

IgE Tests: *In Vitro* Diagnosis

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KEY CONCEPTS

- The presence of food allergen-specific IgE determines the sensitization to a specific food. Sensitization can but may not result in clinical reactions.
- The level of food allergen-specific IgE correlates with the likelihood of clinical reactivity for several foods such as peanut, cow's milk, hen's egg, and fish. Therefore, quantitative measurement of food allergen-specific IgE can be used to obtain diagnostic decision points that help to reduce the requirement of an oral food challenge test. However, these diagnostic decision points are population, age, and disease specific.
- For some food allergens, such as wheat, measurement of specific IgE to individual food allergens and not crude allergen extracts appears to be superior.
- Food allergy is a dynamic process with the majority of children becoming tolerant over time; monitoring food allergen-specific IgE seems helpful in predicting the likelihood of oral tolerance development.
- Measurement of specific IgE antibodies to allergenic peptides seems to enable the prediction of the natural course of the disease.

Introduction

The majority of food allergic reactions are IgE mediated [1]. Therefore, in addition to patient history, the diagnostic work-up of suspected food allergy should include the detection of IgE antibody responses *in vivo* and/or *in vitro*. However, the presence of food allergen-specific IgE does not always correlate with clinical reactivity. Generally, *in vitro* methods have the advantage of being safe, and drug interference (e.g. antihistamines) does not play a role. In the past several years, technological advances have provided new laboratory tools for the quantitation of allergen-specific IgE antibodies in serum [2]. Today, automated and quantitative allergen-specific IgE assays are available and open to improved diagnostic methods. In addition to the measurement of IgE antibodies to crude food allergen extracts, detailed analyses of sensitization profiles to individual allergens in individual patients are possible. This concept has been defined as "Component Resolved Diagnostics" [3]. Moreover, microarray techniques have been adapted in which crude or purified native and recombinant allergens, as well as allergenic peptides, are spotted onto surface-modified glass slides to permit extensive panels of specific IgE measurements to be performed

on small quantities of patient serum. Although the double-blind, placebo-controlled food challenge (DBPCFC) still remains the gold standard in food allergy diagnosis, the procedure is time consuming, costly, and places a great deal of stress on the patient. Therefore, *in vitro* tests for prediction of the outcome of oral challenge tests and the persistence of the food allergic disorders are under development. The latter is especially important to a few children who would benefit from immunotherapy when it is available in the future.

Diagnostic tests for the quantitative measurement of food allergen-specific IgE

Over the past decades, the methods of IgE detection have improved drastically. From the first-generation IgE antibody assays that were only semi-quantitative and labor-intensive to today's quantitative and automated allergen-specific IgE assays, there are several assay methods on the market for the detection of food allergen-specific IgE [2]. Generally, these assays use a liquid or solid phase to capture the allergenic component. Commonly, these allergenic components are allergen extracts of a complex nature and contain allergenic and non-allergenic molecules. Two of the most commonly used FDA-approved third-generation systems are the ImmunoCAP System (Phadia; Uppsala, Sweden) and the Immulite System (Simens Medical Solutions Diagnostics, Tarryton, NY, USA). Generally, the different systems offer

different units that are not comparable with one another, thus making comparison of results difficult.

Interpretation of food allergen-specific IgE

Food allergen-specific IgE concentrations in the serum have been correlated with patient histories and the outcome of oral food challenge tests to generate probability curves that depict the likelihood of patients with a particular food allergen-specific IgE level reacting to the food. In the same way, several groups have described diagnostic decision points for food allergen-specific serum IgE concentration for several foods (Table 21.1) such as peanut, hen's egg, cow's milk, and fish [4–12]. These diagnostic decision points are meant to reduce the requirement for oral food challenge tests. However, it has been observed that the diagnostic decision points varied among the different study populations [13]. Moreover, they appear to be age-dependent [12] and might be different in regard to the presence of atopic dermatitis. Importantly, patients with food allergen-specific IgE levels in the undefined area need to undergo an oral food challenge test.

Owing to the lack of strong correlation between the food allergen-specific IgE levels and the clinical reactivity of the patients, diagnostic decision points for some foods, such as wheat and soy, could not be established. It appears that the crude allergen extracts used in the tests are not optimal. The fact that wheat allergen ω -5 gliadin correlates well with oral challenge results [14] points in this direction. Similar results were found in Brazil nut allergy, where sensitization to Ber e 1 was shown to correlate with the clinical expression of the allergy [15].

Hen's egg

Sampson *et al.* determined the potential utility of the CAP System fluorescent-enzyme immunoassay (FEIA) in the diagnosis of IgE-mediated food hypersensitivity. In this retrospective analysis of 196 children and adolescents with

atopic dermatitis and food allergy, food-specific IgE concentrations were established that could predict clinical reactivity to various food allergens with >95% certainty [16]. One hundred and twenty-three of these children suffered from hen's egg allergy. For hen's egg, a diagnostic level of IgE, which could predict clinical reactivity in this population with >95% certainty, was identified as 6 kU/l. The 90% specificity value was 7 kU/l.

To determine the utility of these 95% predictive decision points in the evaluation of food allergy, a study was carried out 4 years later [5]. Sera from 100 children and adolescents referred for evaluation of food allergy were analyzed for food-specific IgE antibodies by using the Phadia CAP System FEIA. Of these children, 75 had hen's egg allergy; 1/3 of them were diagnosed through DBPCFC and 2/3 by patient's history. Hen's egg-specific IgE values were compared with history and food challenges to determine the efficacy of previously established decision points in identifying patients with increased probability of clinical reactivity. On the basis of the previously established 95% predictive decision points and the 90% specificity value for hen's egg, >95% of food allergies diagnosed in this prospective study were correctly identified by quantifying serum hen's egg-specific IgE concentrations.

Using the same IgE detection method, a similar study was carried out later among a German population. In this study, 227 children underwent an oral food challenge test with hen's egg. The 95% decision points among this German population with 13.0 kU/l was similar to the one in the US, thus confirming the results in a large number of children [12]. In addition, age differences have been observed with lower diagnostic decision points in children under 1 year of age of 11.0 kU/l.

Similarly, Boyano *et al.* performed a prospective study among 81 children under the age of 2 years with suspected egg allergy. Specific IgE antibodies were quantified using the Phadia CAP System. The group found that a level of ≥ 0.35 kU/l for specific IgE antibodies to egg white predicted the existence of reaction in 94% of the cases [7], giving a

Table 21.1 Proposed diagnostic decision points for various foods and age groups

Allergen	Age group	Population	Food-specific IgE	PPV	Reference
Hen's egg	Children and adolescence	US	7 kU/l	98	[5]
	Children and adolescence	German	13 kU/l	95	[12]
	≤ 2 years	Spain	0.35 kU/l	94	[7]
	≤ 1 year	German	11 kU/l	95	[12]
Cow's milk	Children and adolescence	US	15 kU/l	95	[5]
	Children and adolescence	German	–	95	[12]
	≤ 1 year	Spain	5 kU/l	95	[10]
Peanut	Children and adolescence	US	14 kU/l	100	[5]
	Children and adolescence	UK	15 kU/l	92	[17]
	Children and adolescence	France	57 kU/l	100	[6]

much lower level of specific antibodies than reported in other studies.

Cow's milk

Diagnostic decision points for cow's milk have been described by several authors. In most of these publications, the Phadia CAP System was used. In parallel with their studies on hen's egg allergy, Sampson *et al.* retrospectively studied 106 children and adolescents with atopic dermatitis and cow's milk allergy [16]. For cow's milk, a diagnostic level of IgE, which could predict clinical reactivity in this population with >95% certainty, was identified as 32 kU/l. The 90% specificity value was 15 kU/l.

Four years later in their study, to determine the utility of this 95% predictive decision points in a prospective evaluation of food allergy [5], sera from 62 children and adolescents with cow's milk allergy were investigated. One-third of the patients with cow's milk allergy were diagnosed through DBPCFC and two-thirds by patient's history. On the basis of the previously established 95% predictive decision points and the 90% specificity value for cow's milk, 95% of food allergies diagnosed in this prospective study were correctly identified by quantifying serum cow's milk-specific IgE concentrations.

Similar to hen's egg, age-specific difference appears to occur. In a prospective study carried out in Spain on 170 patients under 1 year of age, different cut-off points of the specific IgE for cow's milk were analyzed [10]. The group concluded that 2.5 and 5 kU/l had a positive predictive value (PPV) of 90% and 95%, respectively.

Later, another study was carried out among a German population using the Phadia CAP System. In this study, 398 children underwent the oral food challenge test with cow's milk [12]. A correlation between the challenge outcome and the cow's milk-specific IgE levels has been observed; however, in contrast to the populations of Spain and the US, a 95% diagnostic decision could not be established. The 90% predicted probability gave a much higher value of 89 kU/l than observed in other studies. However, age differences have also been observed with lower diagnostic decision points of 26 kU/l in children under 1 year of age.

A recent study compared the clinical performance of the Immulite 2000 in the diagnosis of cow's milk allergy with that of UniCAP [18]. The authors conclude that both methods were similarly effective in diagnosing cow's milk allergy; however, cut-off levels chosen for the Immulite had to be higher than for the UniCAP.

Peanut

For peanut allergy, diagnostic levels of IgE, which could predict clinical reactivity in a US population with >95% certainty, were identified as 15 kU_A/l [5,16]. These data were confirmed a little later in a UK study that observed 136 children undergoing peanut challenges. The authors

found that a peanut-specific IgE ≥ 15 kU_A/l had a predictive value of 92% for a positive challenge [17]. Rance studied 363 children in France. According to DBPCFC results, 177 children were allergic to peanut and 186 were not. The authors found that specific IgE concentrations of 57 kU_A/l or greater were associated with a PPV of 100% [6].

Tree nuts

To date, no well-defined diagnostic decision points have been established for tree nut allergy. A recent study on 278 patients with tree nut allergy concluded that patients aged 4 years or older with tree nut-specific IgE levels of 5 kU/l or less should be considered for an oral food challenge [19]. However in the study, challenges were only offered to patients with current tree nut-specific IgE and all tree nuts <10 kU/l. Of the 117 eligible patients, 78 declined the challenge, that is only 39 out of 278 patients underwent oral food challenge tests. Similarly, Ridout *et al.* studied 56 patients with the focus on Brazil nut allergy. Of these patients, 43% were diagnosed based on their history, whereas the remaining patients were challenged. The authors concluded from their data that a serum-specific IgE to Brazil nut of <3.5 kU_A/l may require an oral challenge to determine the risk of a Brazil nut allergic reaction [20].

Wheat

The performance characteristics of the CAP System FEIA for wheat appear to be poor [5,11,16]. No correlation between the outcome of oral food challenges and the level of wheat-specific serum IgE has been observed [12]. A diagnostic decision point could not be established. This suggests that the allergen extracts used in the tests are not optimal for diagnosis of allergy. Not only IgE to the water/salt soluble fraction of wheat, but also IgE antibodies to the water-insoluble fractions appear to play a role in wheat allergy [21]. The fact that wheat allergen ω -5 gliadin correlates well with oral challenge results, as shown recently [14], also points in this direction.

Soy

Similar to wheat allergy, the performance characteristics of the CAP System FEIA for soy were poor [5,11,16]. Diagnostic decision points could not be established [12].

Fish

For fish allergy, a diagnostic level of IgE that can predict clinical reactivity in a US population with >95% certainty was identified as 20 kU_A/l [5,16]. The major allergens are parvalbumins [22]. However, the problem of serological and clinical cross-reactivity between different fish species has not yet been solved.

Measurement of food-specific IgE over time

There are still conflicting views on whether the initial level of serum IgE and the changes over time predict the

development of clinical tolerance [23–27]. Niggemann *et al.* concluded from their study of 74 children with atopic dermatitis and various food allergies that specific IgE in serum, although very helpful at the time of the first diagnosis, cannot predict whether a child becomes tolerant after a period of avoidance [27]. In contrast, Vanto *et al.* prospectively studied 95 infants with immediate reactions and 67 with delayed reactions (up to the age of 4 years) to cow's milk [26]. Cow's milk allergy was assessed annually by cow's milk challenges. They were able to show that milk-specific IgE in the serum in children with persistent food allergies appears to have higher levels of milk-specific IgE antibodies initially and are useful prognostic indicators of the development of tolerance to cow's milk in infants with cow's milk allergy. Moreover, recent data showed a relationship between the decrease in food allergen-specific IgE levels, over a specific time period between two challenges, and the tolerance development [24]. A greater decrease in specific IgE levels over a shorter period of time indicated a greater likelihood of tolerance development. Use of these likelihood estimates could aid clinicians in the prognosis of food allergy and in timing of subsequent food challenges, thereby decreasing the number of premature and unnecessary food challenges.

Total IgE

Considering that food allergy is often the beginning of an “allergic march” and is associated with other atopic diseases in many cases, one must take the total IgE into account and should interpret specific IgE levels differently in patients with high total IgE levels compared to those with low levels [28]. This may be especially true for children with atopic dermatitis, who frequently show very high total IgE levels and sensitivity to numerous allergens, often without clinical relevance. However, it was recently shown that the additional determination of total IgE in food allergy is of no advantage. Mehl *et al.* analyzed 992 controlled oral food challenges performed among 501 children and evaluated the utility of the ratio of specific IgE/total IgE compared with specific IgE alone in diagnosing symptomatic food allergy. There was no benefit for the additional determination of total IgE compared with specific IgE alone for cow's milk, hen's egg, wheat, or soy [28].

Component resolved diagnosis

Currently, most diagnostic tests for measurement of food allergen-specific IgE are based on allergen extracts containing a mixture of allergenic and non-allergenic molecules. Through recent advances in proteomics, identification of new allergens for several foods can be performed. Allergen panels from various sources are now available, and detailed analyses of sensitization profiles in individual patients are possible. This concept has been defined as “component

resolved diagnostics” [3]. Observations indicate that molecular analysis of allergen sensitization patterns may serve to enhance the prognostic power of IgE antibody-based allergy diagnostics. The purpose is to establish significant associations between specific subpopulations of specific IgE, measured by the use of individual allergen components or parts thereof, and clinically relevant aspects of the allergic disease. For wheat allergy, measurement of specific IgE to individual wheat allergens appears to be diagnostically superior to measuring whole wheat extract [14,29,30]. Similar results were found in Brazil nut allergy, where sensitization to Ber e 1 was seen to correlate with the clinical expression of the allergy [15]. In pollen-related food allergies also, component resolved diagnostic appears to improve the diagnostic procedure. Comparing sera from 40 carrot-allergic patients, 21 birch pollen-allergic subjects with negative open provocation to carrot and 20 non-allergic individuals for IgE binding to rDau c 1.0104, rDau c 1.0201, rDau c 4, and rLTP, Ballmer-Weber *et al.* found that sensitization to rDau c 1.0201 proved to be highly specific for clinically relevant sensitization [31].

To use component resolved diagnostic, information about the relevant allergens are required. For many foods, such as milk, egg, fish, shellfish, peanut, tree nuts, and seeds, the major allergens have been identified. With recent advances in 2D-gel electrophoresis and proteomics, identification of new allergens has increased successfully [32].

Protein microarray

The latest technology trend is toward microarrays, where crude or purified native and recombinant allergens can be spotted in microdot arrays on silica chips or surface-modified glass slides to permit extensive panels of specific IgE measurements to be performed with small quantities of serum [2]. A rapid development in the area of protein microarray technology has occurred [33]. In one of the first studies, Hiller *et al.* printed 94 purified or recombinant aero- and food-allergens on surface-modified glass slides and labeled them with individual sera from 20 patients. The performance of the allergen microarray was assessed in regard to reproducibility and correlation with skin prick testing or recognition of allergens spotted onto nitrocellulose under conditions of allergen excess. The authors concluded from their study that the allergen microarray allows the determination and monitoring of allergic patients' IgE reactivity profiles to large numbers of disease-causing allergens by using single measurements and minute amounts of serum. This method may change the established practice in allergy diagnosis, prevention, and therapy [34].

Similarly, Kim *et al.* evaluated the usage of protein microarray [35]. House dust mite, egg white, milk, soybean, and wheat were used as allergens, and human serum albumin as negative control. Sensitivity and clinical efficacy of protein chip were evaluated. Comparisons between microarray-based

immunoassays and the Phadia CAP System showed a good correlation for food- and aero-allergens [35].

Microarray assays have several advantages when compared to the currently used *in vitro* determination of IgE. The test can screen hundreds of allergens in parallel with the requirement of only minute amounts of serum. Therefore, the test is attractive especially among pediatric patients. Moreover, microarray assays require much less allergen than what *in vitro* tests currently use. This is important for the use of individual purified or recombinant allergens that are usually more expensive and difficult to obtain. Although these technologies hold promise, their diagnostic performance requires further assessment once their technical details have been optimized. Potential abuses of this newer IgE antibody technology include the use of allergosorbent specificities that lack validation [2].

Measurement of peptide-specific IgE

IgE-binding epitopes have been identified for numerous food allergens including allergens in peanut [36–38], cow's milk [39–42], hen's egg [43–46], wheat [47,48], soy [49], tree nuts [50], fish [51], and shell fish [52,53]. However, their importance in the clinical course of allergic diseases or their roles in cross-reactivity are still not well understood. Generally, *in vitro* cross-reactivity in IgE-binding assays does not correlate well with clinical significance, and currently there is no tool on hand to predict whether a Brazil nut allergic patient would also react to cashew nut.

In cow's milk allergy, differences in the epitope recognition patterns have been observed between younger children, who are likely to outgrow their allergy, and older patients with persistent cow's milk allergy, which suggested that epitope specificity of IgE antibody responses might predict the clinical outcome of cow's milk allergy [42]. Using SPOTs-membrane technology and sera from 10 patients with persistent and 10 patients with transient cow's milk allergy, "informative" IgE-binding epitopes have been identified that were not recognized by any of the patients with transient cow's milk allergy, but showed binding by most of the patients with persistent allergy [54]. Recently, a larger study of 74 patients with challenge-proven cow's milk allergy confirmed that the presence of IgE antibodies to distinct allergenic epitopes of cow's milk proteins can be used as a marker of persistent cow's milk allergy [55]. Importantly, the peptides used were linked to the matrix of a commercial system for IgE measurement. Similar to cow's milk allergy, measurement of peptide-specific IgE seems to be a valuable parameter in predicting the clinical reactivity in peanut allergy [56].

Peptide microarray

Until recently, epitope analysis was carried out with protein digests or SPOTs membrane-based immunoassays. These

methods are time consuming and require large quantities of patient sera. Therefore, similar to protein microarrays, peptide microarrays have been developed recently for the analysis of IgE-binding epitopes [57,58]. Using this newly developed method, Shreffler *et al.* showed that qualitative difference in epitope diversity might provide prognostic information about food allergic patient [58].

Food allergen-specific IgE and the atopic march

Food allergy is one of the first manifestations of the "atopic march," as many of these children will develop asthma and allergic rhinitis later in life. It has been shown that early sensitization to hen's egg is a valuable marker for subsequent allergic sensitization to allergens that cause asthma and allergic rhinitis later in life [59].

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