

Ovalbumin-specific IgE/IgG4 ratio might improve the prediction of cooked and uncooked egg tolerance development in egg-allergic children

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Summary

Background Accurate predictors of natural tolerance development to cooked and uncooked egg are needed in egg-allergic patients.

Objective To compare the diagnostic performance of different immunological tests in relation to egg allergy versus tolerance.

Methods Children aged 5–18 years diagnosed with IgE-mediated egg allergy were prospectively recruited. All followed an egg-free diet. Prick test and specific IgE (sIgE) to ovalbumin, ovomucoid and egg white, ovalbumin-sIgG4 and ovomucoid-sIgG4 were determined. By boiled and raw egg challenges, children were classified as cooked egg allergic (CEA, $n = 50$) or tolerant (CET, $n = 35$), and uncooked egg allergic (UEA, $n = 64$) or tolerant (UET, $n = 21$). Statistics. Comparative analysis (CEA vs. CET and UEA vs. UET). Multivariate logistic regression. Partial receiver operating characteristic curve analysis of tests in relation to CEA and UEA. Negative decision points were defined as cut-offs with sensitivity 95%.

Results Ovalbumin-sIgG4 resulted an independent protective factor for uncooked egg allergy. To identify patients with high probability of egg tolerance, ovalbumin-sIgE/sIgG4 tended to perform better than sIgE and prick, specifically in children with ovalbumin-sIgE < 1.9 kU/L (for UEA) and ovomucoid-sIgE < 2.12 kU/L (for CEA). The most accurate cut-offs to recommend challenges were ovalbumin-sIgE/sIgG4 below 2.49 for cooked egg and 1.45 for uncooked egg, which associated 89.5% and 80% probability of tolerance (negative likelihood ratios 0.08 and 0.06), respectively. These cut-offs identified correctly as tolerant an additional 23% and 14% of children with negative challenges to cooked and uncooked egg, respectively, in comparison with sIgE negative decision points.

Additionally, prick test tended to perform better than sIgE alone in predicting cooked and uncooked egg tolerance for ovomucoid-sIgE < 0.92 kU/L and ovalbumin-sIgE < 1.37 kU/L, respectively.

Conclusions Ovalbumin-specific IgG4 is an independent predictor of tolerance development to uncooked egg. Ovalbumin-sIgE/sIgG4 ratio, followed by skin prick test (SPT), seems to perform better than sIgE in identifying egg-allergic children with high probability of tolerance to cooked and uncooked egg over follow-up.

Keywords diagnostic performance, egg allergy, food allergy, oral food challenge, ovalbumin, ovomucoid, skin prick test, specific IgE/IgG4 ratio, specific IgG4, tolerance

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Introduction

IgE-mediated egg allergy affects up to 8.9% of children in developed societies [1]. Strict avoidance is the accepted management [2, 3], which impairs nutrition and quality of life [3], as egg is a common ingredient.

Nonetheless, baked or cooked eggs are tolerated by 70–80% and 46–55% of egg-allergic children, respectively [1, 4–6]. Most egg-allergic children will outgrow egg allergy throughout childhood and adolescence [7–9], and cooked egg tolerance usually develops years before raw egg [8]. The molecular properties of the two major

allergens in egg white (EW) justify why heated egg has reduced allergenicity. Ovalbumin (OVA) is heat labile. Ovomucoid (OVM) is relatively stable to heat [6], but prolonged heating and interactions with the food matrix can reduce its allergenicity [10, 11].

Due to practical implications, mainly diet liberalization, there is growing interest to characterize these different clinical phenotypes of egg allergy and predict tolerance development. Different cut-offs for sIgE and skin prick test (SPT) have been proposed for egg allergy diagnosis [12–19] and follow-up [5, 8], but these tests are less useful below accepted decision points [20]. Previous evidence suggests that SPT might be helpful in children with low sIgE levels, as EW-SPT was different between tolerant and allergic individuals with EW-sIgE below 7 kU/L [21]. Although OVM-sIgE has shown better diagnostic performance to predict tolerance to cooked egg than EW- and OVA-sIgE [15, 19], the potential added diagnostic value of OVM-SPT has not been studied yet. Additionally, specific IgG4 (sIgG4) has shown a protective role during resolution of cow's milk allergy by blocking sIgE binding [22], but its role in natural tolerance development to egg remains unclear [23, 24]. Recent studies found improved diagnostic performance to estimate tolerance to baked and lightly cooked egg – steamed for 3 min – by combining sIgE and sIgG4 [25, 26].

In this context, we sought to evaluate the usefulness of OVA-sIgE/sIgG4 and OVM-sIgE/sIgG4, along with SPT and sIgE to EW, OVA and OVM to predict tolerance to well-cooked egg (without a wheat matrix) and uncooked egg, which are common foods in our Mediterranean diet, in school-aged children and teenagers on follow-up for IgE-mediated egg allergy.

We hypothesized that (1) allergen-specific IgG4 might be a predictor of tolerance and, accordingly, the sIgE/sIgG4 ratio might perform better than sIgE and SPT in predicting tolerance development and (2) SPT might provide added diagnostic value in children with low sIgE levels.

Methods

Subjects

Children aged 5–18 years on follow-up for IgE-mediated egg allergy, with persistently positive sIgE (> 0.35 kU/L) or SPT (> 3 mm) to EW, OVA or OVM, were prospectively recruited at Hospital Sant Joan de Déu (Barcelona, Spain). Diagnosis had been established within the first 2 years of life based on objective IgE-mediated allergic signs [3] within 2 hours after cooked egg intake plus positive EW-sIgE and EW-SPT. These cut-offs had shown 94% and 93% positive predictive value, respectively, for confirmation of egg allergy by

challenge in an equivalent series of Spanish children [12]. All patients had strictly avoided egg since diagnosis. Clinical data were registered from medical records, including age, gender, and allergic comorbidities (atopic dermatitis, rhinitis, multiple food allergies, and asthma). Asthma severity was assessed according to the Spanish Asthma Guideline [27].

The study was approved by the Ethical Committee of Clinical Research of Sant Joan de Déu Foundation (reference number: PIC-62-11). Written informed consent was obtained.

Procedures

Immunological tests were performed at inclusion, within the month prior to double-blind placebo-controlled food challenges (DBPCFC). SPT was performed on the forearm volar surface following the procedure described by Heinzerling *et al.* [28]. Commercial extracts of EW (4.6 mg/mL of purified protein), OVA (1 mg/mL) and OVM (1 mg/mL) (ALK-Abelló, Madrid, Spain) and 1 mm DHS lancets (Stallergenes Iberica, Madrid, Spain) were used. The mean between the largest weal diameter and its perpendicular diameter was recorded after 15 min using the Prick-film[®] reading method (Inmunotek, Madrid, Spain). sIgE to EW, OVA and OVM as well as sIgG4 to OVA and OVM were determined in serum by ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden). The lower detection limit was 0.1 kU/L for sIgE and 0.01 mg_A/L for sIgG4.

DBPCFC was performed in all cases. First, DBPCFC to cooked EW (boiled at 90°C for 10 min and blended with vegetable puree) was performed with a starting dose of 0.12 g EW protein (equivalent to 1/32 egg) [29]. Doses were doubled consecutively (0.24–0.48–0.96–1.9 g) with hourly intervals until objective IgE-mediated signs appeared [3]. The cumulative dose was 3.7 g EW protein, equivalent to the amount of EW in one egg [29]. If cooked EW was tolerated, then raw egg DBPCFC – masked with chocolate drink – was performed the following day. Consecutive doses were 0.48–0.96–1.9 g EW protein (cumulative dose: 3.4 g).

Children reacting to boiled egg constituted the 'cooked egg allergic' group (CEA), whereas tolerant children constituted the 'cooked egg tolerant' group (CET). Among CET children, those who tolerated raw egg were classified as 'uncooked egg tolerant' (UET), whereas children reacting to cooked or uncooked egg were classified as 'uncooked egg allergic' (UEA) (Fig. 1).

Statistics

Comparative analysis of clinical and immunological parameters was performed between groups (CEA vs.

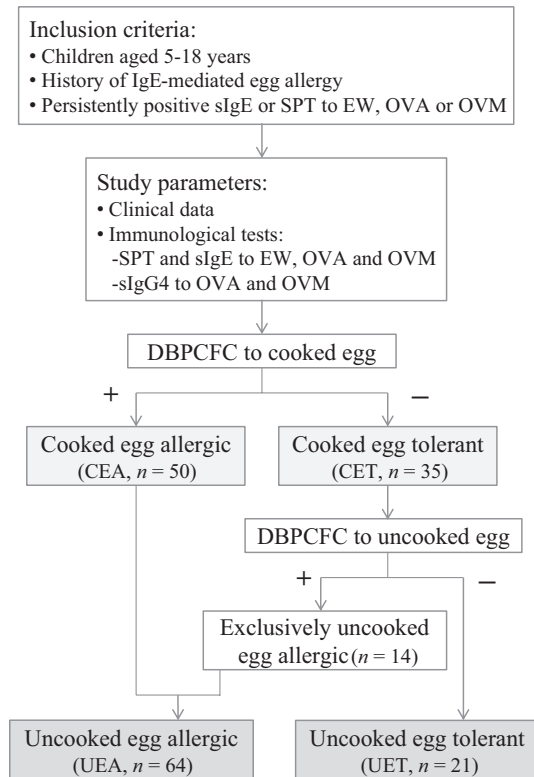


Fig. 1. Study flow chart. CEA: cooked egg allergic, CET: cooked egg tolerant, DBPCFC: double-blind placebo-controlled food challenge, EW: egg white, OVA: ovalbumin, OVM: ovomucoid, sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test, UET: uncooked egg tolerant, UEA: uncooked egg allergic, +: positive outcome at challenge, -: negative outcome at challenge.

CET and UEA vs. UET). Multivariate logistic regression analysis was used to identify independent predictors of reactivity to cooked egg (CEA vs. CET) and uncooked egg (UEA vs. UET). Diagnostic performance of all immunological tests was assessed by receiver operating characteristic (ROC) curve analysis. Challenge outcomes were used as reference parameters. As in previous studies [15, 25], the negative decision point of each test was defined as the cut-off producing a sensitivity of 95%, whereas the positive decision point as the cut-off producing a specificity of 95%. The test with the lowest negative likelihood ratio (NLR) and highest negative predictive value (NPV) at the negative decision point was selected as the most appropriate to predict tolerance. The test with the highest positive likelihood ratio (PLR) and positive predictive value (PPV) at the positive decision point was selected as the most appropriate to predict reactivity. Linear correlation between immunological parameters was studied through the Spearman's coefficient in the entire sample, as well as within groups. PASW Statistics 18 (SPSS Inc., Chicago, IL, USA), pROC-package for R statistics software [30] and GraphPad Prism (GraphPad Software Inc., San Diego,

CA, USA) were used. A two-tailed P value < 0.05 was considered significant.

Results

Eighty-five children were recruited. Mean age was 8.2 years (SD: 2.4). 58.8% had asthma, 67% rhinitis, 68.2% multiple food allergies, and 56.2% atopic dermatitis. Median EW-sIgE was 3.59 kU/L (range: 0.1–75 kU/L), whereas EW-SPT was 8 mm (range: 0–16) (Table S1). Fifty children reacted to cooked egg (CEA, 58.8%), whereas 35 tolerated it (CET, 41.2%). Twenty-one patients tolerated uncooked egg (UET, 24.7%), whereas 64 were considered uncooked egg allergic (UEA, 75.3%) (Fig. 1).

Children with UEA as a group had significantly lower OVA-sIgG4 levels, higher sIgE and SPT to EW, OVA and OVM, higher sIgE/sIgG4 ratios to OVA and OVM, as well as a higher moderate asthma rate compared with UET children. In contrast, no difference was observed for total IgE, OVM-sIgG4, age, gender, atopic eczema, rhinitis, or multiple food allergies between groups (Table S2). Similar differences were found between CEA and CET children, except for OVA-sIgG4 (Table S3).

Ovalbumin-sIgE and Ovalbumin-sIgG4 were identified as independent risk factors for a positive uncooked egg challenge, with odds ratios 4.42 for each increase in 1 kU/L (95% CI: 1.3–15, $P = 0.017$) and 0.46 for each increase in 1 mgA/L (95% CI: 0.23–0.92, $P = 0.028$), respectively. In contrast, OVM-sIgE was found as the only independent risk factor for a positive cooked egg challenge (odds ratio: 1.47 for each increase in 1 kU/L, 95% CI: 1.18–1.83, $P = 0.001$). OVA-sIgG4 showed also a trend towards a protective role in cooked egg allergy, but did not reach statistical significance (odds ratio: 0.48, 95% CI: 0.22–1.02, $P = 0.056$).

For the prediction of uncooked and cooked egg allergy using ROC curve analysis, sIgE and SPT to EW, OVA and OVM as well as sIgE/sIgG4 to OVA and OVM reached statistical significance, whereas OVA- and OVM-sIgG4 did not. EW-SPT did not reach significance either for cooked egg allergy (Tables 1 and 2, Figs 2 and 3 and Figs S1–S2). For uncooked egg allergy, OVA-sIgE/sIgG4 showed the best overall diagnostic performance followed by OVA-sIgE (AUC 0.880 and 0.858, respectively, $P < 0.001$). For cooked egg allergy, OVM-sIgE showed the best performance, followed by OVA-sIgE and OVA-sIgE/sIgG4 (AUC 0.829, 0.828 and 0.825, respectively, $P < 0.001$). Nonetheless, differences between ROC curves were not statistically significant ($P > 0.05$). Likelihood ratio curves for the most useful parameters in relation to challenge outcome are shown in Fig. 4.

Table 1. Diagnostic performance of immunological tests in predicting uncooked egg allergy versus tolerance in children on follow-up for IgE-mediated egg allergy

Predictive properties	Diagnostic test							
	Specific IgE/IgG4		Specific IgE (kU/L)			Skin prick test (mm)		
	OVA	OVM	EW	OVA	OVM	EW	OVA	OVM
Area under ROC curve	0.880	0.850	0.845	0.858	0.828	0.788	0.825	0.798
95% CI	0.789–0.971	0.760–0.940	0.756–0.934	0.773–0.942	0.735–0.921	0.668–0.908	0.698–0.953	0.672–0.924
<i>P</i> value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Negative decision point*	1.45	1	0.69	0.55	0.09	5	5	4.5
Sensitivity, Specificity (%)	94.8, 63.2	94.8, 52.6	94.8, 42.1	94.8, 47.4	94.8, 21.1	94.8, 36.8	96.6, 57.9	94.8, 47.4
PPV, NPV (%)	88, 80	86.7, 76.9	84.5, 75	84.5, 75	92.3, 62.5	80.3, 70	85, 78.8	83.6, 76.9
PLR, NLR	2.6, 0.06	2, 0.1	1.63, 0.12	1.8, 0.11	1.2, 0.25	1.5, 0.14	2.3, 0.08	1.8, 0.86
Positive decision point†	16.57	38.85	3.69	2.01	3.7	10	10	8.5
Sensitivity, Specificity (%)	56.9, 94.7	58.6, 94.7	58.6, 94.7	63.8, 94.7	55.2, 94.7	27.6, 94.7	27.6, 94.7	37.9, 94.7
PPV, NPV (%)	97.3, 43.2	97.4, 42.9	97.5, 44.2	97.7, 47.5	97.3, 41.3	94.7, 32.2	94.1, 31.7	95.6, 35
PLR, NLR	10.7, 0.45	11, 0.44	11, 0.44	12, 0.38	10.4, 0.47	5.2, 0.76	5.2, 0.76	7.1, 0.65

*Negative decision point defined as the cut-off level with 95% sensitivity.

†Positive decision point defined as the cut-off level with 95% specificity.

Cut-off values in bold correspond to the most accurate positive decision point to predict allergy and the most accurate negative decision point to predict tolerance to uncooked egg.

EW, egg white; NLR, negative likelihood ratio; NPV, negative predictive value; OVA, ovalbumin; OVM, ovomucoid; PLR, positive likelihood ratio; PPV, positive predictive value; CI, confidence interval.

Partial ROC curve analyses

We observed that ROC curves of OVA-sIgE/sIgG4 and OVA- or OVM-sIgE crossed at a particular specificity cutpoint, which delimitates two intervals of high and low specificity, respectively (Figs 2 and 3, see vertical line 'A'). This crossing indicates that one test performs better in one interval, whereas the other test performs better in the other interval [30]. In the present study, the tests cut-offs in the high specificity interval help identify children with high probability of allergy. In contrast, the cut-offs in the low specificity interval help identify children with high probability of tolerance. Under these premises, we performed partial area under curve (AUC) analyses to determine the test with the best diagnostic performance in each interval. Also, the crossing cutpoint was identified in each case, as it indicates the value from which one test might perform better than the other test.

For uncooked egg allergy, OVA-sIgE/sIgG4 and OVA-sIgE ROC curves crossed at the cutpoints of specificity 89.5% (Fig. 2), which corresponded to OVA-sIgE 1.9 kU/L. In the high specificity interval (100% to

89.5%), OVA-sIgE showed a trend towards better performance (Fig. 2). Accordingly, the OVA-sIgE positive decision point (2.01 kU/L) associated higher PLR (12) and PPV (97.7%) than any other test (Tables 1 and 3). In contrast, in the low specificity interval, OVA-sIgE/sIgG4 showed a trend towards better performance (Fig. 2) and its negative decision point (1.45) had lower NLR (0.06) and higher NPV (80%) than any other test.

For cooked egg allergy, ROC curves of OVA-sIgE/sIgG4 and OVM-sIgE crossed at the cutpoint of specificity 77.5% (Fig. 3), which corresponded to OVM-sIgE 2.12 kU/L. In the high specificity interval, OVM-sIgE showed a trend towards better performance (Fig. 3) and its positive decision point (3.7 kU/L) reached the highest PLR (10.4) and PPV (92.2%) (Tables 2 and 3). Similarly, in the low specificity interval, OVA-sIgE/sIgG4 showed a trend towards better performance and its negative decision point (2.49) reached the lowest NLR (0.08) and highest NPV (89.5%).

Additionally, the ROC curves of sIgE and SPT crossed as well, showing that SPT tended to have a better diagnostic performance than sIgE for levels below the crossing cutpoint (Figs 2 and 3, see vertical line 'B').

Table 2. Diagnostic performance of immunological tests in predicting cooked egg allergy versus tolerance in children on follow-up for IgE-mediated egg allergy

Predictive properties	Diagnostic test							
	Specific IgE/IgG4			Specific IgE (kU/L)			Skin prick test (mm)	
	OVA	OVM	EW	OVA	OVM	EW	OVA	OVM
Area under ROC curve	0.825	0.813	0.818	0.828	0.829	0.622	0.712	0.712
95% CI	0.722–0.929	0.717–0.91	0.726–0.91	0.737–0.92	0.74–0.919	0.493–0.751	0.591–0.833	0.588–0.837
<i>P</i> value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.07	0.002	0.002
Negative decision point*	2.49	1	0.69	0.55	0.23	5	5	4.5
Sensitivity, Specificity (%)	95.7, 54.8	95.7, 35.5	95.7, 29	95.7, 32.3	95.7, 29	95, 22.6	95.7, 35.5	95.7, 32.3
PPV, NPV (%)	74.6, 89.5	69.1, 84.6	66.6, 81.8	67.6, 83.3	67.6, 83.3	63.4, 70	67.2, 84.6	67.2, 84.6
PLR, NLR	2.1, 0.08	1.5, 0.12	1.3, 0.15	1.4, 0.13	1.4, 0.14	1.2, 0.28	1.5, 0.12	1.4, 0.13
Positive decision point†	81.3	113.3	4.08	2.8	3.74	11	10.5	13
Sensitivity, Specificity (%)	26.1, 95	43.5, 95	67.4, 95	67.4, 95	67.4, 95	15.2, 95	30.4, 95	6.5, 95.8
PPV, NPV (%)	81.2, 45.5	91.6, 52.6	92.1, 66.6	92.1, 66.6	92.2, 65.2	81.8, 44.3	87.5, 48.4	75, 42
PLR, NLR	4, 0.79	6.7, 0.6	10.4, 0.34	10.4, 0.35	10.4, 0.35	2.3, 0.91	4.7, 0.74	2, 0.97

*Negative decision point defined as the cut-off level with 95% sensitivity.

†Positive decision point defined as the cut-off level with 95% specificity.

Cut-off values in bold correspond to the most accurate positive decision point to predict allergy and the most accurate negative decision point to predict tolerance to cooked egg.

EW, egg white; NLR, negative likelihood ratio; NPV, negative predictive value; OVA, ovalbumin; OVM, ovomucoid; PLR, positive likelihood ratio; PPV, positive predictive value; CI, confidence interval.

In summary, the most accurate cut-offs to advise against challenges were OVA-sIgE above 2.01 kU/L for uncooked egg and OVM-sIgE above 3.7 kU/L for cooked egg. Instead, the most accurate cut-offs to identify children with high probability of tolerance and select patients for challenges were OVA-sIgE/sIgG4 below 1.45 for uncooked egg and 2.49 for cooked egg, followed by OVA-SPT below 5 mm and OVM-SPT below 4.5 mm, respectively (Table 3).

Linear correlations between sIgE and sIgG4

No significant linear correlation was found between sIgE and sIgG4 in the entire sample [Rho Spearman (rs): 0.149, *P* = 0.18 for OVA; rs: 0.169, *P* = 0.13 for OVM]. However, a positive linear correlation was found between OVA-sIgE and OVA-sIgG4 in CEA and UEA children (rs: 0.463, *P* = 0.001 for CEA; rs: 0.294, *P* = 0.02 for UEA), but not in other groups. OVA-sIgG4 and OVM-sIgG4 showed a good correlation (rs: 0.707, *P* < 0.001). OVM-sIgG4 levels were lower than OVA-sIgG4 levels (*P* < 0.001).

Discussion

Egg is a complex allergenic source whose allergenicity may be modified by heating plus/minus mixture with

wheat. DBPCFC is the gold standard for food allergy diagnosis, but it may be risky and costly. Thus, more accurate predictors of challenges outcome are under investigation. To our knowledge, this is the first work to compare the diagnostic performance of SPT, sIgE, and sIgG4 to OVA, OVM, and EW in predicting tolerance development to both well-cooked egg (with no wheat matrix) and uncooked egg in school-aged children and teenagers on follow-up for egg allergy. A similar study has been published for baked egg with consistent results [25]. As main contribution, we have shown that the OVA-sIgE/sIgG4 ratio and – to a fewer extent – SPT might predict tolerance to cooked and uncooked egg more accurately than sIgE in children with low sIgE levels. Instead sIgE levels identify more precisely children with high probability of reactivity.

Ovalbumin-sIgE and ovomucoid-sIgE were found as independent risk factors for positive challenges to uncooked and cooked egg, respectively. Accordingly, the most appropriate cut-offs to advise against challenges were OVA-sIgE above 2.01 kU/L for uncooked egg and OVM-sIgE above 3.7 kU/L for cooked egg, on account of 97.7% and 92.2% probability of reaction (PLR 12 and 10.4), respectively. Compared to previous series with equivalent methodology but including younger children, our OVA-sIgE cut-off for uncooked egg challenges coincided with a previous report [5]. In

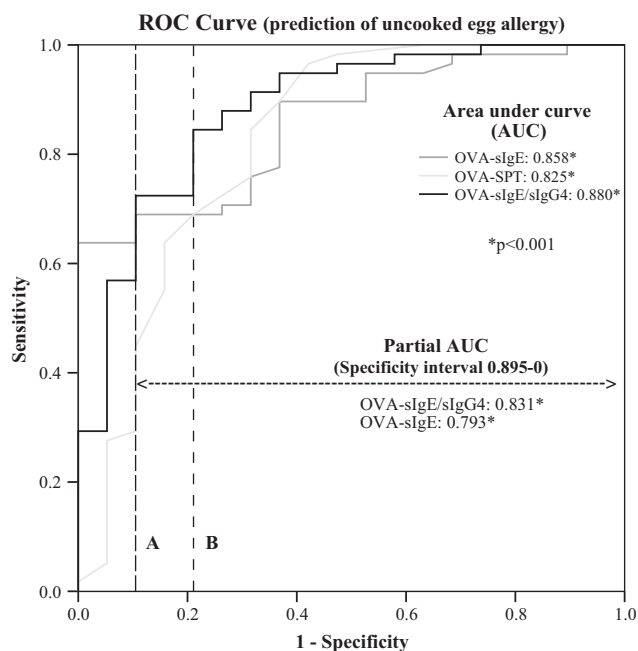


Fig. 2. Receiver operating characteristic (ROC) curve analysis showing the performance ovalbumin-specific IgE (OVA-sIgE), ovalbumin-specific IgE/IgG4 ratio (OVA-sIgE/sIgG4) and ovalbumin skin prick test (OVA-SPT) in relation to the outcome of uncooked egg challenges. Vertical line A showing the crossing point of OVA-sIgE and OVA-sIgE/sIgG4 curves, which delimitates two specificity intervals for partial ROC analysis. Vertical line B showing the crossing point of OVA-sIgE and OVA-SPT at values 1.37 kU/L and 7 mm, respectively, which delimitates two intervals for partial ROC curve analysis. Comparisons between partial ROC curves at each interval did not reach statistical significance. AUC: area under curve.

contrast, Ando et al. [15] proposed a higher cut-off to predict cooked egg reactivity (OVM-sIgE 10.8 kU/L). Different population characteristics, as well as a much longer boiling procedure (60 min), might explain this difference.

We hypothesized that sIgG4 might be a marker or a mechanism of tolerance development in egg allergy. In agreement with this, OVA-sIgG4 was higher in UET than in UEA individuals and OVA-sIgG4 resulted an independent protective factor for uncooked egg allergy. As we had hypothesized, OVA-sIgE/sIgG4 showed a trend towards better diagnostic performance than sIgE and SPT in identifying egg-allergic children with high probability of tolerance, that is, those with low sIgE levels (OVA-sIgE below 1.9 kU/L for uncooked egg and OVM-sIgE below 2.12 kU/L for cooked egg). Accordingly, the OVA-sIgE/sIgG4 negative decision points seem the most accurate to select children for challenges. Our findings support that the functional balance between sIgE and sIgG4 might have practical consequences in clinical tolerance to uncooked and cooked egg, as previously reported for baked and lightly

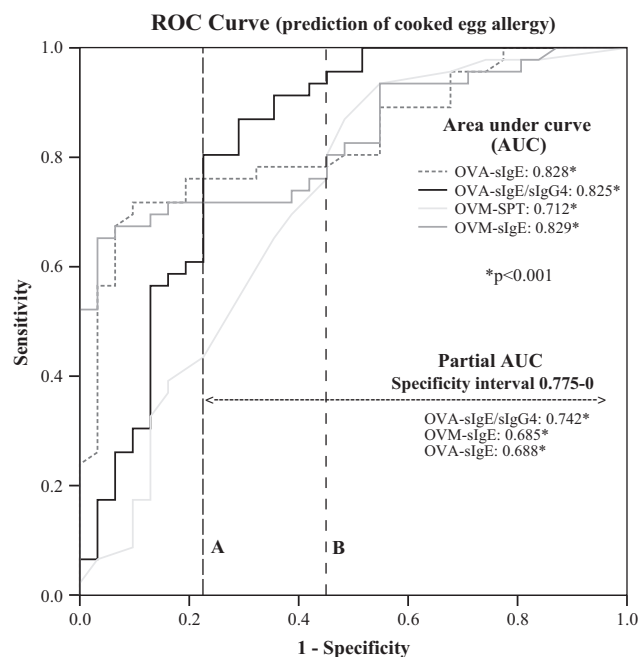
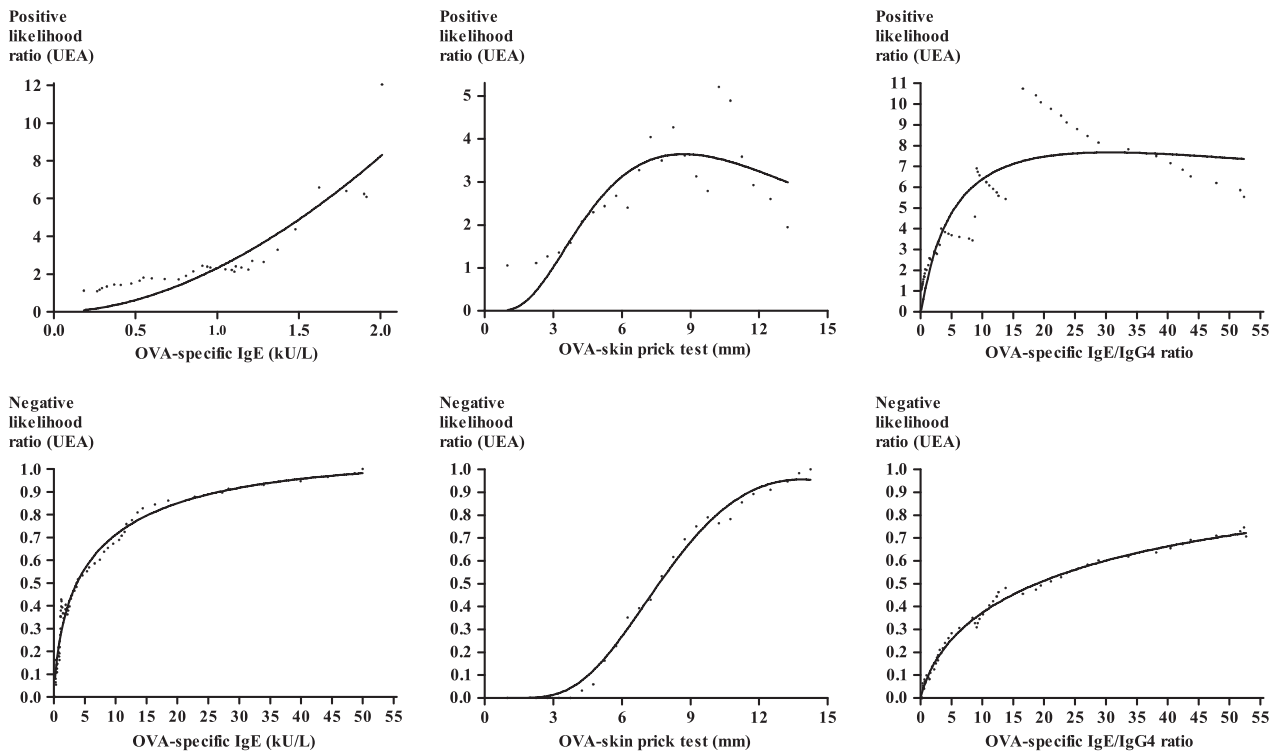


Fig. 3. Receiver operating characteristic (ROC) curve analysis showing the performance ovomucoid-specific IgE (OVM-sIgE), ovalbumin-specific IgE (OVA-sIgE), ovalbumin-specific IgE/IgG4 ratio (OVA-sIgE/sIgG4) and ovomucoid skin prick test (OVM-SPT) in relation to the outcome of cooked egg challenges. Vertical line A showing the crossing point of OVM-sIgE and OVA-sIgE/sIgG4 curves, which delimitates two specificity intervals for partial ROC analysis. Vertical line B showing the crossing point of OVM-sIgE and OVM-SPT at values 0.92 kU/L and 6 mm, respectively, which delimitates two intervals for partial ROC curve analysis. Comparisons between partial ROC curves at each interval did not reach statistical significance. AUC: area under curve.

cooked egg [25, 26]. Additionally, we found that sIgG4 and sIgE levels correlated within the CEA and UEA groups, as previously reported in cow's milk allergic patients [31]. That is, egg-allergic children with higher sIgE levels have also higher sIgG4 levels. This observation might explain why the sIgE/sIgG4 ratio seems to perform better than sIgE alone in children with low sIgE levels, but not in those with higher sIgE levels. This finding also arises the question whether sIgG4 plays any protective role in children with high sIgE levels and/or whether the affinity and epitope recognition pattern of sIgE and sIgG4 differ in them. Further research is needed to clarify this point. Of note, sIgE/sIgG4 to OVM – the dominant allergen in cooked egg – did not perform better than OVA-sIgE/sIgG4 in predicting cooked egg tolerance. The reasons for this remain unclear. First, OVA-sIgE and OVM-sIgE ROC curves for cooked egg allergy almost overlapped, which supports that OVA remains a relevant allergen after boiling egg for 10 min, as shown in a recent study using sera from five egg-allergic children [10]. This fact would also explain the low rate of cooked egg tolerance among

Prediction of uncooked egg allergy (UEA)



Prediction of cooked egg allergy (CEA)

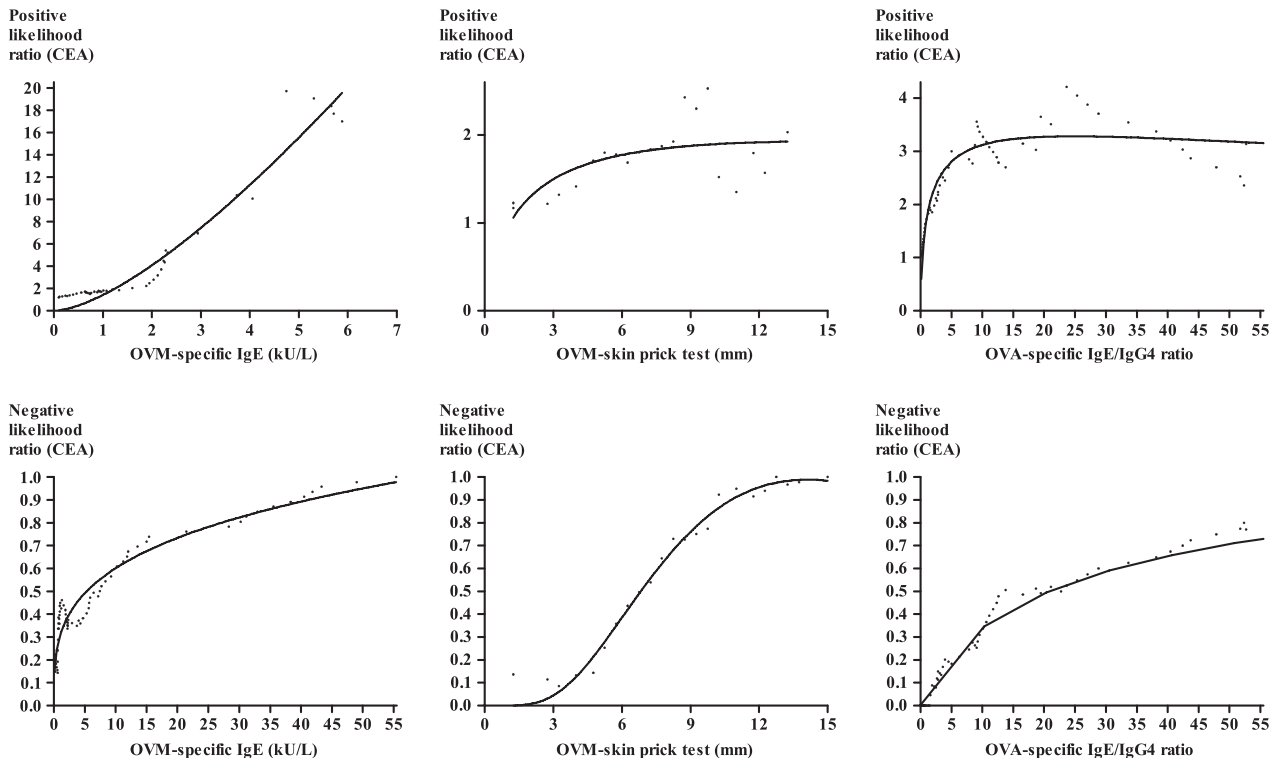


Fig. 4. Logistic regression curves of positive and negative likelihood ratios of the most useful immunological tests in relation to the outcome of challenges to uncooked and cooked egg. Positive likelihood ratio could not be calculated due to specificity 100% for values of OVA-specific IgE above 2.19 kU/L, OVA-skin prick test above 14.5 mm and OVA-specific IgE/IgG4 above 52.7 for uncooked egg allergy, as well as for OVM-specific IgE above 7.17 kU/L and OVM-skin prick test above 13.5 mm for cooked egg allergy. OVA: ovalbumin, OVM: ovomucoid.

Table 3. Proposed cut-offs of immunological tests to predict the outcome of challenges to uncooked and cooked egg in egg-allergic children on follow-up.

Diagnostic properties	Proposed cut-offs of diagnostic tests					
	For Uncooked Egg Allergy			For Cooked Egg Allergy		
	Negative decision point*		Positive decision Point [†]	Negative decision point*		Positive decision Point [†]
	1st option	2nd option		1st option	2nd option	
Cut-off value	OVA-sIgE/sIgG4: 1.45 [‡]	OVA-SPT: 5 mm	OVA-sIgE: 2.01 kU/L	OVA-sIgE/sIgG4: 2.49 [§]	OVM-SPT: 4.5 mm	OVM-sIgE: 3.74 kU/L
Ss, Sp (%)	94.8, 63.2	96.6, 57.9	63.8, 94.7	95.7, 54.8	95.7, 32.3	67.4, 95
PPV, NPV (%)	88, 80	85, 78.8	97.7, 47.5	74.6, 89.5	67.2, 84.6	92.2, 65.2
PLR, NLR	2.6, 0.06	2.3, 0.08	12, 0.38	2.12, 0.08	1.4, 0.13	10.4, 0.35

*Negative decision point defined as the cut-off value with 95% sensitivity.

[†]Positive decision point defined as the cut-off value with 95% specificity.

[‡]OVA-sIgE/sIgE < 1.45 was the most accurate cut-off to identify children with high probability of uncooked egg tolerance and to select children for uncooked egg challenge. This cut-off identified 57% of children (12 out of 21) with negative uncooked egg challenge. Instead, the OVA-sIgE and OVA-SPT negative decision points identified 42.8% and 52.4% of them, respectively ($P > 0.05$).

[§]OVA-sIgE/sIgE < 2.49 was the most accurate cut-off to identify children with high probability of cooked egg tolerance and to select children for cooked egg challenge. This cut-off identified 51.4% of children (18 out of 35) who had negative challenge to cooked egg. In contrast, the negative decision points of OVM-SPT and OVM-sIgE identified 34.3% and 28.5% of those children, respectively ($P > 0.05$).

NLR, negative likelihood ratio; NPV, negative predictive value; OVA, ovalbumin; OVM, ovomucoid; PLR, positive likelihood ratio; PPV, positive predictive value; Ss, sensitivity; Sp, specificity; SPT, skin prick test; sIgE, specific IgE; sIgG4, specific IgG4.

our egg-allergic patients (14 out of 64, i.e. 22%) in comparison with series that used baking or prolonged boiling [1, 4, 15, 25]. Secondly, OVM-sIgG4 levels were lower than OVA-sIgG4 levels. Ovomucoid accounts for 11% of EW weight [29], and this might explain lower exposure to OVM than OVA. Additionally, T-lymphocyte responses to OVM have been found weaker and less frequent than to OVA in egg-allergic individuals [23, 32].

Noteworthy, as we had hypothesized, SPT tended to perform better than sIgE in children with low sIgE levels (OVA-sIgE levels below 1.37 kU/L for uncooked egg and OVM-sIgE below 0.92 kU/L for cooked egg). Globally SPT tended to perform worse than sIgE and sIgE/sIgG4, particularly for cooked egg, but our work helps define in which cases SPT might provide added diagnostic value versus sIgE. As known, IgE is distributed in two pools: a circulating pool and a tissue-fixed pool bound to mast cells and basophils [33]. Due to the high affinity for IgE of Fc γ receptor on these cells, the IgE equilibrium would first favour tissue binding until saturation is reached, relatively depleting serum IgE levels [33]. This mechanism may justify why in children with low sIgE levels SPT might reflect better clinical reactivity than sIgE, supporting that both tests are not interchangeable in egg-allergic patients' follow-up [34].

As immunological tests have limitations within the low range to discriminate allergic versus tolerant individuals – even when including sIgE/sIgG4 and SPT – a diagnostic algorithm that includes clinical data might improve predictive accuracy. Interestingly, DunnGalvin *et al.* [20] found age, gender, and symptoms on previous

exposures (along with sIgE, SPT and IgE-minus-sIgE) as independent predictors of reactivity to egg. Accordingly, a regression model that combined these clinical data improved diagnostic performance to select children for challenges, which seems very useful. However, in our work, age, gender, total IgE, and allergic comorbidities showed no predictive value in egg tolerance development. Different selection criteria might explain these differences. Children in our series were older (8.2 vs. 5.5 years), all had reacted to cooked egg in the past, and all had persistently positive sIgE or SPT. Accordingly, 75% reacted to egg at DBPCFC. Instead, 25–30% of children in DunnGalvin's study were sensitized to egg but had never eaten it and only 39–58% reacted at DBPCFC. Thus, it remains unclear whether DunnGalvin's results are applicable to our population. The potential added diagnostic value of 'symptoms on previous exposure' in our population requires further evaluation.

Finally, we acknowledge some limitations in the present study. First, it has a cross-sectional design. Thus, the relationship between sIgG4 and tolerance development over time should be further addressed through longitudinal studies. Moreover, sIgG4 levels may depend on the amount of previous food exposure [24], which cannot be exactly assessed in our work. All children recruited had eaten egg – leading to allergic reactions – but the processing method, amount, and number of exposures might have been heterogeneous among them. Likewise, all had avoided egg since diagnosis, but unnoticed exposures might have occurred. Of note, previous studies about the role of sIgG4 in egg

tolerance development do not provide either a careful record of previous egg intake [23–26, 35]. Thus, future research should address the exact impact of egg exposure in sIgG4 levels in egg-allergic children and its role in tolerance development over time. Additionally, our results are based on a limited number of individuals, and accordingly, differences in predictive properties among immunological parameters did not reach statistical significance. Thus, our findings should be confirmed and validated in a larger second cohort including younger patients. Of note, Okamoto et al. [26] found no differences between children aged above or below 5 years in the performance of sIgE, sIgG4, and sIgE/sIgG4 to EW in predicting allergy to lightly cooked egg.

In conclusion, OVA-sIgE/sIgG4 tends to perform better than sIgE and SPT in identifying egg-allergic patients with high probability of tolerance, specifically, in patients with OVA-sIgE or OVM-sIgE below 2 kU/L. In them, the most accurate cut-offs to recommend challenges may be OVA-sIgE/sIgG4 below 2.49, which involves 89.5% probability of cooked egg tolerance (NLR: 0.08) and OVA-sIgE/sIgG4 below 1.45, which indicates 80% probability of uncooked egg tolerance (NLR:0.06). SPT tends also to perform better than sIgE alone in children with low sIgE levels.

Conflicts of interest

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Supporting Information

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

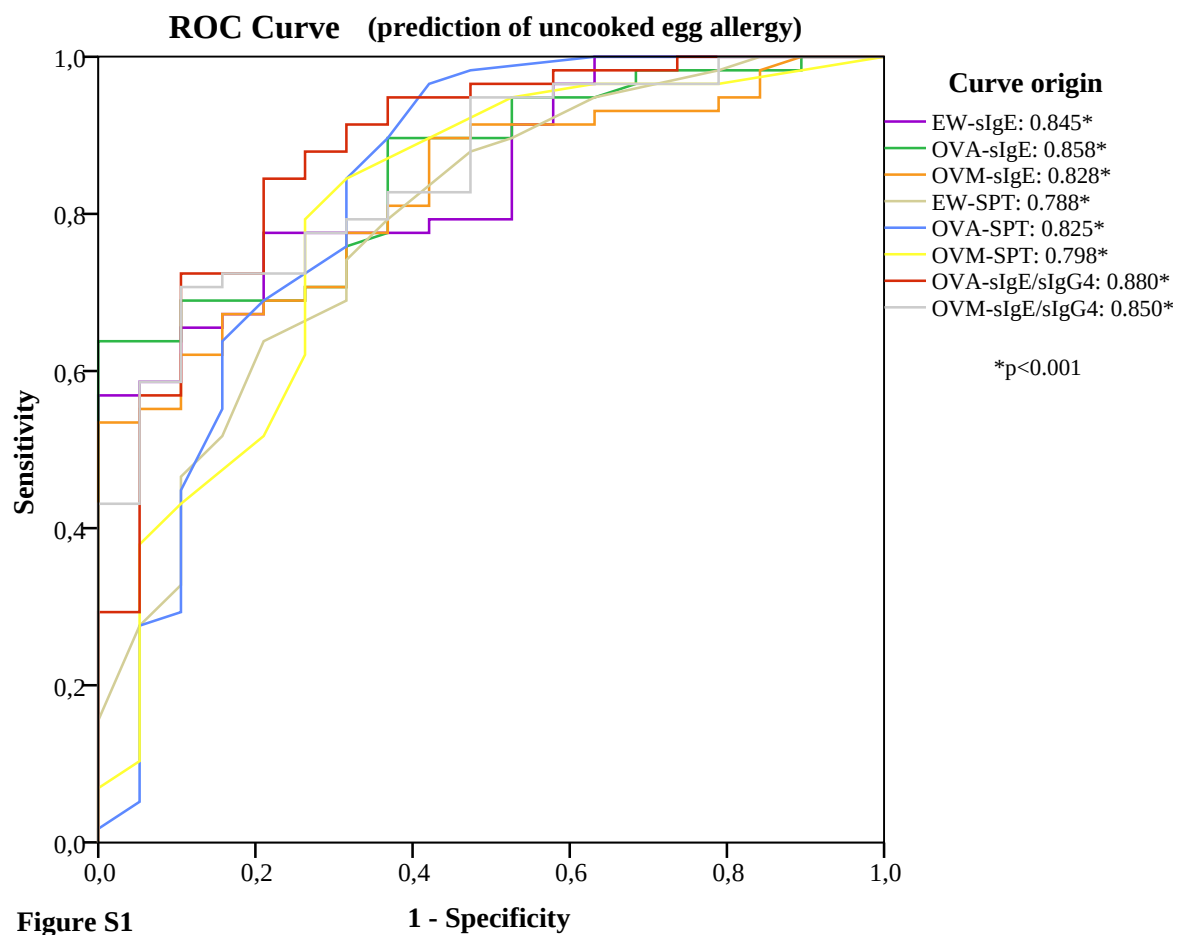
Figure S1. Receiver operating characteristic (ROC) curve analysis showing the performance of immunological tests in relation to uncooked egg allergy.

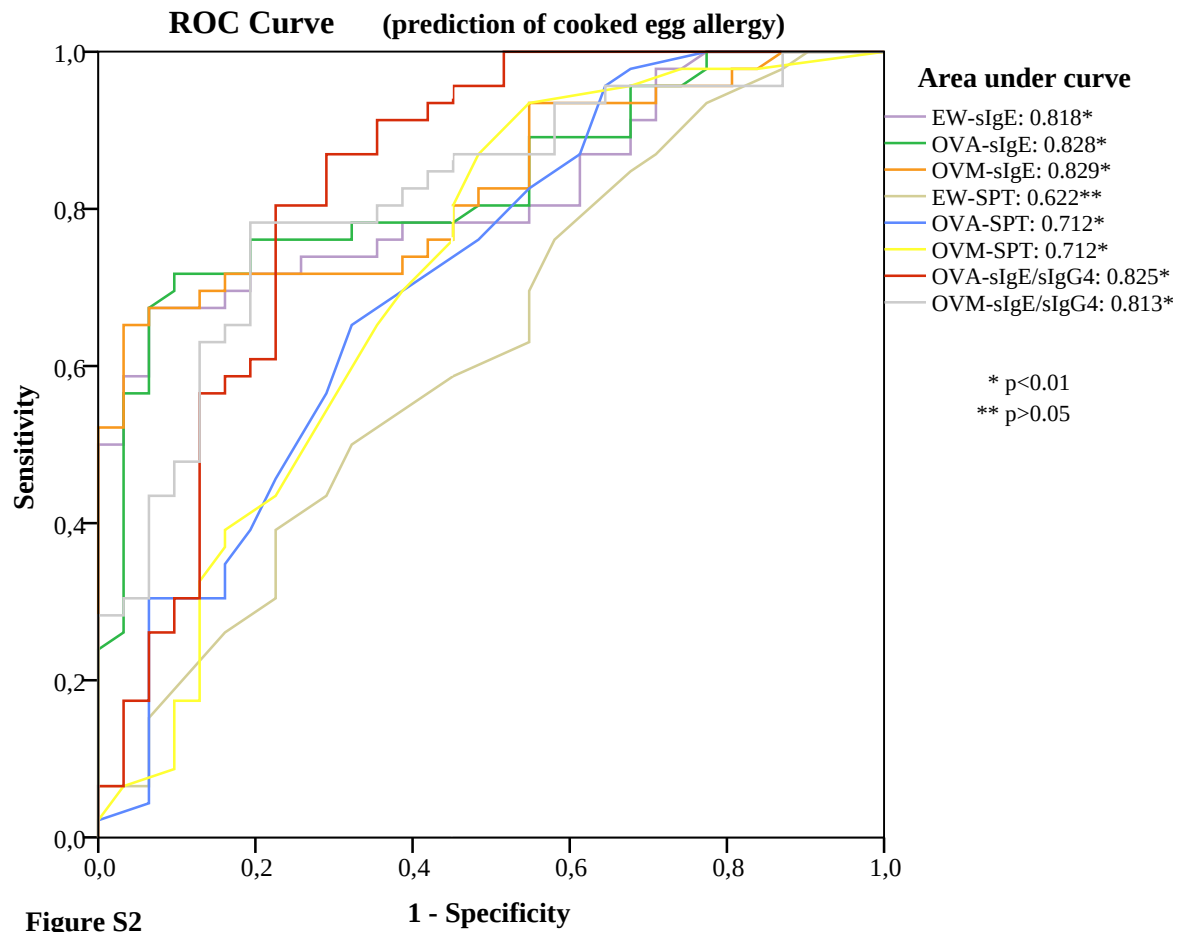
Figure S2. Receiver operating characteristic (ROC) curve analysis showing the performance of immunological tests in relation to cooked egg allergy.

Table S1. Characteristics of subjects recruited.

Table S2. Comparative analysis between uncooked egg-allergic children (UEA) and uncooked egg tolerant (UET).

Table S3. Comparative analysis between cooked egg-allergic children (CEA) and cooked egg tolerant (CET).





Tables for supplementary material

Table S1.

Characteristics of subjects recruited.

Descriptive data	Total sample (n= 85)
Age (years, mean, SD)	8.24 (2.4)
Gender (% males)	51.8%
Atopic eczema	56.5%
Multiple food allergies	68.2%
Asthma	58.8%
Moderate asthma	25.9%
Rhinitis	67%
Immunological tests (median, range)	
Total IgE (KU/L)	550 (44-8816)
EW-sIgE (KU/L)	3.59 (0.1-75)
OVA-sIgE (KU/L)	2.37 (0.1-49)
OVM-sIgE (KU/L)	2.28 (0.1-54.4)
EW-SPT (mm)	8 (0-16)
OVA-SPT (mm)	7.5 (0-15)
OVM-SPT (mm)	7.5 (0-14)
OVA-sIgG4 (mgA/L)	0.23 (0.01-10.4)
OVM-sIgG4 (mgA/L)	0.08 (0.01-9.85)
OVA-sIgE/sIgG4 ratio	12.15 (0.07-1510)
OVM-sIgE/sIgG4 ratio	36.56 (0.05-2720)

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid, SD: standard deviation, sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test.

Table S2.

Comparative analysis between uncooked egg allergic children (UEA) and uncooked egg tolerant (UET).

Descriptive data	Uncooked egg allergic (UEA, n= 64)	Uncooked egg tolerant (UET, n= 21)	UEA vs UET p value
Age (years, mean, SD)	8.3 (2.5)	8 (2.2)	0.66 [§]
Gender (% males)	46.8%	66.7%	0.13 [†]
Atopic eczema	62.5%	38.1%	0.05 [†]
Multiple food allergies	76.5%	52.4%	0.058 [†]
Asthma	57.8%	66.6%	0.57 [†]
Moderate asthma	32.8%	4.8%	0.01 [†]
Rhinitis	73.4%	61.9%	0.5 [†]
Immunological tests (median, range)			
Total IgE (kU/L)	495 (44-8816)	688 (75-7123)	0.19*
EW-sIgE (kU/L)	6.28 (0.51-75)	1.06 (0.1-4.03)	<0.001*
OVA-sIgE (kU/L)	3,89 (0.26-49)	0.81 (0.1-2.1)	<0.001*
OVM-sIgE (kU/L)	5.66 (0.1-54.4)	0.39 (0.1-4.14)	<0.001*
EW-SPT (mm)	8.5 (4-16)	5.5 (0-10.5)	<0.001*
OVA-SPT (mm)	8 (4-15)	4.5 (0-14.5)	<0.001*
OVM-SPT (mm)	8 (0-14)	4.5 (0-13)	<0.001*
OVA-sIgG4 (mgA/L)	0.21 (0.01-10.4)	0.57 (0.03-9.73)	0.02*
OVM-sIgG4 (mgA/L)	0.07 (0.01-2.1)	0.1 (0.01-9.85)	0.1*
OVA-sIgE/sIgG4	23.11 (0.33-1510)	0.93 (0.07-52.5)	<0.001*
OVM-sIgE/sIgG4	54.62 (0.07-2720)	0.76 (0.05-70.2)	<0.001*

Tests used: [§] T student test, [†] Chi-square test and *U-Mann Whitney test.

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid, SD: standard deviation, sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test.

Table S3.

Comparative analysis between cooked egg allergic children (CEA) and cooked egg tolerant (CET).

Descriptive data	Cooked egg allergic (CEA, n= 50)	Cooked egg tolerant (CET, n= 34)	CEA vs CET p value
Age (years, mean, SD)	8.3 (2.1)	8.2 (2.6)	0.63 [§]
Gender (% males)	44%	64.7%	0.06 [†]
Atopic eczema	62%	47.1%	0.15 [†]
Multiple food allergies	64%	38.2%	0.21 [†]
Asthma	62%	52.9%	0.46 [†]
Moderate asthma	40%	5.9%	0.001 [†]
Rhinitis	72%	61.8%	0.32 [†]
Immunological tests (median, range)			
Total IgE (kU/L)	613 (44-8816)	424 (64-7123)	0.58*
EW-sIgE (kU/L)	10.3 (0.51-75)	1.45 (0.1-9.99)	<0.001*
OVA-sIgE (kU/L)	7.4 (0.39-49)	1.1 (0.1-13.4)	<0.001*
OVM-sIgE (kU/L)	7.41 (0.1-54.4)	0.78 (0.1-6.93)	<0.001*
EW-SPT (mm)	8.5 (4-16)	7.5 (0-13.5)	0.02*
OVA-SPT (mm)	8 (4-15)	6 (0-14.5)	<0.001*
OVM-SPT (mm)	8 (0-14)	5 (0-13.5)	0.001*
OVA-sIgG4 (mgA/L)	0.19 (0.01-10.4)	0.31 (0.01-9.73)	0.12*
OVM-sIgG4 (mgA/L)	0.08 (0.01-2.1)	0.08 (0.01-9.85)	0.86*
OVA-sIgE/sIgG4	27.07 (1.5-1510)	2.44 (0.07-228)	<0.001*
OVM-sIgE/sIgG4	66.73 (0.07-2720)	4.61 (0.05-210)	<0.001*

Tests used: [§] T student test, [†] Chi-square test and *U-Mann Whitney test.

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid, SD: standard deviation, sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test.

Figures for supplementary material

Figure S1.

Receiver operating characteristic (ROC) curve analysis showing the performance of immunological tests in relation to uncooked egg allergy.

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid. sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test.

Figure S2

Receiver operating characteristic (ROC) curve analysis showing the performance of immunological tests in relation to cooked egg allergy.

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid, sIgE: specific IgE, sIgG4: specific IgG4, SPT: skin prick test.