Variation in nebulizer aerosol output and weight output from the Mefar dosimeter: implications for multicentre studies

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ABSTRACT: The active aerosol component of nebulizers is less than 100% of output by weight, and may vary between nebulizers in different batches from the same manufacturer. A measure of bronchial responsiveness to methacholine, which can overcome this problem, is required.

One hundred and sixty nebulizers from 21 centres in the European Community Respiratory Health Survey (ECRHS) were calibrated for aerosol and weight output. Methacholine challenge data were obtained for 1,021 subjects in three English centres of the ECRHS. The dose producing a 20% fall in forced expiratory volume in one second (PD20), and log-slope, the regression slope of percentage decline in FEV1 with log (dose), were calculated, with and without calibration of nebulizers by weight.

Within-centre variation in nebulizer percentage aerosol output had a coefficient of variation of less than 10%. Unlike PD20, log-slope is unaffected by constant percentage overestimation of nebulizer output. Variation in output by weight of nebulizers of 10% had little affect on log-slope. It is, however, affected by the scheduled range of doses.

Log-slope shows advantages in analysis, and is less affected by variation in nebulizer output. It can be used for multicentre comparisons, with restriction to a common dose protocol.
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Bronchial responsiveness in epidemiology has recently been measured either by the dose of provocative agent estimated to cause a 20% fall in forced expiratory volume in one second (PD20) or by dose-response slope, calculated as the rate of percentage fall in forced expiratory volume in one second (FEV1) with increasing dose [1]. The two measures give essentially the same information, but have different problems associated with their analysis [2].

The European Community Respiratory Health Survey (ECRHS) is a multicentre study of the variation in the prevalence, risk factors and management of asthma throughout the European Union and elsewhere [3]. The study design, including methacholine challenge using the Mefar dosimeter, was finalized in 1990. In 1992, DENNIS and co-workers [4] reported a nearly twofold variation in aerosol output between two batches of nebulizers manufactured for use with the Mefar dosimeter, which if repeated in the ECRHS could affect between-centre comparison of PD20. Prior measurement of the aerosol output of the nebulizers supplied for the study was not possible, and a change in the manufacturing process at the time could have caused different centres to receive nebulizers from different batches.

This report describes the variation in the nebulizer output in the ECRHS, and proposes a summary of bronchial responsiveness that is less affected by nebulizer variation than PD20, which may be of general use in multicentre studies.

Methods

Output by weight of a nebulizer consists of aerosol and vapour. Aerosol output can be expressed as a percentage of the weight output. Knowledge of this and the weight output for each nebulizer would enable calculation of the dose administered more precisely than by using the manufacturer's nominal weight output.

Nebulizer aerosol output

Nebulizers used in the ECRHS were returned to the co-ordinating centre and then sent to W.A. Arossa in Turin for measurement of aerosol output. Nebulizers were weighed before and after triplicate activation lasting one second. Aerosol output was measured using a fluoride tracer, as described by DENNIS and co-workers [5], and expressed as percentage of weight output. Nebulizers were identified by centre, but not by the concentration of methacholine or dates for which they were used.

Nebulizer weight calibration

The ECRHS protocol [6] specified that monthly calibrations of nebulizer output by weight should be performed, and the data returned to the co-ordinating centre. Weight output of each nebulizer was determined by filling the nebulizer with 3 mL of distilled water and weighing it. Ten inhalations were simulated by firing the dosimeter 10 times for 1 s duration and the nebulizer reweighed. This was then repeated. The average output in grams per inhalation was then calculated.

Measurement of bronchial responsiveness

As dose delivered to each subject could not be calculated from data on aerosol output, presenting a potential problem for the comparison of bronchial responsiveness between-centres using PD_{20}, an alternative summary measure was calculated, log-slope, which is independent of a constant increase or decrease in true output of the nebulizers used at one time (see Appendix). The effects of variation in the output by weight on PD_{20} and log-slope were compared, by estimating the measures in two ways: firstly, with dose being calculated as if all nebulizers had an output of 0.01 g throughout the study; and, secondly, under the assumption that the dose delivered by each nebulizer was directly related to the weight calibration for that nebulizer subsequent to the test. Data from the English arm of the study were used for this analysis. The full protocol for the selection of subjects and methacholine challenge have been published previously [6].

Two ranges of doses were permitted by the protocol, one with a minimum nominal dose of 0.00195 mg and maximum of 1.0 mg, the other a minimum of 0.0078 mg and maximum of 2.0 mg. A long schedule, starting with the lowest dose and increasing in doubling doses until a 20% fall in FEV_1 had occurred, was used for subjects who reported symptoms suggestive of asthma. A short schedule, starting with double the lowest dose and continuing in quadrupling doses until a 10% fall had occurred after which the subject continued with doubling doses, was used for those denying symptoms. The lower range of doses was used in the English centres. In order to maximize the data available for comparison of PD_{20} and log-slope, PD_{20} was estimated by fitting an exponential curve to decline in maximum FEV_1 with log-dose [7], with extrapolation by one doubling dose to 2 mg (10.2 µmol).

Relationship of log-slope to symptoms

Subjects were randomly selected for the study, and an additional sample of symptomatic subjects was chosen [2]. The two samples are combined in this paper. Analysis of variance (ANOVA) was used to determine the relation of log-slope to the three questions used to select the symptomatic sample. For this analysis, log-slope was calculated from the data restricted to doses common to both schedules allowed in the ECRHS, from 0.0078 to 1 mg.

Results

Nebulizer aerosol output

Not all nebulizers could be returned for aerosol calibration, as some were broken in use. Of 225 nebulizers returned from 29 centres in the ECRHS, five from one centre were lost in the post, and 27 were broken or leaked and so could not be calibrated. For the remaining 193 nebulizers from 27 centres, aerosol output, as a percentage of output by weight, varied significantly between centres ($p<0.001$), with an overall average of 43%, and range between centres of 40–52%. The pooled within-centre coefficient of variation (CV) of percentage output was 5.1%, so that true nebulizer output varied by up to about ±10% of the mean output for each centre. The maximum CV for any centre was 8.6%, and the minimum was 1.7% for those with at least five calibrated nebulizers. Output by weight varied significantly between centres, but this was due entirely to one outlying centre; after exclusion of the five nebulizers for that centre, output by weight did not vary significantly between centres. The mean output, with this exclusion, was 0.011 g, with a CV of 5.8%. There was little relationship between output by weight and aerosol output as a percentage of weight output, the correlation being 0.16.

Table 1. – Distribution of PD_{20} and log-slope with and without adjustment for weight calibrations

	Minimum mg	≤2.0 mg n	>2.0 mg n		
PD_{20}					
Without adjustment	0.0036	346	695		
With adjustment	0.0047	302	719		
	Minimum	25th centile	Median	75th centile	Maximum
Log-slope					
Without adjustment	-2.751	1.288	2.754	6.778	79.521
With adjustment	-2.969	1.261	2.769	6.653	79.521
100/(log-slope + 10)					
Without adjustment	1.11	5.96	7.84	8.86	13.80
With adjustment	1.11	6.00	7.83	8.88	14.22

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

Monthly weight calibration for English centres

All calibrations were greater than the nominal output of 0.010 g, the mean being 0.0137 g, with a COV of 10.4% between nebulizers in use in a centre at any one time.

Effect of adjustment for monthly weight calibration

Data were obtained from 1,021 subjects in the English centres, who were given at least two doses of methacholine enabling calculation of PD_{20} and log-dose. As the calibrated output was greater than the nominal output, adjustment increased PD_{20}, thus reducing the number of subjects with a value estimated to be ≤ 2.0 mg (table 1). However, log-slope was only slightly affected

Table 2. – The relationship of log-slope calculated from data for doses 0.0078 to 1 mg, not adjusted for weight calibration, to reported symptoms and asthma medication

	100/(log-slope + 10) Mean	(SD)
Subjects not woken at night by shortness of breath, no asthma attack(s), and no medication for asthma	7.4	(2.0)
Decrease for:	Mean	(SE)
Subjects woken at night by shortness of breath	-0.5**	(0.2)
Subjects with asthma attack(s)	-1.0***	(0.3)
Subjects with medication for asthma	-1.8***	(0.2)

**: p<0.01; ***: p<0.001, compared to subjects not reporting these symptoms.

by the variation in output by weight of the nebulizers. As shown by the quartiles, the distribution of log-slope was skewed, but a reciprocal transformation (Appendix) gave values that were approximately normally distributed, with the same variance. On this transformed scale, the mean difference between adjusted and unadjusted values was 0.005, with a SD of 0.055; the between-subject SD was 2.007.

Relationship of log-slope to symptoms

Table 2 shows the relationships of transformed log-slope to the selection criteria for the symptomatic sample. Subjects who reported "waking at night with shortness of breath" had a statistically significantly ($p<0.01$) lower mean transformed log-slope than those without; and those reporting an attack of asthma in the last 12 months and those reporting current use of medication for asthma had highly significantly ($p<0.001$) lower mean transformed log-slope than those not so reporting. Each of these significant differences was independent of the other two.

The relationship of log-slope calculated from the restricted doses to PD_{20} calculated from all the data is shown in figure 1. The correlation between the two measures for subjects with an estimate of PD_{20} was 0.78. Values of 2, 4 and 6 of 100/(log-dose + 10) correspond to PD_{20}s of about 0.01, 0.1 and 1 mg, respectively.

Discussion

Log-slope was investigated because of the possibility that between-centre comparisons of PD_{20} in the ECRHS would be affected by variation in nebulizer output. It has been shown directly that the variation in weight output between nebulizers in use at one time, of approximately 10%, has a small effect on log-slope. The within centre variation in nebulizer aerosol output was less than this, so that although the effect on log-slope could not be studied directly it can be concluded that the effect would be small. The significant variation between centres affects PD_{20}, but not slope (Appendix).

Log-slope also has the advantage over PD_{20} that it has a value for all subjects challenged with at least two doses. Like other measures of slope, it requires transformation, but as 100/(log-slope + 10) has a reasonably normal and homoscedastic distribution it can be analysed by standard statistical methods, in contrast to other slope measures [2]. Figure 1 shows that the information in PD_{20} and log-slope is broadly equivalent, and table 2 shows that log-slope has the expected relationships to symptoms and
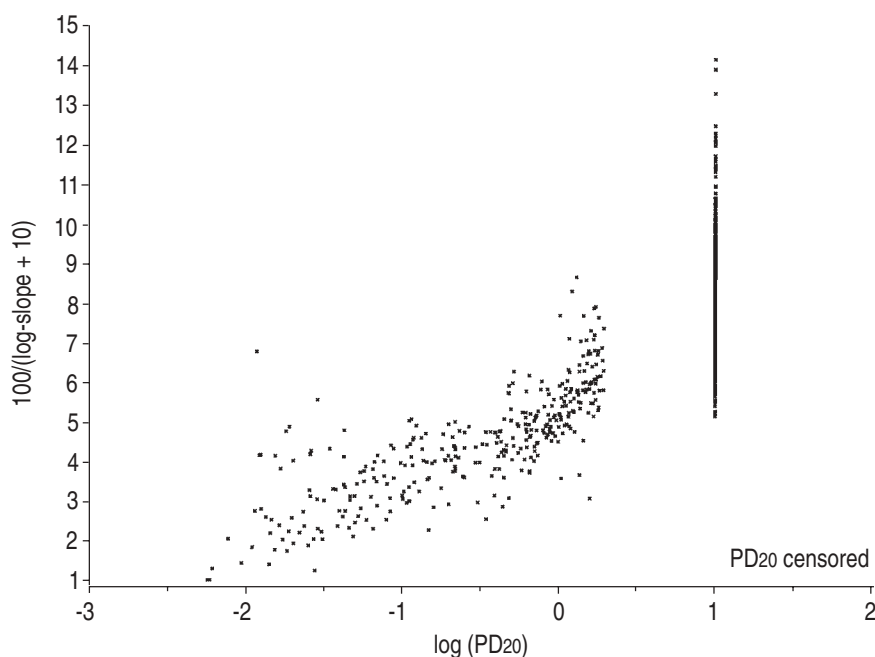

Fig. 1. – The relationship between 100/(log-slope + 10) estimated from doses 0.0078 to 1 mg and log (PD_{20}) estimated from all dose data, using the unadjusted values. PD_{20}: provocative dose of methacholine producing a 20% fall in forced expiratory volume in one second.

diagnosis of asthma. Log-slope is not "reactivity" as defined in some early studies [8–10], as it is estimated from all doses rather than those beyond a threshold at which decline in FEV1 is established.

The disadvantage of log-slope is that percentage fall in FEV1 does not show a good linear relationship with log-dose over the whole dose range, the regression line with dose in milligrams fitting better than that with log-dose. The nonlinearity of the percentage fall in FEV1 with log-dose caused an increase in log-slope when restricted doses were used, as a steeper part of the curve was selected for some subjects. Therefore, it is necessary to restrict data to the nominal doses used by all centres in a multicentre comparison. In the English data, omission of the data for the two lowest doses caused little loss of information. The relationship to symptoms of log-slope calculated from the restricted doses is shown, as this slope will be used in subsequent analyses.

With hindsight, there would have been benefits from the ECRHS using a long schedule of doses for all subjects, although this would have been very time-consuming and expensive. The effect of selecting a short or a long schedule on estimates of log-slope cannot be investigated, as a symptomatic subject given the long protocol is expected to have a larger slope (lower transformed slope) than one given the short schedule. The differences in log slope as estimated from full and restricted dose schedules were largely due to the inherent variability of FEV1 and the lack of precision of any estimate of bronchial responsiveness, but a common schedule of doses could be expected to provide some increased discrimination between subject groups.

DENNIS and co-workers [4] showed that there could be considerable between-batch variation in nebulizer aerosol output, with an approximate twofold difference between the two batches they analysed, and a CV of around 10% for each batch, with aerosol output expressed in mg·s^{-1}. Expressed as a percentage of weight loss output, the mean aerosol output for their two batches was 81 and 63%, with CV of 13 and 10%. They concluded that "provided all nebulizers were purchased together, it is likely that only one batch will be represented within each centre, and so the mean rate of aerosol output could be used in calibration". As the maximum CV for any centre in the ECRHS was less than 10%, it is unlikely that more than one batch was used in any centre, and the range of mean output over the centres suggests, that even if more than one batch was used across centres, the variation was less than was suggested by DENNIS and co-workers [4]. However, as log-slope is unaffected by a constant adjustment in calibration, its use renders it unnecessary to know the mean rate of aerosol output. Even if the aerosol output of each nebulizer is known, the dose of methacholine delivered to the lung or bronchi depends on many unknown characteristics of the subject and nebulizer, so that such a calibration may lend unjustified accuracy to the PD20.

Potential nebulizer batch variation could arise in long-term clinical trials, as well as in multicentre studies. As reproducibility will affect within-subject comparisons to a greater extent than between-subject comparisons, the repeatability of log-slope would need to be assessed before recommending its use in this context. Insufficient data were available to investigate reproducibility in the current study.

Log-slope and PD20 are not totally equivalent. They provide slightly different information, and each measure has advantages and disadvantages. In a multicentre study, in which prior calibration of the aerosol output of every nebulizer and recording of exactly when it is used is not achieved, log-slope can be used as an alternative measure to PD20 to remove any doubts about comparability of the findings.

Appendix

If fall in FEV1 is related to true cumulative dose of methacholine (x) by:

$$\% \text{ fall in FEV1} = a + b \log_{10}(x) \qquad (1)$$

and nebulizer aerosol output is 0.01 AW g, where A is aerosol output as a proportion of weight output and W is the ratio of weight output to the nominal 0.01 g, then nominal dose of methacholine (x') is related to actual dose x by x = AWx', and:

$$\% \text{ fall in FEV1} = a + b \log_{10}(AWx')$$
$$= a + b \log_{10}(AW) + b \log_{10}(x')$$

Thus, log-slope, defined by b, is independent of a constant percentage change in nebulizer output, but PD20 would be under- or overestimated by the factor 1/AW. Dose-response slope as previously defined [2], the regression of % fall in FEV1 on dose in milligrams, *i.e.* b' where:

$$\% \text{ fall in FEV1} = a + b' (x) \qquad (2)$$

is also affected if x is replaced by x' = x/AW.

Because of this, and as found previously [2], no transformation of b' could be found to satisfy the assumptions of standard statistical techniques, namely homogeneity of variance and normality, b' is not considered further, or the two-point slope proposed by O'CONNOR *et al.* [11], which shares both disadvantages. In contrast, the shifted reciprocal transformation 100/ (log-slope + 10) produced constant variance within symptom groups and normality [12], the multiplier of 100 being used to give values in the range 1–15.

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