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Predictive values of egg-specific IgE by two commonly used assay systems for the diagnosis of egg allergy in young children: a prospective multicenter study

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egg allergy; oral food challenge; predictive value; probability curve; specific IgE.

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Abstract

Background: Specific IgE (sIgE) is often used to predict oral food challenge (OFC) outcomes in food allergy, but interpretation of the results may vary depending on the assay method employed and the patient population tested. The aim of this study was to use two commercial assay systems to determine egg-sIgE values predictive of allergy within the most common populations treated at pediatric clinics.

Methods: In a multicenter prospective study, 433 children with suspected or confirmed egg allergy underwent oral challenge (OFC) using cooked egg (CE) and raw egg (RE) powders to diagnose either true allergy in 1-year-old (group A, $n = 220$) or tolerance in 2- to 6-year-old (group B, $n = 213$). Egg white (EW)- and ovomucoid (OM)-sIgE values were measured using the ImmunoCAP® sIgE (ImmunoCAP) and the IMMULITE® 2000 3 gAllergy™ (3gAllergy) systems. Children were recruited from six primary care clinics and 18 hospitals in Japan.

Results: Receiver-operating characteristic (ROC) curve analysis yielded similar areas under the curve (AUC) for the two assays (0.7–0.8). The optimal cutoff values and the probability curves (PCs) of the sIgE by the two assays to predict CE and RE OFC outcomes were determined for both groups. Values for 3gAllergy were higher than for ImmunoCAP; however, correlation of sIgE and predicted probability calculated by PCs were strong between the two methods.

Conclusions: Cutoff values and PCs for egg-sIgE established using both ImmunoCAP and 3gAllergy may be useful for predicting egg allergy in early childhood patient populations.

Recent advances in specific IgE (sIgE) assay technology (1) has enabled quantitative interpretation of serum IgE levels of defined allergen specificity instead of dichotomous (qualitative) results determined following allergen exposure. Quantification is especially helpful for diagnosis, and also for monitoring young children during ongoing care to determine whether food allergy persists or whether it has been outgrown (2, 3). However, present assays do not quantitate the physiological sIgE titer, but only measure relative abundance of sIgE bound to an allergen immobilized on a solid surface. Binding is dependent on the assay's allergen extract source and physical properties of the immobilized allergen that affect epitope conformation. Thus, results expressed as units do not generate the same value using different manufacturer's methods, leading to the occurrence of considerable discrepancy (4–6). Because of this, it is important that physicians interpreting the results understand the predictive sIgE levels of the particular assay they use. Several studies have reported the predictive sIgE values for a variety of food allergens (7–13) but mostly using only one assay system: ImmunoCAP® specific IgE (ImmunoCAP; Phadia, Thermo Fisher Scientific, Inc., Uppsala, Sweden). Similar data are essential for meaningful use of the IMMULITE® 2000 3gAllergy™ system (3gAllergy; Siemens Healthcare Diagnostic Inc., Tarrytown, NY, USA), a popular, widely used sIgE assay system with an extended measurement range.

Although oral food challenge (OFC) is the gold standard for the diagnosis of food allergy, it is inherently accompanied by the risk of inducing severe symptoms, including anaphylaxis (14). It is also considerably time-consuming, and these factors can hinder its application at primary care clinics where a large number of children with suspected food allergy typically present. To avoid unnecessary OFC, the use of 90 or 95% positive decision points has been proposed. These points are based on probability curves determined using quantitative sIgE measurement (7, 12, 13, 15–17) and may be used in various clinical settings. However, these statistically calculated predictive values can vary significantly among clinics or institutions depending on the assay used and characteristics of the study population, such as age, severity, and prevalence of positive reactions. The treatment of the food used in OFC, that is, raw, cooked, or baked, is also important. Thus, predictive values estimated at a single center (as calculated in most of the previous reports) may not be applicable to patients at other clinics. Discrepancies have been noted among retrospective studies, and such study designs also pose significant risk of bias. Thus to provide reliable and applicable values to clinicians, prospective, multicenter studies are needed using populations representative of those commonly seen by clinicians in daily clinical settings.

Egg is one of the most common causes of food allergy in young children, but rare in adults as most individuals outgrow egg allergy during childhood (18, 19). Because of its high prevalence and changing allergic status in very young children, demand is high for correct and early diagnosis of egg allergy. Two populations are seen most commonly in daily clinics. The first population comprises children with infantile atopic dermatitis who are suspected early of food sensitization, and upon recommendation of the pediatrician have not consumed eggs during the first year of life, and now require sIgE testing to confirm allergy (20). The second population is composed of preschool children practicing egg avoidance due to confirmed egg allergy, and who require testing to determine whether the allergy has been outgrown. In our multicenter Improvement of Proper Allergy Diagnostics utilizing 3gAllergy study (IPAD3g) conducted at primary care clinics, general pediatric hospitals, and pediatric allergy-specialized hospitals, we prospectively analyzed the relationships between egg OFC outcomes and sIgE values using the ImmunoCAP and 3gAllergy assays in these two patient populations.

Methods

Study population

Young children aged 1–6 years who had hen's egg OFC at six private practice clinics and 18 hospitals (six pediatric allergy-specialized hospitals and 12 general pediatric hospitals) in Japan were enrolled in the multicenter study from August 2012 to August 2014. All the patients were consuming an egg-free diet prior to the OFC because of suspected or diagnosed egg allergy and had no apparent history of egg-induced symptoms or had not undergone egg OFC within 3 months before the study OFC. OFC was performed at the time of the study as a requisite diagnostic procedure. Before the OFC, the patients were invited to join the study to test validity and performance of the two sIgE assays (ImmunoCAP and 3gAllergy). The study was reviewed and approved by the Institutional Review Board of Mie National Hospital (principal investigator site). Written informed consent from parents and informed assent from children older than 3 years were obtained before enrollment.

Eligible subjects ($n = 433$) were divided into two study groups. Group A ($n = 220$) consisted of 1-year-old children who had either never eaten egg but who had been tested for egg sensitization during infancy because of infantile eczema/atopic dermatitis, (20) or who had eliminated egg for more than 6 months due to mild egg-induced symptoms and egg sensitization. Group B ($n = 213$) consisted of 2- to 6-year-old children who had eliminated egg for more than 12 months because of a diagnosed egg allergy, confirmed by OFC or apparent egg-induced history with documented egg sensitization, or who had never eaten egg because of egg sensitization. Sensitization to egg was defined as EW-sIgE >0.1 either by ImmunoCAP (kU_A/L) or 3gAllergy (IU_A/mL). Patients were excluded if they had:

- apparent symptoms after ingestion of egg within 3 months before the OFC,
- egg OFC within 3 months before the current OFC,
- uncontrolled atopic dermatitis/asthma,
- other chronic diseases.

Group A patients were investigated for the determination of 'true' egg allergy, and group B patients were tested to determine whether or not they had 'outgrown' their previously diagnosed egg allergy.

Oral food challenges

Every subject underwent a single-blind OFC using cooked egg (CE) powder (Kewpie Corporation, Tokyo, Japan). Powder was produced by boiling hen's egg at 95°C for 15 min, followed by pasteurization at 65°C for 20 min, and then spray-dried. Raw egg (RE) was used to create RE powder by spray-drying followed by pasteurization at 75°C for 4 days in a preservation room. One egg equivalent for both powders was 13 g. Pumpkin, sweet potato or cocoa powders were used to mask color and taste of egg. The total dose of CE powder used in each challenge was 6.5 g for group A (1/2 egg) and 13 g for group B (one egg). The dose of RE powder was 4 g (1/4 egg) for both groups. The challenge food was divided into six graded doses (2/100, 4/100, 8/100, 16/100, 32/100 and 38/100), and each increased dose was administered at 15- to 30-min intervals. The OFC was considered positive if objective clinical reactions were noted, such as urticaria, angioedema, rhinoconjunctivitis, cough, wheezing, vomiting, diarrhea, or a decrease in blood pressure. Intense abdominal pain (self-rated as 1 or 2 using a 5-graded pain intensity face scale) was also considered positive even if other objective signs were not observed. OFC was considered negative if no symptoms were observed for 2 h after ingesting the total amount of the powder at a challenge. Full emergency

equipment and medications were readily available during the course of all procedures. Antihistamine was suspended 72 h before the OFC.

Specific IgE measurements

Serum samples from all subjects were collected on the day of OFC or within 4 weeks before OFC. sIgE to egg white (EW) and ovomucoid (OM) were determined using ImmunoCAP and 3gAllergy. The ImmunoCAP reports quantitative results in kilo-units of antibody per liter (kU_A/L); according to the manufacturer, the lower detection limit (LoD) is 0.1 kU_A/L and the upper limit is 100 kU_A/L. The 3gAllergy assay reports results in international units of antibody per liter (IU_A/mL), and as per the manufacturer, LoD = 0.1 IU_A/mL and the upper limit = 500 IU_A/mL.

Statistical analysis

All data were analyzed according to the intention-to-treat principle. For the baseline variables, summary statistics were constructed employing frequencies and proportions for categorical data, and means and standard deviations (SD) for continuous variables. Predictive accuracy of sIgE for OFC outcome was assessed by receiver-operating characteristic (ROC) analysis to determine the area under the curve (AUC). The AUC was estimated using a form of the trapezoid method, and the 95% confidence interval (CI) for the AUC was estimated by the Wald test statistic (21). The optimal cutoff point was determined to maximize the Youden index (sensitivity + specificity – 1). Sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV), and positive and negative likelihood ratios (LR+ and LR-) were then calculated for the cutoff. Probability of a positive OFC outcome for both cooked and raw eggs was estimated using logistic regression according to the method of Söderström et al., (22) and used to generate probability curves with 95% CIs for EW-sIgE and OM-sIgE for each patient group, on each system. The factors associated with OFC outcomes were also investigated by a stepwise selection procedure in a multivariate logistic regression model. A nonparametric test (Mann–Whitney *U*-test) was used to compare OFC-positive and OFC-negative groups;

$P < 0.05$ was considered statistically significant. All statistical analyses were planned and performed using the SAS software program, version 9.4 (SAS Institute, Cary, NC, USA).

Results

Demographics of the patients

Patient demographics are summarized in Table 1. There were more boys, 64%, than girls. Median ages were 16 and 48 months in groups A and B, respectively. History of egg-induced symptoms was present in 27.3% of group A and 58.7% of group B. Most of the subjects had history of eczema in infancy, which prompted the attending physicians to test egg sensitization of the patients. Comorbid atopic dermatitis was observed in 72.1% and majority of them were controlled and mild in severity. Asthma was found in 22.4%.

OFC outcomes

Of the 433 patients who underwent CE OFC, 243 patients passed (negative OFC) and 190 failed (positive OFC). Among the patients who passed the initial OFC, 130 patients declined to have RE OFC, while 113 underwent the second OFC: 55 passed and 58 failed. As the patients who failed CE OFC are unlikely to pass RE OFC because of the high

Table 1 Demographics of the study population who had cooked hen's egg oral food challenge

Characteristics		Group A: 12 – 23 months old (*n* = 220)	Group B: 24 – 83 months old (*n* = 213)	Total (*n* = 433)
Gender	M/total (male%)	141/220 (64.1)	136/213 (63.8)	277/433 (64.0)
Age (months)	Median (range)	16 (12–23)	48 (24–83)	23 (12–83)
OFC performed at:				
Hospital	*N* (%)	115/220 (52.3)	154/213 (72.3)	269/433 (62.1)
Clinic	*N* (%)	105/220 (47.7)	59/213 (27.7)	164/433 (37.9)
Egg allergy suspected because of:				
Sensitization to hen's egg*	*N* (%)	220/220 (100)	213/213 (100)	433/433 (100)
History of egg-induced symptoms†	*N* (%)	60/220 (27.3)	125/213 (58.7)	185/433 (42.7)
Previous OFC†	*N* (%)	2/220 (0.9)	36/213 (16.9)	38/433 (8.8)
History of eczema in infancy	*N* (%)	182/216 (84.3)	159/208 (76.4)	341/424 (80.4)
Diagnosis of atopic dermatitis	*N* (%)	165/210 (78.6)	137/209 (65.6)	302/419 (72.1)
Severity of atopic dermatitis				
Mild	*N* (%)	155/210 (73.8)	111/209 (53.1)	266/419 (63.5)
Moderate	*N* (%)	9/210 (4.3)	24/209 (11.5)	33/419 (7.9)
Severe	*N* (%)	1/210 (0.5)	2/209 (1.0)	3/419 (0.7)
Diagnosis of asthma	*N* (%)	27/214 (12.6)	68/210 (32.4)	95/424 (22.4)

*Hen's egg white-specific IgE ≥0.1 (ImmunoCAP or 3gAllergy).

†The present OFCs were performed more than 6 months after a recent history of induced symptoms and previous OFC.

allergenicity of raw egg (23), they were included in the analysis for RE OFC as positive (Fig. 1). Symptoms induced by OFCs were predominantly cutaneous, such as urticaria and itchy erythema. Gastrointestinal and respiratory symptoms such as abdominal pain, vomiting, cough, and wheeze appeared in about 30% of the subjects (Table S1). Epinephrine injections were required during the CE OFC for 17 patients (3.9%), and during RE OFC for four patients (3.5%); all the subjects recovered promptly without sequelae. There were no cardiovascular symptoms (Table S1).

Diagnostic performance of sIgE assays by ImmunoCAP and 3gAllergy

EW-sIgE and OM-sIgE by ImmunoCAP and 3gAllergy in patients who failed corresponding OFCs were significantly

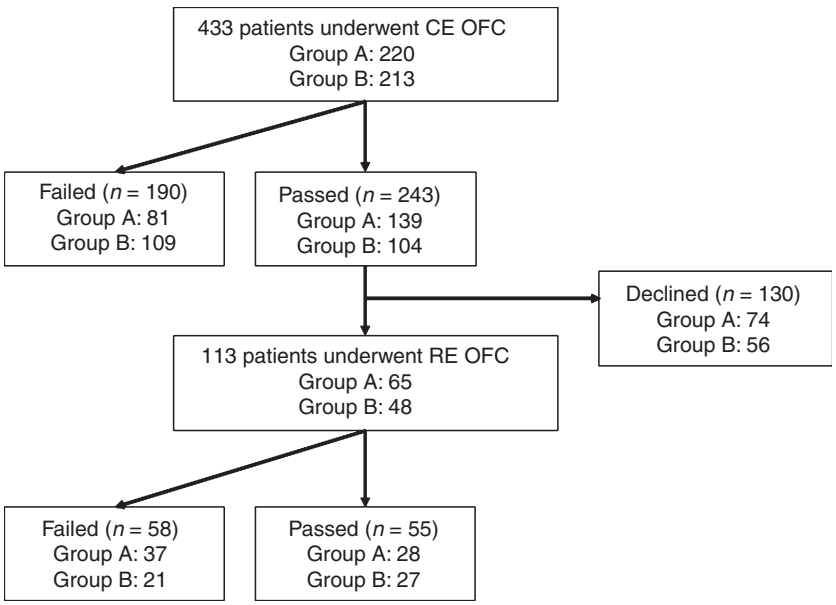

Figure 1 OFC flow diagram showing a cascade of 'Passed' (negative OFC) and 'Failed' (positive OFC) outcomes.

Table 2 Diagnostic performance of sIgE assays in predicting OFC outcomes

Patient group		sIgE assay	AUC	(95%CI)	Optimal cutoff point*	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR–
(a) CE performance											
Group A	EW	ImmunoCAP	0.671	0.599–0.744	7.4	75.3	56.1	50.0	79.6	1.72	0.44
	EW	3gAllergy	0.690	0.618–0.762	54.2	61.7	72.7	56.8	76.5	2.26	0.53
	OM	ImmunoCAP	0.778	0.712–0.843	3.1	72.8	77.7	65.6	83.1	3.27	0.35
	OM	3gAllergy	0.791	0.726–0.856	21.5	66.7	85.6	73.0	81.5	4.63	0.39
Group B	EW	ImmunoCAP	0.786	0.724–0.847	9.1	78.0	67.3	71.4	74.5	2.39	0.33
	EW	3gAllergy	0.797	0.737–0.856	28.5	85.3	62.5	70.5	80.2	2.28	0.23
	OM	ImmunoCAP	0.828	0.773–0.883	9.0	66.1	89.4	86.7	71.5	6.25	0.38
	OM	3gAllergy	0.848	0.796–0.899	45.4	65.1	92.3	89.9	71.6	8.47	0.38
(b) RE performance											
Group A	EW	ImmunoCAP	0.671	0.534–0.808	3.7	75.7	60.7	71.8	65.4	1.93	0.40
	EW	3gAllergy	0.678	0.542–0.814	15.4	73.0	60.7	71.1	63.0	1.86	0.45
	OM	ImmunoCAP	0.574	0.432–0.717	0.5	54.1	67.9	69.0	52.8	1.68	0.68
	OM	3gAllergy	0.634	0.492–0.776	3.0	56.8	71.4	72.4	55.6	1.99	0.61
Group B	EW	ImmunoCAP	0.742	0.632–0.852	3.0	84.7	60.7	90.1	48.6	2.16	0.25
	EW	3gAllergy	0.762	0.660–0.865	22.9	74.6	71.4	91.7	40.0	2.61	0.36
	OM	ImmunoCAP	0.734	0.629–0.839	0.6	72.0	71.4	91.4	37.7	2.52	0.39
	OM	3gAllergy	0.766	0.663–0.869	3.0	78.8	71.4	92.1	44.4	2.76	0.30

AUC, area under the curve; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR–, negative likelihood ratio.

*Units are kU_A/L for ImmunoCAP and IU_A/mL for 3gAllergy.

higher than those who passed (Table S2). Predictive accuracy of the two sIgE tests for OFC outcomes was evaluated using ROC analysis. Overall, the AUCs were higher in group B than in group A, indicating that *in vitro* sIgE predictive ability is lower when conducted at a younger age (Tables 2a and 2b). In terms of the test methods employed, ImmunoCAP and 3gAllergy showed equivalent accuracy in respective OFCs based on the AUC. Optimal cutoff points were higher with 3gAllergy than ImmunoCAP. In CE OFC, the AUC for OM-sIgE was higher than for EW-sIgE, as has been shown previously (Table 2a) (24). Conversely, in RE OFCs, EW-sIgE AUCs were slightly higher than AUCs for OM-sIgE (Table 2a). LR+s for OM-sIgE in CE OFC were satisfactorily high for both ImmunoCAP (6.25) and 3gAllergy (8.47, Table 2b).

Predictive probability for positive OFC

The relationships between OFC outcomes and sIgE levels by ImmunoCAP and 3gAllergy were estimated using logistic regression and illustrated as probability curves (Figs 2 and 3). The curves clearly demonstrate risk of a positive OFC dependent on the sIgE level. To determine the utility in clinical decision-making, EW- and OM-sIgE levels were calculated for each of the two methods at 90%, 80%, and 10% predicted probability for a positive OFC (Table 3). Overall, slopes of the probability curves were more gradual and their 95% CIs were broader for group A than group B, suggesting that diagnostic performance of these tests is not as good for children <2 yrs as it is for older children. However, considering that the predefined total challenge dose in CE OFC for group A was half of a whole egg equivalent, whereas the dose for group B was one whole egg equivalent, the lower predicted probability at higher sIgE levels in group A indicates that 1-year-old infants in this study population are more likely to tolerate egg in small amounts than their older counterparts (Fig. 2).

A multivariate logistic regression analysis was applied to specify a factor that independently affects OFC outcomes. OFC outcome was applied as the response variable; age, gender, comorbid atopic dermatitis and asthma/wheezing, and

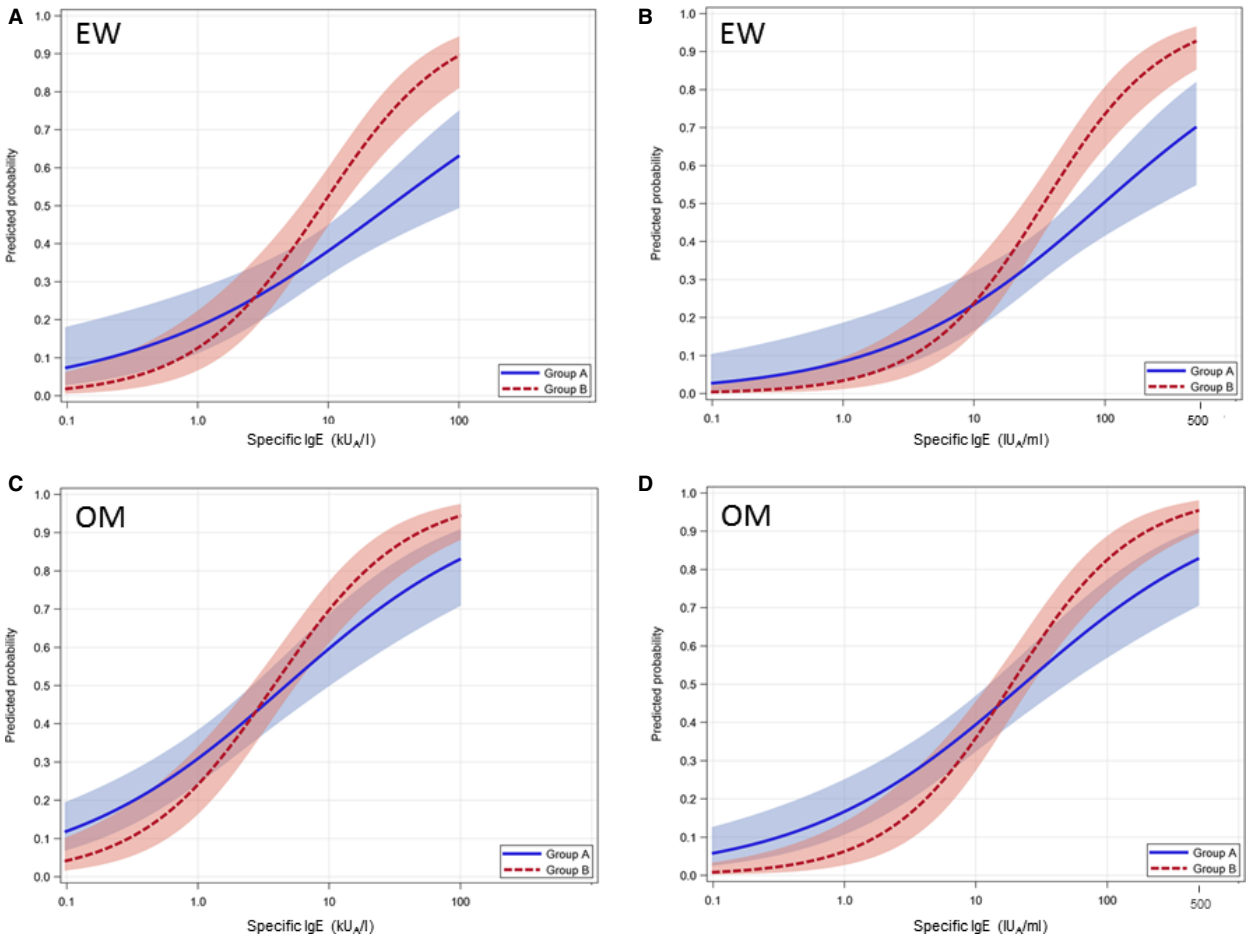

Figure 2 Predicted probability derived from logistic regression for CE OFC outcomes. Estimated probability curves for failing CE OFC at a given EW-sIgE level by ImmunoCAP (A) and 3gAllergy (B), OM-sIgE level by ImmunoCAP (C) and 3gAllergy (D) are depicted. Shaded areas indicate range of 95% CI. Blue lines and shades indicate group A and red lines and shades indicate group B.

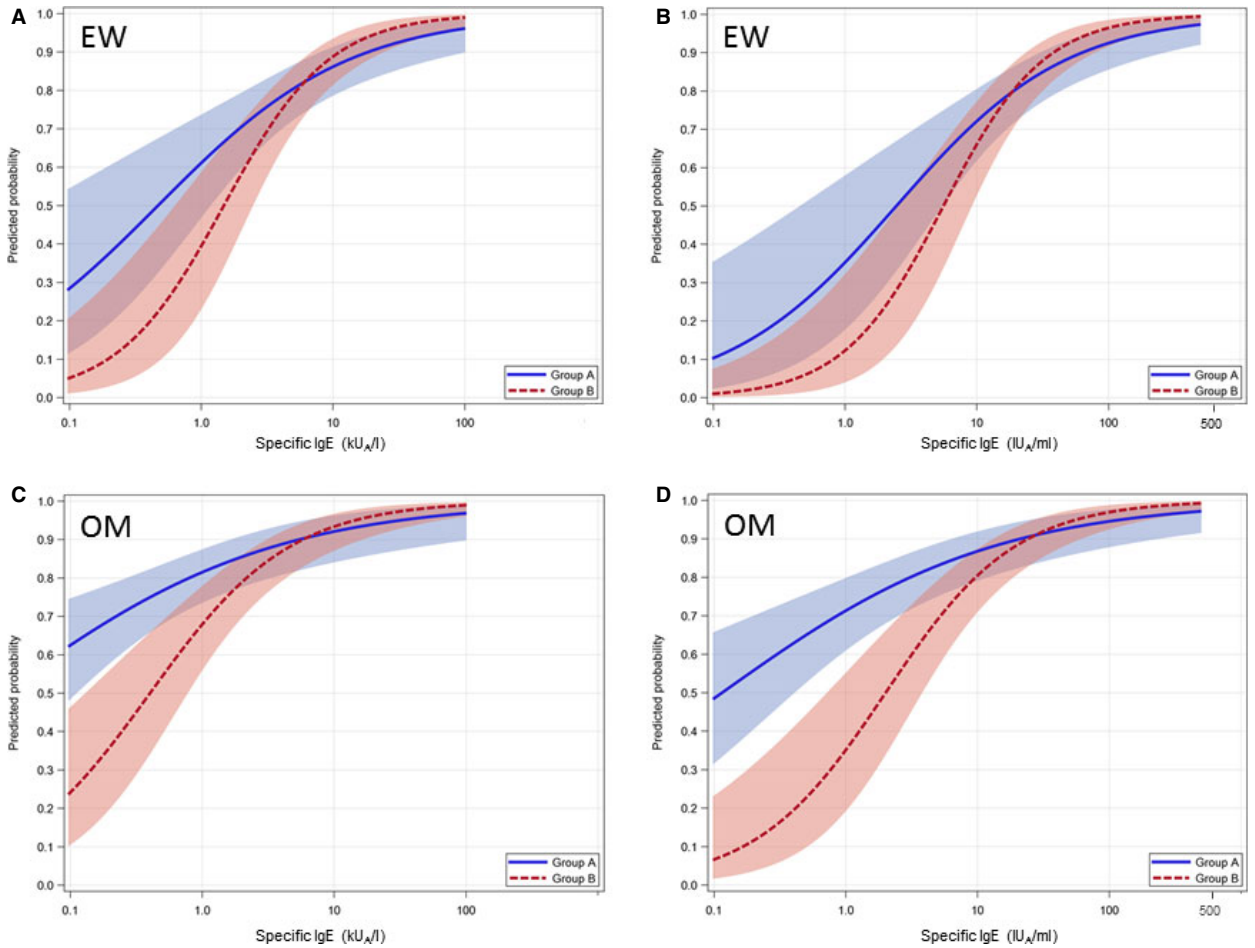

Figure 3 Predicted probability derived from logistic regression for RE OFC outcomes. Estimated probability curves for failing CE OFC at a given EW-sIgE level by ImmunoCAP (A) and 3gAllergy (B), OM-sIgE level by ImmunoCAP (C) and 3gAllergy (D) are depicted. Shaded areas indicate range of 95% CI. Blue lines and shades indicate group A, and red lines and shades indicate group B.

EW- or OM-sIgE by one of the two assays were applied as the explanation variables. None of the factors were independently associated with OFC response except for each of the sIgE outcomes (data not shown).

Correlation of the two sIgE tests

As the probability curves for ImmunoCAP and 3gAllergy were very similar in shape (Figs 2 and 3), we looked at the correlation between EW- and OM-sIgE values by the two assays and found that they were strongly correlated; Pearson's correlation coefficient was 0.97 for EW and 0.95 for OM (Fig. S1). We also examined correlation between each predicted probability by ImmunoCAP and 3gAllergy for individual patients and found that probability of response was also strongly correlated between the two systems (R = 0.98 for EW and 0.93 for OM, Fig. S2). Because log-transformed data of the sIgE values by ImmunoCAP and 3gAllergy showed linear correlation, equations for transformation could be established and a conversion table was also constructed (Table S3).

Discussion

In this prospective multicenter study, we identified predictive values for OFC outcome in young children with suspected egg allergy for EW- and OM-sIgE measured by two commonly used quantitative sIgE assay systems (ImmunoCAP and 3gAllergy). Predictive values like 95% positive decision points and probability curves are highly dependent on characteristics of a study population, such as prevalence of positive outcome, severity of the disease and the presence of comorbid diseases. For this reason, we recruited subjects from a variety of primary care and specialized clinics. The majority of patients are very likely to be examined at daily clinics, where a physician has to ponder whether or not a toddler has a 'true' egg allergy, and likewise, whether or not a preschool-aged child has 'outgrown' egg allergy. The predictive values that we have established in this study fit these clinical needs.

Allergenicity of the food used in an OFC must also be considered when interpreting the results to apply them correctly to the daily diet recommended to food-allergic patients.

Table 3 Egg-sIgE antibody levels with estimated 90%, 80%, and 10% predicted probabilities for positive OFC

		Assay method	90% predicted probability*	80% predicted probability	10% predicted probability
Group A					
CE	EW	ImmunoCAP	n.e.	n.e.	0.2
OFC	EW	3gAllergy	n.e.	n.e.	0.2
	OM	ImmunoCAP	n.e.	71.1	n.e.
	OM	3gAllergy	n.e.	355.0	0.3
RE	EW	ImmunoCAP	18.7	4.9	n.e.
OFC	EW	3gAllergy	63.2	18.9	0.1
	OM	ImmunoCAP	5.7	0.9	n.e.
	OM	3gAllergy	20.6	3.1	n.e.
Group B					
CE	EW	ImmunoCAP	n.e	43.5	0.8
OFC	EW	3gAllergy	355.0	150.0	3.3
	OM	ImmunoCAP	50.0	18.7	0.3
	OM	3gAllergy	211.0	81.9	1.8
RE	EW	ImmunoCAP	11.5	5.3	0.2
OFC	EW	3gAllergy	37.7	18.9	0.9
	OM	ImmunoCAP	6.1	2.1	n.e.
	OM	3gAllergy	24.5	9.5	0.2

n.e., not estimated.

*$_{K}U_{A}$/L for ImmunoCAP, IU_{A}/mL for 3gAllergy.

Allergic potency of egg in particular vastly changes by cooking or processing because heating reduces or denatures allergen epitopes (25). For this reason, we estimated the ability of EW- and OM-sIgEs to predict a positive response when a patient is exposed to either cooked or raw egg in an OFC.

Another important aspect of this study was our identification of EW- and OM-sIgE titers predictive of egg allergy in young children using 3gAllergy. This is the first study to determine these values using this system, which is a quantitative sIgE assay with an extended measurement range. Until now, the predictive values of sIgE in food allergy have been determined mostly using the ImmunoCAP system, making interpretation of the values measured by other assays difficult. Some reports argue that 3gAllergy overestimates IgE values (4, 6) However, assays from different vendors commonly produce disparate values, and in any assay comparison, it is expected that there will be differences between the reference assay and the comparison assay. In one study, for example, 3gAllergy appeared to underperform ImmunoCAP (4). However, at least one study has shown that in terms of assay performance – including accuracy and reproducibility – the 3gAllergy system appears to be comparable to the ImmunoCAP system (1). 3gAllergy utilizes a solid-phased liquid-phase reaction system that might have several potential advantages; 1) the amount of allergen can be optimized within the reaction system, 2) the biotinylated allergen epitopes within the liquid phase are less likely to be blocked by the solid phase, possibly resulting in better reaction kinetics and thermodynamics, and 3) the system exhibits less nonspecific absorption of antibodies (26, 27). Ultimately, the real question is whether the results of the assay are reliable for guiding clinical decisions. We have clearly shown in this study that both commercial assays have comparable performance in predicting OFC outcomes in egg allergy, although 3gAllergy has the slightly higher AUCs and LR+s than ImmunoCAP.

Recently, a systematic review reported on the diagnostic accuracy of skin prick testing (SPT) *vs* sIgE in the clinical diagnosis of food allergy (28). The authors comprehensively reviewed the risk of bias in the literature and proposed cutoff values at which SPT and sIgE measured by ImmunoCAP were diagnostic for EW allergy. In this study, which was conducted using children <2 years of age, allergy to raw egg seems very likely when, using EW extract, SPT generates urticaria ≥4 mm, or when sIgEs are ≥1.7 kU_{A}/L. In children ≥2 years, OFC could be avoided when SPT wheals with EW extract are ≥10 mm, prick by prick wheals are ≥14 mm, or specific IgE is ≥7.3 kU_{A}/L (28). Compared with these reported values, cutoffs found in our study were rather high: for ImmunoCAP, EW-sIgEs ≥18.7 kU_{A}/L and OM-sIgEs ≥5.7 kU_{A}/L were predictive of egg allergy in children <2 years, while in children ≥2 years, EW-sIgEs ≥11.5 kU_{A}/L and OM-sIgEs ≥6.1 kU_{A}/L were predictive (Table 3). However, this discrepancy does not indicate our results are inaccurate. We used different target doses of the challenge food in OFC: instead of using one whole egg equivalent of egg as in the former review, our target dose was set at 1/4 of whole egg. The OFCs in the former study were designed to test for complete tolerance to egg, while we aimed for investigating partial but reasonable tolerance of egg consumption. In reality, children who have outgrown their egg allergy do so in a gradual and continuous manner. During this process, a child in the progression of outgrowing egg allergy might not be able to tolerate the amount of protein found in a whole egg, but it can be beneficial for the child if small amounts of CE can be consumed. Because the criteria for a positive OFC were broad, the sIgE cutoff was higher.

It is notable that sIgE values measured by ImmnoCAP and 3 gAllergy are strongly correlated. Variability in values observed using assays from manufacturers might be attributable to the use of different capture antibodies and/or detection chemistries. This might result in different absolute values, and the heterogeneity of allergens used in the assays may also contribute to the disparity in values. Interestingly, however, we found good agreement between the two assays not only in the absolute values detected, but also in the probabilities estimated for OFC outcomes except for small discrepancy in OM-sIgE at low range (Figs S1 and S2). As we included the subjects who had documented egg sensitization based on positive EW-sIgE, and not on OM-sIgE, negative OM-sIgE results were more frequently observed with ImmunoCAP than 3gAllergy, which may indicate the latter assay is more sensitive than the former in the detection of OM-sIgE. Nevertheless, the findings have shown strong correlation between the two assays, indicating that both the ImmunoCAP and 3gAllergy accurately represent the clinical status of allergy, at least, with respect to egg allergy in children.

This study did have a limitation. We did not perform double-blind placebo-controlled food challenges (DBPCFC), the gold standard in the diagnosis of food allergy. The single-blinded OFC without placebo used in this study may be prone to bias. However, we considered the OFC to be positive only when objective clinical reactions were confirmed. Our intention was to find the predictive values in 'real-world' settings where DBPCFC is usually difficult to perform as a routine diagnostic procedure because of its time-consuming nature.

In conclusion, we evaluated two commercial *in vitro* methods (ImmunoCAP and 3gAllergy) to determine the predictive values of EW-sIgE and OM-sIgE in young children who require a confirmed diagnosis of egg allergy/tolerance. The value estimated to be predictive of OFC response in patients may be useful as a supportive diagnostic tool in egg allergy.

Conflicts of interest

Takao Fujisawa, Mizuho Nagao, and Setsuko Ito received lecture fees from Thermo Fisher Scientific and Siemens Healthcare Diagnostics. Yasunori Sato received lecture fees from Siemens Healthcare Diagnostics. Kanae Furuya declares no conflicts of interest.

Author contributions

Takao Fujisawa and Mizuho Nagao conceived the study. Takao Fujisawa, Setsuko Ito, and Yasunori Sato designed the study. Kanae Furuya made significant contributions to the acquisition and analysis of the data. Yasunori Sato performed statistical analysis. Kanae Furuya, Mizuho Nagao, and Takao Fujisawa wrote the manuscript. All authors made substantial contributions to the design, collection and interpretation of data; they all critically reviewed and approved the final manuscript.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Correlation between log-transformed values of EW sIgE (A) and OM sIgE (B) by ImmunoCAP and 3gAllergy.

Figure S2. Correlation between probabilities predicted by ImmunoCAP and 3gAllergy.

Table S1. Symptoms provoked by OFCs.

Table S2. Comparison of specific IgE levels between OFC positive and negative patients.

Table S3. Conversion table for ImmunoCAP to 3gAllergy.

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