

Article Type: Letter to the Editor

Title

Ovalbumin-specific IgE/total IgE ratio improves the prediction of tolerance development in egg-allergic children aged ≥ 5 years.

Authors

Vazquez-Ortiz M, MD PhD; Machinena-Spera A, MD; Giner MT, MD; Alvaro M, MD PhD; Piquer M, MD; Dominguez O, MD; Lozano J, MD; Jimenez-Feijoo, R, MD; Plaza AM, MD PhD.

Affiliation

Paediatric Allergy and Clinical Immunology Section. Hospital Sant Joan de Déu. Universitat de Barcelona. Spain.

Corresponding autor

Marta Vazquez-Ortiz, MD PhD

Address: Passeig de Sant Joan de Déu, 2. Esplugues de Llobregat. 08950. Barcelona. Spain.

Email: marta.vazquez.ortiz@gmail.com

Telephone number : +34 932532100

Fax number: +34 936009782

Running title

Specific IgE/total IgE ratio in egg allergy

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12417

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Abbreviations

DBPCFC: double-blind placebo-controlled food challenges

EW: egg white

OVA: ovalbumin

OVM: ovomucoid

sIgE: specific IgE

sIgE/total IgE: the ratio of specific IgE to total IgE

SPT: skin prick test

Key words

Egg allergy, children, tolerance, allergy diagnosis, specific IgE/total IgE ratio.

To the editor,

Egg allergy is amongst the most common food allergies, affecting 0.2%-2.5% of children in Europe (1) and 8.9% in Australia (2). Fortunately, most children develop natural tolerance during childhood. Identifying tolerance development is key for removing “the label” of egg allergy, introducing the food into the diet safely and reversing the adverse impact on quality of life and nutrition. Tolerance is best confirmed by oral food challenge, but these are costly and can be risky. Several parameters have been proposed to be helpful in predicting tolerance and selecting likely-tolerant children for challenges, such as specific IgE (sIgE), skin prick testing (SPT) and the sIgE/sIgG₄ ratio (3,4). However, the diagnostic utility is limited by high false positive rates (5) with many children falling in the “grey zone” between the positive and negative decision points (6). Therefore, more accurate predictors of tolerance development are needed.

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Total IgE might cause artifactual elevation of specific IgE levels, possibly leading to misdiagnosis of food allergy. Although a study published 10 years ago reported that the egg-sIgE/total IgE ratio was not superior to sIgE alone in young children with suspected egg allergy (7), more recent evidence suggests that total IgE influences allergen-specific IgE levels (8) and challenge outcome (9). Accordingly, the sIgE/total IgE ratio was found to have better diagnostic performance than sIgE alone for persistent food allergens – particularly for nuts (10). From age 5 years onwards, total IgE and sIgE levels run in parallel, whereas their correlation in younger children is poor (11). In this context, we evaluated the usefulness of the sIgE/total IgE ratio to egg white (EW), ovalbumin (OVA) and ovomucoid (OVM) (in comparison to sIgE and SPT alone) to predict raw egg tolerance development in children aged ≥ 5 years with IgE-mediated egg allergy.

We prospectively recruited 95 children aged 5–18 years with a diagnosis of IgE-mediated egg allergy which had been established within the first 2 years of life based on a previous immediate reaction to egg plus evidence of sensitization (positive EW-sIgE and/or EW-SPT). Patients were on an egg-free diet. Clinical data (including age, gender, atopic dermatitis, rhinitis, multiple food allergies and asthma) were recorded. Immunological tests (SPT and sIgE to EW, OVA and OVM) were performed within the month prior to the double-blind placebo-controlled food challenge (DBPCFC) as described elsewhere (4). All children underwent DBPCFC to raw egg masked in a chocolate drink (cumulative dose: 3.4 g EW protein) as previously described (4), with outcome assessed according to PRACTALL consensus. Ethics approval and written informed consent were obtained.

We performed comparative analysis of clinical and immunological parameters between egg-allergic and tolerant groups. Diagnostic performance was assessed by receiver operating characteristic (ROC) curve analysis, with challenge outcome as the reference parameter. From ROC analyses, the cut-off producing a sensitivity of 95% (i.e. negative decision point) and the cut-off producing a specificity of 95% (i.e. positive decision point) were obtained as in previous studies (4).

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 and GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) were used. A two-tailed P value < 0.05 was considered significant.

23.2% of children passed the raw egg DBPCFC (22/95, egg-tolerant group), whereas 76.8% reacted (73/95, egg-allergic group). Patients' clinical and immunological parameters are summarized in Table 1 for the entire sample and for groups. Mean age was 7.9 years (SD: 2.4).

Egg-allergic children had significantly higher sIgE, SPT and sIgE/total IgE levels, higher atopic eczema rates and lower total IgE levels than egg-tolerant children. Total IgE and OVA-sIgE showed moderate linear correlation in the entire sample (R_s : 0.475, $p < 0.001$), whereas very good correlation in the egg-tolerant group (R_s : 0.868, $p < 0.001$) and good in the egg-allergic group (R_s : 0.611, $p < 0.001$). Similar results were found for EW-sIgE and OVM-sIgE in relation to total IgE.

OVA-sIgE/total IgE obtained the highest AUC in ROC curve analysis (AUC: 0.984, $p < 0.001$, Fig 1). The difference between the AUC for OVA-sIgE/total IgE and the AUC for sIgE and SPT to EW, OVA and OVM was statistically significant ($p < 0.01$ for all comparisons). OVA-sIgE obtained better results than EW-sIgE and OVM-sIgE. A similar observation was found for OVA-SPT in relation to EW-SPT and OVM-SPT (Fig 1). The diagnostic properties of OVA-sIgE/total IgE, OVA-sIgE and OVA-SPT were compared (Table 2). The positive decision point for OVA-sIgE/total IgE (value: 0.0023) was associated with higher diagnostic accuracy than that for OVA-sIgE and OVA-SPT (92.6% vs 67.4% and 37.9%, respectively, $p < 0.001$ for both comparisons). Accordingly, the positive decision point for OVA-sIgE/total IgE allowed the correct classification of an additional 54.7% and 25.6% of patients above that for OVA-SPT and OVA-sIgE respectively. Similar results (although less pronounced) were obtained for OVA-sIgE/total IgE negative decision point (Table 2, $p < 0.05$ for both comparisons).

Our results show that the diagnostic performance of OVA-sIgE/total IgE is excellent, and superior to sIgE or SPT to EW, OVA or OVM alone in predicting raw egg tolerance development in school-aged children and teenagers with IgE-mediated egg allergy. The proposed positive and negative decision points of OVA-sIgE/total IgE have better predictive accuracy, assisting the clinician in predicting who is likely to tolerate egg at formal challenge.

Our observation that egg-tolerant (as opposed to egg-allergic) individuals have higher total IgE levels which correlate strongly with sIgE, support the hypothesis that higher total IgE titers might contribute to elevated but clinically irrelevant sIgE levels. In agreement, in a recent study children with the highest total IgE levels (>75th centile) were less responsive in challenges to cow's milk and egg than those with lower total IgE, even for similar values of sIgE (9). Federly et al found that total IgE levels significantly correlated to sIgE levels to cow's milk, egg, wheat and soya. Indeed, a predictive model was developed to determine sIgE based on total IgE (8). Gupta et al reported improved diagnostic performance of sIgE/total IgE to predict tolerance to persistent food allergens (nuts, seeds and shellfish) (10). However, in contrast to our report, similar results were found for sIgE and sIgE/total IgE for less persistent food allergens (milk, egg, wheat, soy) (10), as Mehl et al had reported for egg (7) and our group for milk (12). This might be partly explained by differences in study populations: in our milk study, patients were younger (median age 3.4 years) (12), and while the ages are not described in the study by Gupta et al., the authors do suggest that children undergoing milk/egg/soya/wheat challenges were younger than those undergoing challenges to nuts/seeds/shellfish (10). Likewise in Mehl's study, challenges were generally performed to confirm or rule out egg allergy diagnosis in much younger children (median age 13 months) with suspected food allergy, ie including egg-sensitized children without previous reactions to egg (7). In contrast, this report addressed tolerance development in school-aged children and teenagers with egg allergy diagnosed in infancy. Future studies should confirm whether the usefulness of the sIgE/total IgE ratio varies in these two different cohorts and/or depending on age, since total IgE and sIgE levels are known to correlate poorly below age 5 years (11). Based on recent evidence, including our

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results, we believe that the usefulness of the sIgE/total IgE ratio to predict tolerance to food allergens needs to be revisited in larger studies which can control for factors potentially influencing the results.

In conclusion, in this cohort, the diagnostic performance of the OVA-sIgE/total IgE ratio was superior to sIgE or SPT to EW, OVA or OVM alone in predicting raw egg tolerance development in school-aged children and teenagers with IgE-mediated egg allergy.

Vazquez-Ortiz M, MD PhD

Machinena-Spera A, MD

Giner MT, MD

Alvaro M, MD PhD

Piquer M, MD

Dominguez O, MD

Lozano J, MD

Jimenez-Feijoo, R, MD

Plaza AM, MD PhD

Paediatric Allergy and Clinical Immunology Section. Hospital Sant Joan de Déu. Universitat de
Barcelona. Spain.

Acknowledgments

The authors would like to thank Dr Paul Turner, MRC Clinician Scientist at Imperial College London, for his valuable comments on this letter.

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Table 1.

Characteristics of subjects recruited and comparative analysis between raw egg-allergic and egg-tolerant children.

Abbreviations: EW: egg white, OVA: ovalbumin, OVM: ovomucoid, sIgE: specific IgE, sIgE/total IgE: the ratio of specific IgE to total IgE, SPT: skin prick test.

Descriptive data	Entire sample (n= 95)	Egg-allergic (n= 73)	Egg-tolerant (n= 22)	p value
Age (years, mean, SD)	7.9 (2.4)	7.7 (2.3)	8.4 (2.6)	0.280
Gender (% male)	53.7%	50.7%	63.6%	0.286
Atopic eczema	57.9%	64.4%	36.4%	0.020
Multiple food allergy	58.9%	64.3%	52.4%	0.325
Asthma	48.4%	45.2%	59.0%	0.325
Immunological tests (median, range)				
Total IgE (KU/L)	382 (14-7123)	350 (14-6972)	633 (54-7123)	0.079
EW-sIgE (KU/L)	2.59 (0.02-75.00)	4.30 (0.14-75.00)	0.76 (0.02-4.03)	<0.001
OVA-sIgE (KU/L)	1.69 (0.02-49.00)	2.98 (0.16-49.00)	0.67 (0.02-2.11)	<0.001
OVM-sIgE (KU/L)	1.62 (0.01-54.40)	2.29 (0.1-54.4)	0.33 (0.01-4.14)	<0.001
EW-SPT (mm)	8 (0-16)	8 (4-16)	5.25 (0-10.5)	<0.001
OVA-SPT (mm)	7.5 (0-15)	8 (0-15)	4.25 (0-14.5)	<0.001
OVM-SPT (mm)	7 (0-14)	7.5 (0-14)	3.5 (0-13)	<0.001
EW-sIgE/total IgE	0.0069 (0.0002-0.2777)	0.0123 (0.001-0.2777)	0.0011 (0.0002-0.0143)	<0.001
OVA-sIgE/ total IgE	0.0059 (0.0003-0.2881)	0.0083 (0.0015-0.2881)	0.0009 (0.0003-0.0025)	<0.001
OVM-sIgE/ total IgE	0.005 (0.0001-0.3138)	0.007 (0.0001-0.3138)	0.0006 (0.0001-0.0031)	<0.001

Table 2.

Predictive properties of immunological tests in predicting raw egg allergy versus tolerance in children on follow-up for IgE-mediated egg allergy.

¹Negative decision point defined as the cut-off level with 95% sensitivity.

²Positive decision point defined as the cut-off level with 95% specificity.

*p<0.05 in comparison to OVA-sIgE negative decision point.

[#]p<0.001 in comparison to OVA-sIgE positive decision point and OVA-SPT positive decision point.

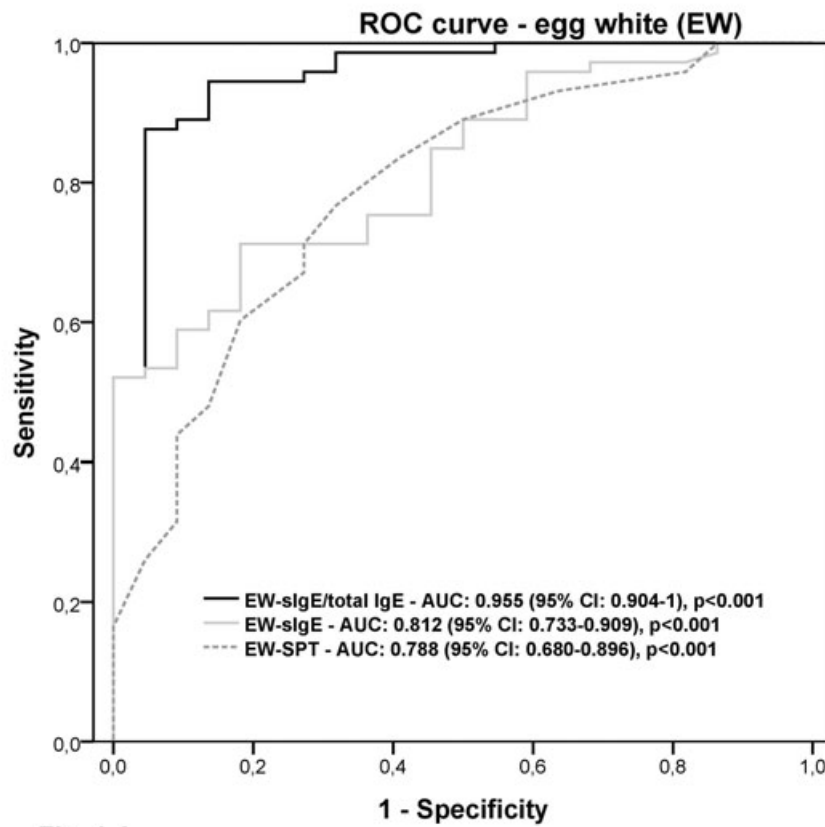
Abbreviations: NLR: negative likelihood ratio, NPV: negative predictive value, OVA: ovalbumin, PLR: positive likelihood ratio, PPV: positive predictive value, ROC: receiver operating characteristics, sIgE: specific IgE, sIgE/total IgE: the ratio of specific IgE to total IgE, SPT: skin prick test.

Predictive properties	Diagnostic test		
	OVA-sIgE/total IgE	OVA-sIgE	OVA-SPT
Area under ROC curve	0.984	0.828	0.822
CI 95%	0.966-1	0.744-0.912	0.708-0.937
p value	<0.001	<0.001	<0.001
Negative decision point ¹	0.0019	0.295	4.25
Sensitivity, Specificity (%)	94.5, 81.8	94.5, 27.3	94.5, 50
PPV, NPV (%)	94.5, 81.8	81.2, 60	86.3, 73.3
PLR, NLR	5.2, 0.067	1.3, 0.2	1.89, 0.1
Diagnostic accuracy (%)	91.6*	78.9	84.2
Positive decision point ²	0.0023	2.01	10.25
Sensitivity, Specificity (%)	91.8, 95.5	58.9, 95.5	20.5, 95.5
PPV, NPV (%)	98.5 , 77.8	97.7, 41.2	93.8, 26.6
PLR, NLR	20.2 , 0.086	12.96, 0.43	4.5, 0.83
Diagnostic accuracy (%)	92.6[#]	67.4	37.9

Figure 1.

Receiver operating characteristic (ROC) curve analysis showing the performance of specific IgE (slgE), specific IgE/total IgE ratio (slgE/total IgE) and skin prick test (SPT) to egg white (EW, Fig 1. A), ovalbumin (OVA, Fig. 1. B) and ovomucoid (OVM, Fig. 1. C) in relation to the outcome of raw egg challenges.

The differences between the area under curve (AUC) for OVA-slge/total IgE and the AUC for slgE and SPT to EW, OVA and OVM were statistically significant ($p < 0.01$ for all comparisons).



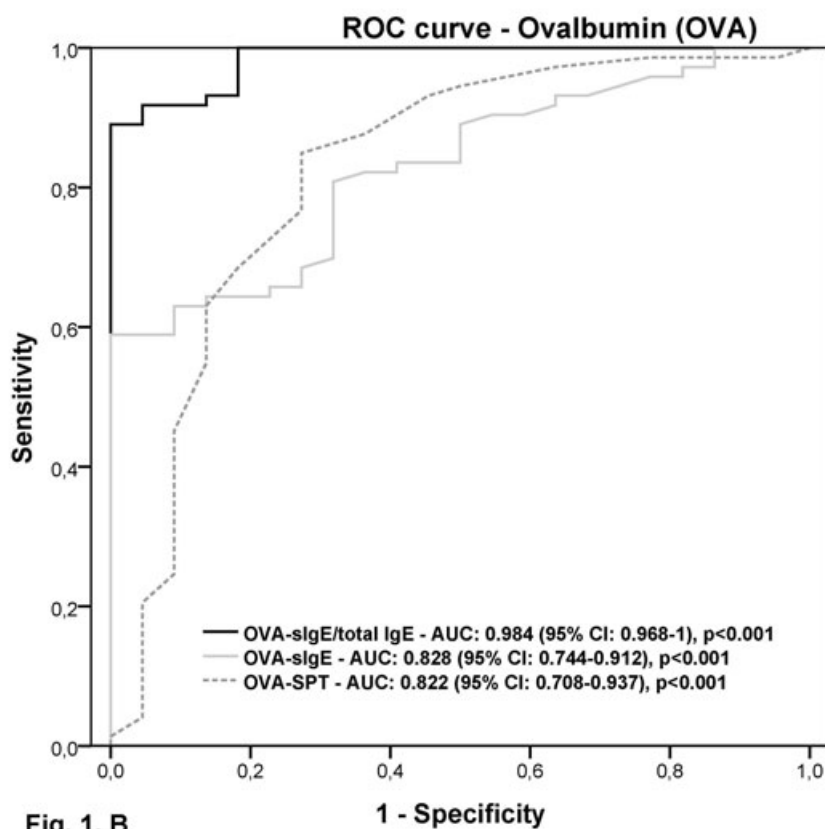


Fig. 1. B

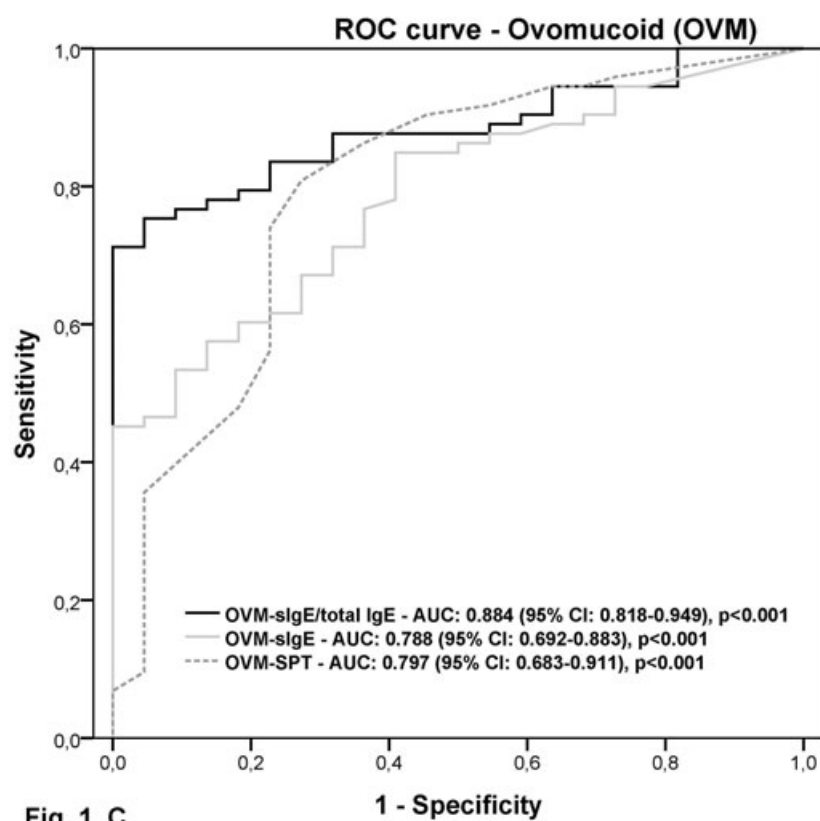


Fig. 1. C