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Molecular diagnosis of egg allergy: an update


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Hen’s egg allergy affects up to 2.5% of young children and is potentially life-threatening. Several phenotypes of egg allergy have been identified, including those who tolerate extensively heated egg in bakery products. Diagnosis and monitoring for resolution often requires oral food challenges, which can result in anaphylaxis. Newer approaches, such as component-resolved diagnostics, microarray analysis and epitope mapping, are being evaluated to determine if these strategies can replace or reduce the need for oral food challenges. Studies suggest that elevated levels of ovomucoid IgE indicate an inability to tolerate extensively heated forms of egg. Egg protein-specific IgE/IgG4 ratios may be helpful in predicting tolerance. Additionally, patients with conformational epitopes to hen’s egg are more likely to resolve their allergy compared with those with IgE binding to sequential epitopes. The pairing of microarray technology to epitope mapping is a potential tool to improve diagnosis. This review examines the current body of literature on these tools.

**KEYWORDS:** component ● diagnosis ● egg allergy ● hypersensitivity ● microarray ● ovalbumin ● ovomucoid ● skin prick test ● specific IgE

Food hypersensitivity is a life-threatening condition that affects 5–8% of children [1–3]. Hen’s egg allergy (HEA) is the second most common food allergy among young children with a prevalence estimated at 0.5–2.5% [4]. HEA can lead to anaphylaxis, a severe and life-threatening allergic reaction [5]. Food hypersensitivity is associated with decreased quality of life in children and families with food allergies [6–8]. Currently, the standard of care in the treatment of HEA includes strict avoidance of the food.

As hen’s egg is a common ingredient in many foods, avoidance can be difficult. This often leads to frequent accidental ingestions and reactions [9]. While strict avoidance has typically been recommended, recent studies demonstrate that a large percentage of HEA patients tolerate heated forms of egg [10–13]. Additionally, HEA is often outgrown, with about 50% of children experiencing resolution of their allergy within 2–6 years [14,15]. Accurate diagnosis of HEA is imperative; however, the current gold standard test for diagnosis of food allergy is the double-blind, placebo-controlled oral food challenge (DBPCFC). These can be burdensome in clinic settings and are associated with a risk of anaphylaxis [16]. Non-blinded oral food challenges (OFC) are acceptable alternatives; however, these are likewise resource consuming and may result in anaphylaxis [16,17]. Given the risks associated with these testing strategies, molecular diagnostic tools are being sought to reduce the need for OFC.

**Current standard testing: egg IgE levels & skin testing**
Currently, the diagnosis of HEA begins with a thorough clinical history and physical examination. If there is a suspicion of HEA, egg-specific IgE levels (elgE) are typically obtained along with skin prick testing (SPT).

**Skin prick testing**
SPT involves the epicutaneous introduction of an allergen to detect specific IgE bound to skin mast cells [18]. A positive SPT is typically defined as a wheal 3 mm greater than the negative control [19]. SPT has a high negative predictive value (NPV) of >90%, and it can be helpful in ruling out food allergy [19,20]. In contrast, a positive SPT has limited specificity, and positive predictive values (PPV) can vary based on age and size of the skin test wheal [19]. In a systematic review and meta-analysis, it was determined that the pooled
sensitivity and specificity of egg SPT were 92 and 58%, respectively [21].

Several studies have attempted to establish a 95% PPV cutoff for SPT size to predict HEA. In one Australian cohort of 467 children (median age, 3 years), they found that an egg SPT above 7 mm had a 100% PPV [22]. In the Australian Healthnuts study which followed 5276 unselected infants, egg SPT sizes of 4 mm or greater had a 95% PPV [23]. In one retrospective study of 385 mainly atopic children (median age, 22 months), it was demonstrated that a wheal of 13 mm using fresh hens egg white had a 95% PPV [24]. Hill et al. [25] found that an SPT cutoff of 7 mm in children greater than 2 years old and 5 mm in children 2 years of age and younger had a diagnostic PPV of 100%. These studies all differed in median ages of patients, percent of patients with atopy and methods of challenging (DBPCFC vs OFC); however, this demonstrates the marked variability in SPT predictive values. An additional limitation of positive and NPVs is that they are dependent on the prevalence of disease, which may not be equivalent in all countries and regions. Overall, while useful particularly in ruling out food allergy, judicious evaluation is needed in the interpretation of positive results.

**Food-specific IgE levels**

Food-specific IgE levels are serum tests that are generally obtained through the use of fluorescence-labeled antibody assays [1]. Similar to SPT, higher IgE levels correlate with an increased risk of reactions [26]. In a systematic review and meta-analysis, it was determined that the pooled sensitivity and specificity of egg IgE were 93 and 49%, respectively [21]. Generally accepted 50 and 95% positive predictive cutoff levels for egg-specific IgE have been calculated based on several studies. In atopic children with a mean age of 5 years, 50% react at an egg IgE of 2 kUA/l and 95% at an egg IgE of 7 kUA/l [19,27]. However, similar to SPT, the PPV of these cutoffs may vary based on age and population selected. For example, in the Healthnuts study previously discussed, an egg IgE of 1.7 kUA/l had PPV of 95% [23].

While these tests are useful in the diagnosis of food allergy, and may eliminate the need for OFC in a subpopulation of egg allergic patients, there remains a need for tests with better predictive values for the large percentage of children who do not have negative testing or testing above the 95% PPV ranges. Additionally, as demonstrated above, these 95% PPV are highly variable among populations and appropriate application of these cutoffs is lacking.

**Tolerance of extensively heated egg: a phenotype of egg allergy**

Recently, studies have demonstrated that 65–80% of egg allergic children are able to tolerate extensively heated or baked egg [10–13]. Those able to consume extensively heated forms of egg can liberalize their diet and have less concern about small exposures. It is thought that baking or extensively heating egg reduces allergenicity by destroying conformational epitopes; the wheat matrix of baked goods may also diminish access to epitopes [28]. This decrease in allergenicity of the egg protein is thought to allow children, who would typically react to egg, to tolerate it in certain baked forms. Additionally, tolerance to extensively heated egg seems to occur before tolerance of partially cooked egg [29]. Not only does ingestion of heated egg allow for broadening of the diet and reduced cross contamination concerns but also longitudinal studies have demonstrated that incorporation of extensively heated egg in the diet may hasten egg allergy resolution [12,30]. Thus, addition of heated egg in the diet of HEA patients offers multiple advantages.

Currently, there is difficulty in predicting which patients with HEA will be able to tolerate baked or extensively heated egg products. Studies with heated egg have utilized several different methods of heated egg preparation with the most marked difference being the utilization of a wheat matrix in some. To distinguish studies utilizing a wheat matrix from those that did not, heated egg products without a wheat matrix will be referred to as extensively heated egg whereas those with a wheat matrix will be referred to as baked egg. Egg SPT and egg IgE levels have limited accuracy in diagnosing which patients can tolerate baked or extensively heated egg. One study challenging HEA children to baked egg reported that an egg SPT less than 10 mm had 100% NPV; however, this was based on nine patients with SPT less than 10 mm [31]. This finding has not been reproduced in other studies [10,13,29,32–34]. More conservatively, a completely negative skin test may be helpful in predicting those who will tolerate baked egg. A negative egg white skin test or skin test using egg muffin has been shown to have a greater than 90% NPV [10,35,36]. The method of skin testing is also important, as it has also been found that skin testing with commercial egg extracts is not equivalent to raw egg skin testing. A retrospective chart review which compared egg extract skin testing versus raw egg skin testing determined that while both were poor predictors of passing baked challenge, extract skin testing was slightly more accurate than raw egg skin testing [37]. Similar to predicting tolerance to unheated forms of hen’s egg, while a negative skin test is highly predictive of tolerating baked or extensively heated egg, a positive skin testing remains less informative.

Egg IgE levels appear to have more utility than SPT in predicting tolerance to baked egg [33,34,36]. Some studies have attempted to establish egg IgE cutoffs for predicting outcomes for baked and extensively heated egg OFCs. In one study with baked egg, an egg white IgE of 6 kUA/l had a >90% NPV, whereas an egg IgE of 9.65 kUA/l had a >95% PPV [36]. In contrast, other studies with baked and extensively heated egg have demonstrated that an egg IgE of 5 kUA/l had a NPV of 70–80% [33,34]. Caubet et al. [38] suggested that an egg IgE of 2.6 kUA/l had 91% NPV to baked egg. In Lieberman’s study of 100 children who underwent baked egg challenges, they established NPV of 89, 77 and 71% for egg IgE levels of 2.5, 5 and 10 kUA/l, respectively [34]. Additionally, a level of 10 kUA/l had a specificity of 94% suggesting that perhaps
challenging children with egg IgE 10 kUA/l and below would capture many more baked egg tolerant patients. While OFC remains the diagnostic test of choice to identify HEA patients who tolerate baked egg, the utilization of negative egg SPT along with some general egg IgE cutoff values may be helpful in identifying which patients would most likely benefit from these challenges. There remains a need for more accurate identification of this subgroup of HEA patients particularly without the need for an OFC. Recently, studies have begun to evaluate the utility of molecular diagnostic testing in this role.

Allergenic egg proteins

The majority of allergenic proteins arise from the egg white rather than the yolk portion of hen’s egg [39]. Egg white contains over 30 different glycoproteins, and four have been recognized to be implicated in HEA: ovomucoid (Gal d 1/OVM), ovalbumin (Gal d 2/OVA), ovotransferrin (Gal d 3) and lysozyme (Gal d 4) (Table 1) [40,41]. Two more newly implicated allergens of unclear significance are lipocalin-type prostaglandin D synthase (L-PGDS) and egg white cystatin. These latter two proteins were found to have IgE reactivity in egg allergic patients [41]. While OVA is typically the most abundantly present egg white protein, OVM is thought to be the dominant allergen in egg white [40,42–44].

Table 1. Major egg allergens [40,70].

<table>
<thead>
<tr>
<th>Allergen</th>
<th>Common name</th>
<th>Heat stability</th>
<th>Location</th>
<th>Allergenicity</th>
<th>Clinical implication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gal d 1</td>
<td>Ovomucoid</td>
<td>Stable</td>
<td>Egg white</td>
<td>+++</td>
<td>Dominant allergen in HEA</td>
</tr>
<tr>
<td>Gal d 2</td>
<td>Ovalbumin</td>
<td>Unstable</td>
<td>Egg white</td>
<td>++</td>
<td>HEA</td>
</tr>
<tr>
<td>Gal d 3</td>
<td>Ovotransferrin/ conalbumin</td>
<td>Unstable</td>
<td>Egg white</td>
<td>+</td>
<td>HEA</td>
</tr>
<tr>
<td>Gal d 4</td>
<td>Lysozyme</td>
<td>Unstable</td>
<td>Egg white</td>
<td>++</td>
<td>Occupational asthma, preservative hypersensitivity, HEA</td>
</tr>
<tr>
<td>Gal d 5</td>
<td>Alpha livetin/ chicken seru albumin</td>
<td>Unstable</td>
<td>Egg Yolk</td>
<td>++</td>
<td>Bird-egg syndrome</td>
</tr>
</tbody>
</table>

HEA: Hen’s egg allergy.

Ovomucoid

OVM is a fairly heat stable molecule that has relative resistance to digestion by proteinases [43,46]. These features contribute to it being the dominant allergen in egg white. An elevated level of OVM IgE is thought to be a negative prognostic marker in egg allergy [42]. Elevated levels of OVM IgE have been associated with persistence of egg allergy [42]. Additionally, studies have shown that elevated levels of OVM IgE may be associated with the inability to tolerate baked or extensively heated egg products [10,38,43,47,48]. Given that OVM is not readily degraded by heat, it follows that patients heavily sensitized to OVM are more likely to react to heated egg. However, its utility for diagnosis in clinical practice is controversial.

Studies have been conducted evaluating the utility of OVM IgE levels in predicting tolerance to raw and heated egg. Ando et al. [47] conducted DBPCFCs to freeze-dried and extensively heated egg in HEA children. They obtained ImmunoCAP levels to egg white, OVM and OVA in 41 patients who were egg tolerant, 29 who were heated egg tolerant and 38 that were allergic to both forms of egg white. In their study, IgE to egg white was better than component testing in distinguishing raw egg allergic patients. However, they also found that in differentiating heated egg allergic versus tolerant, OVM IgE levels were slightly superior to egg white IgE levels. They suggested that an OVM IgE of 0.37 kUA/l had a NPV of 96% and an OVM IgE of 10.8 kUA/l had an 88% PPV. Similarly, a recent study by Benhamou Senouf et al. [49] compared levels of IgE to egg white, OVA and OVM in both native as well as reduced and oxidized forms among three groups of children: raw egg tolerant (group A), hard boiled egg tolerant but raw egg allergic (group B) and all forms of egg allergic (group C). They found that OVM IgE levels had the best receiver operating characteristic (ROC) curve in distinguishing group B patients from group C patients. OVM was not superior in distinguishing raw egg tolerant patients from egg allergic patients. Haneda et al. [50] challenged 100 hen’s egg sensitized patients who had never consumed egg to boiled egg. They found that overall OVM IgE levels had better predictive values than egg white in predicting outcomes of boiled egg challenges. Interestingly, they had a much higher OVM IgE cutoff for a 95% PPV at 26.6 kUA/l; however, their patient population was unique in that it was based on a sample of patients who had never ingested egg previously. A recent study by Vazquez-Ortiz et al. [33] in which 85 children underwent challenges to boiled and raw egg, compared OVA, ovomucoid and EW skin testing along with IgE levels. They found that OVM IgE had the best PPV with levels above 3.7 kUA/l having a 92% predictive value in identifying those who would fail an OFC to boiled egg. Although these studies suggest that
Table 2. Selected studies of note.

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Demographics</th>
<th>Assay</th>
<th>Results</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prediction of unheated HEA</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>Standard testing</strong></td>
<td></td>
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</tr>
<tr>
<td>Sporik et al. (2000)</td>
<td>Atopic Australian children, median age 3 yrs</td>
<td>Unheated egg</td>
<td>Egg SPT, Wheal of 7 mm with PPV 100%</td>
<td>[22]</td>
</tr>
<tr>
<td>Hill et al. (2004)</td>
<td>Atopic Australian children, median age 13.1 mos and 3 yrs</td>
<td>Unheated egg</td>
<td>Egg SPT, Wheal of 7 mm in children &gt;2 y/o and 5 mm in children &lt;2 y/o with 95% PPV</td>
<td>[25]</td>
</tr>
<tr>
<td>Verstege et al. (2005)</td>
<td>Atopic German children, median age 22 mos</td>
<td>Unheated egg</td>
<td>Egg SPT, Wheal 13 mm with 95% PPV</td>
<td>[24]</td>
</tr>
<tr>
<td>Peters et al. (2013)</td>
<td>Australian birth cohort</td>
<td>Unheated egg</td>
<td>Egg SPT, Wheal of 4 mm with 95% PPV</td>
<td>[23]</td>
</tr>
<tr>
<td>Sampson et al. (2014) and Sicherer et al. (2014) (based on multiple studies)</td>
<td>Atopic children, median age 5 y/o</td>
<td>Unheated egg</td>
<td>Egg IgE, 2 kUA/l with 50% PPV and 7 kUA/l with 95% PPV</td>
<td>[19,27]</td>
</tr>
<tr>
<td><strong>Component testing</strong></td>
<td></td>
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<tr>
<td>Vazquez Ortiz et al. (2014)</td>
<td>Spanish children, mean age 8.2 yrs</td>
<td>Unheated egg</td>
<td>OVA IgE/IgG4 ratio, 1.45 with 80% NPV</td>
<td>[33]</td>
</tr>
<tr>
<td>Benhamou Senouf et al. (2015)</td>
<td>Swiss children, median age 2.1 yrs</td>
<td>Unheated egg</td>
<td>OVA IgE, OVA IgE levels had the best ROC for predicting tolerance to uncooked egg</td>
<td>[49]</td>
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<tr>
<td><strong>Microarray analysis</strong></td>
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<tr>
<td>Alessandri et al. (2012)</td>
<td>Italian children, median age 4.1 yrs</td>
<td>Unheated egg</td>
<td>Microarray, Gal d 1 positive 95% PPV</td>
<td>[48]</td>
</tr>
<tr>
<td>Ott et al. (2008)</td>
<td>German children, median age 14 mos</td>
<td>Unheated egg</td>
<td>Microarray, No single allergen on ISAC was superior to standard testing; however, the combination of the allergens on the ISAC was comparable with SPT</td>
<td>[83]</td>
</tr>
<tr>
<td>D’Urbano et al. (2010)</td>
<td>Italian children, median age 4.9 yrs</td>
<td>Heated and unheated egg</td>
<td>Microarray, Gal d 1 0.86 with PPV of 94% and NPV of 79%</td>
<td>[84]</td>
</tr>
<tr>
<td><strong>Prediction of heated HEA</strong></td>
<td></td>
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<tr>
<td><strong>Standard testing</strong></td>
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<tr>
<td>Cortot et al. (2012)</td>
<td>US children, median age 7.2 yrs</td>
<td>Baked egg</td>
<td>Egg SPT, SPT &lt;10 mm with 100% NPV (based on 9 children)</td>
<td>[31]</td>
</tr>
<tr>
<td>Bartnikas et al. (2013)</td>
<td>US children, median age 4.7 yrs</td>
<td>Baked egg</td>
<td>Egg IgE and Egg SPT, 6 kUA/l and wheal of 11 mm with 90% NPV, &lt;3 mm with 100% NPV; 9.65 kUA/l and 25 mm wheal with &gt;95% PPV</td>
<td>[36]</td>
</tr>
<tr>
<td>Lieberman et al. (2012)</td>
<td>US children, median age 5.9 yrs</td>
<td>Baked egg</td>
<td>Egg IgE, 2.5 kUA/l with 89% NPV, 5 kUA/l with 77% NPV, and 10 kUA/l with 71% NPV</td>
<td>[34]</td>
</tr>
</tbody>
</table>

HEA: Hen’s egg allergy; ISAC: Immuno solid phase allergen chip; mos: Months; OVA: Ovalbumin; OVM: Ovomucoid; ROC: Receiver operating characteristic; SPT: Skin prick test; yrs: Years.
OVM IgE levels may be superior to egg IgE levels to predict extensively heated egg tolerance, it is important to note that these studies used heated egg in a form without a wheat matrix. This is important as the wheat matrix may help reduce allergenicity of the egg and thus alter the predictive values of OVM. Kato et al. [51] demonstrated that when OVM is baked with wheat, it polymerizes with gluten and leads to the decreased solubility of OVM. Additionally, PPV and NPV cutoffs in these studies as a whole had a wide range, thus making it difficult to generalize the results to clinical practice.
Studies utilizing a wheat matrix have not as clearly demonstrated the diagnostic utility of measuring OVM IgE. Lemon-Mule et al. [10] evaluated HEA patients to baked and regular egg. Thirty-three tolerated egg, 64 baked egg and 27 reacted to all forms of egg. They determined that OVM IgE compared with OVA IgE, egg IgE and egg SPT was the most predictive of outcomes of OFC to baked egg; however, all were poor predictors. Only at high levels, an OVM IgE of 50 kUA/l, were OVM IgE levels >90% predictive of a positive challenge. They recommended that OFC was the only conclusive test to determine tolerance to baked egg. In Bartnikas’ study of 169 patients who underwent challenges to baked egg, OVM IgE levels were predictive of outcome of baked egg challenges, but was not superior to standard testing [36]. Although an undetectable OVM level was associated with >90% NPV, the overall predictive value of OVM IgE was not superior to egg white SPTs and egg white IgE levels. In Tan’s study of 143 children who underwent a baked egg challenge, they compared SPT with OVM and egg muffin in predicting baked egg tolerance [52]. They determined that SPT is not very helpful in establishing a NPV cutoff, and they found that an OVM SPT of >11 mm was highly predictive of baked egg reactivity. In Caubet et al. [38] study of 117 children who underwent OFC to baked egg, an OVM IgE level of 12.8 kUA/l had a specificity of >95%, but was not superior to EW or OVA IgE testing. Overall, in baked egg challenges, OVM may be helpful but does not seem to offer much more clinical utility than standard testing. The addition of a wheat matrix may allow patients with higher levels of OVM IgE to tolerate baked challenges, and thus, reduce its ability to predict baked challenge outcomes.

Comparisons between these studies should be made with caution, as there were different methods utilized for each study. Taken as a whole, OVM testing offers no value above standard testing in distinguishing patients with raw egg tolerance; however, it may be useful in the setting of diagnosing heated egg tolerance. While OVM testing may be helpful in the guidance of who may tolerate heated egg products, particularly those who may tolerate heated egg without a wheat matrix, it should not be utilized alone in the diagnosis of heated egg tolerance. From a practical standpoint, it should be used as an adjunct to currently used allergy tests to avoid performing some OFCs, but it is not ready to be used on its own in diagnosing heated egg tolerance.

**Ovalbumin**

OVA is the most abundant protein present in egg white [42]. Initially, there was debate over whether OVA versus OVM was the dominant allergen in egg white. However, it has been shown that the use of commercial OVA contaminated with OVM likely led to an overestimation of its allergenicity [42,53]. OVA is heat unstable, and in the presence of heat, OVA becomes a likely less allergenic form, s-OVA [54].

There are few studies evaluating the effectiveness of measuring OVA IgE for predicting egg allergy. The previously mentioned study by Vazquez-Ortiz et al. [33] proposed that an OVA IgE level above 2.01 kUA/l was 97% predictive in identifying those who cannot tolerate raw egg and that it was superior to testing egg white IgE. Similarly, in Benhamou Senouf et al. [49] study of egg tolerant, hard boiled egg tolerant but raw egg allergic, and all forms of egg allergic, they found that OVA IgE levels had the best ROC curve in distinguishing egg tolerant patients from those who were allergic to all forms of egg. However, in their study all tests had high diagnostic accuracy in distinguishing these two groups. Other studies have not supported OVA IgE as superior to egg white testing in predicting tolerance to uncooked egg [47,55,56]. Currently, there is insufficient evidence to suggest that OVA testing adds significantly to standard testing for HEA.

**Other allergenic components of egg white**

Conalbumin or ovotransferrin is a heat-labile protein that accounts for approximately 12% of the protein in egg white [39,57]. It is similar in structure to human ferritin, and it can bind two iron molecules at a time [57]. Ovotransferrin is used in industry as a metal transporter, antimicrobial agent or in anticancer therapies [58]. Studies have shown that egg allergic children can be sensitized to ovotransferrin, and it is considered a major egg allergen [59]. However, studies are limited regarding component testing for ovotransferrin, and its specific role in egg hypersensitivity.

Lysozyme (Gal d 4) is a thermostable enzyme that represents approximately 3.4% of egg protein [60]. Although a third of egg allergic individuals may be sensitized with lysozyme, there are limited studies regarding its clinical relevance in egg allergy [61]. Lysozyme is utilized as a preservative in the food and pharmaceutical industry [62]. There are reports of hypersensitivity to lysozyme in the literature, and patients with egg allergy who are sensitized may react to preservatives in these products [63-66]. Additionally, lysozyme is a cause of occupational asthma [67].

A recent study found that a subset of children with HEA had detectable IgE to two novel egg proteins: egg white cystatin and lipocalin-type prostaglandin D synthase [68]. Chicken egg white cystatin is a cysteine proteinase that makes up 0.05% of egg white protein [68]. L-PGDS is a member of the lipocalin family that is thought to synthesize PGD2, which is related to allergic inflammation *in vivo* [41]. The clinical relevance of these two candidate allergens is still undetermined. Larger studies specifically characterizing the reactivity of these two proteins in egg allergic children are needed.

**Allergenic components of egg yolk**

Alpha livetin (Gal d 5) also known as chicken serum albumin is the major allergen identified in egg yolk; patients sensitized to Gal d 5 may experience bird-egg syndrome, which is characterized by respiratory symptoms, such as allergic rhinitis or asthma with egg ingestion [69,70]. It is thought that primary sensitization occurs with aeroallergen exposure to...
IgE/IgG4 ratio
The role of IgG4 in inducing or reflecting tolerance in food allergy is controversial. These antibodies are thought to compete with IgE binding to CD23 and ‘block’ IgE function and subsequent allergenic responses [74,75]. In aeroallergen studies, tolerance to allergens is associated with increasing IgG4 levels [74]. In research studies of immunotherapy for food allergies, increasing IgG4 levels are often associated with development of tolerance [76–78]. However, these studies evaluated IgG4 levels in inducing tolerance with immunotherapy rather than naturally developed tolerance. Studies evaluating IgG4 levels in natural tolerance are limited. Overall, studies have not demonstrated significant utility in the measurement of IgG4 levels to OVA, OVM or hen’s egg in determining natural resolution of HEA [38,79,80]. Thus, at this time, the use of IgG4 levels to hen’s egg, OVA and OVM alone does not seem to add substantial value in the diagnosis of HEA.

Further studies have attempted to evaluate whether the ratio of IgE and IgG4 to OVA, OVM or hen’s egg may have clinical utility in the diagnosis of HEA. Some studies suggest that IgG4/IgE ratios may be more accurate in predicting natural resolution of HEA. One study limited by not using DBPCFCs demonstrated that infants with higher OVA IgG4/IgE ratios were more likely to tolerate egg over time [81]. In a longitudinal study, Leonard et al. [30] longitudinally followed egg allergic children who tolerated baked egg; they demonstrated that these children had significantly decreasing ratios of OVA and OVM IgE/IgG4 over time.

Other studies have attempted to compare IgE/IgG4 ratios with other tests. Caubet et al. [38] previously mentioned study also evaluated IgE/IgG4 ratios of OVA and OVM. They found that children who reacted to baked egg tended to have higher OVA and OVM IgE/IgG4 ratios as compared with those who tolerated baked egg. They showed that a logistic regression model utilizing IgE and IgG4 to OVA and OVM had better accuracy than utilizing IgE levels to any of the three alone. However, they also indicated that neither the ratio nor logistic regression model were ready to be used in clinical practice. In contrast, Vazquez-Ortiz et al. [33] study proposed that OVA IgE/IgG4 ratios were more accurate than standard testing or component testing in predicting those who are able to tolerate extensively heated and raw egg. Further studies are needed before clinical application of IgG4/IgE ratios.

Microarray
Advancing technology has led to the development of protein microarray for measuring antibodies for diagnostic purposes in the setting of food and aeroallergy. The ImmunoCAP-ISAC or Immuno Solid phase Allergen Chip (VBC Genomics-Vienna, Austria; Phadia, Uppsala, Sweden) utilizes microchip technology to analyze over 100 component allergens in a single assay [39,82]. It has the additional benefit of conducting this assay with a very small amount of serum or plasma [39,82]. While this technology would be advantageous, particularly in children, given its ability to test several allergens on a small amount of blood, few studies have been conducted evaluating its utility in HEA.

In a retrospective study, Ott et al. [83] evaluated 60 patients with suspected HEA, who underwent DBPCFC or OFC to egg. There was no evaluation of extensively heated or baked egg. SPT and ImmunoCAP to egg along with ISAC microarray to Gal d 1, 2 and 4 were performed. They found that no single allergen on ISAC was superior to standard testing. However, they did note that utilizing the combination of the allergens on the ISAC was comparable with hen’s egg extract testing. Overall, they found that ISAC testing was not superior to standard testing, but given the small amount of blood required and the ability to utilize capillary blood samples, they suggested that this may be useful in small children and infants where large blood volumes cannot be drawn. They concluded, however, that this testing methodology is unable to replace the need for OFC in most cases [83].

In contrast, a prospective study evaluating microarray in the diagnosis of HEA conducted by D’Urbano et al. [84] demonstrated that the ISAC may be useful. They conducted OFCs in 46 infants and children with HEA to extensively heated and raw egg. SPT and ImmunoCAP to egg white and yolk along with ISAC microarray to Gal d 1, 2, 3, 4 and 5 were performed. Interestingly, they found that an egg white IgE of 25.27 kUA/L, a higher cutoff than is typically found in the literature, had an 86% PPV and 59% NPV. In contrast, they found that IgE to Gal d 1 yielded a PPV of 94% and an NPV of 79% at a cutoff of 0.86 ISU. While the specificity of this test was high, it had a poor sensitivity. They did not find that either the sequential use of both tests or utilizing the sum of the IgE reactivities on the ISAC was superior to measurement of Gal d 1 alone. While this suggests that OFCs could be deferred for Gal d 1 levels above 0.86 ISU. OFC are still needed to evaluate tolerance to egg in patients with levels below this. While the authors found this technology promising, given the high cost, limited availability and low sensitivity of the testing, the authors recommended that from a clinical perspective a two-tier approach should be utilized. Standard eIgE should be performed initially to screen patients with a high probability of true HEA. Microarray technology can be utilized on the remaining patients. An additional limitation of this study is that while they challenged patients to both boiled and raw forms of egg, no comparisons were made regarding the accuracy in the testing of distinguishing the two groups.

The only study thus far utilizing microarray technology to evaluate HEA to differentiate patients with hypersensitivity to heated and unheated forms of egg was conducted by Alessandri et al. [85]. Sixty-eight HEA Italian children (median age 4.1 years)
underwent DBPCFC to boiled and raw egg. They obtained skin testing to boiled and raw egg white and yolk, commercial extracts to egg white and yolk, egg white IgE by ImmunoCAP and ISAC microarray analysis of Gal d 1, 2, 3 and 5. While they noted that the best ROC curve was associated with boiled egg SPT, Gal d 1 on ISAC had 97% and 90% specificity for both boiled and raw egg allergy, respectively. They found that 94% of Gal d 1 negative patients tolerated boiled egg, and 95% of Gal d 1 positive patients reacted to raw egg. No other egg allergen discriminated responses to challenges. Similar to D’Urbano et al. study, they found that while specificity was high for Gal d 1, sensitivity on ISAC was poor. They concluded that while Gal d 1 testing could be a useful tool in identifying subjects who may tolerate boiled egg, it does not eliminate the need for OFC.

Although these studies exhibited different findings, it has been proposed that this may be secondary to the uniquely large cohort of atopic eczema in Ott et al. study (>95%) [83]. Additionally, in their study, they indicated that eczema exacerbation was indicative of a positive food challenge, which was not considered a positive challenge reaction in the other two studies [48,83,84]. It is also difficult to compare Alessandri et al. study with the others as their study differentiated those patients who were heated egg tolerant. It is important to note that no studies to date on HEA utilizing microarray technology have evaluated extensively heated egg with a wheat matrix. While microarray testing is a promising tool given its ability to evaluate multiple allergens utilizing small volumes of blood, further, larger studies are needed to determine its clinical utility. Additionally, studies utilizing baked egg challenges are needed.

**Epitope mapping**

Current standard testing as well as component-resolved diagnostics evaluates responses to proteins as a whole. In contrast, epitope mapping evaluates IgE binding to specific segments of proteins. Just as testing with individual allergens may have benefits over evaluating mixtures of multiple allergens, it is proposed that assaying IgE binding to individual epitopes within proteins may add utility for diagnostics. There are two major types of epitopes important in HEA: conformational and sequential epitopes. Sequential epitopes have neighboring amino acids, whereas conformational epitopes have amino acids from different regions of the allergen that are brought together by protein folding [85]. Patients with IgE binding to sequential rather than conformational epitopes of OVA and OVM tend to have more persistent HEA; in contrast, those who bind primarily conformational epitopes tend to have more transient HEA [85,86]. It is thought that the extensive degradation process before absorption and uptake of proteins in the gut means that linear epitopes are more likely to cause reactions. In contrast, conformational epitopes are more likely to be disrupted by this process, which reduces allergenicity [28].

The few studies evaluating IgE binding to linear epitopes in OVM have reported that the binding sites seem to resemble each other; in other words, patients tend to react to the same subset of OVM linear epitopes [85–87]. Thus, if it is possible to identify which subset of these linear epitopes results in more persistent or severe allergy, this could be used as a screening tool in HEA. Past studies evaluating IgE binding to OVM epitopes used SPOT membrane-based immunoassays, which are time consuming, labor intensive and expensive [39,87]. Additionally, large volumes of blood are needed, which makes it impractical for use in a clinical setting [39]. It has been hypothesized that utilizing protein microarray instead of SPOT immunoassays to identify epitopes would be more clinically feasible as it allows the evaluation of several epitopes utilizing a small volume of blood. Recently, a pilot study of 50 HEA children utilized peptide microarray in evaluation of IgE and IgG4 binding to OVM [87]. They found that approximately one-third of their patients recognized no sequential peptides.

**Other diagnostic tools**

A variety of other diagnostic tools have been evaluated in their potential application toward diagnosis of food allergies. Polymorphisms or mutations in several genes have been associated with an increased risk of food allergies: IL-10, HLA class II, FOXP3, STAT6, LRP1, CD14 and others [88]. Basophil activation tests utilize flow cytometry to measure the expression of activation markers, CD 203c and CD63, on basophils [89]. Some studies report that basophil activation tests may be helpful in the diagnosis of HEA [89,90]. Additionally, decreased CD63 expression may be associated with development of egg tolerance [91]. Biomarkers such as platelet activation factor or platelet activation marker acetylhydrolase have been associated with risk of anaphylaxis in research animals as well as peanut allergic children [26]. Presently, biomarkers, genetic testing and basophil activation testing is not recommended for clinical use in the diagnosis of HEA.

**Expert commentary**

Standard testing leaves a large population of patients requiring OFC for accurate diagnosis of HEA. Based on studies to date, component testing for egg allergy seems to offer little value in the diagnosis of raw egg allergy.
However, there is potential use of OVM testing for identifying patients with baked egg or heated egg tolerance. Unfortunately, given different methods of egg preparation along with different populations, comparison of studies is difficult. Overall, component testing may offer additional diagnostic information, and it can help predict those patients who may tolerate heated egg products. Unfortunately, it does not eliminate the need for OFC in most patients. In the future, utilizing combination of testing methods, such as ratios of IgE/IgG4 or logistic regression methods could help prediction of tolerance of HEA.

Microarray technology is a newly evolving and interesting field. While studies are limited, it offers the potential of evaluating multiple allergens with a small amount of blood. This offers a great benefit particularly in small children where obtaining venous blood can be cumbersome. However, larger scale studies are needed to truly evaluate whether this would be superior to standardized testing. Studies evaluating baked and extensively heated forms of egg would also be of interest. Given that Gal d 1 testing has shown to be most useful in the setting of evaluating heated egg tolerance, it would follow that perhaps microarray technology that evaluates multiple allergens including Gal d 1 may be most useful in that setting as well. Additionally, the marriage of utilizing epitope mapping through microarray technology is quite appealing. Theoretically, it could help individualize diagnosis and therapy for egg allergic patients. It could be utilized to predict those patients who should likely outgrow their HEA, those with more conformational epitopes. It could also be used to identify those patients who are less likely to resolve their egg allergy, and thus those patients who should be enrolled in desensitization studies or other therapies aimed at building tolerance to HEA. It would be unique in offering patients a chance to predict prognosis of their allergy rather than simply their risk of reaction. While promising, neither of these microarray tools is ready for prime time.

**Five-year view**

Goals for the future of HEA involves development of testing that can predict tolerance of HEA and heated HEA. Additionally, diagnostic testing that predicts the severity of reactions to HEA is needed. In the next few years, more studies utilizing microarray with and without epitope analysis comparing heated egg tolerant with heated egg allergic individuals will help distinguish if these tools will offer benefit in predicting heated egg tolerance. Further studies looking at natural resolution of HEA with IgE and IgG4 ratios should offer clarity regarding their efficacy as well. Additionally, further identification of specific IgE epitopes of egg allergy and its clinical correlation will be helpful not only in diagnostics but also in regards to possible therapeutics for HEA. The utilization of more comprehensive analysis of data such as through the combination of bioinformatics and microarray analysis may offer greater accuracy in prediction of allergy. Perhaps novel techniques evaluating HEA will be discovered. There is a paucity of accurate testing methodologies in HEA, and food allergy is a rapidly expanding region of research. The next few years will likely add greatly to our current body of knowledge regarding the pathophysiology, diagnosis and treatment of HEA.

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**Key issues**

- Standard testing for hen’s egg allergy (HEA) (skin prick testing and eIgE) offers good negative predictive value along with high positive predictive values; however, a large portion of patients requires oral food challenge (OFC) for definitive diagnosis.
- The ability to tolerate heated egg products allows the HEA patient to greatly expand their diet along with potentially accelerating their resolution of egg allergy.
- Elevated levels to ovomucoid, the dominant allergen in egg white, is a negative prognostic marker in HEA and can indicate the inability to tolerate heated egg products.
- Ovomucoid IgE may be helpful in the diagnosis of patients who tolerate heated egg, and it may eliminate the need for an OFC in a subset of patients.
- IgE/IgG4 ratios may offer some benefit in the diagnosis of HEA.
- Microarray technology offers the ability to evaluate multiple allergens with a small amount of blood.
- The identification of IgE-binding epitopes may offer prognostic value. IgE binding to sequential epitopes are associated with persistent allergy, whereas conformational are associated with transient allergy.
- The pairing of microarray technology with epitope mapping is a promising diagnostic tool.
- Molecular-based testing may be helpful in a subpopulation of hen’s egg allergic patients, but does not obviate the need for OFC for a large portion of egg allergic patients.
- Testing strategies are needed to predict severity of reactions and prognosis.
References
Papers of special note have been highlighted as:
• of interest
•• of considerable interest

• In this study of 117 children, the authors demonstrated that the majority of hen’s egg allergy (HEA) children were able to tolerate baked egg, and that continued ingestion of heated egg was associated with immunologic changes indicative of tolerance.


•• This longitudinal study demonstrated that the incorporation of baked egg products into the diet of HEA children may hasten resolution of egg allergy.


- This study established negative predictive values of 89, 77 and 71% for baked hen’s egg tolerance.


- Ando et al. found that the utilization of ovomucoid IgE levels was superior in the diagnosis of allergy to extensively heated egg.


- Ando et al. found that the utilization of ovomucoid IgE levels was superior in the diagnosis of egg allergy.

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epitopes have more persistent allergy and those reacting against primarily conformational epitopes have more transient allergy.


- This study is the first utilizing the combination of epitope mapping and microarray technology in the evaluation of HEA patients.


This study demonstrates that HEA patients reacting to sequential