

Diagnosis of food allergy: tests *in vivo* and *in vitro*

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Introduction

Until recently the origin of atopic dermatitis, gastrointestinal problems, etc. induced by foods have been regarded among paediatricians to be mainly IgE-mediated. However, during recent years, it has been found that about half of children with cow's milk allergy have no cow's milk-specific IgE as measured by skin tests or s-IgE (1). Furthermore, Isolauri and Turjanmaa (2) found that about half of children with challenge-proven cow's milk allergy, mainly those with late reactions, in fact showed a positive atopy patch test (APT) and recently Niggemann published data suggesting that children with atopic dermatitis (AD) and positive DBPCFC with cow's milk show positive APT with cow's milk (3).

Thus, all food allergy in infancy is not IgE-mediated. However, in the early acute stages of food allergy in children less than 6 months, the clinical picture is not the same as that seen in older children and adults, but has an urticarial, acute appearance. My hypothesis is that AD starts with IgE sensitisation with mast cell degranulation as the basic mechanism, reflected by an acute appearance, and gradually converts into a T-cell-mediated disease, mediated by Langerhans cells coated with specific IgE, stimulating/boosting T-cells, combined with the chronic features of atopic dermatitis (AD). Some patients with late reactions to foods may sometimes when challenged also develop immediate reactions.

This paper will mainly discuss the use of skin tests and s-IgE in the diagnosis of IgE-mediated food allergy.

Tests for allergen specific IgE measure circulating IgE. It may be that in some cases IgE is produced locally and only found in the shock organ, e.g. the gut (4). Skin tests, on the other hand, show that IgE molecules are reacting with allergens present in the allergen mixture used for testing. There is a close correlation between titres of specific IgE present in the skin, conjunctiva (5) and bronchi (6), but there are no data on the relation between IgE-mediated skin test reactions and AD. In laboratories, methods such as determination of specific IgE are checked continuously, using duplicate tests, calculating the precision (CV), etc. There are methods available which, followed in detail, are why clinicians are guaranteed reproducible results.

The skin prick test (SPT) is a simple procedure, but for the use of the test it is of great importance to follow the same methodological rules as used in the laboratory (7). In most departments, the prick test is a speciality of the nurse and the doctor is not interested in how it is performed, there are no written instructions available but the test is performed 'as usual' or according to tradition and there are no protocols for checking the precision (CV) or otherwise supervising the testing to be able to correct methodology.

Skin tests

There is no indication to use other tests than the SPT in the diagnosis of IgE-mediated food allergy in infants and children. Intradermal skin tests are used in some areas to increase the sensitivity of skin tests. To obtain similar sensitivity using the SPT, the potency of the extract should be increased by 1000 times (8).

The skin prick test method

Allergen preparations. The allergens used in SPT should be standardised, i.e. their composition and potency should be known. The content of major allergen corresponds largely to the biological activity of allergen extracts (9). If not properly standardised, data obtained cannot be used, neither in scientific comparisons between (10) or within centres (11), nor in diagnostic trials (12, 13) nor in clinical practice. It is recommended to use standardised food allergen extracts with constant composition and known content of major allergen as measured by a given method for major allergen determination, taking into consideration the methodological problems involved (see below) (14).

Unfortunately, standardised food extracts are not available at present on the European market. To overcome this difficulty, we proposed the use of the prick-prick test method (15), which has later become a standard in many clinics interested in food allergy (16). Fresh or fresh frozen food should be used, since it is probably much more relevant with regard to both potency and composition (15, 17). Thus, fresh milk, egg white, etc. can be used (18), since these materials have a relatively well-known composition. Non-diluted cow's milk has higher sensitivity and a still high specificity for both SPT and APT than has cow's milk diluted 10 times (3).

We proposed to fill NUNC vials with a mix from several batches of fresh milk or egg white, mark the vials, and freeze a number of vials sufficient for 1 year's use. The variation in composition and potency probably varies very little between years, but must be kept under control. It has therefore been recommended to test a number of individuals with duplicate or quadruplicate prick-prick tests with the new and the old material in parallel, to obtain reasonable information on the difference in potency. By using controls and comparing the response in the control group, over time the control group will give valuable information (19).

It is also possible to use whole nuts, apples, etc. (15), probably with a wider range of potency and composition of allergens (20, 21). However, in a reported, but non-published trial, we found that

hazelnut sensitive children used as controls reacted to the same degree to two different hazelnuts used for prick-prick testing in two consecutive years (19).

One complicating factor that has not been investigated sufficiently is the presence of foreign protein, e.g. fish allergen, in milk and or hen's egg white.

Method

The best documented method for SPT is that of Østerballe and Weeke (22). According to that method, the lancet should be pressed through a drop of test solution, at 90° against the skin surface for 1 second (count 1001). The pressure should be as similar as possible between test occasions (23). The best feeling for pressure is on the volar surface of the fingertip (Fig. 1).

To supervise the precision continuously, duplicate tests with the same solution should be performed. However, when testing infants and young children it is difficult to do duplicate tests, since the testing personnel are afraid of causing the child pain. Therefore, there are seldom false negative tests in this age group (personal experience). When single tests are used, there will be false negative tests to a higher extent than in older children and adults.

Evaluation of skin prick tests

Fifteen minutes after the test has been performed, the contours should be encircled using a fine filter-tip pen or ball-point pen (23), drawing a fine line on the erythema, close to the weal edge without crossing the weal at any point (Fig. 2). The drawing should be transferred by means of a translucent tape to a record sheet. The size can be measured with a transparent ruler measuring the longest diameter and the midpoint orthogonal diameter and use the mean of these for further calculations. It is also possible to calculate the area of the weal, either with planimetry, which is time-consuming and less precise, or by means of a computerised scanning method (24, 25). One scanning programme, which can be recommended, has been developed by a Danish group and is available from the UCB Institute of Allergy (24, 26).

How to express a skin test response

Before there were any standardised extracts on the market, Aas and Belin (27) proposed to relate the allergen weal response to that of histamine 1 mg/ml (Fig. 3).

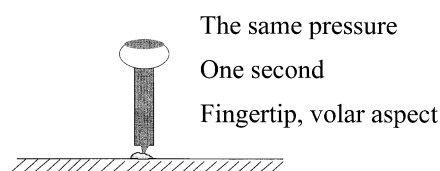


Fig. 1. The tip of the lancet with 1 mm tip should be pressed at 90° against the skin surface, applying the same pressure every time for 1 second (count 1001). The most effective feeling is on the volar surface of the finger tip (33).

The skin test response is best expressed by giving the mean weal diameter or the weal area. Results from provocation tests in other organs such as the eye, nose, bronchi or in food allergen challenges are expressed by the threshold concentration, i.e. the concentration eliciting a positive response, e.g. PC₂₀ or by the total amount of allergen or irritant eliciting a defined response, e.g. PD₂₀. Skin test results were, for many years, expressed as the endpoint, i.e. the concentration eliciting a positive response. However, three decades ago most paediatricians changed from the intradermal skin test to the less painful SPT. Most investigators also began to use one concentration of allergen in the routine, and therefore gave only the size of the weal, not the endpoint concentration. Furthermore, the endpoint of SPT is less precise than that of intradermal testing (28).

It is possible to calculate the endpoint concentration, i.e. the concentration eliciting a weal of the same size as that of the cut-off point, 3 mm mean weal diameter, or 7 mm² area. This method should be used to a higher degree in clinical trials, diagnostic trials, etc. By using the median slope (b) of the allergen dose-response relationship $^{10}\log D = a + b \times ^{10}\log(\text{conc})$ (29) (30) it is possible to use a simple formula for calculation of the endpoint concentration (30).

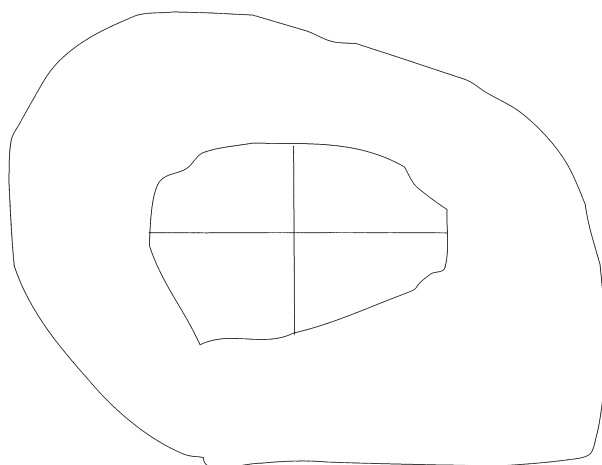


Fig. 2 The weal and surrounding erythema (darker grey). The longest and the midpoint orthogonal diameters should be measured, and the mean used for calculations (33).

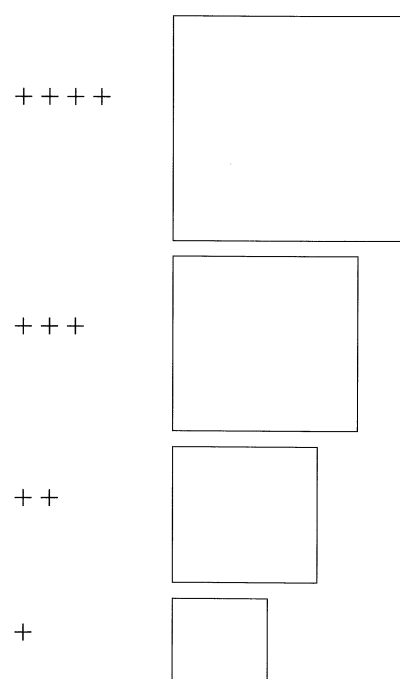


Fig. 3. In the +system according to Aas and Belin, a weal with an area similar to that of histamine is given +++, a weal with double the area a ++++ and one with half the area of the histamine reaction is designated ++ (27).

The slope of the allergen dose-response relationship in the log/log model is similar within the dose/sensitivity-range which is normally used. However, if a lin/log model is used, which is often the case in studies (11), then the slope differs within different parts of the curve and the curve flattens out at low response levels, i.e. the slope will be lower (7).

The dose-response relationship for allergen is very flat (b for area 0.38, for diameter 0.19) (29, 31, 32). By increasing the allergen concentration, the weal diameter increases with a factor of 1.5, i.e. from 2.0 to 3.0 to 4.5 to 6.8 mm in diameter when the allergen concentration is increased from 1 to 10 times, to 100 times and to 1000 times that of the first dose (7). The same difference in weal size between patients indicates a 1 to 10 to 100 to 1000 times difference in sensitivity (Fig. 4).

The problem is that standardised extracts of food allergens are not available. However, the methodology described can even be used with fresh/frozen food, giving the fresh material an arbitrary unit of, e.g. 1000 U.

Cut-off and background

When measuring weals, it is practically impossible to measure diameters less than 3 mm in diameter. In non-published trials, we found that even if we tried our best, the variation was too large to give an acceptable precision. Therefore, the EAACI posi-

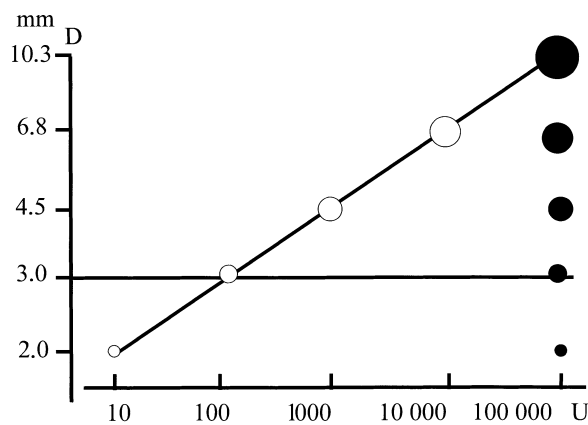


Fig. 4. The rings to the left indicate increasing weal responses to 10-fold increasing concentrations in the same individual. The dots to the right show the response in different individuals tested with the same concentration of allergen, which is most common nowadays, but with 10-fold interindividual increasing sensitivity .

tion papers recommend that 3 mm diameter is used as cut-off (25, 25, 33).

It is better to use the response in individuals not fulfilling the diagnostic criteria. Limits should be set to exclude these individuals from a positive diagnosis. Thus, Eigenmann and Sampson found the upper limit for the variation (95% percentile) to be different for different allergens, 3 mm for wheat and soy, but 4 mm for egg and 5 mm for milk (34), i.e. the difference in sensitivity in DBPCFC-negative children was at least 50-fold between these allergens. Unfortunately the group used non-standardised extracts, which is why the data can not be used in the same or other clinics or in future diagnostic clinical trials.

Precision

To check the precision of the personnel it is advised to perform 10 tests with histamine dihydrochloride 10 mg/mL on the volar surface of the forearm of at least five people and that the results are noted and the CV ($SD \times 100 / \text{mean}$) is calculated.

The CV should be $<20\%$ using the diameter, and for mathematical reasons $<40\%$ using the area of the weal. These figures have been obtained in adults (30, 32). Thus, still higher levels may be found in infants and small children, since the tester often hesitates to perform the test on a crying child. As a rule of thumb, a histamine weal with a mean diameter of 6 mm with a range of ± 1 mm, should be obtained (7).

In vitro IgE tests

The first *in vitro* tests for determination of specific IgE, developed by Wide et al. (35), used sepharose beads as solid phase. Later a number of different

materials for the solid phase and even soluble systems have been developed. All these systems have in common that there must be allergen with specific epitopes present, patient material, e.g. serum, with IgE specific for the allergen tested, and sufficient and suitable antibodies against human IgE. The better the system, the lower levels of IgE antibodies can be detected. The most reliable manufacturers have a product control system assuring that the binding is specific, i.e. that only IgE antibodies are measured and that IgE antibodies directed against other specificities than that of the allergen to be measured are not interfering. It is common to show that high levels (many thousands of kU/L) of non-specific IgE do not give positive results. Positivity can be defined as a level of IgE significantly higher than that of the background. Thus, all results above the background indicate the presence of IgE-antibodies specific to the tested allergen, or allergens with cross-reacting epitopes. However, IgE antibodies or positive SPT does not always indicate disease.

There is little documentation on the properties of these tests with regard to diagnosis of clinical food allergy. New tests are most often compared with the standard RAST test, which only shows whether or not they are able to detect as much IgE as the standard test (36). Also very common is to compare to skin test results, without giving enough data on the SPT method and the potency and composition of the allergens used, e.g. (37). These authors correctly mention that the lack of a gold standard is the major problem. However, there is a gold standard in food allergy diagnosis – the double-blind placebo-controlled food challenge (DBPCFC) (3, 38, 39). Details how to perform this test and the limitations of the DBPCFC will be discussed in other communications in this supplement (38, 40).

Evaluation of test results

Common diagnostic parameters

Most often the sensitivity and specificity of test results (41) are given. Less often the positive and negative predictive values of the test are mentioned. It is most important to understand the meaning of these measures of diagnostic properties (Fig. 5). In most cases the confidence intervals are not given, i.e. the authors do not show whether given data for different test procedures are significantly different, despite nomograms for such estimations being available since the 1930s (5, 13, 23, 42).

To illustrate results from diagnostic tests, ROC curves are often used. Furthermore, to illustrate

Disease	Test		
	+	-	
+	True pos	Fase neg	TP+FN
-	False pos	True neg	FP+TN
	TP+FP	FN+TN	TP+FN+FP+TN

Sensitivity = TP in % of diseased
 Specificity = TN in % of healthy
 Pos. predictive value = TP in % of test pos.
 Neg. predictive value = TN in % of test neg.

Fig. 5. Definition of sensitivity, specificity, positive and negative predictive values (41).

the diagnostic value of tests at different degrees of positivity, Gerhard and Keller developed a system that illustrates the diagnostic properties at different levels of positivity (43). Many find these diagrams difficult to understand, but they give valuable information (Fig. 6) (44), since not only a figure on the sensitivity and specificity (and other measures of diagnostic capacity), a feature common with the ROC curves, but also the numbers of individuals with positive and negative test results at different levels.

In all types of diagnostic tests, it is of great importance to choose the controls correctly. 'Normals' are seldom suitable. The best information on the diagnostic features of a test is obtained when those who have a suspicion to be diseased, but do not fulfil the diagnostic criteria, are used, i.e. mimic real life. The diseased in our case are those with proven clinical food allergy, i.e. those with a positive DBPCFC (open challenge in infants). The reference population should be those who do not fulfil the diagnostic

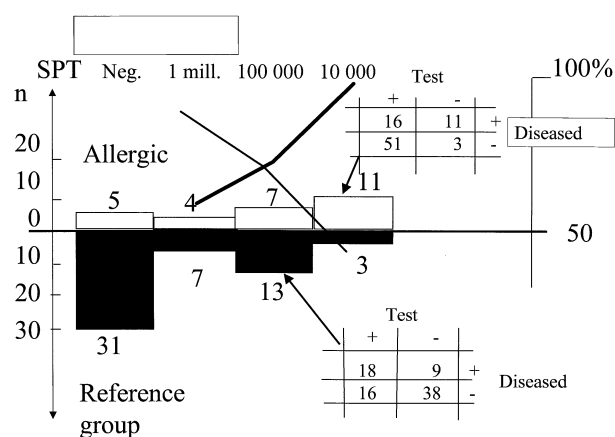


Fig. 6. Data presented according to Gerhardt and Keller (43). SPT performed with 10-fold dilutions of extract and disease defined as symptoms at exposure to dog and positive conjunctival provocation test (CPT). Above the horizontal line are cases fulfilling the diagnostic criteria, below those not fulfilling the criteria set up in the trial (44).

criteria, i.e. in our case children with possible food allergy but without positive oral food challenge. That is the clinical situation we are in when evaluating the diagnosis.

Likelihood ratios

Other parameters for defining the diagnostic properties of tests are the likelihood ratios (LR) (45). The LR+ is defined as sensitivity/(1-specificity) and the LR- as (1-sensitivity)/specificity, describe the change in odds favouring disease given a positive and a negative test result, respectively (45). The LR+ and LR- both refer to test results before the disease status is known, and are therefore more useful to clinicians evaluating patients. Similar to sensitivity and specificity, the confidence intervals can be calculated. Values of LR+ significantly more than 1 indicate that there is a significance for positive outcome of the test in diseased and the LR- and LR significantly <1 should be present for a correct diagnosis in healthy individuals.

Unfortunately, there are no data on LR+ and LR- within the area of food allergy diagnosis.

Prognostic diagnosis

IgE-mediated tests are also of value for estimating the risk of future IgE-mediated diseases. Thus, Hattevig et al. found a positive specific IgE at 8 months of age to predict the development of atopic disease during childhood (3, 46). These results have been confirmed recently (47). Furthermore, s-IgE towards grass and mite and/or cat allergen at 18 months of age in a child with atopic dermatitis is a risk factor for the development of asthma within the next 18 months, according to recent data from the ETAC study (48).

Conclusion

Tests for allergen-specific IgE only allow evaluation of children/infants with possible IgE-mediated allergy. As mentioned, about half of the cow's milk allergic infants in a cohort do not have IgE-mediated but cell-mediated disease, probably best diagnosed by APT. However, until further evidence is established, DBPCFC/open oral challenges should be performed in these cases to confirm the diagnosis.

The IgE-tests are of value, especially at an early stage of the disease, for diagnosing IgE-mediated allergy.

In vitro tests for IgE diagnose IgE sensitisation, whereas skin tests diagnose IgE-mediated mast

cell release. IgE sensitisation is always time limited. In diagnostic trials, sensitivity and specificity data should be accompanied by confidence intervals and likelihood ratios should be utilised regularly.

Last but not least, positive IgE-tests in infancy and early childhood indicate future risk of developing IgE-mediated diseases.

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