

Utility of Gal d 1-Specific IgE Levels in Predicting Reactivity to Boiled Egg in Chinese Children

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Keywords

Boiled egg allergy · Component-resolved diagnostics · Oral food challenges · Children

Abstract

Background: Many researchers have reported predicting the outcome of oral food challenges (OFCs) on the basis of specific IgE (sIgE) levels. However, the clinical usefulness of the determination of IgE antibodies to egg allergen components in Chinese children with suspected boiled egg allergy is not well studied. **Objective:** Our objective was to assess the diagnostic performance of sIgE to egg white and Gal d 1, 2, 3, and 5 based on the open challenge outcome for boiled egg. **Methods:** A total of 48 child patients with a suspect of boiled egg allergy were included. Serum egg white and Gal d 1, 2, 3, and 5 sIgE were measured by ImmunoCAP. Diagnostic value was assessed by area under the receiver operating characteristic curves (AUC). **Results:** Using the OFC results as the reference parameter, Gal d 1 sIgE had the highest AUC (0.84) compared with egg white (0.77) and other investigated components (ranging from 0.51 to 0.71). The clinical sensitivity and specificity for the sIgE to Gal d 1 at optimal cutoff (6.15 kU_A/L) were 73.7% and 96.7%, respectively. Sensitization to Gal d 1 with a cutoff value of >7.48 kU_A/L indicated a 90% probability of positive challenge. **Conclusion:** Quantita-

tive measurements of Gal d 1 sIgE antibodies using ImmunoCAP are useful in the management of boiled egg allergy in Chinese children.

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Introduction

Hen's egg is one of the most common food allergens, with a prevalence of up to 9% in infants and young children worldwide [1, 2]. While egg allergy is transient and often resolves by school age in most children, approximately 20% of allergic children are at continuing risk of potentially severe reactions into adulthood [3, 4]. Persisting egg allergy is associated with a more severe clinical course and allergy to both raw and boiled egg [4, 5]. By contrast, studies have also shown that the ability to tolerate boiled egg offers a potential predictor of transient egg allergy, and 60–80% of children allergic to raw or lightly cooked egg tolerated boiled forms of egg [6].

Diagnosing an egg allergy affects children and their families economically and socially, making an accurate diagnosis necessary. Frequently used diagnostic techniques include skin prick tests, measuring sIgE to egg ex-

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tract, and food challenges for confirmation [7–10]. Food challenges are the gold standard diagnosing egg allergy but are time-consuming, costly, and potentially dangerous [11]. While egg skin prick tests and sIgE can be used for diagnosis, the specificity and sensitivity of these tests are relatively low [12, 13].

The growing availability of allergenic molecules has introduced a new phase in diagnostics, allowing for greater management of the allergic disease. Component-resolved diagnostics (CRD) has been a very promising tool to increase the diagnostic accuracy in food allergy [14]. The approach can be used to detect the type of allergic reaction (i.e., boiled egg allergic vs. tolerant), which is important for correct patient management [15]. However, studies regarding use of CRD in the diagnosis of boiled egg allergy are limited and have shown conflicting results [16–19]. On the other hand, established cutoff values of egg allergen component sIgE for predicting outcomes (as positive/negative) of the challenge test to boiled egg vary widely depending on the study population of different geographical locations or from different ethnic backgrounds [18, 20, 21].

The major egg allergens proposed as candidates for both sensitization without clinical relevance and true allergy are thought to be ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (Gal d 3), and lysozyme (Gal d 4) [22]. Although Gal d 2 is the most abundant and comprises 54% of the total proteins in egg white, Gal d 1 has been shown to be the most dominant and allergenic protein due to its thermo and acid-stable properties, compared with other egg components [23]. However, the clinical importance of Gal d 3 and egg yolk allergen Gal d 5 remains unclear. The aim of this study was to assess the clinical performance of sIgE to egg white, as well as sIgE to Gal d 1, Gal d 2, Gal d 3, and Gal d 5 in the diagnosis of children with suspected boiled egg allergy in our geographical region, which is located in northern China. Furthermore, we sought to determine cutoff levels of different egg allergens in predicting boiled egg challenge outcome.

Materials and Methods

Patient Selection

From May to November 2019, 48 children with suspected allergy to hen's egg based on sensitization (defined as an egg-specific IgE level >0.35 kU_A/L) and/or reaction history (including skin, gastrointestinal tract, respiratory tract, and/or cardiovascular symptoms following egg ingestion) who were referred to Tianjin Hospital were consecutively enrolled in the study. Upon study entry, each child underwent a complete physical examination and detailed medical review for evaluating egg allergy. All patients were

eligible for OFCs for egg allergy confirmation and were put on a hen's egg-free diet prior to boiled egg challenge. Moreover, oral corticosteroids were not allowed in the preceding 2 weeks and leukotriene receptor antagonists and antihistamine drug in the preceding 1 week. The final inclusion criterion was a conclusive boiled egg OFC result, irrespectively of a hypersensitivity history, and sIgE result.

Oral Food Challenges

All patients were challenged in a hospital setting and supervised by physicians in accordance with the international consensus protocol [24]. The OFCs were performed openly with cooked egg (boiled at 100°C for 15 min) in stepwise-increasing doses (100, 250, 500, and 1,000 mg, totaling 1,850 mg of egg protein) given every 30 min, if no reaction was observed with the previous dose. Patients were monitored throughout and remained for at least 2 h after the completion of the challenge. Challenges were interrupted at the first objective sign of IgE-mediated acute reaction. Then, patients were treated according to the European Academy of Allergy and Clinical Immunology's (EAACI) food allergy and anaphylaxis guidelines [25]. The cumulative eliciting dose was determined as the total dose of boiled egg (in milligrams) eliciting an allergic reaction resulting in challenge cessation. A negative challenge (passed) was confirmed by the absence of any symptoms after ingestion of the maximum amount (1,000 mg) of boiled egg. The challenge was considered positive (failed) when allergic reaction developed with manifestations from the skin, gut, respiratory tract, and cardiovascular system. Severity of egg allergic reactions at challenge was scored from 1 to 5 according to the Sampson severity score [26].

IgE Measurements

Serum samples were collected from all study subjects prior to OFCs and frozen at -20°C until use. Serum IgE levels to egg white, Gal d 1, Gal d 2, Gal d 3, and Gal d 5 were measured using ImmunoCAP (Phadia, Uppsala, Sweden) according to manufacturer's instructions.

Statistical Analysis

The independent group *t* tests were used for comparing normally distributed continuous variables, whereas Mann-Whitney *U* tests were used for comparing nonnormally distributed variables. Pearson's χ^2 test was used to compare categorical data of different patient groups. The degree of relationship between quantitative variables was analyzed using the Spearman rank correlation test. Taking the results of the provocation test as the gold standard, the diagnostic performance of tests was assessed using receiver-operator characteristic (ROC) curve analysis. The best cutoff values that would distinguish the subjects with tolerance or allergy to boiled egg were found using ROC curve analysis, and the corresponding sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) were calculated. The area under the curve (AUC) from ROC analysis was calculated to estimate the accuracy of all tests and was compared using the Delong test [27]. The relationship between sIgE titers and egg challenge outcome was analyzed with logistic regression. Samples with specific IgE values of <0.35 kU_A/L were coded as 0.35 kU_A/L in the statistical calculations. Statistical significance was assigned for 2-tailed $p \leq 0.05$. Statistical analysis and graphs were generated and plotted using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA).

Table 1. Baseline patient characteristics in the allergic group and the tolerant group and OFC outcome

Characteristics	Total (n = 48)	Allergic at OFC (n = 19)	Tolerant at OFC (n = 29)	p value
Age, median (range), years	4.6 (0.8–9.5)	3.8 (0.8–9.5)	5.1 (0.8–8.2)	0.438
Sex, n (%)				
Male	27 (56.3)	10 (52.6)	17 (58.6)	0.77
Female	21 (43.7)	9 (47.4)	12 (41.4)	
Family history of food allergy, n (%)	11 (22.9)	5 (26.3)	6 (20.7)	0.732
Physician-diagnosed other food allergies, n (%)	20 (41.7)	11 (57.9)	9 (31.0)	0.08
sIgE, median (range), kU _A /L				
Egg white	2.22 (<0.35–72.56)	8.96 (<0.35–72.56)	1.25 (<0.35–22.0)	0.001
Gal d 1	0.78 (<0.35–53.84)	6.65 (<0.35–53.84)	<0.35 (<0.35–17.33)	<0.001
Gal d 2	1.69 (<0.35–61.1)	6.87 (<0.35–61.1)	1.36 (<0.35–26.6)	0.0132
Gal d 3	0.39 (<0.35–8.73)	0.63 (<0.35–8.73)	0.39 (<0.35–2.88)	0.0245
Gal d 5	0.35 (<0.35–6.02)	<0.35 (<0.35–5.32)	<0.35 (<0.35–6.02)	0.377

OFC, oral food challenge.

Results

Patient Characteristics and Oral Egg Challenge Outcome

Subject characteristics are detailed in Table 1. The median age was 4.6 years (range, 0.8–9.5 years) and 56.3% (27 of 48) were male. Of the 48 children, 22.9% had a family history of food allergy and 41.7% had physician-diagnosed other food allergies. Thirty-eight of the 48 children were sensitized to egg, whereas 7 children had egg-specific IgE antibodies less than the limit of detection. The median levels of antigen-sIgE antibodies for egg white, Gal d 1, Gal d 2, Gal d 3, and Gal d 5 were 2.22 kU_A/L, 0.78 kU_A/L, 1.69 kU_A/L, 0.39 kU_A/L, and <0.35 kU_A/L, respectively.

A total of 29 of 48 children (60.4%) were egg tolerant and 19 (39.6%) were egg allergic in OFCs. Of the 19 positive challenges, skin symptoms were most commonly observed (15 patients, 78.9%) during challenge followed by gastrointestinal (14 patients, 73.7%), respiratory (4 patients, 21.1%), and cardiovascular (1 patient, 5.3%). Multisystem reactions were observed in 13 (68.4%) patients, 4 of whom experienced anaphylaxis. The cumulative dose of boiled egg protein required to provoke allergic symptoms varied from 100 mg to 1,850 mg (median dose: 350 mg) (Table 2). There were no significant differences among the patients in the allergic group versus the tolerant group in terms of sex, age, family history of food allergy, and physician-diagnosed other food allergies. The egg allergic group had significantly higher concentration of sIgE against egg white, Gal d 1, Gal d 2, and Gal d 3 compared to the egg tolerant group (*p* values 0.001, <0.001, 0.013, and 0.024) (Table 1). The frequency of sen-

Table 2. Symptoms, severity, and cumulative eliciting doses in 19 egg allergic patients at OFC

	n	%
Symptoms at OFC		
Skin	15	78.9
Gastrointestinal	14	73.7
Upper or lower respiratory	4	21.1
Cardiovascular	1	5.3
Neurological	0	0
Anaphylaxis	4	21.1
Severity		
1	1	36.8
2	7	42.1
3	3	15.8
4	8	5.3
5	0	0
Total amount of reacting dose of egg protein		
100 mg	3	15.8
350 mg–850 mg	11	26.3
1850 mg	5	57.9

OFC, oral food challenge.

sitization to Gal d 1 and Gal d 2 was significantly higher among the egg allergic children than the tolerant children (84.2% vs. 48.3% and 89.5% vs. 58.6%) (Fig. 1). The levels of IgE, doses of egg ingested, severity, and symptoms of each patient with an allergic reaction during the egg OFC are specified in online supplementary Table E1 (see www.karger.com/doi/10.1159/000519324 for all online suppl. material).

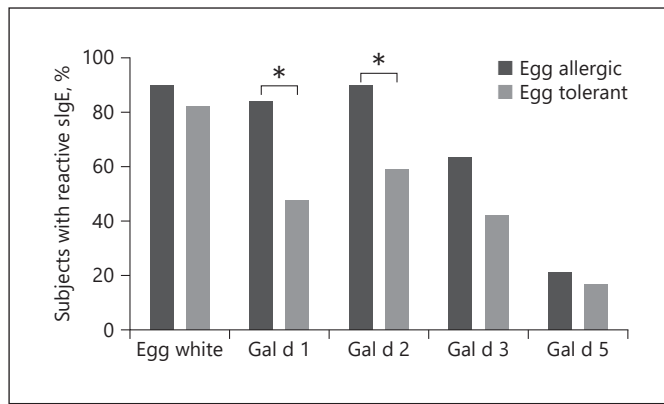


Fig. 1. Frequency of sensitization (>0.35 kU_A/L).

Correlation between Serology, Cumulative Eliciting Doses, and Symptom Severity Scores at OFC

sIgE to egg white significantly correlated with Gal d 1 ($r = 0.710$, $p < 0.001$) and Gal d 2 ($r = 0.624$, $p < 0.001$). We also found that sIgE to Gal d 1 correlated with Gal d 2 ($r = 0.375$, $p = 0.009$), but not with Gal d 3 or 5. For positive challenges ($n = 19$), symptom severity scores and cumulative eliciting doses were not associated with the level of sIgE against egg white or any egg components (all p values >0.05). In addition, symptom severity scores were not correlated with cumulative eliciting doses.

Diagnostic Value of sIgE Reactivity to Egg Components

The outcome of the OFC was considered as the reference parameter. Egg white, Gal d 1, and Gal d 3 sIgE were significantly associated with boiled egg challenge outcome ($p = 0.012$, 0.008 , and 0.019 , respectively) by logistic regression. However, it is worth noting that some patients with egg white and egg component sIgE levels <0.35 kU_A/L failed boiled egg challenges. Plots of the predicted probability of boiled egg allergy based on egg white, Gal d 1, and Gal d 3 sIgE titers are displayed in Figure 2A–C. The diagnostic ability of the in vitro tests to discriminate between nonallergic and allergic children was evaluated by ROC analysis. The ROC analysis for egg white, Gal d 2, and Gal d 3 yielded comparable AUC values, ranging from 0.71 to 0.77. AUC for Gal d 5 was poor (0.51). In contrast, Gal d 1 sIgE generated the best ROC with an AUC of 0.84, indicating that it is the most accurate way to diagnose boiled egg allergy in children (Fig. 2D).

Table 3. Specificity, sensitivity, and predictive values for various serum egg white and component sIgE levels

Cutoff point, kU _A /L	Specificity, %	Sensitivity, %	PPV, %	NPV, %
Egg white sIgE				
≥4.09	82.8	63.2	70.6	77.4
≥14.07	96.7	42.1	88.9	71.8
Gal d 1 sIgE				
≥3.25	96.7	73.7	93.3	84.8
≥7.48	96.7	47.4	90.0	73.7
Gal d 2 sIgE				
≥6.15	86.2	57.9	73.3	75.8
≥15.63	96.7	21.1	80.0	65.1
Gal d 3 sIgE				
≥0.87	89.7	52.6	78.8	76.5
Gal d 5 sIgE				
≥2.9	96.7	10.5	66.7	62.2

PPV, positive predictive value; NPV, negative predictive value.

Performance Characteristics of sIgE Tests at Various Cutoff Values

The optimal cutoff values, whereby equal weight is given to specificity and sensitivity, were 4.09 kU_A/L for egg white, 3.25 kU_A/L for Gal d 1, 6.15 kU_A/L for Gal d 2, 0.87 kU_A/L for Gal d 3, and 2.90 kU_A/L for Gal d 5. In addition, we identified >95% specificity, proposed by some as a negative decision point below which challenge should not be considered [28], for an egg white sIgE of 14.07 kU_A/L, Gal d 1 sIgE of 3.25 kU_A/L, Gal d 2 sIgE of 15.63 kU_A/L, Gal d 3 sIgE of 1.34 kU_A/L, and Gal d 5 sIgE of 2.90 kU_A/L, with a corresponding sensitivity of 42.1%, 73.7%, 21.1%, 31.6%, and 10.5%, respectively. Moreover, the calculated sIgE titer that indicated a 90% probability of reaction (90% PPV) was 7.48 kU_A/L for Gal d 1. However, 90% probability for a negative challenge (90% NPV) could not be established in this study (Table 3).

Discussion

We attempted to identify immunologic predictors of boiled egg allergy. In this study of infants and children with suspected allergy to boiled egg, the quantification of Gal d 1 sIgE had a better discriminative ability in comparison with both egg white sIgE and other component sIgE. An experimental ImmunoCAP Gal d 1 sIgE test was developed with a sensitivity and specificity of 73.7% and 96.7% at the optimal cutoff value (3.25 kU_A/L) obtained

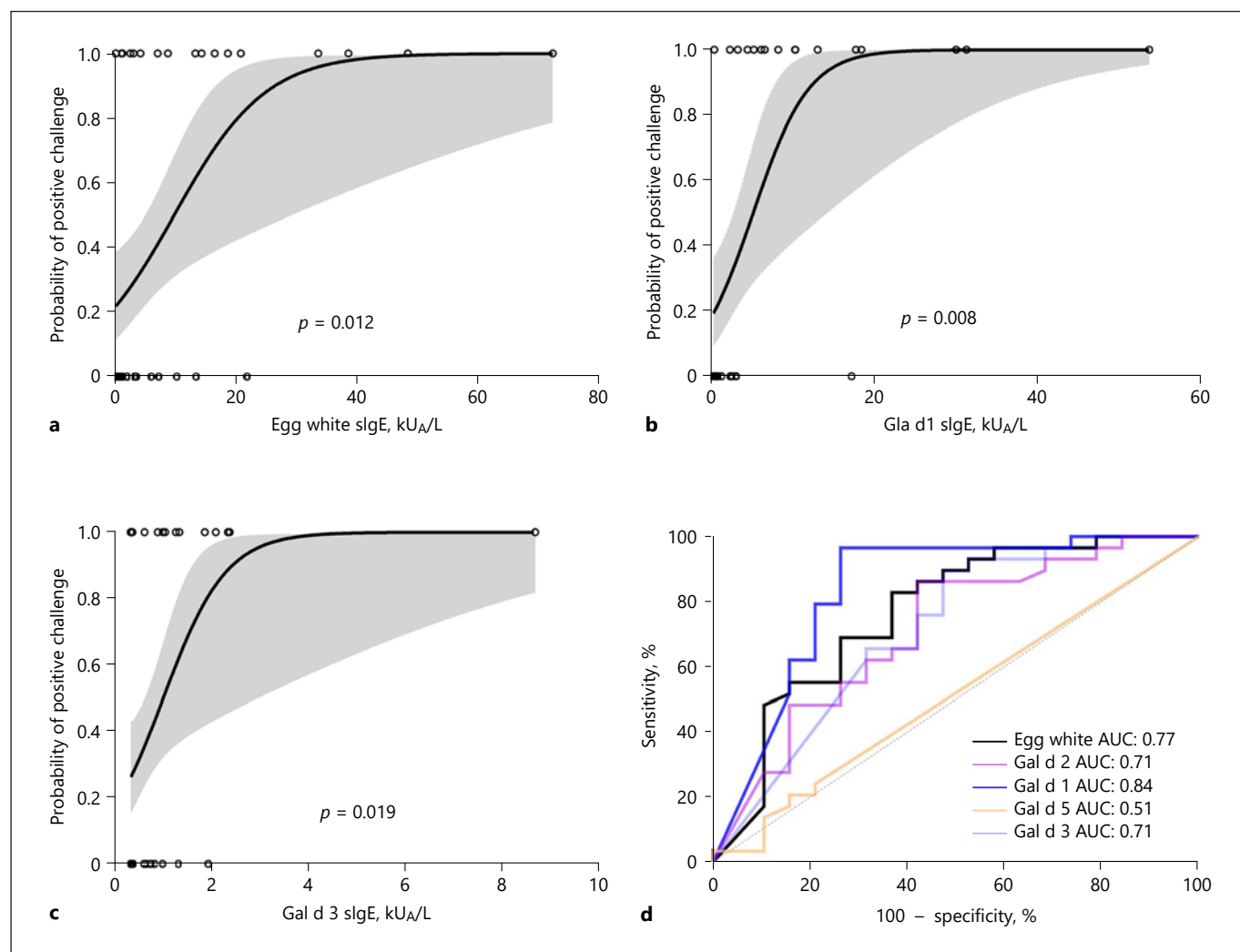


Fig. 2. a–c Predictive probability curves of egg white, Gal d 1, and Gal d 3 in determining boiled egg allergy. Shaded regions denote 95% prediction limits. Open circles represent sIgE titers for each child. d ROC for egg white and component sIgE testing along with their AUC. ROC, receiver-operator characteristic; AUC, area under the curve.

from ROC analysis. Gal d 1 sIgE with a threshold of 7.48 kU_A/L predicted a positive boiled egg challenge outcome with 90% probability. Moreover, none of the investigated egg allergens was correlated with cumulative eliciting doses or severity of allergic reactions, which is in accordance with the study performed by Palosuo et al. [18].

In line with our findings, previous studies have supported the role of Gal d 1 sIgE titer as a useful tool to predict clinical phenotypes (boiled allergic vs. tolerant) of children with egg allergy [18, 19]. This is likely due to the fact that Gal d 1 is highly resistant to heat and digestion by proteinases, as opposed to other major egg proteins

[23]. However, contradictory results have been published. Recent studies investigated whether egg CRD could improve current methods for egg allergy diagnosis and found Gal d 1 sIgE was not superior to whole egg sIgE testing in distinguishing patients with clinically reactive egg allergy from those tolerant of boiled egg [16, 17]. The regional location, dietary habits, age, and other characteristics of the study population that have a significant impact on the usefulness of the disease-specific tests are likely to explain the discrepancy in results [29]. Further, it is important to note the nonstandardized and different OFC protocols used in different studies. Some studies

used boiled egg in a form with a wheat matrix, which may affect the allergenicity of egg proteins [30] and result in differences in egg challenge outcomes, thereby altering the predictive values of Gal d 1 sIgE [6]. We challenged patients openly to the whole egg boiled at 100°C for 15 min without the addition of wheat. Therefore, immunologic predictors of outcomes of boiled egg challenges in the present study may be different from those reported in previous studies.

While the clinician-supervised OFC remain the gold standard for diagnosing food allergy, it has limited availability and is stressful for both the patients and physicians. Therefore, the so-called diagnostic decision points that estimate the outcomes of OFC are very useful for risk stratification when evaluating candidates for an OFC in practice. Although various cutoff levels of sIgE to egg white and egg allergen components have been analyzed [18, 21, 31–33], no common decision points have been achieved. We defined sIgE levels producing a sensitivity of 95% as the positive decision point and levels producing a specificity of 95% as the negative decision point. We were unable to reach a 95% sensitivity. However, the negative decision point for a very low risk of reacting to boiled egg was established in our study. This means patients with lower egg white sIgE and egg allergen components sIgE levels were likely to pass a boiled egg challenge and boiled egg products should not be avoided, compared with those with higher levels. In addition to improving the quality of life by diminishing the extent of an egg avoidance diet, there is also some evidence that suggest consumption of boiled egg could change the natural evolution of egg allergy by promoting development of tolerance to all forms of egg [34, 35]. Nevertheless, we noted that undetectable sIgE levels did not exclude boiled egg allergy. Our 90% PPV decision point for Gal d 1 sIgE titer was 7.48 kU_A/L, suggesting that in patients with sIgE to Gal d 1 higher than this value, the OFC may not need to be performed. It should also be pointed out that IgE levels for the 90% probability of a positive challenge is dependent on the prevalence of the disease and may vary in different populations. Consequently, diagnostic decision points need to be validated for each population separately before applying them in clinical practice.

There are some limitations in our study. First, this is a study conducted by a single pediatric academic institution. The OFC was usually offered to those patients who were considered to have a low risk of egg allergy, thus creating a selection bias. Therefore, the validity of our results should be evaluated further in another cohort. Sec-

ond, open food challenges were used rather than double-blind, placebo-controlled food challenges. This may increase the risk of bias caused by clinicians or patients' expectations of possible outcomes. However, the sufficiency and validity of the use of open challenges in pediatric populations has been previously independently confirmed [36, 37]. In conclusion, the measurement of Gal d 1 sIgE antibodies seems to be associated with responsiveness to the challenge test and will improve the diagnosis of boiled egg allergy.

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Statement of Ethics

The study was approved by the Institutional Review Board of the Tianjin Hospital (TJHMR2019025), and written informed consent was obtained from all parents before their children's enrollment.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

R.Z. contributed to conceptualization, writing – review and editing, and supervision; D.M. contributed to methodology, software, and writing – original draft preparation. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Table E1. Clinical characteristics of patients with an allergic reaction during the boiled egg OFC

Patient	Sex/Age (year)	egg white sIgE (kU _A /L)	Gal d 1 sIgE (kU _A /L)	Gal d 2 sIgE (kU _A /L)	Gal d 3 sIgE (kU _A /L)	Gal d 5 sIgE (kU _A /L)	Total reacting dose (mg)	symptoms during OFC	severity scores
#1	m/2.4	3.25	5.33	7.26	0.35	0.35	350	E, N, Dy	4
#2	m/0.8	13.51	10.39	0.38	0.63	0.35	100	LSI, Di	4
#3	f/3.6	38.78	31.4	6.87	1.28	3.44	350	U, AP	2
#4	m/9.2	14.5	13.15	1.62	2.36	0.35	1850	N	2
#5	f/1.2	0.35	0.35	0.8	0.35	0.35	1850	U	2
#6	f/1.0	48.6	30.16	61.1	0.35	0.35	350	LSI, U, Dy	4
#7	m/6.5	2.75	4.55	8.63	1.01	0.35	100	U, V	3
#8	m/4.3	1.33	2.39	2.41	1.35	1.06	850	A	2
#9	f/8.4	8.96	0.35	12.5	1.06	0.35	100	F, CW	4
#10	m/9.5	7.25	6.65	0.77	8.73	0.35	1850	LSI, V	3
#11	f/0.9	33.82	17.86	28.6	0.38	1.35	850	LIS, U, Di	4
#12	f/1.1	0.35	0.35	0.35	0.35	0.35	350	U, AP, Di	4
#13	m/1.8	1.25	6.23	0.35	0.9	0.35	350	U, V	3
#14	m/5.4	72.56	53.84	30	0.37	0.35	850	U, Di	4
#15	f/3.8	18.88	10.42	18.33	2.4	0.35	350	V, Dy, DBP	4
#16	m/6.7	4.43	3.35	1.69	0.35	0.35	850	AP	2
#17	m/4.5	16.7	8.3	7.99	0.35	0.35	1850	U	1
#18	f/7.6	20.98	18.5	6.49	1.88	3.84	350	U	2
#19	f/2.9	1.44	0.52	7.38	2.11	0.35	1850	A, AP	2

m: male; f: female; A: angioedema; AP: abdominal pain; CW: cough /wheezing; DBP: decreased blood pressure; Di: diarrhea; Dy: dyspnea; E: erythema; F: flushing; LSI: lip swelling/itching; N: nausea; P: pruritus; U: urticaria; V: vomiting.