

Comparison of Standardized Skin and Provocation Tests with RAST and Histamine Release from Leukocytes in Bronchial Asthma

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One reason for conflicting results in comparative studies on different methods in clinical allergology is the lack of standardization of the diagnostic procedures. However, standardization of the methods is the only way to improve diagnostic procedures as long as allergen extracts remain unsatisfactory. There seem to be two main sides to the problem:

1. standardization of the test procedures,
2. standardization of the test evaluation.

The way chosen in our laboratory to overcome these problems as far as possible will be presented. The results of comparative studies on these clinical tests and RAST or histamine release from leukocytes will be presented in the second part of this paper.

METHODS

Allergic history

A specially developed questionnaire (6) was introduced in 1974, which may be evaluated by the aid of a computer. Today, with some modifications, this questionnaire is used by many allergologists in our country, as a first step of standardization.

Skin prick test

We regard the prick test as the best routine skin test method, due to its simplicity. In our laboratory the skin reactions are evaluated by measurement of the weal diameter. The relation of allergen effect, positive control with histamine (5.4 mmol), and negative control with diluent are calculated according to the following formula(7):

$$\text{Relative weal diameter} = \frac{D_{\text{allergen}} - D_{\text{diluent}}}{D_{\text{histamine}} - D_{\text{diluent}}}$$

(D=weal diameter)

Thereby a histamine standard (=1) has been introduced and non-specific skin test reactions eliminated. This procedure is in some ways comparable to the proposal of Aas and coworkers (1). As 'positive' we regard reactions of at least 0.6 (approx. 3 mm weal diameter), as these reactions have been found to be reliable in repeated tests.

Intradermal skin tests

In our laboratory these tests are performed only for scientific reasons. The evaluation of intradermal skin tests with 10-fold increasing allergen concentrations is carried out in the same way as the prick test. As only reactions of at least 85% of the histamine control (0.54 mmol/l) were regularly followed by larger reactions with increasing allergen concentrations, these reactions were regarded positive (8). The allergen dose required for the 85% reaction (relative weal diameter=0.85) was used in comparative studies to express intradermal skin sensitivity.

Bronchial provocation tests

Bronchial provocation tests are performed as described by Gonsior et al. (3), by inhalation of 10-fold increasing allergen concentrations with a conventional nebulizer (Heyer, Bad Ems). The initial allergen concentration is estimated from allergic case history, skin sensitivity, and histamine challenge tests. Bronchial obstruction is measured by body plethysmography (Jaeger, Würzburg), which needs less cooperation by the patient than spirometry and avoids artefacts by the measurement itself, e.g. spirometry-induced asthma. For a positive test an increase of specific airway resistance SR_{aw} above 2 kPa·s is required. This value represents the 'dyspnoea threshold' (5), where the patient usually feels a slight ventilatory discomfort. In addition an increase of SR_{aw} of at least 50% above the baseline is required.

By introduction of the threshold value the clinical significance of allergen-induced bronchial obstruction seems to be established better than by use of an arbitrary 'percent change' alone. As a parameter for a positive test in comparative studies we use the amount of allergen inhaled for a positive bronchial reaction (8). A further improvement might be obtained by the determination of an allergen threshold concentration, i.e. the allergen concentration at the intersection of the dose-response curve with the dyspnoea threshold value.

The *in-vitro* techniques used in this comparative study will only be described very briefly.

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Table 1
Parameters and criteria in skin and bronchial provocation tests

Test	Measurement	Controls	Parameter	Positive
Prick Test	weal diameter (mm)	1. histamine (5.4 mmol/l) 2. diluent	relative weal diameter (cf. text)	≥ 0.6
Intradermal test	weal diameter (mm)	1. histamine (0.54 mmol/l) 2. diluent	relative ^a weal diameter (cf. text) allergen ^b threshold dose for 0.85-test	≥ 0.85
Bronchial provocation test	body plethysmography	diluent inhalation	Specific ^a airway resistance SR) allergen ^b dose at 'dyspnoea threshold' (cf. text)	SR _{aw} ≥ 2 (kPa·s) and increase $\geq 50\%$

^aParameter used to describe size or intensity of the reaction.
^bParameter used to describe sensitivity to allergen.

Determination of specific IgE

The RAST was performed with the same batch of allergen used in the *in vivo* tests, coupled to special discs. RAST-results were expressed in terms of specific activity of the samples (counts per minute).

Histamine release from leukocyte suspension

Allergen-specific histamine release from leukocytes of the allergic subjects was determined by a modified automated fluorimetric (Perkin-Elmer) histamine assay (9). In a proportion of patients only low histamine concentrations were released by allergen (2). Therefore it was impossible to evaluate these tests using an allergen threshold concentration (e.g. at 50% release). Instead, the area below the allergen dose-response curve was regarded as suitable for evaluation (8).

In the next part of this paper some of the results of a comparative study (8) of our methods will be described. This study was performed mainly for two reasons:

1. A correlation of the tests was thought necessary to prove the clinical significance of the parameters and

criteria chosen.

2. We wanted to investigate the possibility of a replacement of bronchial provocation tests by skin or *in vitro* tests (4).

The results of all these tests were compared in two ways, namely in a qualitative way, comparing the agreement of positive and negative reactions of each test, and in a more quantitative way, by correlating the parameters of test. Although for practical purposes the classification 'positive/negative' is important, we have to recognize that with allergic reactions there is no 'either-or', but a more or less clear dose-response relationship. There is no distinct point on these dose-response curves which might indicate the clinical significance of a reaction. Therefore the classification into 'positive' or 'negative' is necessarily arbitrary. For a comparison of different methods the evaluation by classifications or scores should be replaced by continuous scales.

RESULTS

As an example of a qualitative comparison the discrepancies between bronchial provocation tests with six

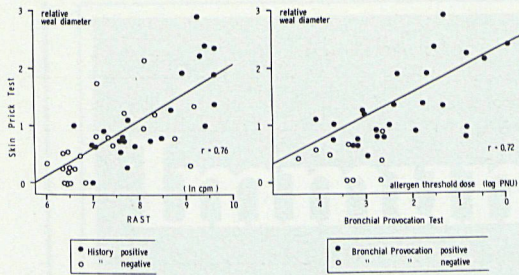


Fig. 1. Correlation between skin prick test and RAST of bronchial provocation test. Methods and parameters: cf. text.

allergens (hazelnut and rye grass pollen, horse and cat dander, house dust, and alternaria ten.) and skin tests or *in vitro* tests are described. Following our criteria we found a good agreement between skin prick tests and bronchial provocation tests (91%). The agreement with RAST was comparable (90%). Histamine release agreed quite well with negative provocation tests, but was negative in a proportion of positive provocation tests, due to low histamine release in a proportion of patients. In patients with good histamine release the agreement between all the tests was excellent.

The correlations between RAST, prick test, and bronchial provocation test are shown in Fig. 1. There was a significant correlation between prick test and RAST ($r = 0.76$), however, small prick tests were occasionally associated with high RAST activity and vice-versa. Prick test results correlated with RAST ($r = 0.72$). Prick tests of more than 1.0 (i.e. the size of histamine control) were always correlated with positive provocation tests. A good correlation was also present between RAST and bronchial provocation tests ($r = 0.71$). At a RAST-class of 3 or 4 all the bronchial provocation tests were positive. Also a correlation of histamine release and prick test was observed ($r = 0.67$), but as mentioned above the agreement of both tests was not satisfactory because of patients with insensitive leukocytes.

In summary, these investigations demonstrate a good correlation of *in vivo* allergy tests, indicating the clinical significance of the parameters and criteria chosen. However, a number of discrepancies between *in vivo* and *in vitro* tests underline that in allergic bronchial asthma a combination of several tests remains necessary to establish the correct diagnosis. At the same time it is confirmed that neither the RAST nor histamine release will be suitable to

replace biological tests. *In vitro* tests should, however be used to analyze allergic reactions as an additional tool in the diagnosis of bronchial asthma.

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