

The Precision of the Conjunctival Provocation Test

CHRISTIAN MÖLLER,¹ BENGT BJÖRKSTÉN,^{2,3} GÖRAN NILSSON² and STEN DREBORG^{2,3}

Dept. of Pediatrics, ¹ University of Umeå, ² Pharmacia Diagnostics, Uppsala and
Dept. of Pediatrics, ³ University of Uppsala, Sweden

Repeated conjunctival provocation tests (CPT) were done in 20 children with rhinoconjunctivitis due to birch pollen allergy. Compared with the first open challenge, three successive blinded CPTs were reproducible within an allergen strength difference of one 10-potency in 92% of the tests. Based on the data, a power function was constructed, making it possible to determine the number of patients needed to discriminate CPT sensitivity of a given magnitude between two populations. During the study period specific serum-IgE increased in only three of 19 patients, indicating that stimulation of IgE antibody production is not a common consequence of repeated tests. We conclude that CPT is useful in clinical research. When indicated in clinical routine, CPT is a safe and easy test with good precision.

Key words: allergic conjunctivitis; atopic hypersensitivity; conjunctival provocation test; hay fever; IgE.

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CLINICAL ASPECTS

The conjunctival provocation test (CPT) is a simple, safe and rapid challenge test. We have found that the test has good precision. Thus, even moderate differences can be detected in the conjunctival sensitivity to an allergen. Repeated tests do not affect the sensitivity of the conjunctiva. Although some patients have increased specific serum-IgE levels after repeated tests this is not a common consequence. We conclude that CPT, when indicated in clinical routine, is a safe and easy test with good precision.

Reliable tests are needed to objectively confirm a case history suggesting allergy. Circulating specific reaginic antibodies can be demonstrated with *in vitro* tests, e.g. radioallergosorbent test (RAST). Tissue-fixed specific antibodies can be demonstrated by skin test, e.g. prick or intradermal tests. To establish the diagnosis in questionable cases and to evaluate the efficacy of specific immunotherapy, demonstration of antibodies in the shock organ is of particular interest. This is mainly done by provocation tests. The bronchial provocation test has a series of disadvantages: it is time-consuming and involves a risk of systemic anaphylactic reaction. The nasal provocation test is difficult to evaluate unless a very clearcut positive reaction is obtained. Unspecific hyperreactivity may in both tests give false positive

results. The tests are at best only semiquantitative (1, 4).

In recent years the conjunctival provocation test (CPT) has been increasingly employed, at least in Sweden. The test is easy to perform and without any appreciable risk of systemic reactions. Although the CPT has been used for decades (5) and evaluated by other means (6) the precision of the test has not been studied. We have therefore investigated the precision of the CPT with end-point titration in 20 allergic children, using a well-characterized pollen extract.

As provocation tests may, theoretically, sensitize patients to new allergens, or enhance previous specific IgE production, we also studied the influence of repeated CPT on serum levels of specific IgE antibodies to the allergen used.

MATERIAL AND METHODS

Patients

Twenty children, 9–16 years old (mean 11.7 years) (16 boys and 4 girls) were divided into two groups of 10 children. All the patients had rhinoconjunctivitis due to allergy to birch. The diagnosis was established through positive case history, positive skin prick test (SPT), at least 2+ according to Aas & Belin (2), and positive CPT (according to our method). In addition to birch allergy some children also had other allergies but were all free of symptoms during the test period. The children had not been hypersensitized previously and were otherwise healthy. Medication was suspended at least 1 month before and during the test period.

Material

The same freeze-dried, highly purified and biologically standardized birch pollen allergen preparation (Spectralgen®, Pharmalgen®, Pharmacia Diagnostics, Uppsala, Sweden) was used for SPT and CPT. An unbuffered saline solution with 0.03% human serum albumin (Albumin diluent®, Pharmacia Diagnostics) was used as diluent. Specific IgE antibodies were determined with Phadebas RAST® (Pharmacia Diagnostics) and expressed as Pharmacia RAST units (PRU).

Methods

The first group of 10 children was tested in March 1980, and the second in March 1981, well ahead of the pollen season. The birch pollen allergen preparations were reconstituted and diluted 1–8 days before the test and stored at +4–+8°C. The dilution factor for the allergen solutions in different vials was 3.2, i.e. factor 10^{0.5}.

The CPT was performed by administering one drop of the allergen dilution in the conjunctival sac. If no reaction was noted within 20 min the next concentration of the test solution was applied in the conjunctival sac of the other eye.

Criterion for positive CPT was congestion of the conjunctival mucosa and itching of the eye.

The children were initially challenged openly with increasing strengths of the test solutions. The CPT were then repeated double blind three times with 1 week intervals. All vials were coded by the manufacturer and the investigator was not aware of the coding principle. Each group of 10 children was given successively higher doses of the allergen. However, in the first group the initial strength varied, and the continuity was randomly interrupted by giving one or several vials with lower concentrations. In the second group, each child on one occasion started with active material, on one occasion with a vial containing albumin diluent, and on one occasion with two vials of the diluent.

Blood samples for specific IgE were collected immediately before the first challenge and 1 week after the last challenge.

Statistical methods

For all statistical calculations the potencies of the allergen solutions giving positive CPT results were given in Biological Units/ml (BU/ml) expressed as 10 logarithmic values. This also applies to the calculations of IgE.

To evaluate the precision of the method, the standard deviation (SD) between the threshold concentration of the initial CPT and the following blinded tests were calculated for each patient. Based on a pooled estimate of these standard deviations a power function for Student's *t*-test was constructed.

The calculations were based on the assumption that the CPT in scientific work is usually intended for comparison of changes, e.g. the effect of a certain therapy in two groups with *n* patients in each. If σ is the SD in single provocation tests the SD of the difference between two provocations is $\sqrt{2} \times \sigma$. For the difference between the mean changes in the two groups the standard error is

$$\sqrt{2}\sigma \sqrt{\frac{1}{n} + \frac{1}{n}} = \frac{2\sigma}{\sqrt{n}}.$$

The hypothesis of equal change in the two groups is tested by Student's *t*-test with

$$t = \frac{\sqrt{n}|\bar{d}_1 - \bar{d}_2|}{2\sigma},$$

where $\bar{d}_1$ and $\bar{d}_2$ are the observed mean changes in the groups. To test the hypothesis on the 5% significance level the obtained *t*-value is compared with the appropriate percentile (95 for a one-sided and 97.5 for a two-sided test) of a *t*-distribution with *n*-2 degrees of freedom. The significance level is the probability to reject the hypothesis when it is true. More interesting, however, when planning a study, is the probability to reject the hypothesis when it is false, i.e. to discover a true difference between the groups. This probability depends on the magnitude of the true difference and the number of patients in the two groups. For given values of σ and *n*, this probability can be calculated as a function of the true difference (the power function of the test). Thus, if a certain difference is expected, the power function gives the probability to discover a true difference of that size with the test. Usually, experiments are planned in such a way that the probability to discover a certain true difference can be defined in advance. If the power function is calculated for several values for *n*, the required size of *n* in a planned study can easily be identified.

RESULTS

Fifty-nine paired differences from the initial values (¹⁰log of threshold concentrations) were obtained from the results and used for calculations (1 CPT resulted in reaction to the albumin diluent due to unknown reasons, and was excluded). The differences were in 85% of the cases 0.5 or less, in 92% within 1.0 and in 97% within 1.5 (Fig. 1).

The pooled SD of the CPT results within patients was 0.47 with 59 df. Assuming $\sigma = 0.5$, the power function was calculated for *n* = 9, 19 and 40 (Fig. 2). As indicated in the figure there is a 95% probability to reject the hypothesis of no difference between two groups of 40 patients if the difference in change of CPT threshold concentration between the two groups is ap-

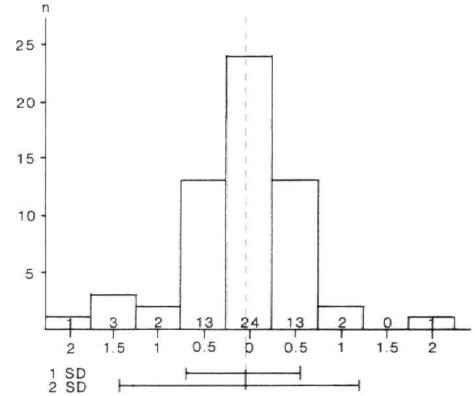


Fig. 1. Differences in threshold concentrations giving positive CPT between the first and each of the subsequent three blinded tests in 20 patients (59 differences – 3 in all but 1 patient). The differences are expressed in 10-log steps.

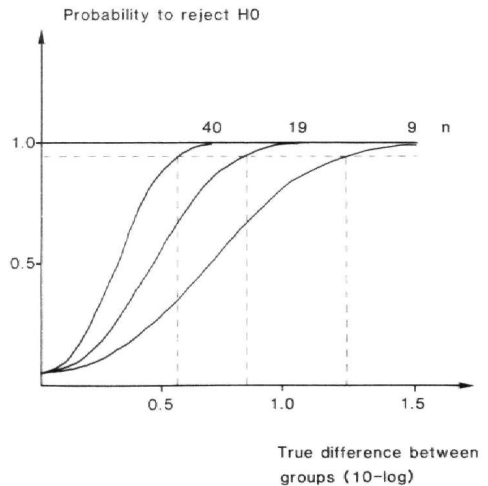


Fig. 2. Power function demonstrating probability for correct discrimination of differences in CPT sensitivity between two populations. The figure gives the probability for three different sample sizes (*n* = 40, *n* = 19 and *n* = 9). The differences are expressed in 10-log steps of the threshold concentrations giving positive CPT. The dotted lines indicate 95% probability to reject the hypothesis of no difference between the two groups.

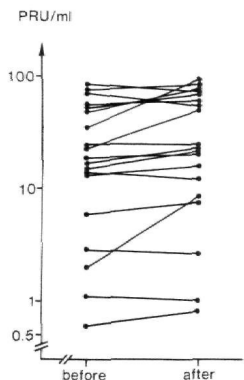


Fig. 3. Levels of IgE antibodies to birch pollen in 19 patients before and 1 week after four weekly CPTs.

prox. 0.5, i.e. half a 10-fold dilution step. Similarly, there is approx. 99% probability to detect a difference between two groups of 19 patients if the difference in CPT threshold concentration is 1.0.

Birch pollen-specific IgE in serum was examined in 19 cases before and after the four CPT. In 16 patients the value of the second determination deviated less than factor $10^{0.19}$ from the first (Fig. 3). The median specific IgE value for these patients was 18.5 PRU/ml (range 0.6–85) before the challenges and 22.0 PRU/ml (range 0.8–82) after the repeated CPT. In three patients the specific IgE level increased with factor $10^{0.33}$ – $10^{0.62}$. The values in these patients before CPT were 2.0, 35.5 and 22.8 PRU/ml and after 8.4, 92.5 and 49.0 PRU/ml respectively. The increased serum IgE was not associated with increased CPT sensitivity.

DISCUSSION

In all, we performed four CPT with 1-week intervals in each of 20 patients. One patient reacted once to the albumin diluent. He had no obvious infection and, as far as he knew, had not been exposed to any allergen to which he was sensitive. In an open study the test result would have been excluded as non-valid. This test result was therefore excluded in the statist-

ical analysis. The difference between extract concentrations giving positive challenge on two different occasions (i.e. comparing paired differences) was in 85% of the cases within factor $10^{0.5}$, in 92% within factor $10^{1.0}$ and in 98% within factor $10^{1.5}$. On two occasions there was a difference in threshold concentration between individual CPT of factor $10^{2.0}$. The explanation for the single outlying observations in the two patients remains unexplained. Possibly, host factors, e.g. a sub-clinical allergic reaction to another allergen, or the presence of an infection may explain these two CPT results. Technical factors could also be involved. However, the test extracts are stable in freeze-dried condition and, when reconstituted, in Albumin diluent® (7). Thus they contained the same allergenic activity throughout the study period. The variation in CPT results could therefore be explained by variations in test conditions.

The data permitted us to construct a power function by which it is possible to determine the number of patients needed to discriminate CPT sensitivity of a given magnitude between two populations, and also to determine whether a found difference in CPT sensitivity between two groups of patients is statistically significant or not.

Apart from a slight nasal secretion – possible tears – no patient had any allergic reaction from any organ other than the eyes in connection with the CPT. This demonstrates the safety of the test and is in accordance with other studies (6). There was no indication of altered sensitivity by repeated CPTs, i.e. the children were neither more nor less sensitive by the end of the test period.

The levels of specific IgE antibodies in serum usually increase during the pollen season, when the patient is exposed to the allergen (3). Similarly, IgE levels usually increase in the early phase of immunotherapy (3). It is not clear if this is an expression of increasing allergenic sensitivity or not. Our test procedure, in which the patients were exposed to the allergen on four occasions during 1 month, could also conceivably raise the specific IgE. However, this was not the rule, although there was a two-fold increase in three of 19 patients.

We conclude that CPT is useful in clinical research, e.g. for studies of altered reactivity during hyposensitization therapy. When clinically indicated, CPT is a safe and easy test with good precision.

REFERENCES

1. Aas, K.: The bronchial provocation test. Charles C. Thomas, Springfield, 1975.
2. Aas, K. & Belin, L.: Standardization of diagnostic work in allergy. *Acta Allergol.* 29, 237-240, 1974.
3. Foucard, T. & Johansson, S. G. O.: Allergen-specific IgE and IgG antibodies in pollen allergic children given immunotherapy for 2-6 years. *Clin. Allergy* 8, 249-257, 1978.
4. Mygind, N.: Nasal allergy. Blackwell Scientific Publications, pp. 185-192, 1979.
5. Peshkin, N. M.: A dry pollen ophthalmic test in pollen asthma and hay fever patients negative to cutaneous tests. *J. Allergy* 3, 20-29, 1932.
6. Tuft, L., Ettelsson, K. N., Gupta, K. & Kreuger, C.: Eye tests with inhalant allergens. *J. Allergy* 30, 492-505, 1959.
7. Zetterström, O., Öhman S., Nilsson, G. & Dreborg, S.: The stability of freeze-dried and reconstituted timothy pollen allergen extract and hymenoptera venoms, reconstituted in saline with and without albumin. *Allergy* 37, Suppl. 1:25, 1982.

Address:

Christian Möller

Barnkliniken

S-901 85 Umeå

Sweden

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