Comparison of \( PD_{20} \) with two alternative measures of response to histamine challenge in epidemiological studies


ABSTRACT: Bronchial responsiveness to histamine or methacholine provides a useful objective measure for epidemiological studies of airways disease, but most people in a community population do not have a 20% fall in forced expiratory volume in one second (FEV\(_1\)) with the highest dose administered. Histamine challenge data were analysed to compare the repeatability, Normality and separation of symptom groups of the early dose-response slope with provocative dose producing a 20% fall in FEV\(_1\) (PD\(_{20}\)).

Tests were continued until a 20% fall in FEV\(_1\) occurred, or 4 \( \mu \)mol had been given. Data were available for 510 randomly selected subjects, and for an additional 283 with wheeze. A repeat test was obtained in 104 individuals. PD\(_{20}\) was estimated by curve fitting, with extrapolation to 8 \( \mu \)mol. Least-squares slope of percentage decline in FEV\(_1\) on histamine dose was calculated, using all the measured points and two-point slope as the fall from the post-saline measurement to the maximum cumulative dose divided by the maximum dose.

Log transformation of PD\(_{20}\) and shifted reciprocal transformations of slope produced constant variance. Over all subjects the three measures had similar repeatability; in subjects with PD\(_{20} \geq 8 \mu \)mol the intraclass correlation for two-point slope was only 0.26, but was 0.66 for least-squares slope. Neither measure of slope was normally distributed; but the distribution of log(PD\(_{20}\)) was consistent with a censored normal distribution.

In conclusion, least-squares slope is preferable to two-point slope for epidemiological studies. Either PD\(_{20}\) or least-squares slope can be analysed; the former requires methods for censored data, but the latter requires transformation with an arbitrary constant, +10\% \( \mu \)mol\(^{-1}\) being recommended, and methods requiring normality should be used with caution.

Eur Respir., 1993, 6, 670–679

Whilst provocation testing has become established in studies of airway disease [1, 2], there has been continued debate over how the results should be expressed. Complete description of the histamine or methacholine dose-response curve requires at least two parameters, one for position and one for maximal responsiveness, and may require a third parameter for "slope" [3]. However, estimation of maximal response, or plateau, requires higher doses of drug than can be administered in a community setting. A single measure of response, such as provocative dose producing a 20% fall in forced expiratory volume in one second (PD\(_{20}\)), has been used in epidemiological studies [4, 5].

Many subjects do not achieve a 20% fall in forced expiratory volume in one second (FEV\(_1\)) from baseline at the highest dose of histamine or methacholine that can be administered, so that their PD\(_{20}\) is "censored", in that it is known only to be not less than the highest dose administered. This causes problems in studies of the general population carried out to ascertain risk factors for bronchial hyperresponsiveness, for example, since PD\(_{20}\) is estimable only in a minority of individuals. Although the problem can be partially overcome by analysis of PD\(_{20}\) as a two-group variable, "reactors" and "nonreactors", [5] this is wasteful of information.

In order to provide a solution, attempts have been made to see whether the early part of the dose-response curve could provide a useful measure for epidemiological studies. O'Connor et al. [6] measured the slope of the early part of the dose-response curve as the percentage fall from the post-saline FEV\(_1\) to the FEV\(_1\), at the total cumulative dose, divided by the total cumulative dose, hereafter described as the two-point slope, to avoid confusion with other measures of slope. Abramson et al. [7] compared this two-point slope with PD\(_{20}\) and with the early dose-response slope calculated from all points on the curve, here denoted least-squares slope. Both methods used data from studies in which the challenge was terminated when a 20% fall in FEV\(_1\) occurred, or a maximum predetermined dose of methacholine had been
given. O'CONNOR et al. [6] studied only nine normal and 10 asthmatic volunteers, whereas ABRAMSON et al. [7], by omitting slopes not significantly different from zero and censored PD20 values, compared the repeatability and normality of the measures in different groups. Their conclusion, therefore, that the two-point slope of O'CONNOR et al. [6] should be used in epidemiological studies, is open to question. Comparison has been made using data from small samples of volunteers [6, 8], where censored PD20 values have been omitted [6, 7, 9], and no author [6-9] has considered all of the criteria.

We report a comparison of PD20 and least-squares slope, and the two-point slope of O'CONNOR et al. [6], in terms of normality and, as a measure of validity, separation of symptom groups. Histamine dose-response data were used from a population sample comprising two groups, totalling nearly 800 individuals [10]. Repeatability was assessed on a sub-sample of 104 subjects. The relation between the measures was also investigated, to provide a means of translating one measure to another, if that is found to be appropriate.

Methods

Subjects

In a study of all subjects aged 18–64 yrs in two villages and a market town in Southern England, a 20% random sample of 4,277 subjects, who returned a completed questionnaire, was invited for an initial histamine challenge test. Of 855 invited, 522 agreed to the test, and 510 (60%) were given at least two doses of histamine. Exclusions were due to difficulty in complying with instructions or initial or post-saline FEV1, less than 60% predicted. In addition, all remaining 470 subjects who answered "Yes" to the question "Have you had wheezing or whistling in your chest at any time in the last 12 months?" were also invited, of whom 311 responded and 283 (60%) were given at least two doses of histamine. A sub-sample of 170 out of the total of 793 individuals was invited to return for a second histamine challenge test, of which 130 individuals had recorded a fall of 20%, or nearly 20%, in FEV1, at the initial challenge test [10]. 112/170 (66%) responded, but two or more doses of histamine were administered to only 104 on the second occasion.

Subjects who had taken theophyllines or antihistamines in the previous 24 h, or a bronchodilator in the last 6 h, were asked to return later after omitting treatment. Ethical approval was obtained from the Local Ethics Committees, and the test was explained to all subjects, who then signed a consent form before the test was carried out.

Measurements

Height was measured, and predicted FEV1, calculated as recommended by Cotes [11]. FEV1 was measured using a dry spirometer (Vitalograph). Initial FEV1 was recorded as the maximum of three consecutive readings that agreed to within 5%. Subjects whose initial FEV1 was less than 60% of the predicted value were not challenged with histamine.

The protocol of YAN et al. [12] was followed, with doubling doses of histamine from 0.03 to 4 µmol administered to subjects with a history of wheezing, or whose post-saline FEV1 was less than 90% predicted. All other subjects were given 0.06 µmol histamine, followed by quadrupling doses, until their FEV1 had fallen by at least 10%, when the schedule was changed to doubling doses. The test was stopped when the FEV1 had fallen by 20% or more from the post-saline value, or the 4.0 µmol dose had been given, or at the subject's request.

Estimation of PD20

In order to make maximum use of the data, PD20 was estimated by a curve-fitting method, which O'Connor et al. [10] showed to have some advantages but no disadvantages for epidemiological studies, over linear interpolation. At relatively low doses, at which very few subjects reach a plateau, the relation of FEV1 to log(dose) is one of increasing rate of decline, which can be described by the exponential curve:

\[
\log_{10}(c-FEV1) = a + b \log_{10}(dose)
\]

where "c" represents mean FEV1 before administration of histamine, "b" describes rate of change of log(FEV1) with log(dose), and "a" defines the position of the curve. The curve was fitted to the data of each subject to whom two or more doses of histamine were administered. PD20 was estimated from the fitted curve, as the dose producing a 20% fall from the post-saline FEV1. Extrapolation to a dose of 8 µmol was used, as this had been shown [10] to increase the number of estimates without reducing repeatability. Values greater than 8 µmol were regarded as "censored", and set to 8 µmol for the purposes of repeatability calculations and graphic output.

Two-point slope and least-squares slope

The two-point slope as proposed by O'CONNOR et al. [6] was calculated as the percentage decline in FEV1 from the post-saline value to that at the total cumulative dose administered, divided by the total dose. The least-squares slope was calculated from all data except the post-saline FEV1. All subjects for whom a PD20 could be calculated had at least two doses of histamine, so the line:

\[
\% \text{ fall in } FEV1 = \alpha + \beta(dose)
\]

could be fitted by least squares, and the least-squares slope \(\beta\) thus estimated. \(\alpha\) is the intercept, i.e. the % fall in FEV1 at zero dose.

Criteria for comparison of measures

Each of the three measures examined is a summary of decline in FEV1, with increasing dose, so that validity, the
The most important criterion for any measurement, is not a good candidate for distinguishing between them. However, the relation of each to self-reported asthma and wheeze in the last 12 months was investigated. The next most important criterion is that of good repeatability, total lack negating any previous assertion of validity. Repeatability cannot be summarized as a single value, unless variation is constant, and for statistical analysis this is the most important criterion [13, 14], as it is an assumption required for analysis of variance and regression. The second criterion for analysis is that of normality of the underlying distribution(s). This is the second criterion rather than the first, as analysis of variance is more robust to non-normality than to unstable variance.

These criteria cannot be considered in isolation from the scale of measurement, and this can be chosen for each measure so that one of the criteria for analysis is satisfied. As constant variance is the most important of these, this was used to determine the transformation of each measure [13], as follows: PD$_{20}$ and dose-response slope are in different units, μmol and μmol$^{-1}$, respectively, and therefore the scale on which each is analysed can be chosen independently. The within-subject standard deviation of log$_{10}$ (PD$_{20}$) plotted against mean value is shown in figure 1 for the 86 retested subjects with at least one estimated PD$_{20}$, censored PD$_{20}$ being set to 8 μmol. A similar plot, showing little relation between standard deviation and mean value, was found for the 73 subjects with two estimates. The two-point slope and least-squares slope are in the same units, and it is desirable to apply the same transformation, so that they can be compared directly. Within-subject standard deviation of the two-point slope and of the least-squares slope was found to increase with mean value. As the two-point slope and least-squares slope can take zero and negative values, unmodified reciprocal and logarithmic transformation were excluded. Transformations of the form log(slope+constant) did not result in constant variance, but standard deviation plotted against mean of 1/(slope+10) showed quite reasonable independence for both the two-point slope and the least-squares slope, as illustrated for the latter in figure 2. This modified reciprocal transformation was, therefore, used for each measure of slope.

Repeatability

Repeatability was estimated from the data from the sub-sample who were invited for a second histamine challenge test. As PD$_{20}$ and the two measures of dose-response slope were analysed on different scales, the appropriate measure of repeatability for comparison was the intraclass correlation coefficient [13]. This was calculated for each measure on its chosen scale for all retested subjects, for those with PD$_{20}$ estimated at both occasions, and for the dose-response slopes for subjects with one or both PD$_{20}$s censored. The between and within subject components of variance of each measure were calculated as described by Armitage and Berry [14]. The intraclass correlation coefficient is the ratio of estimated between-subject variation to total variation, i.e. the sum of between and within subject variances. Values range from around zero, for a measure which is random data, to one, for a perfectly repeatable measure which has no within-subject variability.

![Fig. 1. The relationship between standard deviation and mean for log$_{10}$(PD$_{20}$) in 86 subjects with at least one uncensored value. PD$_{20}$: provocative dose producing 20% fall in forced expiratory volume in one second.](image-url)
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**Results**

Of the total 793 subjects given at least two doses of histamine, 593 had a PD\textsubscript{20} estimated to be >8 \textmu mol, and two of <0.03 \textmu mol. Of the 104 individuals tested twice, 73 had an estimate of PD\textsubscript{20} on both occasions, and 18 had two censored PD\textsubscript{20}s.

**Repeatability**

Table 1 shows results from the retested sample. The mean difference (a) of between repeat measures was small for all three variables, and not significantly different from zero (standard errors not shown). The within-subject standard deviation (c), the estimate of variation in a single measurement, is the standard deviation of the difference (b) divided by \sqrt{2}. The between-subject standard deviation (d) is an estimate of true between-subject variation, i.e. with the within-subject variation removed, and the intraclass correlation coefficient (e) is \frac{d^2}{d^2+c^2}. Comparing measures on all 104 subjects, 1/(least-squares slope +10), with an intraclass correlation coefficient of 0.89, was the most repeatable. Restricting results to the 73 subjects with both PD\textsubscript{20}s <8 \textmu mol, i.e. not censored, reduced the repeatability of all measures. For the 31 subjects with one or both PD\textsubscript{20}s \geq 8 \textmu mol, the repeatability of the two-point slope was little better than expected by chance, but the least-squares slope was reasonably repeatable.
Table 1. – Between- and within-subject variation and intraclass correlation coefficient of PD<sub>20</sub>, two-point slope, and least-squares slope for all subjects in the repeatability sample and those with and without two estimates of PD<sub>20</sub>

<table>
<thead>
<tr>
<th>Measure of response</th>
<th>n</th>
<th>Mean difference (a)</th>
<th>SD of within subject difference (b)</th>
<th>Within subject SD (c)</th>
<th>Between subject SD (d)</th>
<th>Intraclass correlation coefficient (e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>log&lt;sub&gt;10&lt;/sub&gt;(PD&lt;sub&gt;20&lt;/sub&gt;)</td>
<td>104</td>
<td>-0.028</td>
<td>0.357</td>
<td>0.252</td>
<td>0.596</td>
<td>0.85</td>
</tr>
<tr>
<td>1/(two-point slope+10)</td>
<td>104</td>
<td>0.001</td>
<td>0.019</td>
<td>0.013</td>
<td>0.020</td>
<td>0.84</td>
</tr>
<tr>
<td>1/(least-squares slope+10)</td>
<td>104</td>
<td>0.002</td>
<td>0.014</td>
<td>0.010</td>
<td>0.029</td>
<td>0.89</td>
</tr>
<tr>
<td>Subjects with both PD&lt;sub&gt;20&lt;/sub&gt; &lt; 8 µmol</td>
<td>73</td>
<td>-0.047</td>
<td>0.405</td>
<td>0.287</td>
<td>0.489</td>
<td>0.74</td>
</tr>
<tr>
<td>log&lt;sub&gt;10&lt;/sub&gt;(PD&lt;sub&gt;20&lt;/sub&gt;)</td>
<td>73</td>
<td>-0.000</td>
<td>0.015</td>
<td>0.011</td>
<td>0.020</td>
<td>0.78</td>
</tr>
<tr>
<td>1/(two-point slope+10)</td>
<td>73</td>
<td>0.000</td>
<td>0.016</td>
<td>0.011</td>
<td>0.020</td>
<td>0.77</td>
</tr>
<tr>
<td>1/(least-squares slope+10)</td>
<td>73</td>
<td>0.000</td>
<td>0.016</td>
<td>0.011</td>
<td>0.020</td>
<td>0.77</td>
</tr>
<tr>
<td>Subjects with one or both PD&lt;sub&gt;20&lt;/sub&gt; ≥ 8 µmol</td>
<td>31</td>
<td>0.003</td>
<td>0.025</td>
<td>0.018</td>
<td>0.010</td>
<td>0.26</td>
</tr>
<tr>
<td>1/(two-point slope+10)</td>
<td>31</td>
<td>0.001</td>
<td>0.011</td>
<td>0.008</td>
<td>0.010</td>
<td>0.66</td>
</tr>
<tr>
<td>1/(least-squares slope+10)</td>
<td>31</td>
<td>0.001</td>
<td>0.011</td>
<td>0.008</td>
<td>0.010</td>
<td>0.66</td>
</tr>
</tbody>
</table>

PD<sub>20</sub>: provocative dose producing a 20% fall in forced expiratory volume in one second.

Fig. 3. – Normal plot of log<sub>10</sub>(PD<sub>20</sub>) in the random sample of 510 subjects (Censored data shown by line — — — —). PD<sub>20</sub>: provocative dose producing 20% fall in forced expiratory volume in one second.

Normality of measures

The Normal plot of log<sub>10</sub>(PD<sub>20</sub>) for the 510 subjects in the random sample, with censored PD<sub>20</sub> set at 8 µmol, is shown in figure 3. As the uncensored values lie approximately on a straight line, the assumption of a Normal distribution for between-subject variation in log(PD<sub>20</sub>) is justified. This was confirmed by the correlation of 0.993 with Normal scores for the uncensored values, which was not significantly different from the value 1.0 denoting perfect Normality. A similar approximation to a straight line Normal plot was also obtained when the data of all 793 subjects were used.

The Normal plot for (least-squares slope + 10) was not linear, as shown in figure 4, and an almost identical plot was obtained for the two-point slope showing similar curvature. The correlation coefficients were 0.897 and 0.873, respectively, significantly different from 1.0 at the 5 and 1% levels. The curvature, i.e. non-Normality, is evident in the lower part of the curve, where values of 1/(dose - response slope+10) correspond to measurable PD<sub>20</sub>s.

Separation between symptoms groups

Mean and standard deviation are shown in table 2 for the measures for subjects with and without "wheeze" in the last 12 months, and for subjects with or without "asthma ever". Eight subjects did not answer the question about asthma. The censored PD<sub>20</sub>s were set to 8
μmol, and this reduced the means and standard deviations. The smaller standard deviation of the asymptomatic groups may, to some extent, be a consequence of this, but the same was found with least-squares slope and two-point slope. The difference in means divided by the common standard deviation, which summarizes separation between groups [14], was therefore invalid. A standard error of the difference in means was calculated for 1/(two-point slope+10) and 1/(least-squares slope+10) using the separate standard deviations, as shown in table 3, with the corresponding estimates for log₁₀(PD₂₀) derived using the method of WOLYNETZ [16]. The ratio (z) of difference to standard error of means for "wheeze" and "no wheeze", i.e. the large sample statistic for testing significance of the difference of means, was greatest as displayed by 1/(least-squares slope+10); for asthma, similar separation was achieved by the three measures.

Relation between measures

As expected, there was a close relationship between the two-point slope and the least-squares slope. The relation between 1/(least-squares slope+10) and log₁₀(PD₂₀) is shown in figure 5. The relation, for uncensored PD₂₀, showed slight curvature, but was reasonably well described by the regression relations:

\[ \frac{1}{\text{least-squares slope} + 10} = 0.040 + 0.043 \log_{10}(\text{PD}_{20}) \quad R^2=0.902 \]

\[ \log_{10}(\text{PD}_{20}) = -0.843 + 21.1/\left(\text{least-squares slope} + 10\right) \]

The corresponding relations for the two-point slope were:

\[ \frac{1}{\text{two-point slope} + 10} = 0.040 + 0.043 \log_{10}(\text{PD}_{20}) \quad R^2=0.897 \]

\[ \log_{10}(\text{PD}_{20}) = -0.823 + 20.7/\left(\text{two-point slope} + 10\right) \]

![Fig. 4. - Normal plot of 1/(least-squares slope+10) in the random sample of 510 subjects. Not all points have been plotted at the dense part of the curve.](image)

Table 2. - Mean and standard deviation of PD₂₀, two-point slope, and least-squares slope for subjects with and without "wheeze" in last 12 months, and for asthmatic and nonasthmatic subjects, in the total sample

<table>
<thead>
<tr>
<th>Wheeze last 12 months</th>
<th>Asthma ever</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>\log_{10}(\text{PD}_{20})</td>
<td>443</td>
</tr>
<tr>
<td>1/(two-point slope+10)</td>
<td>443</td>
</tr>
<tr>
<td>1/(least-squares slope+10)</td>
<td>443</td>
</tr>
</tbody>
</table>

PD₂₀: provocative dose producing a 20% fall in forced expiratory volume in one second.
Table 3. - Mean difference of PD\textsubscript{20}, two-point index, and least-squares slope between symptomatic and asymptomatic subjects

<table>
<thead>
<tr>
<th></th>
<th>Wheeze Yes-No</th>
<th></th>
<th></th>
<th></th>
<th>Asthma Yes-No</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>Standard</td>
<td>z</td>
<td>n</td>
<td>Mean</td>
<td>Standard</td>
<td>z</td>
</tr>
<tr>
<td></td>
<td></td>
<td>difference</td>
<td>error</td>
<td></td>
<td></td>
<td>difference</td>
<td>error</td>
<td></td>
</tr>
<tr>
<td>log\textsubscript{10}(PD\textsubscript{20})</td>
<td>793</td>
<td>-1.467*</td>
<td>0.125*</td>
<td>-11.78</td>
<td>785</td>
<td>-1.593*</td>
<td>0.109*</td>
<td>-14.64</td>
</tr>
<tr>
<td>1/(two-point slope+10)</td>
<td>793</td>
<td>-0.0259</td>
<td>0.0022</td>
<td>-11.62</td>
<td>785</td>
<td>-0.0437</td>
<td>0.0030</td>
<td>-14.76</td>
</tr>
<tr>
<td>1/(least-squares slope+10)</td>
<td>793</td>
<td>-0.0252</td>
<td>0.0019</td>
<td>-13.30</td>
<td>785</td>
<td>-0.0425</td>
<td>0.0029</td>
<td>-14.71</td>
</tr>
</tbody>
</table>

*: calculated using method due to WOLYNETZ [16]. PD\textsubscript{20}: provocative dose producing 20% fall in forced expiratory volume one second. z: ratio of difference of standard error.

Fig. 5. - Relationship between 1/(least-squares slope+10) and log\textsubscript{10}(PD\textsubscript{20}) in 793 subjects. Not all censored PD\textsubscript{20} have been plotted. PD\textsubscript{20}: provocative dose producing a 20% fall in forced expiratory volume one second.

Discussion

A number of criteria have been examined for each of the proposed measures, including those of repeatability, normality and relations to wheeze and asthma. In order to compare the measures using data from the same individuals, only those who were given at least two doses of histamine have been included, although the two-point slope can be calculated from post-saline FEV\textsubscript{1} and FEV\textsubscript{1} at a single dose, and the method of WOLYNETZ [16] can be used when the data include PD\textsubscript{20} less than the lowest dose. There were only four subjects in the total population sample who were given a single dose for whom the least-squares slope could not be calculated; three of these had a greater than 20% fall in FEV\textsubscript{1} at the lowest dose of histamine.

If simplicity of calculation were the only criterion, then the two-point slope would be favoured, but repeatability and the assumptions of standard analyses are more important considerations. Although the two-point slope and least-squares slope can be measured for almost all subjects, this does not justify the use of standard statistical techniques if the assumptions of constant variance and normality are violated, and the necessity of using methods for censored data for analysis of PD\textsubscript{20} should not exclude the latter from consideration. The data for each subject limit the measures that can be considered, but because a computer will almost inevitably be used in the analysis of epidemiological data ease of calculation is not a criterion that is likely to influence greatly the choice of measure.

Transformations

The ideal transformation of each measure would produce stable variance and normality. Log transformation of PD\textsubscript{20} was justified on these two grounds, that below the censoring limit within subject variation was independent of the mean PD\textsubscript{20} and that between-subject variation...
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below the censoring point could be described by the lower part of a Normal distribution curve. These are the assumptions required to use the method of WOLYNETZ [16], which enables analysis of variance or regression to be carried out on censored data. It is not claimed that this is simple, but it has been shown to be valid by the results presented. The method, as published, can be implemented on any machine with a Fortran compiler, with data input determined by the user.

No ideal transformation was found for either two-point slope or least-squares slope. Transformations used by others for the two-point slope include the log transformation [8], and the shifted log transformation [7, 9]. The constant in the shifted log transformation \( \log(\text{two-point slope} + \text{constant}) \) is necessary as some zero or negative values are found, except in small samples, as used by SEPPALA [8]. ABRAMSON et al. [7] concluded that \( \log(\text{two-point slope} + 1.5) \) was non-normal, and the histogram of PRAT et al. [9] for subjects with chronic airflow limitation showed a very positively skewed distribution. For normal subjects, PRAT et al. [9] found a non-normal distribution due to outliers at the extremities; longer tails than the normal distribution [15] were found in our data, as shown by the shape of the normal plot in figure 4. Our data showed that no transformation of the form \( \log(\text{two-point slope} + \text{constant}) \) would produce a normal distribution, or the more important property of constant variance, not considered by other authors. On the criterion of stable variance \( 1/(\text{two-point slope} + 10) \) was preferable to \( \log(\text{two-point slope} + 10) \).

The shifted reciprocal transformation was tried following ABRAMSON et al. [7], who discarded nonsignificant least-squares slopes. However, the existence of a slope for all tested subjects was the reason for its use being assessed, so that any advantage was lost. We confirm their finding that the distribution of \( 1/(\text{least-squares slope} + \text{constant}) \) is non-normal, but because \( 1/(\text{least-squares slope} + 10) \) showed constant variance, we did not explore their inverse cube root transformation, which would also require an arbitrary constant. Although choice of transformation was based on a group partially selected by PD_{20}, this should not affect the relation between variation and mean value, only the density of points on the scatter plots in figures 1 and 2; whilst a larger sample size is desirable it is rarely practicable in repeatability studies. The transformation chosen was the simplest within a range of possibilities, rather than estimated precisely from the data.

In our data, the minimum value of two-point slope was -7.2, and of dose-response slope -6.4, i.e. decreases of 7.2 and 6.4\% \text{mmol}^{-1} of post-saline FEV_{1}. The larger the sample, the greater the largest chance increase that will be found. The constant 10 was chosen as a "round" figure, and in the expectation that it would suffice for most data, as well as satisfying the criterion of constant variance.

Repeatability

Any advantage of the two-point slope or the least-squares slope will relate to its ability to provide a measure in the group of subjects with a censored PD_{20}. In this group of 31 subjects, the intraclass correlation of 0.26 for the two-point slope was little better than expected by chance. SEPPALA [8] found an intraclass correlation of 0.50 for \( \log(\text{index}) \) in 14 nonresponsive subjects, but compared to an intraclass correlation of 0.99 in 16 subjects with a methacholine \( \text{PD}_{20}; \text{FEV}_{1}; \) the higher values may be due to inappropriateness of the log transformation, or the use of volunteers rather than a population sample. Our data for 31 subjects are too few to be totally conclusive about repeatability in the nonresponsive group, but provide the best estimate to date. The intraclass correlation coefficient of 0.66 showed that the least-squares slope was reasonably repeatable in this group and, therefore, least-squares slope did provide information extra to that given by PD_{20}. As \( 1/(\text{two-point slope} + 10) \) and \( 1/(\text{least-squares slope} + 10) \) were on the same scale, it can be seen that the lower intraclass correlation coefficient of the former was due entirely to greater within-subject variation (table 1), as might be expected from the fact that the two-point index is based on less data than the least-squares slope. The two-point slope gives little information beyond that given by PD_{20}, and the slope of the dose-response curve based on all data points, therefore, should be preferred to that based on just two.

Association with symptoms

As the standard deviations of the measures of slope differed between subjects with symptoms and subjects without, the difference in means divided by the common standard deviation could not be used as a measure of separation. However, the standard error of the difference in means could be calculated for each measure. The ratio of difference in means to its standard error is not an absolute measure of separation because it decreases with sample size, but can be used to compare separation of different measures on the same samples. The least-squares slope appeared to be more strongly associated with recent wheeze than PD_{20}, and no less so with asthma. Two-point slope and PD_{20} showed similar separation. BRUSCH et al. [17] found better separation with \( \log(\text{PD}_{20}) \) than with two-point slope between subjects with a clinical diagnosis of asthma and those without, but did not examine PD_{20}. We did not use receiver-operating characteristic curves, because they could not be constructed for PD_{20} across the whole scale, and there was no intention of choosing a cut-off point for diagnostic use.

Relations between alternative measures

Within the range of estimated PD_{20}, the information given by the least-squares slope and PD_{20} was almost equivalent, as shown by the reasonably linear relationship between the indices. The regression equations permit translation from PD_{20} to least-squares slope, or vice versa for purposes of comparison with authors using the alternative index.
General discussion

PD_{20} values obtained by curve-fitting rather than by linear interpolation were used because Chinn et al. [10] showed that this allowed extrapolation by one doubling dose, without loss of repeatability.

Sigmoid shaped curves have been found in normal subjects at doses higher than can be given in epidemiological studies [3, 8, 18]. Woolcock et al. [18] gave equations for the dose-response curve in 10 normal subjects, from which it can be calculated that the dose producing a fall of 90% of the maximum fall achieved, ranged from 9.4 μmol histamine to 16.7 μmol in nine subjects, with the tenth having an outlying value of 51.8 μmol. Seppala [8] found a plateau in only four subjects out of 19 given concentrations of methacholine up to 256 mg·ml⁻¹, one at 64 mg·ml⁻¹, the other three at 128 mg·ml⁻¹. The purpose of this paper is to compare the alternative measures for data from low doses only, at which a plateau will rarely be seen. Whilst some normal subjects may reach a plateau at higher doses, and not achieve a PD_{20}, this does not preclude the use of PD_{20} as described here.

The PD_{20} values obtained from histamine and methacholine challenge tests are closely related in adults [19]. Thus, it seems unlikely that our findings with histamine will differ from those of Abramson et al. [8], who used methacholine. We analysed histamine results, because data from a large population sample were available. The intraclass correlation coefficients in table 1 should be treated with some caution, as they are based on components of variance from the sample selected to test repeatability, and this was biased towards reactive subjects. This is almost inevitable in studies of repeatability of PD_{20}, for which the sample was originally selected. The effect was to increase between-subject variation over that found in a truly random sample, which would be dominated by subjects with censored PD_{20}, and so the intraclass correlation coefficients may be overestimated. They are, however, appropriate for comparison of the measures as used here.

Conclusions

PD_{20} has the recognized drawbacks of all censored data, but log(PD_{20}) otherwise satisfies usual statistical assumptions of analysis of variance, and will can be analysed as a continuous variable, using the method of Woolynetz [16]. The results in table 3 show that very similar results are obtained using this method for log(PD_{20}) and conventional analysis for transformed slopes. Burney et al. [5] obtained comparable results using the method of Woolynetz [16] and analysis of PD_{20} as a two-group measure. Transformation of either slope measure requires addition of a constant, which is to some extent arbitrary, although 10% μmol⁻¹ was chosen in the hope that it would prove suitable for all data.

It is not surprising that PD_{20} and the two measures of slope give much the same information, as they are derived from the same data. Least-squares slope gives slightly more information than PD_{20}, but the non-normality of its distribution must not be overlooked. The two-point slope, however, gives little more information than PD_{20}, despite taking a value for all subjects, and the only justification for its use in preference to least-squares slope would be lack of computing facilities, which is unlikely in an epidemiological study. Given the widespread use of PD_{20} and the availability of methods to cope with censoring, there is not a good case for a measure of slope completely replacing PD_{20}; the relations found between PD_{20} and each measure of slope can be used to compare results obtained by different authors. It is hoped that comparisons will be made by other researchers, using all relevant criteria.

Acknowledgements: The authors would like to thank M. Kelson, F. Anderson and D. Corfield for their help with the challenge tests, K. Snowden, L. Neville, J. McNicol, E. Thomas and R. Wills for help with fieldwork and T.E. Wilson, D. Davies, J. Davies, R. Lorge, R. Coplin, N. Norwell and D. Pollard for their assistance in their General Practices.

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