Allergen Component Testing for Food Allergy: Ready for Prime Time?

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Abstract Food allergies can cause life-threatening reactions and greatly influence quality of life. Accurate diagnosis of food allergies is important to avoid serious allergic reactions and prevent unnecessary dietary restrictions, but can be difficult. Skin prick testing (SPT) and serum food-specific IgE (sIgE) levels are extremely sensitive testing options, but positive test results to tolerated foods are not uncommon. Allergen component-resolved diagnostics (CRD) have the potential to provide a more accurate assessment in diagnosing food allergies. Recently, a number of studies have demonstrated that CRD may improve the specificity of allergy testing to a variety of foods including peanut, milk, and egg. While it may be a helpful adjunct to current diagnostic testing, CRD is not ready to replace existing methods of allergy testing, as it not as sensitive, is not widely available, and evaluations of component testing for a number of major food allergens are lacking.

Keywords Food allergy · Diagnosis · Skin prick testing · Food-specific IgE · Sensitivity · Specificity · Oral food challenge · Component-resolved diagnostics · Microarray · Milk allergy · Hazelnut allergy · Egg allergy · Peanut allergy · Shrimp allergy

Introduction

Food allergy has been defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food [1•]. When the adverse reaction is IgE mediated, symptoms typically occur within 2 h of ingestion of the trigger food. Symptoms of an IgE-mediated reaction can vary greatly, from a mild reaction with symptoms such as localized urticaria or oral pruritus to a severe reaction including life-threatening anaphylaxis. While any food can trigger an allergic response, a majority of allergic reactions are caused by ingestion of cow's milk (CM), hen's egg (HE), wheat, soy, peanut, tree nuts, fish and shellfish. The prevalence of food allergy has been estimated as more than 1–2 % but less than 10 % of the general population based on objective diagnostic methods [2]. A review of 23 studies showed the prevalence of self-reported food allergy to cow's milk, hen's egg, peanut, fish, and shellfish to be 12 % in children and 13 % in adults [3]. The prevalence has been increasing in recent decades [4, 5].

Accurate diagnosis of food allergies is necessary both to prevent severe, life-threatening allergic reactions and to avoid unnecessary dietary restrictions. Diagnosis of IgE-mediated food allergy is typically made using the clinical history in combination with skin prick testing (SPT) and/or immunoassays of serum food-specific IgE (sIgE) levels. These tests tend to be sensitive tools in the detection of IgE-mediated food allergy, but have a number of disadvantages, including that positive test results to tolerated foods are not uncommon; results can be affected by factors such as age, reagents used, or cross reactivity between proteins found in pollens and foods; and test results do not accurately predict the severity of an allergic reaction.

Double-blind placebo-controlled oral food challenge (OFC) is currently the gold standard test for diagnosing food allergy. Due to the time and labor-intensive nature of

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these tests, open food challenges are typically performed in the clinical setting. These are usually performed when the SPT or sIgE is positive but less than the designated 95 % positive predictive value (PPV) or 95 % specificity for a particular food. They are also performed when the history does not correspond to the SPT or sIgE results. Even open OFC can be time consuming though, and there is a risk of immediate allergic reaction and anaphylaxis. Lieberman et al. [6] reported that 132 (18.8 %) of 701 open OFCs to a variety of foods elicited a reaction in their university-based, outpatient practice over about a 2-year period. Of those reactions, 12 (9.1 %) were treated with epinephrine, with one that required two doses of epinephrine.

There is clearly room for improvement in testing to differentiate asymptomatic sensitization from true clinical allergy prior to OFC. Allergen component-resolved diagnostics (CRD) have garnered a lot of attention in recent years in the diagnosis of food allergy, offering the possibility of a more accurate assessment while requiring less patient serum. Instead of using crude allergen extracts consisting of a mixture of allergenic and non-allergenic components, CRD uses pure allergen proteins, produced by purification from natural allergen sources or recombinant expression of allergen-encoding complementary DNA. In recent years, a number of studies on a variety of major allergens have demonstrated that CRD could improve the specificity of allergy testing. These studies are discussed below.

Milk Allergy

In the last 1–2 years there have been a number of reports detailing the clinical performance of allergen microarray-based diagnostics for food allergy. Protein microarrays allow specific IgE against multiple molecules to be detected in the same assay using small amounts of sera. For CM allergy, Ott et al. [7] reported the first study on the clinical performance of a component-based microarray with respect to the outcome of OFC in suspected CM allergy in 2008. They concluded that while allergen microarrays provide a new tool to diagnose symptomatic CM allergy, testing with singular allergen components was comparable, but not superior, to current extract-based fluorescence enzyme immunoassays.

This conclusion differed somewhat from a recent report by D'Urbano et al. [8], who did find additional benefit in using the microarray to predict CM OFC results. In this study, infants and children referred to their Allergy Unit in Italy for suspected IgE-mediated food allergy were enrolled. Patients had a history of severe and/or immediate reactions to CM. They performed SPT, sIgE (ImmunoCAP and microarray), and open OFC in all patients. OFCs were positive in 32 of 58 (55 %) patients. IgE reactivities to casein (Bos d 8, 46.5 %) and α -lactoglobulin (Bos d 4,

27.6 %) were more frequent, while few patients showed sIgE to IgG bovine (Bos d 7, 10.3 %) or lactoferrin (Bos d lactoferrin, 10.3 %).

The authors reported the utility of using the Bos d 8 component to predict OFC outcomes. Use of the milk sIgE 95 % clinical decision point (CDP) (≥ 16.6 kU/l) resulted in a positive predictive value of 93 % and a negative predictive value of 57 % compared with the Bos d 8 microarray 95 % CDP [> 0.60 ISU (ISAC standardized units)], which resulted in a PPV of 96 % and an NPV of 78 %. The authors concluded that component-based allergen microarray provides improved PPV and NPV in the diagnosis of CM allergy when compared with standard sIgE testing, which could reduce the number of OFCs that need to be performed. Of note, a high number of false-negative results were observed, suggesting that in patients with sIgE to Bos d 8 lower than the 95 % CDP the OFC should still be performed.

Egg Allergy

In the study described above, D'Urbano et al. also examined the use of microarray for the diagnosis of HE allergy. In this arm of the study, 46 patients with a history of severe and/or immediate reactions to HE were recruited. SPT, sIgE (ImmunoCAP and microarray), and OFC were performed for all patients. OFCs were positive in 22 of 46 (48 %) patients. Ovomucoid (Gal d 1, 43.5 %), ovalbumin (Gal d 2, 52.1 %), and lysozyme (Gal d 4, 36.9 %) showed the highest frequencies of IgE reactivity, while reactivity to conalbumin (Gal d 3, 13.0 %) and serum albumin (Gal d 5, 4.3 %) was less frequent.

In applying the use of this data to the results of OFCs, the authors reported that for suspected HE allergy, the 95 % CDP for sIgE to egg white (25.3 kU/l), measured with ImmunoCAP, yielded a PPV of 86 % and an NPV of 59 %. The 95 % CDP for sIgE to Gal d 1 yielded a PPV of 94 % and an NPV of 79 %. Similar to the results seen for Bos d 8 in CM in this study, the use of the 95 % CDP for sIgE to Gal d 1 resulted in a high number of false negatives (21 %), suggesting that OFCs should be performed for patients with sIgE to Gal d 1 lower than the 95 % CDP. Given the only modest additional accuracy of the microarray testing, as well as the limited availability and relatively high cost, the authors suggest that this technology should be used only after standard specific IgE testing has been performed and that it be performed in centers where the OFC is going to be conducted.

Alessandri et al. [9] also evaluated the usefulness of the molecular diagnostic approach in children with suspected HE allergy, examining the response to specific IgE to HE allergens, including Gal d 1, Gal d 2, Gal d 3, and Gal d 5. They studied 68 Italian children, ranging from 1 to 11 years

of age. All patients were referred for suspected HE allergy and were following a HE elimination diet. They underwent double-blind, placebo-controlled food challenges with boiled and raw egg white and yolk. Specific IgEs to Gal d 1, Gal d 2, Gal d 3, and Gal d 5 were measured using immunosolid phase allergen chip (ISAC) 103 microarray test (Thermo Fisher Scientific, Portage, MI).

Forty-four of 47 Gal d 1-negative patients tolerated boiled egg (94 %). Conversely, 20 of 21 Gal d 1-positive patients reacted to raw egg (95 %). No other HE allergen test was able to discriminate patients' response to HE challenge. They concluded that since Gal d 1-negative children showed a high frequency of tolerance to boiled egg, and Gal d 1-positive children showed a high frequency of raw HE allergy, Gal d 1 IgE testing may be a useful tool to predict oral tolerance to boiled eggs. It should be pointed out that while Gal d 1 performance could be a helpful tool in deciding which patients should undergo OFCs, it was less than 90 % sensitive in identifying subjects allergic to boiled egg. The Gal d 1 would, therefore, not likely be a definitive test obviating the need for physician supervised oral food challenge in a patient with suspected HE allergy.

Caubet et al. [10] recently reported that the interaction between IgE and IgG₄ to Gal d 1 and Gal d 2 may be helpful to better predict reactivity to baked HE. Patients with documented IgE-mediated HE allergy were enrolled (mean age = 6.9 years), and they underwent open OFCs to baked HE, followed by regular HE in tolerant subjects. They measured egg white, Gal d 1, and Gal d 2 sIgE levels, as well as Gal d 1 and Gal d 2 IgG₄ levels using the UniCAP system (Thermo Fisher Scientific). Of 117 children, 64 tolerated baked HE, 23 tolerated regular HE, and 27 reacted to baked HE (3 baked HE challenges were inconclusive because of refusal to eat the entire challenge food). In 2008, the authors reported that heated HE-reactive subjects had larger skin test wheals and greater egg white-, Gal d 1-, and Gal d 2-sIgE levels compared with baked egg- and egg-tolerant subjects [11]. There was no significant difference between Gal d 1- and Gal d 2-sIgG₄ levels between subjects reactive and tolerant to baked HE.

More recently, they reported that Gal d 1- and Gal d 2-sIgE/IgG₄ ratios were significantly higher in baked HE-reactive subjects than in baked HE-tolerant or HE-tolerant subjects ($p=0.001$ and $p=0.003$ respectively). Baked HE-reactive patients with levels of sIgE of more than 7 kU_A/l to egg white had significantly higher IgE/IgG₄ ratios compared with tolerant patients with similar levels of sIgE to egg white ($p=0.03$). Also, the majority of children with more severe reactions during the OFC requiring treatment with epinephrine had a high IgE/IgG₄ ratio to Gal d 1 and/or Gal d 2. They reported that the combination of sIgE and IgG₄ performed better than sIgE alone in predicting reactivity to baked egg, with both the highest positive and negative predictive values. The authors do point out that further

studies must still be conducted before the Gal d 1- and Gal d 2-sIgE/IgG₄ ratio is ready to be used in clinical practice.

Peanut Allergy

A recent study by Nicolaou et al. [12•] demonstrated the high rate of false-positive SPT and specific IgE to peanut. In a population-based birth cohort of 933 children in the UK, they found that 110 (11.8 %) were peanut-sensitized at 8 years of age. After OFCs were performed on children without a convincing history of reactions on exposure, peanut-specific IgE ≤ 15 kU_A/l, and skin test ≤ 8 mm, the estimated prevalence of clinical peanut allergy was only 22.4 % among sensitized children.

The utility of component-resolved diagnostics has been extensively studied in peanut allergy, and several studies demonstrate that they may improve the specificity of current peanut allergy testing. In 2004, Koppelman et al. [13] first suggested the importance of the peanut component, Ara h 2, in predicting reactivity or tolerance to peanut. They reported that Ara h 2 was the most important peanut allergen in their cohort of 32 patients, with 26 out of 32 peanut-allergic patients recognizing this component.

Similarly, Nicolaou et al. [12•] determined that Ara h 2 was the most important predictor of clinical allergy to peanut in their birth cohort. Among 110 sensitized children, 12 had a convincing history of reaction to peanut and sIgE ≥ 15 kU_A/l and/or SPT ≥ 8 mm. OFCs were performed in 79 who had parental consent to determine whether the children were clinically reactive to peanut. Sensitization profiles between children with peanut allergy and peanut-tolerant children were compared by using a microarray with 12 components from peanut (Ara h 1–3 and 8), grass (Phl p 1, 4, 5b, 7 and 12), and potentially cross-recognizing components [Bet v 1, Pru p 3 and cross-reactive carbohydrate determinants (CCD)].

Serum was available in 29 children with peanut allergy and 52 peanut-tolerant children. Subjects with peanut allergy tended to have higher fold-change values (calculated expression level estimates of the sample against the negative controls) to the major peanut components Ara h 1 to 3, whereas the peanut-tolerant subjects had higher values to CCD and grass components Phl p 1, Phl p 4, and Phl p 5. The groups did not differ for Ara h 8, Bet v 1, Pru p 3, Phl p 7, or Phl p 12. Ara h 2 appeared to offer the best discrimination, as the median fold change for Ara h 2 was 6.06 and 0.28 in the subjects with peanut allergy and the peanut-tolerant subjects, respectively. The researchers concluded that IgE response to Ara h 2 may be a clinically useful tool in predicting peanut allergy.

Dang et al. [14] also reported that Ara h 2 sIgE levels provide higher diagnostic accuracy than whole peanut sIgE levels. As part of the Australian HealthNuts study [15], 11-

to 15-month-old infants were recruited at immunization sessions. SPT to peanut was performed on all infants, and if there was any detectable SPT wheal reaction, the infant was invited for a formal open OFC. A total of 411 peanut-sensitized infants underwent a peanut OFC. Of these, 274 had a negative OFC result, while 137 had a positive OFC result. One hundred forty non-sensitized patients were also brought in for a peanut OFC as negative controls; all of these patients had negative peanut OFCs. One hundred peanut-allergic subjects were randomly selected for Ara h 2 testing. One hundred peanut-tolerant subjects were also randomly selected for Ara h 2 testing, including 58 peanut-sensitized subjects and 42 non-sensitized controls. Whole peanut IgE testing was also performed.

Compared to the currently used whole peanut sIgE levels, Ara h 2 sIgE levels were more accurate in determining peanut allergy. An Ara h 2 sIgE level of 0.46 kU_A/l provided 95 % specificity and 73 % sensitivity, whereas a peanut sIgE level of 6.2 kU_A/l provided 95 % specificity, but only 44 % sensitivity. When using a peanut sIgE level of 15 kU_A/l, providing a 95 % PPV and 98 % specificity, the sensitivity of the peanut sIgE drops to 26 %. An Ara h 2 level of 1.19 kU_A/l was also found to provide 98 % specificity, but offered a much better sensitivity of 60 %. Given the improved accuracy found in the Ara h 2 sIgE diagnostic testing, they concluded that this test should be considered the preferred diagnostic tool for determining peanut allergy.

The peanut component Ara h 6, structurally similar to Ara h 2, has been reported as a relevant allergen in peanut allergy, though it is unclear if assessment of IgE levels to this component would offer additional diagnostic value to that of Ara h 2 as part of CRD [16]. Asaranoj et al. [17] recently reported a case of a 15-year-old boy who was sensitized to Ara h 8 but not to Ara h 1, 2, or 3, who developed anaphylaxis including vomiting, diarrhea, and lower respiratory obstruction. This led to examination of Ara h 2 and Ara h 6 in a blood sample drawn 2 months after the peanut challenge, which demonstrated marked sensitization to Ara h 6 (24 kU_A/l), while Ara h 2 remained below 0.35 kU_A/l (0.12 kU_A/l). Study authors point out that in rare cases, IgE to Ara h 6 may occur in the absence of sensitization to Ara h 2 and can cause a severe reaction to peanut.

While Ara h 1, 2, and 3 have been associated with severe reactions upon exposure to peanut, isolated Ara h 8 sensitization has been reported to be associated with no or mild symptoms among peanut-sensitized subjects [18, 19]. Asaranoj et al. [20•] reported that isolated Ara h 8 sensitization indicates tolerance to peanut in almost all cases. Participants included 144 children in Sweden sensitized to peanut (IgE $\geq$ 0.35 kU_A/l) who were sensitized to Ara h 8 ($\geq$ 0.35 kU_A/l) but not to Ara h 1, 2, or 3 (all $<$ 0.35 kU_A/l). Eighty-two of these children were tolerating peanuts regularly and did not undergo challenge; 62 children were invited for OFCs.

An open OFC with peanut was performed in the subjects who did not consume peanut regularly, while a double-blind, placebo-controlled OFC was performed if the child had a documented history of systemic reactions up to grade I anaphylaxis, defined as a mild anaphylactic reaction by Vetander et al. [21]. Nearly 90 % of these children were either peanut consumers or did not react to peanut challenge. Another 14 (9.7 %) children experienced oral cavity symptoms at the first two but not subsequent challenge doses. One boy, who experienced lip swelling, stomach cramping, and objective tiredness, had sensitization to Ara h 6 (0.45 kU_A/l). This report demonstrated that isolated Ara h 8 sensitization indicates tolerance to peanut in almost all cases, though sensitization to Ara h 6 or thus far unidentified determinants in peanut might cause symptoms in rare cases.

Shrimp Allergy

Gómez et al. [22] examined the utility of recombinant and natural shrimp tropomyosins rPen a 1 and nPen m 1 in the diagnosis of shrimp allergy. Study subjects included 18 with positive OFC to shrimp (shrimp allergic), 18 who had a history of immediate symptoms after ingestion of shrimp that were suggestive of shrimp allergy but negative OFC (shrimp tolerant), and 9 who were tolerant to shrimp who had persistent rhinitis and/or asthma with positive SPT and/or sIgE to mites (dust mite positive). SPT was performed using commercial extracts of *Dermatophagoides pteronyssinus* and shrimp, and prick-by-prick testing was performed with raw red shrimp. sIgE to shrimp, recombinant and natural shrimp tropomyosins rPen a 1 and nPen m 1, recombinant Der p 10, and *Dermatophagoides pteronyssinus* was assessed by fluoroimmunoassay and/or immunoblotting. Double-blind, placebo-controlled food challenges were performed in all patients except in those confirming well-tolerated intake of shrimp in the previous 3 months.

SPT to shrimp was positive in all shrimp-allergic patients, as well as in 61 % of the shrimp-tolerant patients and 11 % of the dust mite-positive patients. Specific IgE to shrimp was also detected in all shrimp-allergic patients, while 55 % and 44 % of the shrimp-tolerant and dust mite-positive patients also had detectable shrimp-specific IgE levels, respectively. Specific IgE to rPen a 1 was detected in 16 of the 18 (89 %) shrimp-allergic patients, while 6 (33 %) of the shrimp-tolerant patients demonstrated this sensitivity. This study demonstrated that SPT and sIgE testing to shrimp are both extremely sensitive (100 %), but not specific. While study authors reported that measuring sIgE to rPen a 1 had similar sensitivity (89 %) to SPT and shrimp sIgE, specificity was improved (77 % vs. 50 %).

Hazelnut Allergy

It is well known that cross-reactivity between proteins found in pollens and foods can account for the positive test results for a variety of plant-derived foods. This issue is particularly problematic in the diagnosis of hazelnut allergy. Systemic reactions to hazelnut are generally mediated by IgE to Cor a 8, a lipid transfer protein, whereas oropharyngeal symptoms to hazelnut, known as pollen-food syndrome, are due to Cor a 1, a heat labile protein that is homologous with the major birch pollen allergen, Bet v 1 [23–25]. Beginning in 2007, a large number of patients had higher than expected serum hazelnut-specific IgE levels when tested with the ImmunoCAP system (Thermo Fisher Scientific). This coincided with the supplementation of the hazelnut allergosorbent with additional recombinant Cor a 1 to improve the test's sensitivity for birch-related reactions to hazelnut [26]. As a result, it has become nearly impossible to distinguish between serious allergy to hazelnut and potential hazelnut tolerance among persons with birch pollen allergy without the performance of an OFC. CRD has the potential to be a very useful tool in the diagnosis of hazelnut allergy, but clinical trials correlating OFC results with component testing for hazelnut must still be performed.

Conclusions

For the diagnosis of food allergy, CRD offers the potential to identify patients with clinical allergy as opposed to patients who are merely sensitized but tolerant. CRD has been extensively studied for use in the diagnosis of peanut allergy, and there are well-known associations between particular peanut allergen components, risk of clinical allergy, and the possibility of tolerance. For other food allergens, the clinical relevance is not well established. In the studies on cow's milk, hen's egg, and shrimp component testing that were published in the last 2 years, CRD offered increased specificity, but decreased sensitivity, when compared to traditional SPT and serum-specific IgE testing. From a practical standpoint, this means that CRD for these foods could be used as an adjunct to currently used allergy tests to avoid performing some OFCs, but that it is not ready to be used on its own. Also, clinical trials using oral food challenges to evaluate component testing for a variety of major food allergens, including fish, wheat, sesame, and most tree nuts, are lacking at this time.

Another factor preventing the use of CRD in the clinical setting is the limited availability of these tests. Currently, one company (Thermo Fisher Scientific) offers CRD to a variety of foods, including panels of selected recombinant and purified native allergens that can be tested on a fluorescence enzyme immunoassay platform (ImmunoCap) or a

microarray-based assay (Immuno Solid phase Allergen Chip [ISAC]) of more than 100 allergen components [27]. These diagnostic tools are widely available in Europe, and many are available in the US (PiRL, Portage, MI), though most component tests have not been approved for use by the US Food and Drug Administration (FDA). The one exception is the peanut allergen component test, which became commercially available in the US in August 2011, after the FDA announced clearance of the individual peanut component tests in May of that year.

For the reasons listed above, CRD it is not yet ready to replace current diagnostic tests, and oral food challenge remains the gold standard in the diagnosis of food allergy. In the next few years, it will be extremely interesting to see if CRD technology will evolve into a useful tool in the diagnosis of a wider variety of food allergies, and whether or not it becomes an instrument that replaces standard SPT and IgE testing or is used as a supplement to them.

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