

Diagnosis of food allergy: the oral provocation test

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Introduction

Diagnosing a food allergy is like working on a jigsaw puzzle, in which the oral challenge test is the key piece. Oral challenges are currently the mainstay in the diagnosis of a food reaction, i.e. in establishing a cause–effect relationship between the ingestion of a given food (or food additive) and the reported reaction (1, 28). Despite improvements in our knowledge of the role of IgE and cellular interactions in the last 20 years, no *in vitro* or *in vivo* laboratory test is diagnostic for food allergy.

Skin tests are characterised by a high negative predictive accuracy and a low positive predictive accuracy (2). Together with the patient's history, they help the physician to select the food (s) to challenge. Exceptions are due to the potency of the extract (3), the type of allergen and the age of the patient (4). IgE-mediated sensitivity to certain fruits or vegetables (apple, orange, banana) frequently go undetected, because of lability of the allergen concerned (5) and children younger than 2 or 3 years are more likely than older children to have a negative skin test and a positive challenge (4, 6).

The *in vitro* measurement of specific IgE was hampered in the past by the same pitfalls as the skin tests, i.e. specificity and negative predictive accuracy were high, while sensitivity and positive predictive accuracy were low (2). Sampson et al. recently tried to establish cut-off levels in order to predict which patients will react to food challenges. They used CAP-FEIA with optimal decision point analysis and receiver operating characteristic (ROC) curves (7).

In the analysis of more than 100 children, they established cut-off levels for egg, peanut, milk and fish, which were associated with more than a 95% probability of reaction to *Double Blind Placebo Controlled Food Challenge (DBPCFC)*. If confirmed, these results will enable the identification of patients most likely to react. This study was unable, however, to identify a cut-off level with a negative predictive accuracy of at least 90%, so there is still a significant risk of reaction even when the IgE dosage is below 0.35 KuA/L. Oral challenges are still crucial in evaluating this new approach.

The positive outcome of an oral challenge does not identify the pathogenic mechanism involved, it merely detects the offending food or foods.

In food-mediated gastrointestinal disorders, circulating IgE are rarely detected (8, 9) and non-IgE-mediated reactions are often involved. The oral food challenge is, at present, the least invasive procedure enabling the diagnosis of a food reaction in food gastroenteropathies.

Food challenge

Oral provocation consists mainly in the administration of a suspect food (s) and the assessment of the reaction. The apparent simplicity of this approach clashes with three main problems:

- the emotional value of food for human beings;
- the lack of symptoms pathognomonic for food allergies; and
- the lack of laboratory parameters for an objective clinical observation of any reaction.

People often associate food with 'allergic' reactions, to such a point that two out of three US housewives believe they suffer from an adverse reaction to some foods, and increasing numbers of school children have dietary restrictions because of reported food allergies (10). The tricky symptoms of food allergy often mingle with those of other disorders and this overlap often causes misdiagnoses.

Although some laboratory tests, e.g. ECP and plasma histamine dosage, eosinophils and neutrophils count, intestinal permeability tests have been proposed to make the clinical observations more objective, no single test or association of tests has yet been validated sufficiently to confirm the positive outcome of an oral challenge (11, 12). Food challenges may be open, single-blind (when only the patient is unaware of what food is ingested) or double-blind (when patient, physician and nurses are all blinded) (1). The accuracy of a food challenge depends on our ability to take into account all the confounding variables, the most important of which is represented by patient and observer bias. Blinded challenges have been found more accurate, reducing errors to a minimum not only in research, but also in daily practice, and confirming only 50% of the positive open challenges (13). The double-blind food challenge is now largely accepted as the best test available to reproduce and confirm food reactions.

Challenge procedure

The basic procedure is the same for all kinds of challenge (1). A detailed history usually helps the challenge to be designed appropriately to reproduce the conditions of the reaction. Since the aim of a food challenge is to determine whether a given food is responsible for a certain reaction, the challenge takes place when the patient is symptom-free or, more frequently, when a stable baseline condition is achieved, so that the symptoms elicited will be as unequivocal as possible. The diagnostic procedure must therefore begin with a diet that eliminates the suspected food, or with a diet containing a limited variety of foods, for 7–14 days or more for chronic diseases, such as atopic dermatitis, or up to 3 months for gastroenteropathies. Symptomatic therapy can be associated if required.

Once any symptoms have regressed completely, or by at least 50%, the patient can be challenged.

For the challenge to serve its purpose it is essential to record a description of any symptoms, the approximate quantity of food needed to produce them, the frequency and reproducibility of the reaction, the most recent occurrence of the

reaction, and the role of any 'effect modifiers' (e.g. exercise). The food challenge must be tailored to each patient, carefully considering the following crucial points (2):

- the timing of the onset of symptoms;
- the quantity of food to use to begin the challenge;
- intervals at which the challenge food should be administered;
- the type of food;
- the length of the observation period.

Challenges usually begin early in the morning, in a fasting state. Antihistamine should be withheld long enough to allow for positive skin prick tests for histamine, bronchodilators, cromolyn and inhaled steroids to be performed 6–12 hours before the challenge. The starting dose should be about half of the minimum quantity estimated by patients to have produced the symptoms: range 25–500 mg. Titrated amounts should be administered and the dose can be doubled at intervals slightly longer than the one reportedly needed for the onset of symptoms, usually 15–30 minutes (30–60, if capsules are used). Doubling of the dose should continue until symptoms have been elicited or until 8–10 g of dried food or 60–100 g of wet food have been administered in a single dose. It has been suggested that a total amount approximating an age-related average daily intake has to be reached. Raw food and the "usual" (or "normal") means of administration are the best choice but the food intake must be quantified in order to control the dosage exactly (1, 18, 19).

In blinded challenges, dehydrated foods are preferred because they are easier to conceal or encapsulate, but freeze-drying or cooking could sometimes alter their allergenic potential. This situation is more common in the oral allergy syndrome elicited by some fruits or vegetables (5), but there are reports of reactions to raw egg following negative challenges with cooked egg, or meat allergies with reactions to raw meat but not to lyophilised meat (14, 15).

In the blind challenge, the means of administration depends on the food concerned and the quantity to administer. Capsules are the best method for blinding, but have their drawbacks: they bypass the mouth; they cannot be used for large amounts of food; they should not be used in cases of anaphylaxis; they are not well tolerated by children, especially the younger ones (although they can be dissolved in a liquid medium).

It is thanks to the imagination of numerous colleagues that, in 20 years of blind challenges, so many vehicles have been developed to suit a patient's age, the type and amount of food involved, and the type of reaction (Table 1). In

the blind challenge, the essential difference lies in the use of a placebo. In theory, this is a meal that is identical in flavour, colour and consistency to the one containing the allergen. The vehicle itself could be used as the placebo, particularly in paediatric age, providing its volume is the same as the one used in the active arm of the challenge. When capsules are used, dextrose capsules can make a good placebo.

Open challenge

The open challenge consists in administering the 'normal', unmasked, food. It has a greater potential for bias, but could be useful *to refute the patient's claims* when the skin test is negative, the history confused and the patient has been excluding the food. Open challenges can be used for a *preliminary screening* at home of foods on a low level of suspicion (by medical history, skin test and/or *in vitro* specific IgE dosage) in a patient with numerous suspected foods, or as part of a programme to restore a regular diet after an elimination diet and before testing a highly suspect food (16).

Open challenges with a negative outcome have a predictive accuracy similar to DBPCFC. Positive outcomes must be evaluated carefully and should be confirmed by a blind challenge, particularly in children with multiple food allergies, to avoid any unjustified dietary restrictions (17). Open challenges can replace DBPCFC in *infants* providing they are performed in 'controlled' settings under the supervision of trained personnel to ensure a standardised clinical monitoring (symptom scoring) and well-documented food reactions (12).

Other problems must be considered when infants are involved. The appearance of small weals due to contact urticaria may tempt the physician to suspend the challenge, although proceeding with the oral intake may not necessarily

trigger systemic symptoms. In addition, children may reject a food because it has a 'strange' taste and their resistance can even lead to vomiting for emotional reasons. In such cases the use of a vehicle, as in blinded challenges, can be helpful (18).

Blinded challenges

When should the placebo portion of the challenge be administered?

In all cases for research purposes and in many clinical food challenges the placebo should be administered to confirm the outcome of a positive open challenge, or when symptoms are complex (chronic disease) or varied and functional (vomiting, abdominal pain or colic). The aim is to reach a final, conclusive diagnosis of food allergy with no misinterpretation on the part of the physician, patient and parents. *Single-blind* trials can be useful in paediatric clinical practice to eliminate parental bias, or in research to select patients for the DBPCFC.

In the DBPCFC the procedure is the same as for an open challenge, except that two coded portions are given with an interval between them of several hours or days of observation. Placebo and food challenge are administered in equal number and in the same amount. The staff preparing the challenge material must be different from the physicians or nurses administering the challenge. The challenge material is prepared in incremental doses as prescribed, suitably labelled and recorded. Contamination between different foods should be avoided by keeping the phases of the challenge separate and using different or disposable kitchen utensils (18). The need to mimic a 'natural' exposure and the difficulty of masking adequate quantities of some foods (e.g. fish, peanut) makes it mandatory to run an open challenge with the raw food in normal age-related quantities after each negative blind challenge. DBPCFC has an excellent diagnostic accuracy, as judged by false negative and false positive results.

Bock and Sampson, respectively, report in their patients an incidence of 1.8% (6) and 4.6% (13) false negatives and of 0.5%–0.9% false positives. *False negatives* are mainly due to dose–response mechanisms, i.e. the allergen dose administered is too low to elicit the reaction obtainable with the larger amounts used in an open challenge. This dose–response mechanism is particularly evident when patients are acquiring a tolerance to a given food. In such cases, the challenge should be repeated at higher dosage. The DBPCFC can therefore be used to determine the threshold of reactivity to foods (19) that are tolerated in small

Table 1 Vehicles that have been used in the double-blind, placebo-controlled food challenge (25)

Capsules
Infant formula
Apple sauce
Neocate
Chocolate pudding
Tapioca fruit mixture
Grape juice
Hamburger
Tuna fish
Ice cream (grape)
Popsicles
Lentil soup
Mashed potato
Cereal

amounts by children who are 'growing out of' their food hypersensitivity.

Other causes of false negative result could arise either from the loss of the allergenic potential due to cooking or processing (5) or from the patient's strong conviction (18). Another indication for the DBPCFC is to evaluate the clinical importance of *cross-reactions* found at the *in-vitro* dosage of food-specific IgE. Several clinical studies evaluating cross-reactions between botanical and animal species have shown that, in most cases, the individual reacts to a single food and tolerates the others, even though they belong to the same family (18–20). It is therefore essential to obtain a certain diagnosis to avoid excluding whole categories of foods from the child's diet, with the consequent risk of malnutrition and/or scarce compliance with dietary restrictions (21).

Since 1980, the use of DBPCFC as a 'gold standard' has led to a common terminology, improving communications between researchers and practitioners, leading to a better classification of clinical phenotypes, preventing the misdiagnosis of the causes of reactions and inappropriate medical therapy.

Safety measures

The main drawback of the oral challenge is the risk of triggering severe reactions, and even anaphylactic shock. Staff trained to deal with anaphylactic reactions and emergency equipment have to be readily available. There are currently no laboratory tests for earmarking patients at greatest risk of anaphylactic reaction to a challenge, so an intravenous access is established before performing the challenge.

Recently, Niggeman and co-workers attempted to combine several indicators for a better prediction of severe clinical reactivity to food challenges (24). According to their findings, a patient's history of allergy to cow's milk, wheat or soy was a reliable indicator of the need for medical support, irrespective of the level of specific IgE, while a level of specific IgE ≥ 17.50 KUA for cow's milk and wheat and an history of allergy to egg associated with a level of specific IgE ≥ 3.50 prompts the need for parenteral medication.

How long should the observation period be?

The timing of clinical observation depends on the symptoms described and the type of reaction suspected.

For immediate-onset symptoms, the observation period could be 2 hours after each challenge

dose and the whole challenge can be carried out in a day (one dose in the morning, one in the afternoon). This approach, although saving time, presents the disadvantage of overlooking slow or delayed-onset reactions so it would be better to distribute the challenge over 2 days. It was suggested that the observation period should be of 24 hours if an IgE-mediated immediate reaction is suspected, of 48 hours in case of an IgE-mediated late reaction, in particular for chronic disease such as atopic eczema. Therefore the challenge should take 2 or 3 days at least (12). Sometimes reactions take longer to occur and two patterns have been described (25):

- a single dose of food elicits symptoms hours or days after the challenge;
- multiple doses of food are needed over a period of days to trigger the symptoms.

These situations are more likely in gastrointestinal disorders, e.g. allergic eosinophilic gastroenteritis or in recently reported 'very delayed' gastrointestinal cow's milk reactions (26). In such cases, it is essential to use a placebo and to repeat the cross-over with the challenge two or three times to rule out any bias, especially if the patient is monitored at home.

When to avoid the challenge?

The challenge should be avoided in cases of certain anaphylactic reaction to a given food. In such cases it must only be performed if the culprit food has not been clearly identified or in children after a period of exclusion diet to ascertain whether the child has recovered.

Several recommendations have emerged recently from the literature for the selection of candidates for the challenge. Sampson and co-workers showed that reactive patients are those with food-specific IgE levels in excess of the 95% positive predictive value, thus reducing the need for oral challenges for certain foods, e.g. egg, milk, fish and peanut by 50% (7). If cut-off levels can be established for other foods, more children could probably postpone their challenge.

Conclusions

The challenge protocols initially used for studying immediate IgE-mediated reactions have gradually been modified to adapt them for the assessment of delayed immunological reactions, be they IgE-mediated or not. So different protocols can be used, depending on the symptoms reported and the type of reaction suspected.

The variety of symptoms and reaction times, especially for gastroenteropathies in children, explain the problems encountered in attempting a standardisation of the procedure. A first step might be to identify patient groups with similar clinical features and immunological reactions and thus standardise the challenge for different patient categories. A proposed protocol has been developed, essentially for patients with IgE-mediated reactions (especially atopic dermatitis (12)). For gastroenteropathies and 'unusual' symptoms, in which reactions may be delayed by days or even weeks (27), no unequivocal protocol has been established as yet, in terms of the amount of food to administer and the observation times, so an accurate description of the challenge procedure is still essential in such cases (25).

Objections concerning the laborious and time-consuming nature of performing a challenge are countered by the opportunity to achieve a certain diagnosis avoiding misinterpretations and the consequent use of diets or medical treatments administered without a proper rationale. The future trend will be to optimise clinical observation with laboratory parameters, thus reducing the need to administer a placebo.

For the time being, oral provocation tests are essential (28):

- to obtain an unequivocal diagnosis, identifying the offending food (s) and excluding any clinical cross reactions, thus avoiding the exclusion of too many foods;
- to establish any persistence or loss of reactivity;
- to compare new diagnostic procedures against a 'gold standard'; and
- to evaluate the effect of new treatments for food allergies.

Golden jubilee

Over the last 20 years, thanks to the use of oral challenges, and double-blind placebo-controlled food challenges in particular, food allergies have emerged from the region of empiricism and scepticism (29). The real prevalence of food allergies, the role of specific foods in different age groups, the symptom patterns at different ages (6) and the relevant associations with different reactions (i.e. oral allergy syndrome and immediate IgE-mediated reaction (5), atopic dermatitis and immediate and late IgE reactions (13), gastrointestinal syndromes disorders and non-IgE mediated reactions (27)) have become clear.

Experience and confidence with the use of oral challenging procedures are of the utmost importance for any paediatric allergist. Fifty years after

the first blinded challenges (30), the DBPCFC is still the reference test for diagnosing food allergies and is used to evaluate progress with laboratory tests and new treatments.

The gold standard celebrates its golden jubilee.

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