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

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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 μmol 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 μmol. 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} >8 μmol 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% μmol^{-1} being recommended, and methods requiring normality should be used with caution.

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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 response, 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 PD_{20} 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], or where censored PD_{20} values have been omitted [6, 7, 9], and no author [6–9] has considered all of the criteria.

We report a comparison of PD_{20} 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 FEV_1 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 FEV_1 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 FEV_1 calculated as recommended by COTES [11]. FEV_1 was measured using a dry spirometer (Vitalograph). Initial FEV_1 was recorded as the maximum of three consecutive readings that agreed to within 5%. Subjects whose initial FEV_1 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 FEV_1 was less than 90% predicted. All other subjects were given 0.06 μmol histamine, followed by quadrupling doses, until their FEV_1 had fallen by at least 10%, when the schedule was changed to doubling doses. The test was stopped when the FEV_1 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 PD_{20}

In order to make maximum use of the data, PD_{20} was estimated by a curve-fitting method, which CHINN *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 FEV_1 to log(dose) is one of increasing rate of decline, which can be described by the exponential curve:

$$\log_e(c\text{-}FEV_1) = a + b\ (\log_{10}\ dose)$$

where "**c**" represents mean FEV_1 before administration of histamine, "**b**" describes rate of change of $\log_e(FEV_1)$ with $\log_{10}$ (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. PD_{20} was estimated from the fitted curve, as the dose producing a 20% fall from the post-saline FEV_1. 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 FEV_1 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 FEV_1. All subjects for whom a PD_{20} could be calculated had at least two doses of histamine, so the line:

$$\%\ \text{fall in}\ FEV_1 = \alpha + \beta(\text{dose})$$

could be fitted by least squares, and the least-squares slope β thus estimated. α is the intercept, *i.e.* the % fall in FEV_1 at zero dose.

Criteria for comparison of measures

Each of the three measures examined is a summary of decline in FEV_1 with increasing dose, so that validity, 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.

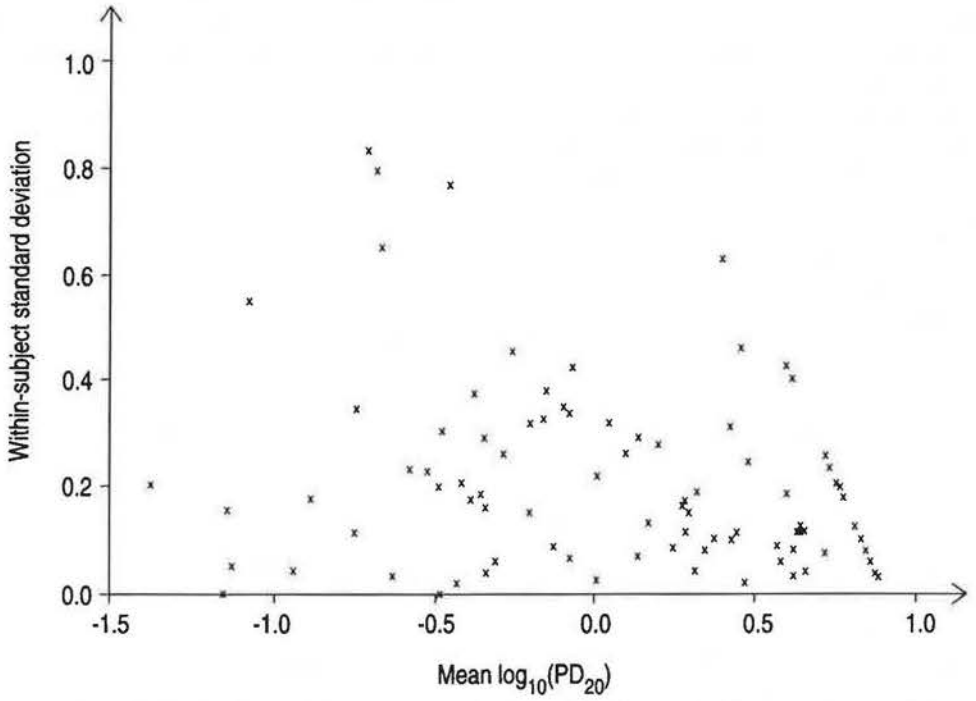

Fig. 1. – The relationship between standard deviation and mean for $\log_{10}(PD_{20})$ in 86 subjects with at least one uncensored value. PD_{20}: provocative dose producing 20% fall in forced expiratory volume in one second.

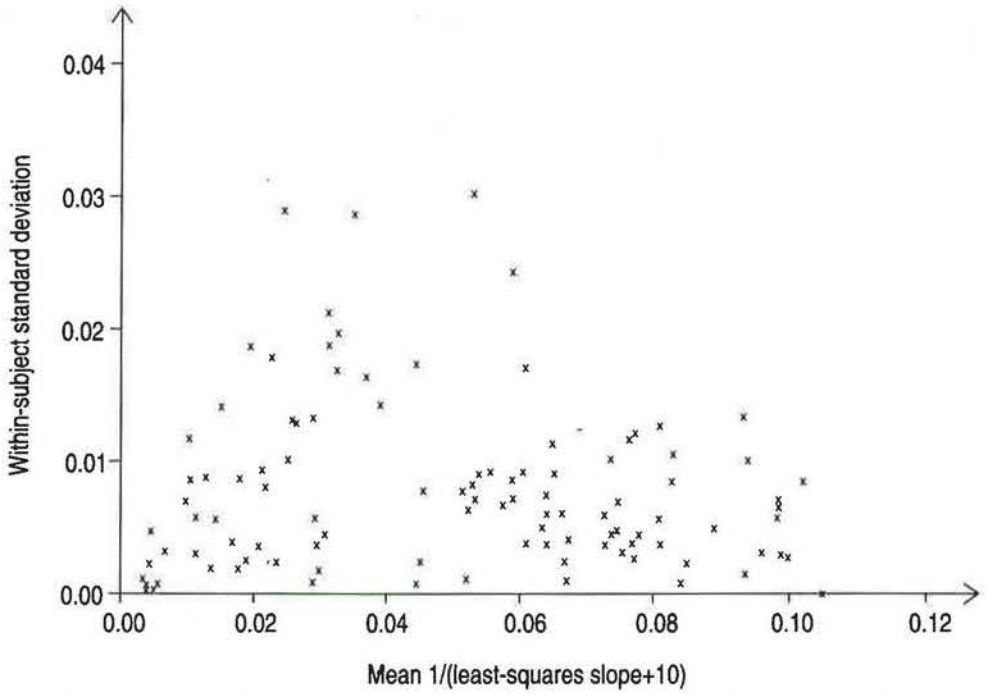

Fig. 2. – The relationship between standard deviation and mean for 1/(least-squares slope+10) in 104 subjects having performed 2 histamine challenges.

Normality

In order to investigate the normality of between-subject variation for all subjects in the random sample, a normal plot, *i.e.* the sorted data plotted against the expected values for a sample of the same size from the standard distribution, was constructed; the first of the two PD_{20}s and slopes were used for subjects with repeat challenges. The correlation of each measure with the corresponding normal score was calculated to give the Shapiro Francia statistic [15].

Separation between symptom groups

The ability of each measure to separate subjects who reported "wheeze" in the last 12 months from those who did not, and subjects who had "asthma ever", as self-reported, from those who had not, was investigated. The mean and standard deviation of each group, and the standard error of the difference in means, was calculated for least-squares slope and the two-point slope on their chosen scale. The mean difference in $\log_{10}$ (PD_{20}) and its standard error were calculated, using the method of WOLYNETZ [16], which takes account of the censored data.

Relations between measures

The relations between the three measures were investigated graphically using data from the total sample. For noncensored PD_{20}, the regression lines of transformed least-squares slope and two-point slope on $\log_{10}$ (PD_{20}) were calculated.

Results

Of the total 793 subjects given at least two doses of histamine, 593 had a PD_{20} estimated to be >8 μmol, and two of <0.03 μmol. Of the 104 individuals tested twice, 73 had an estimate of PD_{20} on both occasions, and 18 had two censored PD_{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 $d^2/(d^2+c^2)$. 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_{20}s <8 μmol, *i.e.* not censored, reduced the repeatability of all measures. For the 31 subjects with one or both PD_{20}s ≥8 μmol, 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_{20}, two-point slope, and least-squares slope for all subjects in the repeatability sample and those with and without two estimates of PD_{20}

Measure of response	n	Mean difference (a)	SD of difference (b)	Within subject SD (c)	Between subject SD (d)	Intraclass correlation coefficient (e)
All subjects in repeatability sample						
$\log_{10}(PD_{20})$	104	-0.028	0.357	0.252	0.596	0.85
1/(two-point slope+10)	104	0.001	0.019	0.013	0.030	0.84
1/(least-squares slope+10)	104	0.002	0.014	0.010	0.029	0.89
Subjects with both PD_{20} <8 μmol						
$\log_{10}(PD_{20})$	73	-0.047	0.405	0.287	0.489	0.74
1/(two-point slope+10)	73	-0.000	0.015	0.011	0.020	0.78
1/(least-squares slope+10)	73	0.000	0.016	0.011	0.020	0.77
Subjects with one or both PD_{20} ≥8 μmol						
1/(two-point slope+10)	31	0.003	0.025	0.018	0.010	0.26
1/(least-squares slope+10)	31	0.001	0.011	0.008	0.010	0.66

PD_{20}: provocative dose producing a 20% fall in forced expiratory volume in one second.

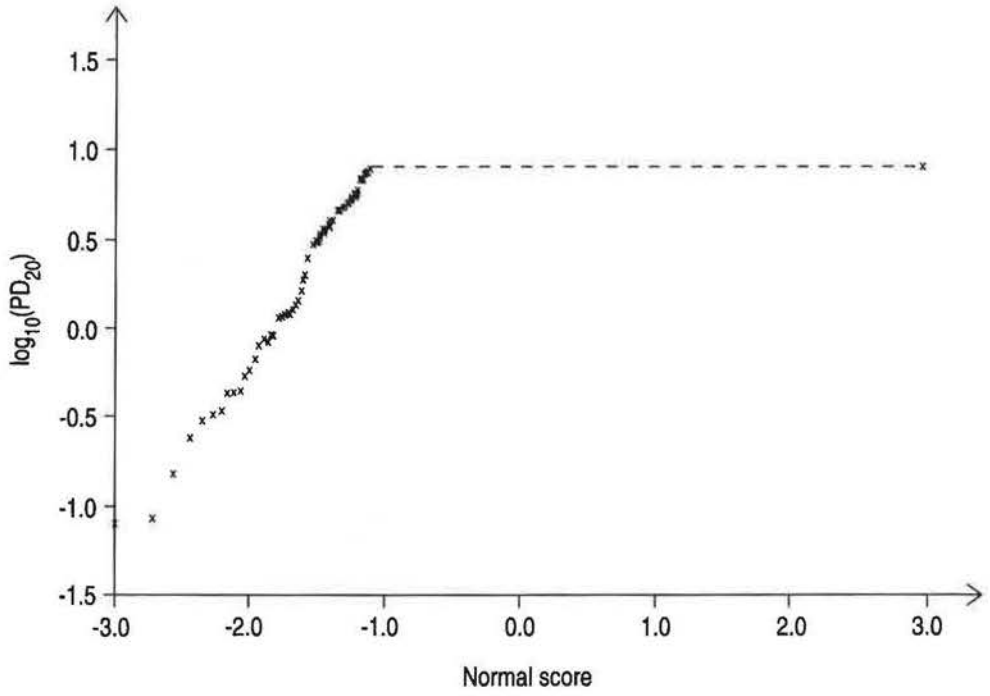

Fig. 3. – Normal plot of $\log_{10}(PD_{20})$ in the random sample of 510 subjects (Censored data shown by line – – – –). PD_{20}: provocative dose producing 20% fall in forced expiratory volume in one second.

Normality of measures

The Normal plot of $\log_{10}(PD_{20})$ for the 510 subjects in the random sample, with censored PD_{20} 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 $\log(PD_{20})$ 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_{20}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_{20}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 $\log_{10}(PD_{20})$ 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 $\log_{10}(PD_{20})$ is shown in figure 5. The relation, for uncensored PD_{20}, showed slight curvature, but was reasonably well described by the regression relations:

$$1/(\text{least-squares slope}+10) = 0.040+0.043\ \log_{10}(PD_{20}) \qquad R^2=0.902$$

$$\log_{10}(PD_{20}) = -0.843+21.1/(\text{least-squares slope}+10)$$

The corresponding relations for the two-point slope were:

$$1/(\text{two-point slope}+10) = 0.040+0.043\ 1\log_{10}(PD_{20}) \qquad R^2=0.897$$

$$\log_{10}(PD_{20}) = -0.823+20.7/(\text{two-point slope}+10)$$

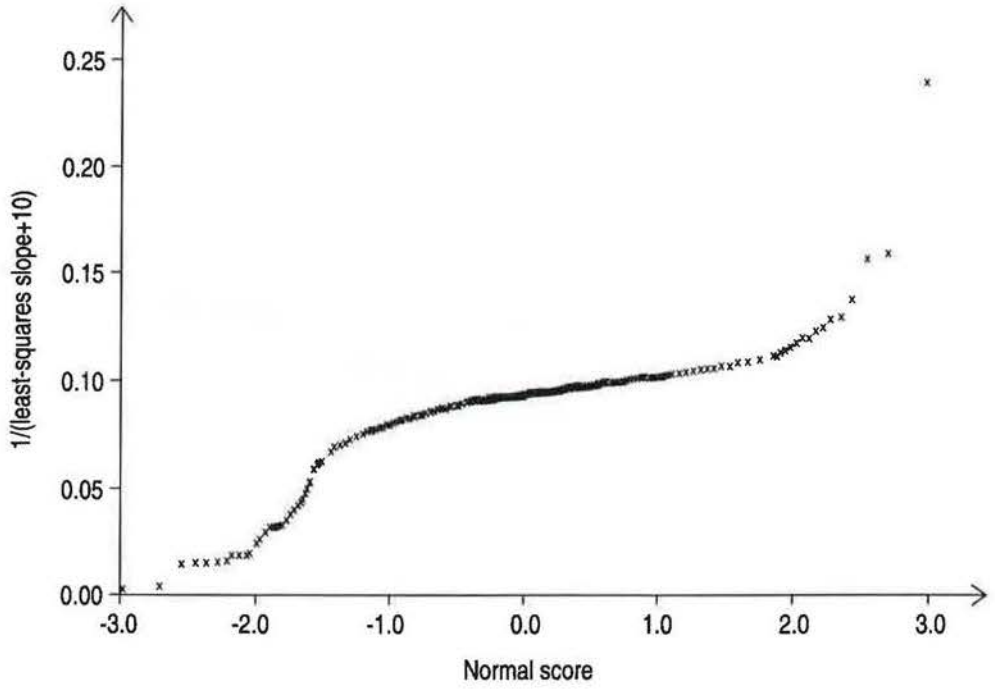

Fig. 4. – Normal plot of 1/(least-squares slope+10) in the random sample of 510 subjects. Not all points have been plotted at the dense part of the curve).

Table 2. – Mean and standard deviation of PD_{20}, 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

	Wheeze last 12 months						Asthma ever					
	No			Yes			No			Yes		
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD
$\log_{10}(PD_{20})$	443	0.87	0.16	350	0.50	0.58	654	0.82	0.23	131	0.10	0.68
1/(two-point slope+10)	443	0.095	0.018	350	0.069	0.035	654	0.091	0.023	131	0.047	0.032
1/(least-squares slope+10)	443	0.094	0.016	350	0.069	0.033	654	0.090	0.020	131	0.047	0.032

PD_{20}: provocative dose producing a 20% fall in forced expiratory volume in one second.

Table 3. – Mean difference of PD_{20}, two-point index, and least-squares slope between symptomatic and asymptomatic subjects

	Wheeze Yes-No				Asthma Yes-No			
	n	Mean difference	Standard error	z	n	Mean difference	Standard error	z
$\log_{10}(PD_{20})$	793	-1.467+	0.125+	-11.78	785	-1.593+	0.109+	-14.64
1/(two-point slope+10)	793	-0.0259	0.0022	-11.62	785	-0.0437	0.0030	-14.76
1/(least-squares slope+10)	793	-0.0252	0.0019	-13.30	785	-0.0425	0.0029	-14.71

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

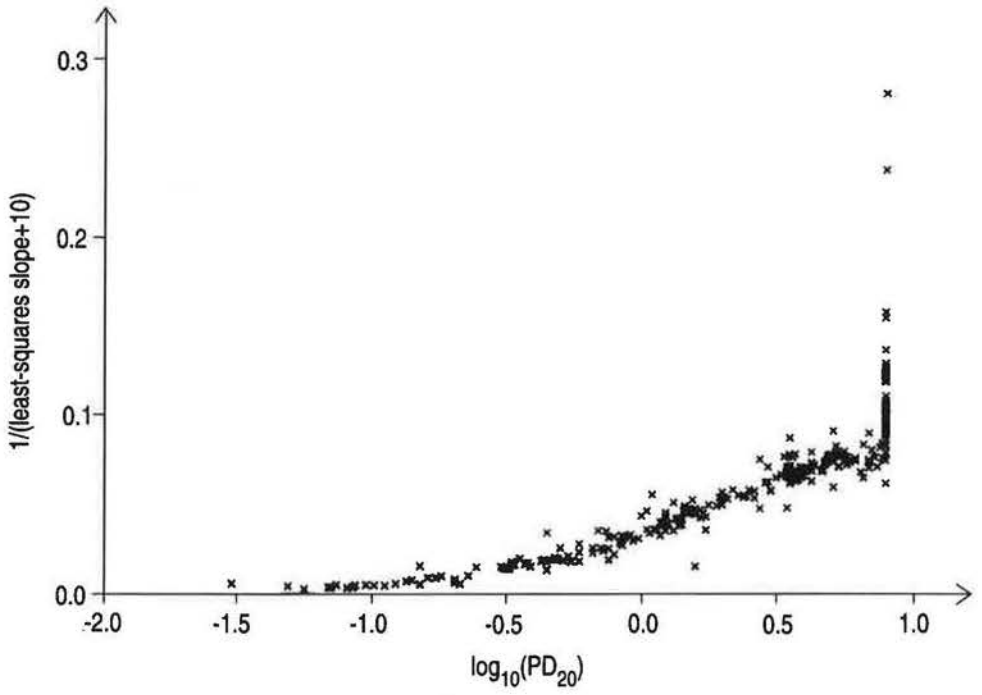

Fig. 5. – Relationship between 1/(least-squares slope+10) and $\log_{10}(PD_{20})$ in 793 subjects. Not all censored PD_{20} have been plotted. PD_{20}: provocative dose producing a 20% fall in forced expiratory volume in one second.

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_1 and FEV_1 at a single dose, and the method of WOLYNETZ [16] can be used when the data include PD_{20}s 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_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_{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_{20} was justified on these two grounds, that below the censoring limit within subject variation was independent of the mean PD_{20}, and that between-subject variation

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(two-point slope+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(two-point slope+1.5) was non-normal, and the histogram of PEAT *et al.* [9] for subjects with chronic airflow limitation showed a very positively skewed distribution. For normal subjects, PEAT *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(two-point slope+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/(two-point slope+10) was preferable to log(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/(least-squares slope+constant) is non-normal, but because 1/(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.* increases of 7.2 and 6.4% μmol^{-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(index) in 14 nonresponsive subjects, but compared to an intraclass correlation of 0.99 in 16 subjects with a methacholine $PD_{20}FEV_1$; 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/(two-point slope+10) and 1/(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. BRUSCHI *et al.* [17] found better separation with $\log(PD_{15})$ 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}s 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 $mg \cdot ml^{-1}$, one at 64 $mg \cdot ml^{-1}$, the other three at 128 $mg \cdot ml^{-1}$. 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 compone-nts 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 can be analysed as a continuous variable, using the method of WOLYNETZ [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 WOLYNETZ [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.

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