} &lt; 12.28 μmol (n = 132)</th>
<th>Negative slope (n = 227)</th>
<th>Non-reactive (n = 450)</th>
<th>Test used</th>
<th>( t ) test (95 = 20)6 &lt;0.0001</th>
<th>Analysis of variance ( F = 484 ) &lt;0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inverse cube root of the slope</td>
<td>1.99</td>
<td>3.14</td>
<td>—</td>
<td>t test</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log dose-response slope</td>
<td>0.826</td>
<td>0.387</td>
<td>0.217</td>
<td>Analysis of variance</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 4 Reproducibility of bronchial reactivity indices

<table>
<thead>
<tr>
<th>Index</th>
<th>n</th>
<th>Intraclase correlation</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpolated PD_{20}</td>
<td>36</td>
<td>0.28</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Exponential PD_{20}</td>
<td>84</td>
<td>0.52</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inverse cube root of the slope</td>
<td>206</td>
<td>0.74</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Log dose-response slope</td>
<td>726</td>
<td>0.73</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Discussion

These results support the use of the dose-response slope\(^1\) 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.\(^16\) 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;\(^26\) 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...
all data and does not assume linearity. It provided an estimate of PD_{20} in a greater proportion of this occupational sample than interpolation, was reproducible and valid, and has been used in other population studies.24 In studies including many asthmatic subjects the results have often been expressed as log PD_{20}. As log transformation did not normalise the distribution of the exponential estimate of PD_{20} in our occupational population the original scale in mmol was retained for analysis. In any case a normal distribution might not be expected in the "hyperreactive" tail of bronchial reactivity measurements. A censored index such as PD_{20}, which can be derived for only a small proportion of the general population, represents only this tail of the distribution.

The transformed slope coefficient is a continuous index of bronchial reactivity applicable to a larger proportion of the population than PD_{20}. The inverse cube root of the slope was estimated for 44% of subjects in this aluminium smelter who showed a significant linear dose-response relationship. There are some concerns about its use. Slopes from regression lines that did not significantly differ from zero were excluded, so that 56% of subjects had censored values. Very small slopes became large outlying values after reciprocal transformation. Although these outlying values could be reduced by the addition of an increment (similar to the one added to the dose-response slope before log transformation), none of our conclusions was altered by including slopes from non-significant regression lines. We thought that this effort added little useful information to the dose-response slope, which was much simpler to calculate.

Another theoretical problem was the undue influence of outlying points on each regression line. In a minimally reactive subject the highest dose given (6.14 mmol) was four times the previous dose (1.54 mmol). Statistical testing did not, however, identify excessive influence on the line of best fit from this outlying point.

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 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 FEV, 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 FEV, 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 FEV, shows little or not 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%/mmol11 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.

The study was sponsored by a grant to the University of Newcastle from Alcan Australia Limited. MAJ was a postgraduate medical research scholar of the National Health and...
Analysis of bronchial reactivity in epidemiological studies

Medical Research Council. We wish to thank Paula Watts for assistance in testing subjects and John Wołodarczyk for project and data management. Susan Chinn kindly provided the Fortran program to estimate PD_{20} and Ira Tager made helpful comments on the analysis of the data. Stephen Parish provided statistical advice.


Analysis of bronchial reactivity in epidemiological studies.

M J Abramson, N A Saunders and M J Hensley

Thorax 1990 45: 924-929
doi: 10.1136/thx.45.12.924

Updated information and services can be found at:
http://thorax.bmj.com/content/45/12/924

These include:

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/