

Precision of biological standardization of allergenic preparations

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Biological standardization (BS) aims at equilibration of the activity of allergen preparations from different types of allergen source materials. The biological unit (BU), proposed in the Nordic guidelines for 20 patients, has been found reproducible among different countries in Europe, but to be relatively imprecise, with a 95% confidence interval of about one power of 10. A more precise estimate of the biological activity of allergens or difference in sensitivity between populations would be of value. We used C_h , i.e. the concentration of allergen eliciting a wheal of the same size as histamine in the individual patient, estimated by regression line analysis. The C_h of 36 patients included in a BS trial was used. One of the 36 C_h -values was drawn randomly, and then sent back to the sample. This procedure was repeated 10, 20, 30, 40 and 60 times to create "samples" of different sizes. Ten samples of each size were produced. With 60 "individuals", the 95% confidence interval of the sample and the confidence interval of the medians were reduced to less than a factor of 2, i.e. to 74 to 128% of the median of the medians.

Clinical aspects

When biologically standardized (BS) allergens are used in clinical practice, the clinician obtains meaningful information about the sensitivity, as compared with others tested with BS material. For 20 patients, the 95% confidence interval of the median, i.e. the unit, is about one power of 10. By including more patients in BS trials, precise data about the potency of the allergen preparation can be obtained. However, the improvement in precision obtained by including more patients in the basic documentation of BS allergenic preparations is not of significance, since the acceptance limit of new batches of allergen preparations is 30–300% of the labelled value. The imprecision of the basic BS product documentation has considerably less influence on routine diagnostic tests than the imprecision of the skin prick test technique.

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In 1978, the Committee on Allergen Standardization, within the Nordic Association for Allergy Research (1), proposed that allergenic preparations should be biologically standardized (BS), i.e. the activity of allergenic preparation, prepared from different types of allergen source materials, should be equilibrated on the basis of skin prick tests. Initially, a sample of 10 patients was proposed (1). Later Dreborg et al. (5, 7–9) and the Nordic guidelines (12) proposed that 20 patients be included in BS trials. According the Nordic guidelines (12), 10000 biological units/milliliter (BU/ml) is defined as the concentration eliciting a wheal of the same size as that of histamine dihydrochloride 10 mg/ml

(54.3 mmol/l) in the median sensitive patient in a sample of 20 patients, referred to a university clinic, clinically sensitive to the allergen in question. The BU, proposed by the Nordic Committee on Allergen Standardization (1) and approved by the Nordic Authorities (12), has been found to be reproducible in different countries in Europe (5, 7, 9), i.e. the unit is valid all over Europe. With a sample of 20 patients the 95% confidence interval was about one power of 10 (3, 4, 5). However, to determine more precisely the potency of allergenic extracts, or to investigate the difference in sensitivity between populations, for the same allergenic preparation, a more precise estimate would be of value (4). No data have been

published about the effect on precision when larger samples are used.

The aim of the present study was to investigate, by a theoretical model, whether BS trials including 10–60 individuals influenced the precision of the BU, i.e. 10 000 BU/ml.

Material and methods

Patients

The study comprised 36 patients, 20 males and 16 females, mean age 31.5 (range 15–50) years, attending the Department of Allergology, Hospital "La Fe", Valencia. They all had a clinical history of wall pellitory (*Parietaria*) hay fever and/or asthma. Results from the original trial were published in 1986 (6).

Allergen preparation and reference solutions

Pollen from wall pellitory, *Parietaria judaica*, was obtained from Allergon AB (Välinge, Sweden). Extraction was performed in 0.125 M ammonium bicarbonate buffer, pH 7.8, followed by ultra-filtration (nominal cut-off 2000 kD.). The partly purified material (intermediate product – IMP) (8) was lyophilized and reconstituted on the day of testing with albumin (0.03%) saline to a top concentration of 3 mg dry weight/ml and then diluted 10-fold five times. Detailed results on the composition of the IMP and results of the BS have been reported elsewhere (6). Histamine dihydrochloride 10 mg/ml (54.3 mmol/l) and Albumin Diluent® (0.03% albumin human, 0.9% saline and 0.4% phenol) was obtained from Pharmacia Diagnostics AB (Uppsala, Sweden).

Method for biological standardization

Biological standardization was performed as previously described (3, 5, 8) and later prescribed by the Nordic authorities (12). In principle, three 10-fold concentrations of the *Parietaria* IMP were individually chosen for each patient, the lowest inducing a wheal of similar size as that of histamine dihydrochloride 1 mg/ml. All three concentrations of allergen preparation and histamine were tested in quadruplicate on the back of each subject. Based on the results, the concentration, in µg dry weight/ml, eliciting a wheal of the same size as that of histamine dihydrochloride 10 mg/ml in the same patient (C_h) was estimated by regression line analysis in each of the 36 patients. Thus 36 C_h values were obtained. The concentration was given µg dry weight/ml.

Model for estimation of the 95% confidence interval in samples of varying sizes

One of the 36 C_h values was drawn randomly, the value was noted and then returned to the pool of 36 C_h values. A new value was drawn, and returned to the pool, etc. In this manner, "BS" was performed 10 times with a sample size of 10 "individuals", 10 times with 20 "individuals", 10 times with 30 "individuals", 10 times with 40 "individuals", and 10 times with 60 "individuals". The median and the 95% confidence limits were read in appropriate statistical tables.

Results

In the original trial, the unit was found to be 19 µg dry weight/ml, with confidence limits 14–24 µg dry weight/ml. The 95% confidence interval in several of the "trials" with 10 patients was more than one power of 10 and the range of medians more than half a log interval. However, with 60 patients the 95% confidence interval was always less than half a log step. By chance the original median happened to fall in the middle of the range of medians using 60 "individuals". The median of the medians was 18 µg dry weight/ml, the 95% confidence interval 15–20 µg dry weight/ml, and the range of medians 14–22 µg dry weight/ml, i.e. 78–121% of the median of the medians. The range of C_h values was about four powers of 10; i.e. the difference in skin sensitivity between the least and the most sensitive patient in the sample was 10 000 times.

Discussion

It has been reported by several authors that, provided the same inclusion criteria are used, results of BS are reproducible across regions in Europe (2, 5, 7, 9, 11).

The variation between the 10 estimates of the median, where the median constitutes the unit, decreased with increasing number of individuals included in the "trials". The precision of the estimate was lower when only 10 individuals were included, as proposed by Aas et al. (1) in 1978, than with 20 patients included, as in our publications (3–8) and as recommended in the Nordic guidelines (12). The precision of the estimate increased further by including 60 patients, which seems to be a suitable sample size for BS and for estimating differences in sensitivity between different populations using the same stable allergen material.

According to the method given in the appendix of the Nordic guidelines for registration of allergenic preparations, 48 skin prick tests should be performed on each patient, which is too laborious to be used in

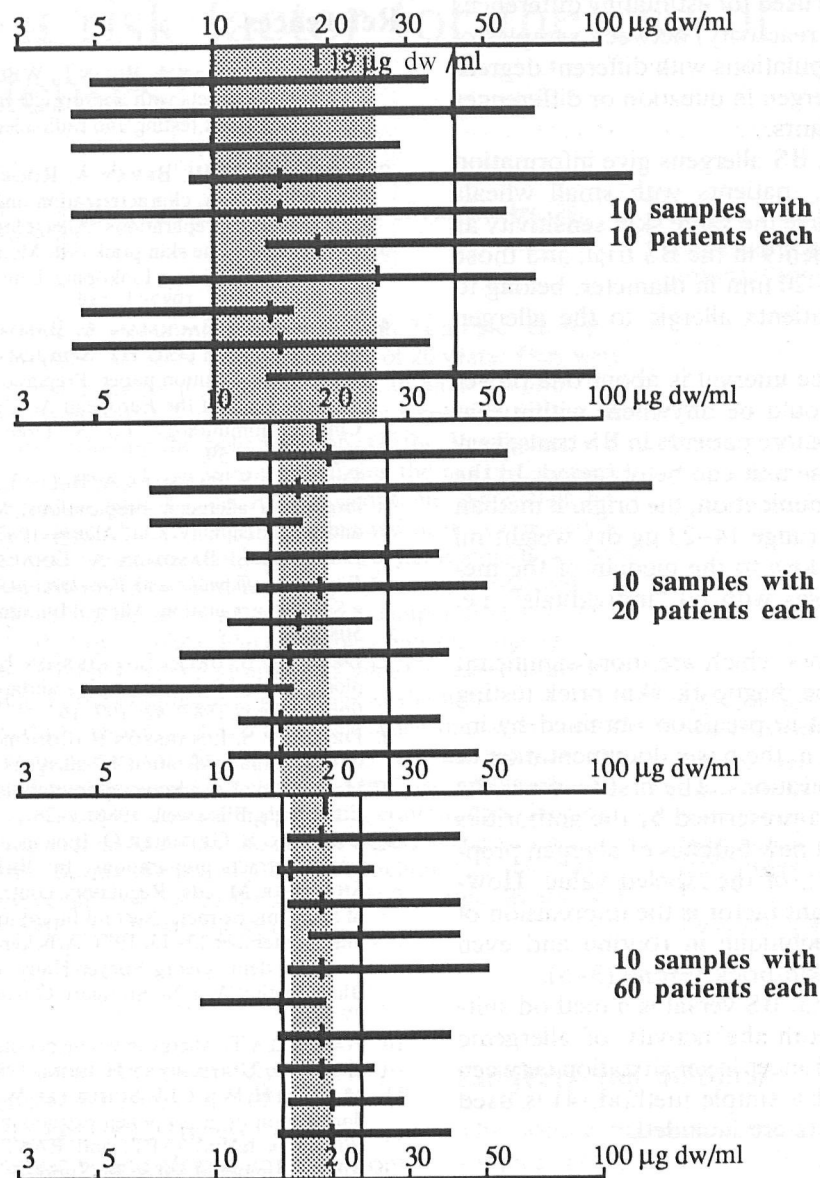


Fig. 1. Data on the median and the 95% confidence limits of the C_h -value for each of 10 samples of 10, 20 and 60 "individuals". Vertical lines indicate the range of medians for each size of sample. Arrow showing 19 µg dry weight/ml indicates the median of C_h values in the original trial on which this communication was based. Shaded area represents the 95% confidence interval of the medians.

population studies. However, according to the Nordic guidelines, other methods correlating to the proposed method may be used. Several authors have been engaged in simplifying the procedure. Haahtela et al. (10) found the *median* slope (b) of the allergen dose-response relationship, in a sample of patients, to be about 0.2, using the diameter of the wheal as an estimate of the response. These data were confirmed by Dreborg & Grimmer (9) and Dreborg et al. (4). Further, Dreborg et al. (5), using the area of the wheal as an estimate of the response and investigating more than 450 patients, found the median slope

to be 0.38 (b calculated by the diameter of the wheal = $0.5 \times b$ using the area of the wheal).

Dreborg (3) proposed a simplified method for BS using the median slope, i.e. $b = 0.2$ (diameter, or 0.4 area), and documented the correlation between this method for estimating the BU and the reference method of the Nordic guidelines (12). This method involves only 4–8 skin prick tests on the forearm of the patient and correlates well with the original more complicated method. Therefore, it can be used, for example, in epidemiological studies.

Provided inclusion criteria are the same, this

simple method can be used for estimating differences in sensitization (skin reactivity) between samples of patients, e.g. from populations with different degrees of exposure to the allergen in question or differences in exposure to pollutants.

In clinical practice, BS allergens give information about sensitivity; i.e., patients with small wheals (3 mm in diameter) have the same skin sensitivity as the least sensitive patients in the BS trial, and those with large wheals, 15–20 mm in diameter, belong to the most sensitive patients allergic to the allergen tested.

The 95% confidence interval is about one power of 10; i.e., the unit could be anywhere within that interval. By including more patients in BS trials, very precise estimates of the unit can be obtained. In the trial used in this communication, the original median value, i.e. 19 and the range 14–23 μg dry weight/ml (6), happened to be close to the median of the medians of the 10 samples with 60 "individuals", i.e. 18 μg dry weight/ml.

There are two factors which are more significant for the result of routine diagnostic skin prick testing than the improvement in precision obtained by including more patients in the basic documentation of BS of allergenic preparations. The first factor is the wide acceptance limits prescribed by the authorities (12) for the potency of new batches of allergen preparations, i.e. 30–300% of the labeled value. However, the most important factor is the imprecision of the skin prick test technique in routine and even scientific diagnostic skin prick testing (3–5).

I would conclude that BS versal is a method suitable for estimating both the activity of allergenic preparations and differences in sensitization between populations, provided a simple method (4) is used and at least 60 patients are included.

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