

Reproducibility of Skin Sensitivity Using a Quantitative Skin Prick Test

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The reproducibility of repeated quantitative skin prick test titrations was evaluated in seven asthmatic patients tested 2-4 times within 6 weeks. The skin sensitivity was estimated as endpoint titration and histamine equivalent reaction using both 1 and 10 mg/ml histamine dihydrochloride. The histamine equivalent reaction had a significantly higher reproducibility compared to endpoint titration, with a coefficient of variation less than 10 % for histamine 10 mg/ml. Further, using this histamine concentration, the deviation in repeated testing was for practical purposes less than 0.5 log step. The same constancy in skin sensitivity was found in one patient tested 3 times and then retested another 3 times 6 months later by a different tester. The results indicate that the quantitative skin prick test and histamine-estimated skin sensitivity have a clinical application in the assessment of pharmacologically or immunotherapy-induced changes in the releasability of skin mast cells.

Key words: endpoint titration; histamine equivalent reaction; reproducibility; skin prick test; skin sensitivity.

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The value of skin testing in the diagnosis of allergy has been documented in several studies (3, 9, 10, 18). Immunotherapy initiates changes in some biological parameters including specific IgE and IgG. That there are conflicting data on changes in skin test sensitivity (4, 6, 11, 16) may partly be due to the reproducibility of the skin test.

Skin test sensitivity can be stated as either endpoint or histamine equivalent reaction (1, 8, 10). Titrated skin tests (quantitative skin test) seem superior to testing with only one concentration of allergen extract (8, 15). Recently, the reproducibility of quantitative skin tests has been documented with the reservation that only tests with a high correlation coefficient and a low variation coefficient are acceptable (8, 15).

The reproducibility of skin sensitivity has been evaluated in only few studies, none using the quantitative skin test. The present study is

an evaluation of the constancy of skin sensitivity estimated by endpoint titration and histamine equivalent reaction with repeated tests over a period of time.

MATERIAL AND METHODS

Patients

Seven adult asthmatics allergic to mould allergens were tested (5 females and 2 males, median age 27 years, range 19-33). The diagnosis of mould allergy was based on history, skin prick test (SPT), RAST and bronchial challenge. All patients were tested outside the Scandinavian mould season and none was treated with drugs which are recognized to interfere with SPT results. Informed consent was obtained and the study was approved by the local Ethics Committee.

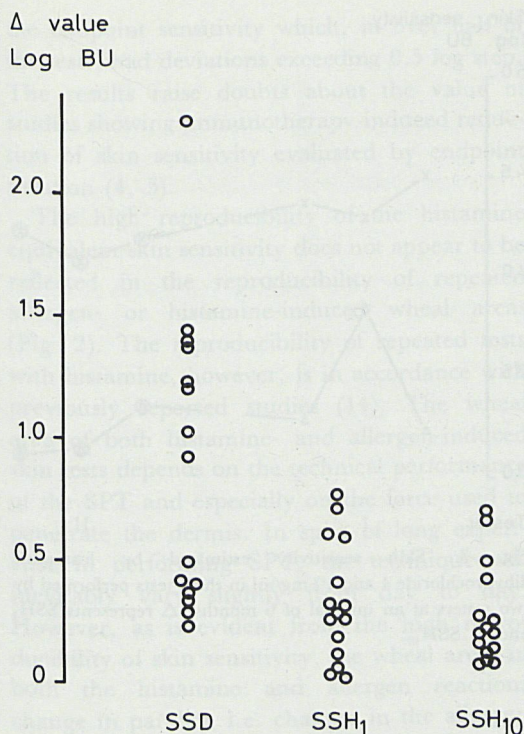


Fig. 1. Deviation of the skin sensitivity of repeated tests from the one with the lowest numeric value. Skin sensitivity is estimated as endpoint = diluent equivalent reaction (SSD), histamine equivalent reaction (SSH₁ and SSH₁₀) using histamine dihydrochloride 1 and 10 mg/ml respectively.

Test extracts

Standardized partly purified lyophilized allergen preparations of *Cladosporium* and *Alternaria* (Pharmalgen®/Spectralgen®, Pharmacia, Uppsala, Sweden) reconstituted in HSA buffer were used. Reconstitution and dilution were performed on the day of SPT, and the same batch was used for all tests. Histamine dihydrochloride 1 and 10 mg/ml and HSA buffer were used as positive and negative reference.

Skin test procedure

Skin prick tests were performed as previously described (7, 8). In short, quadruplicate determinations of a minimum of four 10-fold allergen dilutions (10–100,000 BU/ml), histamine dihydrochloride 1 and 10 mg/ml and HSA buffer were performed on the patients' forearms. A 1 mm SPT-needle (Allergy Pricker,

Dome/Hollister-Stier) was introduced through the extract drop at a right angle and then removed. The skin reactions were recorded at maximum size and the area calculated by planimetry (13). The mean wheal area was plotted as a function of the allergen concentration in a log-log system and linear regression was performed. Tests with a correlation coefficient of < 0.9 were regarded as invalid. The allergen concentration eliciting a wheal equal to the one produced by HSA buffer, histamine dihydrochloride 1 and 10 mg/ml was interpolated from the regression line and termed skin sensitivity (skin sensitivity diluent = endpoint titration (SSD), skin sensitivity histamine 1 and 10 mg/ml (SSH₁ and SSH₁₀), respectively). The reproducibility of individual test sessions was as previously reported (8).

Study design

Patients were retested 2–4 times over a period of 6 weeks with at least a 1-week free interval. All tests were performed by the author except in one patient who was retested three times 6 months later by a nurse.

Statistics

The reproducibility was evaluated as coefficient of variation ($SD\% = SD/\text{mean area} \times 100$).

Non-parametric statistics were applied to evaluate differences in the reproducibility and deviations of sensitivity in the three ways of estimating skin sensitivity (Wilcoxon's signed rank test).

RESULTS

The differences between the numeric least sensitive skin test and the other tests performed on the same patient are evident from Fig. 1. The median deviation is 0.9 log step using endpoint titration (SSD), 0.28 using histamine 1 mg/ml (SSH₁) and 0.19 using histamine 10 mg/ml (SSH₁₀). The difference between SSD and SSH₁ or SSH₁₀ is significant ($P < 0.01$), which is not the case concerning the histamine equivalent reactions. Deviations between indi-

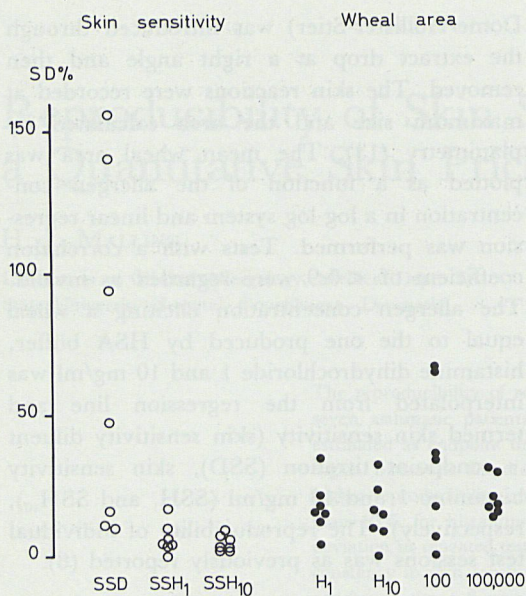


Fig. 2. The reproducibility of skin sensitivity and wheal area in repeated tests on seven patients. The reproducibility is calculated as coefficient of variation ($SD\% = SD/\text{mean} \times 100$). SSD, SSH₁ and SSH₁₀ as in Fig. 1. H₁ and H₁₀ represent histamine dihydrochloride 1 and 10 mg/ml. 100 and 100,000 are the allergen concentrations in BU/ml.

vidual values > 0.5 log step were found in 53 % of the tests using SSD (8/15), 27 % using SSH₁ (4/15) and 13 % using SSH₁₀ (2/15).

Fig. 2 gives the reproducibility of repeated estimates of skin sensitivity stated as coefficient of variation (SD%) using SSD, SSH₁, and SSH₁₀, as well as wheal areas elicited by histamine 1 and 10 mg/ml and allergen concentrations 100 and 100,000 BU/ml. A significantly higher reproducibility (lower SD%) of SSH₁ and SSH₁₀ compared with SSD was found ($P < 0.02$). The difference between SSH₁ and SSH₁₀ was not significant and neither was the reproducibility of the wheal areas.

The constancy of three successive tests repeated after an interval of 6 months by two different testers is shown in Fig. 3. Except for one test using SSH₁ the skin sensitivity deviates less than 0.5 log step. Using endpoint sensitivity the deviations were approximately 3 log steps (data not shown).

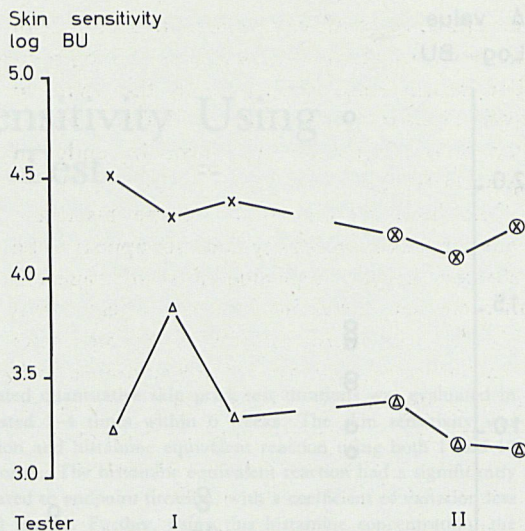


Fig. 3. Skin sensitivity estimated by histamine dihydrochloride 1 and 10 mg/ml in three tests performed by two testers at an interval of 6 months. Δ represents SSH₁ and $\times$ SSH₁₀.

DISCUSSION

The value of using skin sensitivity as a parameter of pharmacologically or immunotherapy induced changes in skin response depends on the reproducibility of the tests. The reproducibility of repeated SPT using histamine has been documented in several studies (2, 12, 14). Decrease in skin sensitivity to allergen extracts initiated by immunotherapy has been reported by many authors (4, 5, 17), but only one study (11) has reported on the reproducibility of repeated tests in untreated patients, using a test less sensitive than the quantitative skin test.

The present study shows that the use of titrated skin test and the histamine equivalent reaction as an estimate of skin sensitivity results in a high reproducibility (Fig. 2), and, furthermore, with histamine dihydrochloride 10 mg/ml the results for all practical purposes deviate no more than 0.5 log step in repeated tests (Fig. 1). The coefficient of variation is less than 10 % for all tests, probably due to the high reproducibility of individual tests (8). More than 70 % of the tests with SSH₁ showed a sensitivity deviation of less than 0.5 log step, in contrast to

the endpoint sensitivity which, in over half of the tests, had deviations exceeding 0.5 log step. The results raise doubts about the value of studies showing immunotherapy-induced reduction of skin sensitivity evaluated by endpoint titration (4, 5).

The high reproducibility of the histamine equivalent skin sensitivity does not appear to be reflected in the reproducibility of repeated allergen- or histamine-induced wheal areas (Fig. 2). The reproducibility of repeated tests with histamine, however, is in accordance with previously reported studies (14). The wheal area of both histamine- and allergen-induced skin tests depends on the technical performance of the SPT and especially on the force used to penetrate the dermis. In spite of long experience in performing SPT, the technique will inevitably vary slightly from day to day. However, as is evident from the high reproducibility of skin sensitivity, the wheal areas of both the histamine and allergen reactions change in parallel, i.e. changes in the allergen wheals due to technical variation are accompanied by corresponding change in histamine wheals. This contrasts with endpoint titration, which only depends on the allergen-induced wheal reaction. As seen in Fig. 2, the small allergen reactions (*in casu* 100 BU/ml) are difficult to reproduce (SD % 0-70 %). Minimal changes in the pressure used in SPT result in variations of the endpoint over several log steps. The problems involved in reproducing endpoint titration have recently been discussed (8).

Furthermore, the study reveals a high degree of constancy in the histamine-estimated skin sensitivity performed by two different testers (Fig. 3). The absolute wheal areas obtained by the two testers differed considerably due to differences in technical performance, but, as earlier discussed, this does not change the skin sensitivity. If endpoint had been used, the differences might have been interpreted as a result of therapy-induced changes.

In summary, quantitative skin tests using the histamine equivalent reaction to estimate skin sensitivity show a high reproducibility. This was especially the case with histamine dihydrochloride 10 mg/ml. Changes exceeding 0.5 log

step are, probably, real providing the reproducibility of the individual tests is high. In addition, skin sensitivity, as evaluated by different testers, appears to be a relatively constant parameter, though the low reproducibility of endpoint titration reduces the clinical value. When using the highly reproducible histamine equivalent sensitivity, quantitative SPT might be valuable for assessing changes in skin mast cell-mediator releasability induced by immunotherapy or drugs.

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