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REVIEW ARTICLE

Advances in Food Allergy Diagnosis

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Abstract: An accurate diagnosis of food allergy is extremely important to guide safe and yet not overly restrictive dietary management. The cornerstone of the diagnosis of food allergy is the clinical history; it allows appropriate selection of the allergens to be tested and interpretation of the results of allergy tests, namely Skin Prick Test (SPT), Specific IgE (sIgE) to allergen extracts and, more recently, specific IgE to allergen components and the Basophil Activation Test (BAT). SPT and sIgE to allergen extracts are very sensitive methods to detect IgE sensitization to a specific food and assess the possibility of spontaneous resolution. Cut-offs have been generated based on the probability of clinical reactivity during oral food challenges and can improve the specificity of SPT and sIgE, helping to confirm the diagnosis of food allergy. Specific IgE to allergen components refines food allergy diagnosis as it allows differentiating species-specific from cross-reactive allergens, aiding the differential diagnosis between a true and potentially severe food allergy from pollen-food syndrome or clinically irrelevant sensitization. The BAT is a new diagnostic test which has high specificity and sensitivity and can complement specific IgE, allowing the deferral of OFC in patients with a positive BAT. Depending on the likelihood of clinical allergy determined based on the combination of the history and the results of allergy tests, an oral food challenge may be indicated to confirm or exclude the diagnosis. Oral food challenge is the gold standard for the diagnosis of food allergy, but is a resource-intensive procedure with some level of risk involved; thus they are reserved for the equivocal cases. This review article discusses the above diagnostic techniques detailing the methods, utility, advantages and disadvantages.

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1. INTRODUCTION

An accurate diagnosis of food allergy is extremely important. Food allergy is a common paediatric condition. Its prevalence has been estimated to range between 1 to 10 % of the general population [1] and is increasing in parallel with the increase in the prevalence of other allergic conditions [2, 3]. The highest prevalence of challenge-proven food allergy has been reported in Australia, where 9% and 3% of infants from the Healthnuts study were allergic to egg and to peanut, respectively [4]. The prevalence of food allergy varies with age, being more common in infants and young children. Some children may "outgrow" their food allergy but, for others, it may be persistent. Cow's milk, egg, soya and wheat allergies tend to be transient whereas allergy to peanut, tree nuts, fish and shellfish tend to be more persistent. There is currently no curative treatment for food allergy and management relies on food allergen avoidance and emergency medication, including self-injectable adrenaline in the severe cases. Food allergy can result in life-threatening reactions and diminish patients' quality of life, with the constant threat of food allergic reactions placing significant dietary and social restrictions on these patients. It is, therefore, crucially important that the diagnosis of food allergy is made correctly to prevent unnecessary dietary restrictions, which unsupervised can lead to malnutrition and failure to thrive and to keep patients safe by identifying the culprit allergen and thereby aiding the prevention of accidental allergic reactions.

The US National Institute of Allergy and Infectious Diseases (NIAID) defines food allergy as an "adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food" [5]. Food intolerances (*e.g.* lactose intolerance and other metabolic, pharma-

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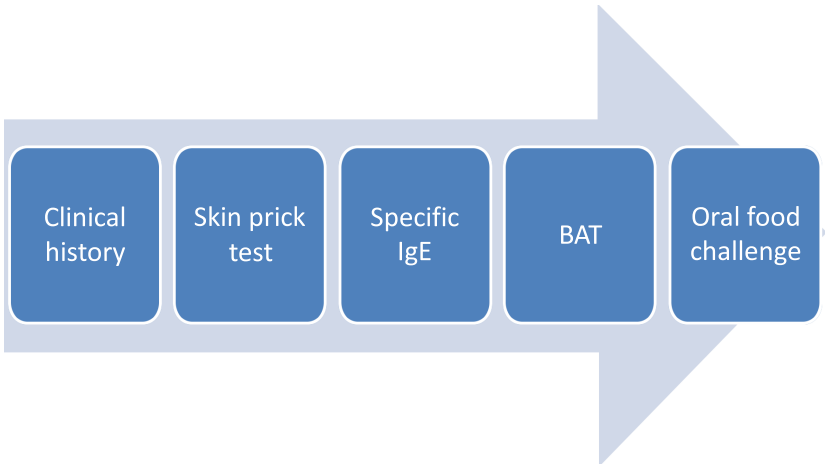

Fig. (1). Diagnostic approach to IgE-mediated food allergy. BAT, basophil activation test.

cologic or toxic conditions) are not considered allergic conditions because they are not immune-mediated. Food allergies can be IgE-mediated or non-IgE mediated, depending on the involvement of IgE antibodies in the pathogenesis. This review will focus on the diagnosis of IgE-mediated food allergy, which is characterized by the acute onset of symptoms (generally within 2 hours of ingesting or being exposed to the trigger food) and can vary from a mild reaction (*e.g.* localized urticaria or oral pruritus) to severe reactions, namely life-threatening anaphylaxis (Fig. **1**).

The diagnosis of IgE-mediated food allergy is enabled by a combination of the clinical history and the results of allergy tests, with Oral Food Challenge (OFC) being the gold-standard [4]. The clinical history is the most important piece of information for an accurate diagnosis of food allergy; however, it is not sufficient to confirm the diagnosis of food allergy as the Positive Predictive Value (PPV) of the history alone is about 50% [6]. This may be due to reported symptoms not being related to the food, being caused by another food or not being immune-mediated. A review from 2010 showed that the self-reported food allergies to cow's milk, hen's egg, peanut, fish, and shellfish were about 12% in children and 13 % in adults [3]. However, the rate of self-reported reactions exceeds that of proven IgE-mediated food allergy.

The diagnosis of immediate-type food allergies requires evidence of allergen-specific IgE, which can be obtained using Skin Prick Test (SPT) or determining allergen-specific IgE (sIgE) levels in the serum. SPT and specific IgE usually give comparable results, assuming use of the same or very similar allergen source [7, 8]; however, since the blood test measures sIgE in the serum and the SPT reflects IgE bound to cutaneous mast cells, their results may not always correlate. Serum sIgE and SPT have high sensitivity to detect IgE sensitization [9]. Sensitization alone is also not sufficient to define food allergy, as the majority of sensitized children do not have a food allergy. For example, only about 1 in 5 school-age children in the UK who are sensitized to peanut have a peanut allergy [10]. In the previously mentioned Healthnuts study, 17% of infants were sensitized to egg, 9% to peanut and 2.5% to sesame, whereas only 9%, 3% and 0.7% had challenge-proven egg, peanut and sesame allergies, respectively [4]. Thus, the diagnosis of IgE-mediated food allergy requires both a history of developing allergic symptoms on exposure to the specific food and IgE sensitization.

In the case of patients with a clear history of an immediate-type reaction to a specific food, the presence of IgE sensitization confirms the diagnosis of IgE-mediated food allergy. However, in the cases where patients do not have any history of oral exposure to the food, either of developing typical allergic symptoms or of being able to eat age-appropriate amounts of the food without developing any symptoms, the results of IgE sensitization tests can be more difficult to interpret. There are also cases where the results of SPT and/or specific IgE are discordant with the clinical history. Whenever it is not possible to conclude whether a patient is allergic or not to a specific food, based on the clinical history and IgE sensitization tests, the patient should be offered a physician-supervised OFC. Double-blind Placebo-Controlled Food Challenge (DBPCFC) is the "gold standard" for the diagnosis of IgE-mediated food allergy; however, open OFC is often suitable for clinical purposes [11]. OFCs are resource-heavy and high-risk as they have the potential to induce acute and potentially severe allergic reactions. In order to improve the diagnostic accuracy and reduce the number of patients that need to undergo an OFC, novel methods to test for food allergy have emerged in the recent years, namely IgE testing to individual allergens (or allergen components) and the basophil activation test.

2. CLINICAL HISTORY

A thorough clinical history is the most important step in the establishment of a diagnosis of food allergy. The clinical history allows to establish a pre-test probability of clinical allergy (which will then be taken into account to determine the post-test probability of clinical allergy), to interpret the results of IgE sensitization tests (*i.e.* to determine the post-test probability and ultimately the clinical relevance of a given allergy test result) and to select the allergens to be tested. A judicious use of IgE sensitization tests is critical to avoid false-positive results and increase the chances of obtaining true-positive and true-negative results that corroborate the clinical history [12].

Important pieces of information to collect as part of the history are: characterization of the food-induced allergic reactions (*e.g.* symptoms, time of onset, the circumstances

immediately before the onset of symptoms), age of onset, detailed dietary history, history of any previous food elimination and therapeutic interventions, co-morbidities and family history of atopy.

Food allergies can have a wide range of manifestations; cutaneous, gastrointestinal, respiratory, cardiovascular and anaphylaxis (Table **1**). As well as focussing on the possible causal food, the history should gather information regarding the form in which the allergen was ingested (raw, loosely-cooked, cooked, or baked) and in what quantity and over what time course [13, 14]. The chronology of symptoms after eating the food is an essential part of the clinical history. Most immediate food allergic reactions occur within 30 minutes of exposure to the allergen, provided that the threshold dose has been met [15]. They can, however, develop within a few minutes or up to 2 hours following exposure to the culprit allergens. Delayed allergic reactions can also occur, in particular with red meat ingestion in patients sensitized to oligosaccharide galactose-alpha-1,3-galactose (alpha-gal) which may follow sensitization by tick bites [16]. The reproducibility of the reactions helps guide the probability of the causative food and the time frame since the last reaction is important when thinking about whether an allergy may have been outgrown. A food allergic reaction more frequently develops following ingestion but also can occur through inhalation of aerosolized allergens or direct contact with the skin [17].

Table 1. Symptoms and signs of IgE mediated food allergy.

System	Clinical Features
Cutaneous	Urticaria, angioedema, pruritis, erythema
Ophthalmologic	Conjunctivitis
Ear, Nose and Throat	Rhinitis, sneezing, itchy mouth and throat
Gastrointestinal	Nausea, vomiting, abdominal pain, diarrhoea
Respiratory / airway (anaphylaxis)	Wheeze, chest tightness, dyspnoea, cough, hoarse voice, swollen tongue
Cardiovascular (anaphylaxis)	Blood pressure drop (pallor and floppiness in a child), cardiovascular shock

It is important to ask about possible co-factors, such as exercise, menstruation, stress, Non-steroidal Anti-inflammatory Drugs (NSAIDs) or alcohol, which are known to enhance an allergic reaction to food [18]. If exercise is the only trigger for an anaphylactic episode, then it is defined as Exercise-induced Anaphylaxis (EIA). Analysis of the anaphylaxis registry of German-speaking countries revealed that 18% of anaphylactic episodes in children and adolescents were associated with exercise [18, 19]. There may be other associated co-factors such as medication intake and illness. If exercise alone is tolerated but when combined with a particular food (before or soon after exercise) [20], anaphylaxis is triggered, then it is known as food-dependent exercise-induced anaphylaxis (FDEIA) [19, 20]. Often, the food alone is tolerated when not associated with exercise. Wheat is the most common food allergen to be involved in FDEIA, although others have been reported, such as shellfish and nuts [21]. The implicated food varies depending on the geographical origin of the patient. Wheat and shellfish are common triggers in the Japanese population [22, 23] tomato, nut as well as wheat [24, 25]. Although alcohol can play a role as a co-factor in adolescents, symptoms due to alcohol could also be due to an allergy to barley or to grapes, sensitivity to vasoactive amines or caused by an enzymatic congenital deficiency of alcohol dehydrogenase. Food additives may also be co-factors in anaphylaxis [13, 14].

Atopic co-morbidities and family history of atopy should be obtained, as the likelihood of food allergy is higher in children with early-onset moderate to severe eczema and in children with parents or siblings with food allergy and in [13, 26]. Children who suffer simultaneously from asthma and food allergy are more prone to have food anaphylaxis [27] as well as uncontrolled and more severe asthma [28].

The physical examination should focus on children's growth and development, with a nutritional status assessment. Excluding foods that contain essential nutrients in the context of food allergy may lead to poor growth and stunting in children. Furthermore, the number of foods avoided is associated with changes in anthropometric parameters, namely low weight and height for age in these children. [29] Hence, the intervention of a dietician, particularly in children with multiple food allergies is required.

3. *IN VIVO* TESTS

3.1. Skin Prick Test

SPT is a simple and safe method of testing for IgE sensitization to a specific food allergen. The procedure of SPT consists of putting drops of commercial extracts of food on skin, which is then pierced with a small lancet, allowing the allergen to come into contact with the skin mast cells. Positive and negative controls, consisting of 10 mg/ml of histamine and saline respectively, are placed for comparison. A positive reaction is shown by the typical "wheal and flare" reaction, which results from the erythema, pruritus and oedema that develop within 10 to 20 minutes. The SPT result is given by the wheal diameter. A wheal diameter of 3mm or greater than the negative control is often arbitrarily considered a positive SPT result [5, 30].

Considering the result of SPT positive if greater or equal than 3 mm, SPT has high sensitivity but low specificity. Compared with OFC, SPTs have low specificity and Positive Predictive Value (PPV) for making an initial diagnosis of food allergy [5]. Thus, the use of SPTs alone, without taking into consideration the clinical history, may lead to false-positive results and to over-diagnosis of food allergy. On the other hand, SPTs have high sensitivity and high negative predictive values (NPV > 90%) [6] and thus, a negative SPT is useful to exclude food allergy. Negative SPTs occasionally occur in patients with IgE-mediated food allergy. Therefore, in cases where the history is highly suggestive of food

allergy and SPTs is negative, further evaluation with other allergy tests and eventually OFC is necessary before ruling out the diagnosis of food allergy.

As the likelihood of being allergic increases as wheal diameter of SPT increases [31, 32] diagnostic decision levels have been defined in different studies for the common allergens, usually using a cut-off with a 95-100% PPV (Table **2**), and can increase the specificity of SPT [17, 33, 34]. For example, for peanut allergy, an 8 mm weal on SPT has a 95% probability of clinical allergy and about 98.5% specificity in the UK and in the US, making SPT more useful to confirm the diagnosis of food allergy [33, 35]; however, the majority of patients tested have SPT results below these cut-offs [36]. In any case, the results need to be interpreted, considering the clinical information obtained in the history, in terms of likelihood of clinical food allergy.

There can be variability in skin reactivity and hence in the measurement and interpretation of the reaction depending on age, time of the day, gender, menstruation and the site on the body where SPT is performed, the examiner's technique and the SPT device and reagents used [7, 17, 37]. Studies looking specifically at histamine reactivity in both atopic and non-atopic subjects show that the size of the SPT to histamine is lowest under 4 years and then increases significantly until 15 to 20 years. Beyond 50 years, there is then a decline in reactivity until a plateau at 60 years [38]. Infants under 24 months have been shown to have smaller SPT reactions to both histamine, food and aero-allergens but they can still be interpreted accurately with a clinical history [39]. 95% Positive Predictive Values (PPVs) are obtained at lower measurements in children under 2 years [40]. Previously, some studies had suggested that results of SPTs can vary depending on the time of day, however, a more recent study showed that there was no statistically significant difference between the mean morning wheal and flare responses and those from the evening [41]. The timing in the menstrual cycle has been shown to have an effect on skin reactivity. One study demonstrated an increase in SPT size for histamine and morphine in both atopic and non-atopic women, as well as an increase in an allergen in the atopic group, on days 12 to 16, which is when oestrogen levels peak [42]. Males have been shown to have increased skin reactivity to histamine [43]. The most common locations to perform skin prick testing are the volar aspects of the forearms and the back. However, the forearm shows less reactivity to histamine and allergen when compared to SPTs on the back [44]. It may even be possible to see variation in reactivity between different areas tested on the back. One study noticed a significant gradient of reaction size on the back, with the middle third of the back being more reactive than either the top or bottom thirds [45]. In one study the reactivity of the forearm was affected by the handedness of the patient and their relatives [46]. There is the potential for significant user variability with SPT. Even when performed according to a standardised technique by a trained person, it can be difficult to reproduce the test result. Within the same panel of tests for one patient the variability may be as high as +/- 30% [47]. One study revealed a large variability in prick test inoculum volume with an Interquartile Range (IQR) of 416 to 82253 pico-litres [48]. There are several different types of equipment for pricking the skin. In 1979, a new, single-use lancet with a 1.0mm length point was trialled and shown to give reproducible results when pressed through a drop of extract at 90 degrees against the skin surface for 1 second [49]. Multi-test applicators have been shown to reduce reproducibility when repeated at weekly intervals compared to single test devices such as lancets [47]. Another study comparing 5 commercial SPT devices concluded that they differ significantly in the size of reactions they induce, rates of false negatives, the size of the negative control and the patient discomfort level [45]. Ideally, criteria for positive and negative tests need to be established for each available device [50]. Health-care professionals should be trained on the correct use of the device used by their department [51]. The allergens used must be standardised with regard to their composition and strength [52].

Skin prick testing has multiple advantages: it is safe, cheap, immediate and the result is easy to understand by patients and families. SPTs are contraindicated in patients with extended dermatitis or dermographism and those on anti-H1 antihistamines. Because it is a form of *in vivo* testing, attention should be paid to the possibility of causing systemic reactions [53] and emergency medications should be available to treat such reactions. In these situations, fur-

Table 2. Predictive value of cut-offs for SPT weal diameter and sIgE level for a positive OFC [32-34, 63, 101] *PPV=86%.

Food	95-100% PPV	
	SPT (mm)	Specific IgE (KU/L)
Egg white	≥ 7	≥7
Cow's milk	≥8	≥15
Peanut	≥8	≥14
Sesame	≥8	≥7
Fish		≥20
Soybean		≥65*
Wheat		≥100

ther testing, namely for sIgE, should be performed. Histamine is the key mediator in the development of the wheal and flare. Therefore, it follows that anti-histamine medication will have an effect on skin reactivity. It takes approximately 3 days for skin reactivity to return to baseline after chlorphenamine [54]. Studies have shown that cetirizine can have a rapid effect on suppressing skin reactivity, with one study showing that over 40% of patients had inhibited wheal responses within 90 min of a single dose of cetirizine [55]. This effect increased and was persistent at 24 hours. Regular long-acting antihistamine has a more sustained inhibition of wheal and flare which is still significant at day 3 after stopping the medication, compared to placebo [56]. Clinically, patients are advised to stop taking long-acting antihistamines 5 to 7 days and short-acting antihistamine 48 hours prior to skin prick testing. Although plasma clearance of long-acting anti-histamine may be 24 hours, the medication remains in the extravascular space for longer and continues to reduce extravasation, hence the prolonged effect beyond one day [55]. Oral steroids do not appear to suppress skin reactivity and can be continued [57].

The American College of Allergy, Asthma and Immunology state in their Practice Parameter for Food Allergy in 2006 that "intracutaneous (intradermal) skin tests for foods are potentially dangerous, overly sensitive (increasing the rate of a false-positive test result), and not recommended. Intracutaneous allergy skin tests with food extracts give an unacceptably high false-positive rate, can elicit systemic reactions (rarely an issue for prick tests), and should not be used."

3.2. Prick-to-prick Tests

When using commercial extracts, false-negative reactions can occur, either due to the absence of the relevant allergens or due to the instability of allergenic proteins [26]. Using fresh foods for SPT can obviate this problem. In a French study of 430 children with a history of immediate hypersensitivity reactions to food, 81.3% had positive tests with a prick to prick testing compared with 40% of those patients to the commercial extract. The results of SPT to fresh foods also reflected better the outcomes of OFC. Prick-to-prick testing or modified SPT is performed by first pricking the fresh food followed by the pricking of skin. In order for modified SPT to be possible when the fruits are not in season, fresh fruits can be frozen and subsequently reused for skin testing with the ''fresh'' fruit [31]. There may be variability in the allergenic quality of the food used for testing. Hence, the degree of skin test reactivity can depend on the cultivar of the fruit or vegetable, the part of the food that is pricked (*e.g*. lipid-transfer proteins are usually located in the peel of the fruits and nuts), on how ripe it is, on the way it is stored prior to its use, and on processing. The risk of systemic allergic reactions is increased with prick-to-prick testing [58].

Modified SPT is indicated in patients with suspected IgE-mediated allergy to fruits and vegetables (*e.g*. apple, banana, pear, melon, carrot, celery) and in patients with pollen-food syndromes, who are primarily sensitized to pollen allergens and later on start developing mild allergic symptoms to related fruits and vegetables. In this situation, the responsible allergen is frequently labile and not present in commercial extracts. Skin prick testing with fresh tree nuts may also be helpful to identify patients who are reactive to oil based proteins (oleosins) which are often absent in aqueous extracts. Oleosins have been described in peanut, hazelnut [59] and sesame. Modified SPT using sesame paste (or tahini) is more sensitive to diagnose sesame seed allergy compared with commercially available allergen extracts [31, 60]. Prick-to-prick testing can also be useful for patients with suspected wheat allergy. The PPV of SPT and sIgE to wheat is less than 75%, particularly in adults due to cross-reactivity with grass pollens. Most commercial extracts have a low sensitivity as they are mixtures of water- and salt-soluble wheat proteins, hence lacking the gliadin fraction, which is insoluble. Performing prick-to-prick testing using wheat flour partially overcomes this problem, although in many cases an OFC is necessary for the final diagnosis of wheat allergy [60].

4. *IN VITRO* TESTS

4.1. Specific IgE to Whole Extracts

Serum sIgE test is another means of detecting allergen-specific IgE antibodies using fluorescent labelled antibody assays to detect the presence of circulating IgE to a suspected food allergen. sIgE levels were originally measured using the Radioallergosorbent Test (RAST), but the term RAST should be abandoned since this test has been replaced by more sensitive fluorescence enzyme-labelled assays. It is important to outline that there are different laboratory methodologies (Phadia ImmunoCAP, Agilent Turbo-MP, Siemens Immulite 2000) available for sIgE measurement and their results may not be comparable. Therefore, the predictive values associated with clinical evidence of allergy may differ according to the method used [61].

Serum sIgE measurement is useful to detect allergic sensitization, but sIgE alone cannot be considered diagnostic of food allergy. In fact, many children with positive tests have no clinical symptoms when exposed to the allergen [5]. Foods selected for testing should be based on the medical history and epidemiology of food allergens. Testing to large panels or multiple allergens without considering the patient's history should be avoided because false-positive test results can lead to unnecessary elimination diets [62]. Serum testing can be particularly convenient when SPTs are contraindicated if there is a discrepancy between the history and the results of SPT (*e.g.* history is suggestive of anaphylaxis and yet SPT is negative) or to confirm the results of SPT when considering referring for an OFC. The disadvantages of using sIgE include the need to obtain blood samples, delayed results and higher cost.

As with SPT, higher sIgE levels are more likely to be associated with clinical reactivity and diagnostic decision levels have been established for common food allergens, such as peanut, egg, milk, fish, soy, and wheat (Table **2**) [32]. These cut-offs are used to determine a probability of clinical allergy that will support the decision as to whether an OFC is warranted or when advising patients about the likelihood of clinical reactivity to the suspected food allergen [63]. Whereas 95% PPV cut-offs are usually used to confirm the diagnosis of food allergy, 50% NPV cut-offs are usually used to decide when to perform an OFC, as such NPV would mean a less than 50% chance that the patient would react

during the OFC [62]. A significant decrease (*e.g.* more than 50% in the last year) is also indicative that the patient may be outgrowing the food allergy [64]. The predictive value of sIgE levels varies across patient populations and might be related to the patient's age, ethnicity, time since last ingestion of the suspected food allergen, and coexistent atopic disorders, such as atopic eczema [32] – these aspects have been recently reviewed [65].

4.2. Specific IgE to Single Components

Specific IgE to single allergens (or components) is a recent advance in testing for food allergy and is known as a Component-Resolved Diagnosis (CRD). While sIgE traditionally uses extracts derived from the whole food, CRD focuses on single allergenic molecules within that food. CRD uses purified allergen proteins, produced by purification from natural allergen sources or obtained from recombinant expression of allergen-encoding cDNA. Some of these proteins are species-specific, while others occur in multiple allergen sources (cross-reactive components). Testing for specific IgE to components is available individually (*e.g.* using ImmunoCap®, Thermofisher) or with multiple allergen components simultaneously (*e.g.* using Immuno Solid-phase Allergen Chip (ISAC) allergen microarray testing, Thermofisher). The names of the allergens are given by the first 3 letters of the genus, the first letter of the species and a number that usually reflects the order by which they were identified and designated a food allergen. For example, Ara h 2 is the second allergen described in peanut whose scientific name is *Arachis hypogaea*.

Component testing enables the identification of specific IgE against not just the major allergens in protein structures but also the "panallergens" or minor allergens. Currently, there are just a few panallergen families including profilins, procalcins, non-specific lipid transfer proteins and Bet v 1 homologues (Bet v 1 is a major allergen in birch pollen). Use of component testing against these panallergens and major allergens can help differentiate species-specific from cross-reactive allergens (Table **3**), determine the risk of a severe reaction, guide the decision around OFC and there is some evidence that it can provide information regarding the potential for the allergy to resolve.

With regard to differentiating species-specific from cross-reactive allergens, this is useful in patients with allergic rhinitis and suspected Pollen-Food Syndrome (PFS). PFS occurs when a patient sensitized to pollen (*e.g.* birch or grass pollen [66]) ingests a plant food such as fresh fruits, nuts or vegetables that contain cross-reacting allergens (most commonly Bet v 1 homologues) triggering localized oropharyngeal symptoms [67]. These allergens are labile which means that acid or heat can change their structure such that the cooked plant food is usually tolerated and that a systemic reaction to the raw food is less likely due to proteolysis in the stomach acid [68]. PFS is also known as Oral Allergy Syndrome (OAS). Profilin is very ubiquitous in all plants and therefore sensitization to profilin can result in reactions to many pollens and foods, usually with the presentation of PFS, but in rare circumstances, systemic reactions have been reported [69]. Cross-reactive Carbohydrate Determinants (CCD) are sugars in plants that cross-react with glycoproteins in plants and invertebrates. Some patients are sensitized to these CCDs but they should not be advised to avoid the cross-reactive foods to which they tested positive because CCD sensitization rarely causes symptoms. This cross-reactivity seen in PFS might contribute to food allergy over-diagnosis because the traditional tests such as SPT and sIgE are often positive but not necessarily clinically relevant if the positive test is due to panallergen or CCD sensitization. Sensitization to cross-reactive pollen components should be suspected when allergy tests to several foods of plant origin are positive, especially if along with a history of pollen allergy.

Seed-storage proteins are generally good markers for systemic reactions to nuts. For instance, Ara h 2 is considered a genuine marker of peanut allergy [70] and, together with Ara h 6 (both 2S albumins) is considered the best predictor of severe peanut allergy [71]. Cor a 9 and Cor a 14 are the best predictors of hazelnut allergy. On the contrary, Ara h 8 and Cor a 1, which are Bet v 1-homolog allergens, indicate tolerance or OAS to peanut and hazelnut, respectively [72]. Sensitization to LTP proteins (*e.g.* Pru p 3, Cor an 8 and Ara h 9) in, Southern European countries is associated with systemic reactions (*e.g.* to peach, hazelnut and peanut, respectively) [73-75]. In Food-Dependent Exercise-induced Anaphylaxis (FDEIA) with wheat as the causative food, omega-5 gliadin is the specific allergen responsible for the reaction and specific IgE to this component should be requested if FDEIA is suspected [32].

CRD is considered to have a role in predicting the likelihood of resolution of food allergies such as milk and egg. In egg, several components can be tested for but the most useful one is ovomucoid (Gal d 1), which can assist in the diagnosis of persistent egg allergy and allergy to baked egg. However, it is not superior to egg white sIgE for the diagnosis of egg allergy. Ovomucoid (Gal d 1) is heat and protease-stable and can elicit reactions at low levels. A study in Japan, showed that children who had prolonged egg allergy had higher ovomucoid IgE levels compared to those children who outgrew their egg allergy before 3 years of age [76] suggesting that raised ovomucoid may be indicative of a more prolonged course of egg allergy. Ovomucoid has also been used to predict the likelihood of a more severe reaction to heated egg [77, 78]. In milk allergy, casein (Bos d 8) is described as being the allergen most predictive of reacting to baked milk and possibly of persistent cow's milk allergy [79, 80].

4.3. Basophil Activation Test

The Basophil Activation Test (BAT) is a novel test for food allergy with high diagnostic accuracy, that is progressively moving from the research laboratory to clinical practice [81]. BAT is a functional assay which assesses whether basophils degranulate when stimulated with the allergen. It has, therefore, the potential to resemble more closely the clinical phenotype of patients than tests that merely quantify the levels of allergen-specific IgE. BAT can be potentially be seen as an OFC *in vitro*, where basophils involved in acute allergic reactions are exposed to the culprit food extract in a test tube [82]. The BAT is a test based on flow cytometry where the expression of activation markers are measured on the surface of allergen-stimulated basophils. Determination

of basophils activation by flow cytometry was first described with CD63. Since then, CD203c and a variety of other activation markers have been described. CD63 is a membrane protein localized in the basophils' secretory lysosomal granules (the same that contain histamine) and is a marker of degranulation. Its translocation to the cell membrane is mediated by allergenic activation of basophils and can be measured by flow cytometry. CD203c is a constitutive and specific basophil marker and can consequently be used as a single identification marker [82]. The basophil activation requires about 1 ml of fresh blood and needs to be processed within a few hours of blood collection. The procedure consists of stimulation with allergen or controls, staining with fluorochrome-conjugated antibodies and red cell lysis followed by flow cytometry [82].

BAT has been studied in the diagnosis of a variety of food allergies and its reported sensitivity ranges from 77 to 98%, and the specificity from 75 to 100% (Table **4**), having higher accuracy than SPT and sIgE [81-91]. In a recent study regarding peanut allergy, BAT showed 100% specificity, suggesting that in patients with a positive BAT peanut allergy could be diagnosed with a high degree of certainty and the OFC could be deferred [92]. Furthermore, BAT has better performance in discriminating between peanut allergic and tolerant patients compared to other diagnostic tests, even in cases where SPT and sIgE measurements are equivocal [92]. BAT can be performed with whole extracts or single allergens, which, depending on the food in question, can be more informative than using extracts [85, 93, 94]. BAT can also be used to diagnose PFS [90, 95, 96], allergy to red meat [16] and FDEIA [97].

In some studies, BAT has shown to be informative in identifying patients with more severe peanut allergy with BAT reactivity reflecting the severity and BAT sensitivity reflecting the threshold of the allergic reactions during the OFC [81, 98]. Basophil reactivity has also been shown to distinguish different phenotypes of milk and egg allergy, namely patients who tolerate extensively heated forms of cow's milk and egg while still reacting to unprocessed cow's milk and egg from patients who react to all forms [99]. As patients who react to extensively heated milk or egg tend to have more persistent food allergy this discrimination using BAT could have prognostic implications. Hence, BAT may be helpful in the evaluation of natural resolution of transient food allergies and to decide when the food can safely be reintroduced into the diet. Finally, BAT has also been in the monitoring of clinical response to immunomodulatory treatment of food allergy in research studies [81].

Table 3. Allergen components useful to diagnose food allergy.

Foods	Components Associated with Clinical Allergic Reactions
Peanut	Ara h 1, Ara h 2, Ara h 3 Ara h 9*
Hazelnut	Cor a 9, Cor 14 Cor a 8*
Cashew Pistachio	Ana o 3
Brazil nut	Ber e 1
Walnut	Jug r 1, Jug r 3
Soya	Gly m 5, Gly m 6, Gly m 8
Wheat	Tri a 19
Cow's milk	Casein**
Egg	Ovomucoid**

*In Southern Europe.
**For baked and persistent milk/egg allergies.

Table 4. The basophil activation test has high specificity and sensitivity to diagnose food allergy.

Food Allergen Extract or Component	Sensitivity	Specificity
Cow's milk extract [88]	89%	83%
Casein [88]	67%	71%
Ovalbumin [86]	77%	100%
Egg white extract [88]	74%	62%
Ovomucoid [88]	80%	73%
Wheat extract [89]	86%	58%
nTri a 19 [89]	86%	58%
rTri a 19 [89]	83%	63%
Peanut extract [92]	98%	96%
Ara h 2 [84]	92%	77%
Hazelnut extract [87]	100%	97%
Peach extract [94]	87%	69%
Pru p 3 [94]	77%	97%

5. ORAL FOOD CHALLENGES

Controlled OFCs are considered the gold standard for the diagnosis of food allergy and patients with equivocal results of SPT, specific IgE or BAT (if available), should be offered an OFC to ultimately confirm or exclude the diagnosis of food allergy. Any OFC involves some level of risk to the patient and it is important to consider the potential severity of a reaction before undertaking the OFC. Medical history is of utmost importance, along with appropriate *in vivo* or *in vitro* tests, when deciding on whether to submit the patient to an OFC since it is used to estimate the probability of allergy and the culprit food(s) involved. In some circumstances, an OFC cannot be avoided in order to make a final diagnosis. Children with a history of adverse reaction to a food should undergo OFC in the following instances: to establish or exclude food allergy diagnosis, to determine the threshold value of sensitivity to a food, to assess tolerance in transitory food allergies such as cow's milk or hen's egg allergies and finally, for research purposes such as clinical trials. Patients

without a specific history of adverse reaction to a food should undergo OFC as well if sensitization to a food is diagnosed and tolerance is unknown.

Exclusion criteria for OFC may include a recent history of anaphylaxis to the specific food to be tested, test results over the 95% PPV cut-offs, acute infections, unstable angina pectoris, chronic, unstable atopic disease, pregnancy and use of medication which may mask, enhance or impair the treatment of a reaction. These drugs include antihistamines, neuroleptics, NSAIDs, ACE-inhibitors, oral steroids (above 5 mg per day) and beta-blockers. Inhaled $beta_2$-agonists, and topical steroids might be continued if kept at a fixed level, since interrupting these medications may jeopardize the interpretation of the outcome of the challenge.

The OFC can be Double-blind Placebo-controlled Food Challenge (DBPCFC) or open food challenge. DBPCFC is the most accurate test for diagnosing food allergy. However, due to the costs and time-consuming character of DBPCFCs, single-blind and open-food challenges are more frequently used in clinical practice. DBPCFC is the recommended method when studying subjective symptoms (including abdominal pain, oral pruritis, migraine or joint complaints) or when investigating late reactions or chronic symptoms (atopic dermatitis, isolated digestive reactions or chronic urticaria). DBPCFC is also used when clinical history and the results of diagnostic tests are conflicting and in research protocols. Open challenges are usually the first approach in clinical practice, particularly when IgE-mediated acute reactions with objective signs are suspected and when the probability of a negative outcome is high. They are also appropriate in young children with immediate-type reactions who are less likely to have psychological reactions and in pollen-related oral allergy syndrome patients since it is difficult to blind fruits and vegetables and maintain their allergenicity [100, 101].

Different dosage schedules are available for performing OFCs which differ in starting dose, incremental scale, the time between doses, and top dose. Schedules also differ depending on the protein content of the food, for instance, fish has a high protein content whereas celery has a low protein content and therefore different amounts would be necessary in order to provide an adequate quantity of food-specific protein to the patient. Generally, beginning the OFC with a starting dose at the low milligram level is safe and results in less severe reactions when compared to higher doses. An interval of at least 15 to 20 minutes between doses is recommended in order to lower the probability of high doses accumulation which may result in severe reactions. The top dose required in order to avoid false negative DBPCFC is not established but appears to be at least 2 g [11].

Oral food challenges start with a low dose, which is supposed to be lower than the threshold dose capable of inducing a reaction. The dose is steadily increased while monitoring for allergic symptoms, until a final dose, which corresponds to a standard portion according to the child's age, is reached [11]. OFCs must be performed by an experienced clinical team in an appropriate supervised environment with the training and resources to treat any reactions including anaphylaxis. The reaction and the dose that provoked the reaction should be well-documented. In a study with 125 children with atopic dermatitis under different food restrictions, 89% of the 364 OFC performed were negative, allowing significant dietary expansion. Thus, challenge testing can help avoiding inappropriate dietary restrictions. It is also important to emphasise that a sufficient quantity of the tested food be must ingested during the OFC in order ensure the safety of subsequent ingestion at home. A positive reaction after passing an OFC could theoretically be due to the fact that the steady escalating food doses during the challenge might lead to transient desensitization [31].

CONCLUSION

There are several new and exciting tests being used in food allergy diagnosis including sIgE to individual allergen components and BAT. However, the clinical history is still the most valuable piece of information to reach an accurate diagnosis of food allergy. *In vitro* and *in vivo* tests (such as SPT, specific IgE and BAT) can be used, together with the clinical history, to reduce the need for resource-intensive, potentially high-risk OFC. The role of a clinician with training and experience in Allergy cannot be overemphasised in order to interpret the results of these allergy tests and to decide the need for OFC.

LIST OF ABBREVIATIONS

BAT	=	Basophil Activation Test
CCD	=	Cross-reactive Carbohydrate Determinants
CRD	=	Component-resolved Diagnosis
DBPCFC	=	Double-blind Placebo-controlled Food Challenge
EIA	=	Exercise Induced Anaphylaxis
FDEIA	=	Food-dependent Exercise-induced Anaphylaxis
ISAC	=	Immuno Solid Phase Allergen Chip
LTP	=	Lipid Transfer Proteins
NPV	=	Negative Predictive Value
NSAIDs	=	Non-steroidal Anti-inflammatory Drugs
OAS	=	Oral Allergy Syndrome
OFC	=	Oral Food Challenge
PFAS	=	Pollen-Food Allergy Syndrome
PPV	=	Positive Predictive Value
PR-10	=	Pathogenesis-Related Proteins
RAST	=	Radioallergosorbent Test
sIgE	=	Specific IgE
SPT	=	Skin Prick Test

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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