

Utility of component diagnostic testing in guiding oral food challenges to milk and egg

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ABSTRACT

Background: Food allergies affect up to 8% of children, and milk and egg allergies are the most common triggers. Accurately diagnosing these food allergies is important to prevent allergic reactions and to avoid unnecessary dietary restrictions. However, positive skin-prick tests and detectable levels of serum food specific immunoglobulin E (IgE) alone may not be diagnostic for food allergy. Advances in the identification of relevant allergens and the development of recombinant proteins now allow assessment of IgE binding to individual proteins within a food. Component-resolved diagnostics (CRD) have the potential to provide more accurate assessments of clinical reactivity to food allergens.

Objective: To examine the available data for CRD for milk and egg allergies.

Methods: This review discussed studies that evaluated the utility of CRD for guiding decisions about food challenges for milk and egg.

Results: Results of studies indicated that CRD may offer increased specificity, but sensitivity was lacking when compared with standard skin-prick testing and measurement of serum food specific IgE levels.

Conclusion: The role of CRD in the diagnosis and management of milk and egg allergies is not well established at this time. Further studies are needed to explore the diagnostic value of CRD for milk and egg allergies.

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Food allergy continues to be an important health concern and now affects up to 8% of children and 2% of adults.^{1,2} Milk and egg are the two most-common triggers of food allergies for children.¹ An accurate diagnosis of food allergies is necessary both to ensure that individuals who are affected are adequately prepared to prevent severe, life-threatening allergic reactions and to avoid unnecessary dietary restrictions that can carry nutritional, growth, and psychosocial impacts.^{3–5} Skin-prick testing (SPT) and measurement of serum food specific immunoglobulin E (sIgE) are routinely used to support a diagnosis of food allergy in individuals in whom their history is suspicious for allergy. These diagnostic tests are highly sensitive tools, so positive test results do not necessarily indicate clinical reactivity.⁶ Thus, interpretation of test results should take into account different factors, including the age of the child, his or her reaction history, and the suspected food allergen.

The criterion standard for a food allergy diagnosis remains oral food challenge (OFC).⁶ In addition to the

risk of an allergic reaction, the time and resource-intensive nature of OFCs pose significant barriers to performing a high volume of OFCs in clinical practice.⁷ Therefore, interest in developing diagnostic tests that are highly sensitive and specific and that can accurately predict clinical reactivity and potentially reduce the number of OFCs needed would be an important contribution in the field of food allergy. Advances in the identification of relevant allergens and the development of recombinant proteins now allow measurement of IgE binding to individual proteins within a food; this is known as component-resolved diagnostics (CRD). By measuring sIgE to individual protein allergens instead of to crude allergen extracts that contain a mixture of allergenic and nonallergenic components, CRD offers the possibility of increased sensitivity and specificity, and thus improved diagnostic accuracy, which could assist in the decision-making process for pursuing OFCs. This would be highly desirable, particularly for milk and egg allergies because these are the most common allergies that affect young children.

MILK ALLERGY

Milk allergy is the most common food allergy in young children and affects ~2% of children <4 years of age.¹ Although allergy to milk is transient in the majority of affected individuals, with 52.6% becoming tolerant at a median of 5.3 years of age,⁸ one-third still retain their allergy past 12 years of age.⁹ Milk exposure can trigger severe reactions in individuals with allergies.¹⁰ In addition, avoidance of milk has been noted to

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be associated with poor growth.^{3,4} Therefore, an accurate diagnosis is necessary to ensure optimal outcomes for affected children. Milk is composed of several proteins, with casein being the major allergen.¹¹ Casein accounts for 75–80% of all cow's milk proteins, and the presence of IgE specific to casein has been noted to be associated with more persistent milk allergy and more severe reactions to milk.¹²

Early studies examined whether detecting IgE to specific milk proteins could better predict clinical reactivity to milk. In 2008, Ott *et al.*¹³ examined the clinical performance of a component-based microarray in predicting the outcome of OFC in children with suspected milk allergy. This retrospective study compared OFC outcomes with results from the component microarray test (ISAC version CRD 51; Thermo Fisher Scientific, Portage, MI) from stored serum obtained before OFC for 85 children. The results demonstrated that, although allergen microarrays allowed detection of IgE to an array of individual milk components by using only small volumes of serum, testing with individual allergen components was comparable with but not superior to standard IgE testing in predicting outcome of OFC to milk.

In contrast, D'Urbano *et al.*¹⁴ reported an added benefit of component testing in predicting milk OFC outcomes for children. Results of SPT, sIgE to milk, sIgE to individual milk proteins (ImmunoCAP and microarray; Thermo Fisher Scientific, Portage, MI) and OFC were examined for 58 infants and children with a history of severe and/or immediate reactions to milk. OFCs were positive for 55% of these children. Although sIgE testing to individual milk proteins did not improve the overall accuracy for predicting OFC outcome compared with routine milk sIgE testing, there is a potential role for sequential testing, in particular, with sIgE to casein in those individuals with routine milk sIgE of <95% positive predictive value. These conflicting results may be due to different subject selection criteria.

Because the majority of children with milk allergy are able to tolerate baked milk,¹⁵ studies also examined whether CRD has a role in predicting OFC outcome to baked milk. In a prospective study of children with milk allergy who underwent a series of challenges to heated milk products that contained differing milk protein content subjected to varying heating temperatures and durations (muffin, pizza, rice pudding) as well as unheated milk, IgE testing to casein was comparable with standard sIgE to milk for predicting baked milk tolerance.¹⁶ When using combined data from two clinical trials for baked milk, cutoff levels were determined and the optimal level that gave equal weight to sensitivity and specificity was 4.95 kU_A/L for sIgE to casein and 9.97 kU_A/L for sIgE to milk.¹⁷ Thus, the investigators indicated that OFCs to baked

milk should be considered for those individuals with casein sIgE of ≤ 5 kU_A/L and milk sIgE of ≤ 10 kU_A/L.¹⁷ Casein sIgE of <1 kU_A/L would indicate that the individual has a high chance of tolerating baked milk (95% negative predictive value).

A retrospective study that examined outcomes of baked milk OFC found SPT to milk to be the best predictor for baked milk OFC outcomes; sIgE testing to casein did not provide added diagnostic value.¹⁸ Of note, the subjects in this study did not undergo OFC to unheated milk, and standard criteria for offering baked milk OFC were not provided. Although CRD for milk proteins is already approved by the U.S. Food and Drug Administration, additional studies would be beneficial to define the role of CRD in diagnosing milk allergy and in determining optimal cutoff levels for interpreting CRD results.

EGG ALLERGY

Egg allergy is the second most-common food allergy that affects infants and young children.¹⁹ Similar to milk allergy, tolerance naturally occurs for the majority of children with egg allergy, with 49.3% becoming tolerant at a median of 6 years in one study¹⁹ and 68% outgrowing egg allergy by 16 years of age in another study.²⁰ Egg white is composed of >20 different glycoproteins. Ovomucoid (OVM) (Gal d 1) is a heat-stable protein and is the dominant allergen, whereas the heat-labile ovalbumin (Gal d 2) is the most abundant protein.²¹ IgE binding to specific sequential epitopes of OVM has been identified as a potential marker of persistent egg allergy.²²

Microarray analysis of IgE binding to component proteins of egg has been examined for the diagnosis of egg allergy. In the study by Ott *et al.*,¹³ 60 children with suspected egg allergy underwent OFC. The performance characteristics of sIgE to microarray components (ISAC version CRD 51) were comparable with standard IgE testing to egg in predicting OFC outcome (fresh whisked egg) but did not seem to provide added value. Different conclusions were drawn in another study that examined the use of microarray for the diagnosis of egg allergy by using data from 46 children with a history of severe and/or immediate reactions to egg.¹⁴ SPT, sIgE (ImmunoCAP and ISAC version CRD89; Thermo Fisher Scientific, Portage, MI) and OFC (egg boiled at 100°C for 10 minutes, and raw egg) were performed for all study subjects, and 48% had positive challenge results. The sIgE to OVM was frequently detected in those with a positive challenge result. By using this data set, sIgE to OVM was a better predictor for OFC outcome than sIgE to egg white. These contrasting results may be due to differences in patient populations, selection criteria, and/or challenge procedures.

Because different phenotypes of egg allergy exist, studies have explored whether CRD may distinguish those who are able to tolerate certain forms of heated egg from those who are reactive to egg in all forms. Alessandri *et al.*²³ assessed 68 children who were avoiding egg due to a suspicion of allergy based on previous reactions and positive testing results. These children were challenged sequentially with boiled egg (100°C for 10 minutes) and then raw egg. Specific IgE to egg components were tested by using both ImmunoCAP and ISAC 103 microarray (Thermo Fisher Scientific, Portage, MI). Ninety-four percent of children who tolerated boiled egg at OFC had an undetectable sIgE level to OVM, whereas 95% of those who reacted to raw egg had elevated sIgE levels to OVM. These results indicated that sIgE to OVM can be a reliable marker of clinical reactivity to boiled and raw egg.

Additional studies examined the utility of sIgE to egg components by using ImmunoCAP in predicting OFC outcome. In a study from Japan, the challenge outcomes for 108 children with suspected egg allergy were compared with test results from sera obtained before the OFC²⁴; sIgE to egg white and OVM performed similarly when distinguishing those reactive to raw egg from children who were not allergic. However, sIgE to OVM was a better predictor for outcome with challenge to heated egg (90°C for 60 minutes). By using these results, the investigators proposed that OFC to egg can be avoided for those with an egg white sIgE value of >7 kU_A/L (sIgE egg white of 7.38 kU_A/L had 95% specificity for reactivity to raw egg), whereas those with an sIgE value of <0.60 kU_A/L were unlikely to have egg allergy (95% sensitivity). For sIgE to OVM, a level of <1 kU_A/L indicated a low risk of reaction to heated egg (1.16 kU/L had a 97% negative predictive value, 97% sensitivity) whereas a level of >10.8 kU_A/L was highly indicative for heated egg reactivity.

Approximately 70% of children with egg allergy are able to consume egg cooked in a wheat matrix (baked egg) without reactions.²⁵ Inclusion of baked egg in the diet allows expansion of food choices, improves quality of life, and may hasten the development of tolerance to unbaked egg, thus reliable markers of clinical reactivity to baked egg have been explored. In a prospective study, children with egg allergy sequentially underwent OFCs to varying degrees of heated egg: muffin, waffle, then French toast or scrambled egg (unless sIgE to egg white showed a $>95\%$ positive predictive value for clinical reactivity).²⁵ The best predictor of baked egg tolerance in the article by Lemon-Mulé *et al.*²⁵ was a negative SPT result. The sIgE OVM was the best serologic predictor (probability curves pictured on page 980 and text page 979²⁵). Of note, only a highly elevated level of sIgE to OVM (>50 kU_A/L) was highly predictive of clinical reactivity to baked egg. A recent study included three different groups of

children: children in group A were able to consume egg in all forms, children in group B reacted to raw egg but were tolerant to cooked egg (hardboiled egg or egg in produce heated at 180°C for at least 30 minutes), and children in group C were reactive to egg in all forms.²⁶ Results of this study indicated that IgE to OVM was the best discriminator between those tolerant to cooked egg and those allergic to both raw and cooked egg (groups B and C).²⁶

Results from a retrospective study in which the subjects included those who had sIgE to OVM measured and who underwent baked egg OFCs indicated that sIgE to OVM was not superior to standard SPT or sIgE to egg white in predicting the outcome of baked egg challenges.²⁷ This study included data from 169 in-office and home OFCs; none of these patients underwent OFC to unbaked egg.²⁷ The investigators found that sIgE to egg white of ≥ 9.65 kU_A/L and OVM sIgE of ≥ 3.38 kU_A/L were levels at which food challenges could be excluded because of a high probability of clinical reactivity to baked egg.²⁷ At OVM sIgE of ≤ 0.35 kU_A/L and egg white sIgE of ≤ 6.00 kU_A/L, there was a very high chance for negative challenge to baked egg (90% pass rate).²⁷

Because there is evidence that IgG₄ may play a role in the acquisition of tolerance, the ratio of sIgE to sIgG₄ may provide useful information regarding clinical reactivity. This was explored by using the data from the study by Lemon-Mulé *et al.*²⁵ of 117 children with suspected egg allergy. The study group included 64 children who tolerated baked egg, 23 who tolerated all forms of egg, and 27 who reacted to baked egg (3 baked egg challenges were not completed due to the child's refusal).²⁵ Children who reacted to baked egg had high IgE:IgG₄ ratios to OVM and ovalbumin, whereas those who tolerated baked egg had low IgE:IgG₄ ratios.²⁸ In addition, high IgE:IgG₄ ratios to OVM and ovalbumin were associated with more-severe reactions (anaphylaxis that required treatment epinephrine) during the OFC to both baked and unbaked egg. The investigators reported that a statistical model that used a combination of sIgE and sIgG₄ was a better predictor for clinical reactivity to baked egg than sIgE alone.²⁸

More recently, another group sought to develop a model to predict severe reactions at OFC to boiled egg (20 minutes).²⁹ A prediction model was developed by using a retrospective data set of 198 positive OFCs and validated with prospectively acquired data from 140 consecutive OFCs. In addition to age (≥ 5 years old), complete avoidance of egg, and a total IgE value of <1000 IU/mL, OVM sIgE was found to be independently associated with severe reactions at OFC.²⁹

Similar to milk, CRD for egg proteins is already approved by the U.S Food and Drug Administration. However, additional studies would be helpful to determine the role of CRD in the routine diagnosis of egg

allergy and for providing guidance on decision points to consider when applying CRD results to patient management. It is important to note that reactions, including anaphylaxis, have been reported in some patients who had undetectable OVM sIgE levels, so OFC remains the criterion standard for diagnosing egg allergy.

CONCLUSION

The role of CRD in the diagnosis and management of milk and egg allergies is not well established at this time because studies do not uniformly find sIgE to milk and egg components to consistently outperform standard sIgE testing to milk and egg in predicting OFC outcomes. These differing results are likely due to variations in patient selection and food preparation for challenges. Analysis of the results indicated that CRD may offer increased specificity, but sensitivity was lacking when compared with standard SPT and sIgE testing. Therefore, additional studies are needed to determine the clinical utility of CRD for milk and egg allergy. Food challenges remain the criterion standard for food allergy diagnosis. Expanded roles of CRD to provide prognostic information regarding the severity of reactions to milk and egg are intriguing but remain to be seen.

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