

## EDITORIAL

# *In vitro* tests in the diagnosis of food allergy

The perception that food triggers a large variety of symptoms creates an increasing pressure on the health care system to investigate possible causal relationships between food intake and disease. Questionnaire studies have clearly demonstrated this perception in diseases with a well-known relation to allergy such as asthma, where up to 60% of patients have tried to modify their diet [1]. Also, in other disorders such as chronic inflammatory bowel disease, there exists a clear perception among 65% of the patients that intake of certain foods are related to the activity of their disease [2]. Even in the general population up to 15–20% report having adverse reactions to food [2,3]. In sharp contrast with this, widespread general belief the prevalence of food hypersensitivity as diagnosed by objective methods such as the double-blinded placebo-controlled food challenge (DBPCFC) is much lower, and restricted to a more limited number of conditions [4]. In a large cross-sectional survey where 20.4% of the respondents reported suffering from food intolerance, less than 2% actually produced symptoms when subjected to DBPCFC [3]. The European Academy of Allergy and Clinical Immunology (EAACI) has stated in a recently published position paper [5] that adverse reactions to food are almost exclusively expressed by 'classical' allergic symptoms such as anaphylaxis, urticaria, asthma, rhinitis, the oral allergy syndrome, and gastro-intestinal symptoms.

In a situation where doctors are confronted with a high number of candidates for food hypersensitivity reactions it is tempting to look for other objective diagnostic tools than the DBPCFC. Especially if the suspicion is not confined to a single food item, the resources for challenge testing may be overwhelming, and much time and money would be saved if a single blood sample could be conclusively screened for reactivity to a large number of foods by an *in vitro* allergy test<sup>1</sup>. In this issue of *Clinical and Experimental Allergy* Michael Jenkins and Andrew Vickers report a study of a combined IgE and IgG4 specific antibody test to 95 different food items. Nine months after its introduction in the clinic the test was subjected to a small reproducibility study which demonstrated that 30–45% of the results could not be reproduced by a different test of the same blood sample [6]. If the clinical actions in form of a diet are based exclusively on the test results the patients may find themselves deprived of various food items on an almost random basis. This sad story of serious flaws in a test is far from isolated, but do we have any *in vitro* tests that can be used for a meaningful diagnosis?

A fundamental but sometimes overlooked requirement

for the development and validation of a diagnostic test is a definition of the relevant disease entity. If the food–disease relationship cannot be recognized by independent methods, it is impossible to investigate the value of a diagnostic test. Since disorders such as multiple chemical sensitivity, chronic fatigue syndrome, rheumatoid arthritis and psychological disturbances have not been convincingly associated with food intake [4]<sup>2</sup>, it is not worthwhile even to consider the use of *in vitro* tests for replacement of DBPCFCs in such situations. Secondly, if any *in vitro* tests should be used for a defined disorder, three basic conditions should be fulfilled (a more extensive discussion is given in [7]):

- 1 the test should measure the new quantity with sufficient specificity and precision, i.e. a technical validation must be performed;
- 2 abnormal values of the new quantity should be associated with clinical disease, i.e. a clinical validation must be performed including the definition of age- and sex-matched normal values (reference intervals); and
- 3 other physiological or pathological conditions should be identified which may produce responses outside the reference interval.

When sufficient information is available to meet these three conditions, data will often be expressed in both qualitative and quantitative form but the latter should be encouraged. For example, the technical validation may be expressed by the degree of interference from other analytes and by the accuracy and reproducibility. Basic parameters for the clinical validation are the clinical sensitivity and specificity [7].

Technical validation of the test is the responsibility of the manufacturer rather than the end user, it could be argued. The article by Jenkins and Vickers clearly demonstrates, however, that it may be worthwhile to perform at least a

<sup>1</sup>An allergy test is understood to be a test aiming at identifying one or more agents to which a patient may react with allergic or allergy-like symptoms and where an immunologically hyperresponsiveness has been induced. The underlying nature of the immunological disease process is not restricted to an IgE response, and need not be clearly defined. This definition does not include *ex vivo* investigations where mediators, cells or cellular functions are measured subsequent to natural or experimental *in vivo* exposure of the patient to allergens. Not included either are: *in vitro* tests where cellular functions are investigated without challenge or upon a challenge of a non allergen specific nature, such as determination of CD4 or CD8 T lymphocytes, or cellular stimulation by cytokines or complement products, etc.

<sup>2</sup> Manuscript in preparation: EAACI adverse reactions to food subcommittee position paper on controversial aspects of adverse reactions to food by Ortolani C, Bruijnzeel-Koomen C, Bengtsson U, Bindslev-Jensen C, Bjorksten B, Høst A, Ispano M, Jarish R, Madsen C, Nekam K, Paganelli R, Poulsen LK, and Wuthrich B.

smaller part of the technical validation such as a reproducibility study [6]. Chances are that if a test is analytically out of control it will show up as poor reproducibility. Regarding the information provided by the manufacturer one should carefully scrutinize the available documentation. There are examples of 'FDA [Food and Drug Administration] approved tests', where the FDA approval was related either to the allergen extracts *per se* or was related to a test design used for quite different purposes (e.g. ELISA for determination of specific antibodies). Even when a test has moved into the daily clinical practice, monitoring of the quality should not be neglected. Routine quality control programmes are now becoming increasingly used for specific IgE tests both on national and international levels [8], and it is conceivable that an increased effort will take place for IgE tests in the future.

Only when the first condition has been met, the clinical validation can take place. A clinical trial must be conducted where the DBPCFC outcome is used as the true diagnosis. Performing retrospective studies using sera or other samples from patients that have previously been diagnosed by challenge, is tempting. This is indeed possible, but due to the natural history of the disease, development of clinical tolerance may occur — especially in children — and it is essential to use serum samples that have been drawn close to the time of the diagnosis. Use of an appropriate control group is also important. Healthy individuals may not be the optimal control since they would not be likely candidates for testing in the daily clinical practice. Rather it is recommended to use a group of patients suspected of food allergy but where the suspicion of an adverse reaction to a particular food item has been excluded by challenges. Unfortunately, the large amount of food items and thus potential allergens makes the task of fully validating a test for any allergen almost impossible. Allergens may be very different and great care should be taken in transferring the result from one allergen system to another, even if the foods in question seem to be closely botanically or zoologically related. The fulfilment of the second condition greatly reduces the number of tests available for the diagnosis of food allergy. At present only measurements of specific IgE and leucocyte histamine release for allergens such as milk, egg, cod, cereals, hazelnut, apple and kiwi fruit have been investigated in patients subjected to DBPCFC (reviewed in [7]).

The third and final condition is the identification of other disease states which may give abnormal, i.e. positive, test results, and this may further reduce our armamentarium of *in vitro* diagnostic tests. Cross-reactions between an inhalant and a food allergen or between two food allergens may be of serological but not of clinical importance [9]. A majority of grass-allergic patients express IgE-serological but not clinical positivity to cereals [10] and the same may be true for peanuts [9] [11]. For various species of fish,

however, there seems to be a shared clinical and serological cross-reactivity [12]. As discussed by Jenkins and Vickers, the case for the use of IgE/IgG4 as a diagnostic tool in food allergy is quite weak, among other things due to the substantial levels of food-specific IgG4 in all subjects that have eaten the food in question [6,13].

Thus, it seems that we have a long way to go, before we can make good use of *in vitro* tests in replacing challenges for the diagnosis of adverse reactions to food. *In vitro* tests may be used from different perspectives, however. The *in vitro* test may be used for the selection of food items for challenge. If a case history is not clearly suggestive of a single food, a screening, e.g. of the various ingredients in a pizza, may be of great help in selecting which foods that should be included in a challenge programme. However, it is important to be aware of the widely occurring cross-reactivities between various inhalant and food allergens to avoid unnecessary challenge procedures. Another use of the tests are for classification: after a clinical reactivity to a food has been determined by DBPCFC, our differentiation between IgE-mediated food allergies and other forms of food hypersensitivity relies with the demonstration of specific IgE antibodies either suggested by a positive skin test or histamine release test, or directly measured by serological test [14]. One argument is that the mere demonstration of specific IgE in a food hyperreactive person does not imply an absolute proof of the mechanism behind the disease. Our present knowledge of the pathophysiology of the disease, however, clearly points to classical type 1 IgE-mediated reaction in the majority of the clinically proven (i.e. DBPCFC positive) patients that have been described in the literature.

The great focus on the relation between food and health has made the diagnosis of food allergy and related diseases a cause of great concern and controversy, both in the daily clinical practice and in academic quarters. Much of this controversy stems from the limited amount of scientifically sound knowledge in the area, including quite a few publications on validation of diagnostic tests for food hyperreactivity according to the principles described above [15]. A publication bias could possibly exist for negative results of test validations, and since dissemination of technically unsound *in vitro* tests may have profound impact on both the quality and the costs of the allergy diagnosis, contributors and editors of scientific journals should be encouraged to publish independent and well-performed technical and clinical validations of new test systems.

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L. K. POULSEN  
*Laboratory of Medical Allergology*  
*Allergy Unit 7542*  
*National University Hospital*  
*Tagensvej 20*  
*DK-2200 Copenhagen*  
*Denmark*