

Are there any biomarkers that can predict tolerance to baked egg in egg allergic children younger than 6 years?

To the Editor,

The prevalence of food allergy is increasing worldwide. Egg allergy is of growing importance in early childhood; it is estimated that around 2% of preschoolers suffer from food allergy.¹ Five years after diagnosis, spontaneous tolerance is only achieved in 66% of egg-allergic children.² A strict elimination diet is an accepted clinical option, but avoidance does not prevent adverse events and its consequences. Egg is a common allergen in processed foods, specially baked goods; perception of children's quality of life is affected by the duration of the exclusion diet.³ New strategies can help making every day's life easier for children and guardians. The introduction of baked egg (BE) increases food variety, improving quality of life. Recently published data suggest that daily introduction of BE products in an oral immunotherapy protocol induced desensitization to lightly cooked egg and IgG4 levels increased significantly after 6 months of treatment.⁴ However, there are controversial data on daily introduction accelerating natural tolerance, despite the evidence showing that children who tolerated BE are more likely to pass egg challenges compared with those avoiding BE.⁵ Major allergens in egg white (EW) are ovalbumin (OVA, Gal day 2) and ovomucoid (OVO, Gal day 1) proteins. OVA is the predominant protein, is heat-labile, and has conformational epitopes, while OVO is heat-resistant and shows sequential epitopes. Egg allergenicity is modified by the destruction of conformational epitopes dependent on both heating temperature and cooking time.^{3,6} The wheat matrix is also important as OVO polymerizes with gluten to form large insoluble aggregates affecting digestibility by making the epitopes less accessible and recognizable.⁶

A great variability has been found in the literature for cutoff values for skin prick test (SPT) or specific IgE (slgE) that could assist the clinician for more accurately predicting tolerance to egg. A systematic review shows that in children ≥ 2 years, OFC to raw egg could be avoided when EW SPT is ≥ 10 mm or EW-slge is ≥ 7.3 KUA/L, and a diagnosis of heated egg allergy can be done with a EW SPT > 5 and > 11 mm in children < 2 and ≥ 2 years, respectively.⁷ For BE, results have shown a great variability and there is a risk of severe reactions even with low cutoff values.^{5,7} Accordingly, a supervised oral food challenge (OFC) before the introduction of BE in allergic patients continues to be the gold standard.^{8,9}

We aimed to evaluate tolerance to BE in egg-allergic children younger than 6 years. A single-center, prospective study was performed, including children over 12 months and up to 6 years who

had been referred to our outpatient clinic with suspected egg allergy. Egg allergy was diagnosed by a suggestive clinical history, SPT > 3 mm and/or slgE > 0.35 KU/L for egg white (EW) and/or OVO, and a positive OFC to extensively heated egg (EHE). A 6-step protocol with EHE-OFC: 0.3, 1, 3, 9.5, and 30 g of weighted hard-boiled EW followed by one well-cooked egg omelet (7.5 g of protein). When a

TABLE 1 Patients' characteristics and immunologic parameters

Age (months, median, IQR)	38.5 (30-50)
Gender (%)	
Female	45
Male	55
SPT [mm, mean (SD)]	
Yolk	6.61 (4.39)
EW	9.61 (6.33)
OVA	7.44 (4.37)
OVO	8.76 (4.74)
slgE [KU/L, mean (SD)]	
Yolk	1.49 (2.63)
EW	8.60 (15.01)
OVA	3.83 (7.38)
OVO	8.59 (14.47)

Abbreviations: EW, egg white; OVA, ovalbumin; OVO, ovomucoid; SD, standard deviation; slgE, specific IgE; SPT, skin prick test.

TABLE 2 Differences in SPT and specific IgE between the tolerant and non-tolerant groups

BE-tolerant group	Non-BE-tolerant group	P value
slgE (KU/L, mean [SD])		
Yolk 0.99 (1.47)	2.05 (3.91)	.021
EW 4.91 (7.98)	16.27 (22.08)	.005
OVA 2.27 (2.94)	7.11 (11.64)	.016
OVO 5.14 (10.99)	15.95 (18.45)	.002
SPT (mm, mean [SD])		
Yolk 6.09 (4.80)	7.98 (3.45)	.022
EW 9.26 (6.54)	10.97 (5.99)	.245
OVA 6.73 (4.19)	9.08 (4.44)	.031
OVO 8.80 (4.76)	9.19 (4.86)	.758

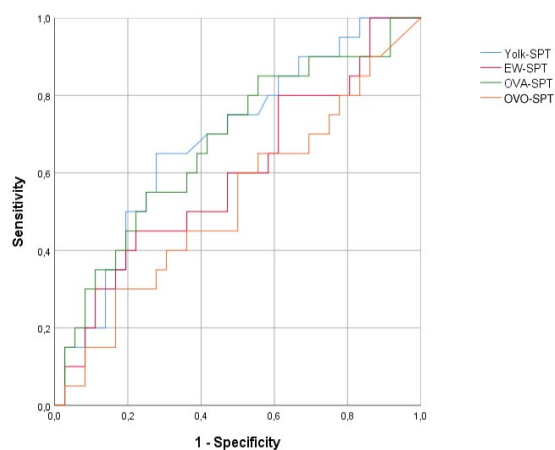
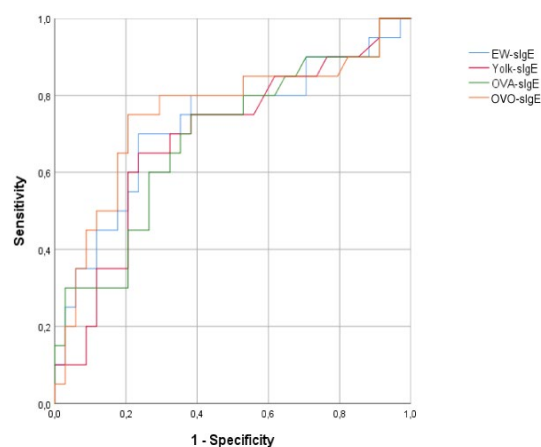


FIGURE 1 ROC curves for SPT and sIgE in all patients included and challenged with BE

	Area under the curve (CI 95%)	Standard error	P value
Yolk-SPT	0.68 (0.542 - 0.829)	0.073	.022
EW-SPT	0.59 (0.436 - 0.753)	0.081	.245
OVA-SPT	0.67 (0.526 - 0.824)	0.076	.031
OVM-SPT	0.52 (0.364 - 0.686)	0.082	.758



	Cut-off value	Sensitivity	Specificity	Area under the curve (CI 95%)	Standard error	P value
EW-sIgE	≥ 3.38 KU/L	80%	63.9%	0.731 (0.583 - 0.878)	0.075	.005
Yolk-sIgE	≥ 0.27 KU/L	80%	41.2%	0.689 (0.538 - 0.840)	0.077	.021
OVA-sIgE	≥ 0.87 KU/L	80%	47.1%	0.688 (0.538 - 0.838)	0.077	.022
OVM-sIgE	≥ 3.51 KU/L	80%	72.2%	0.756 (0.610 - 0.903)	0.075	.002

positive EHE-OFC was confirmed, we invited the patients' family to participate in the study. When accepted, all patients underwent an open BE-OFC, in a day hospital, using store-bought biscuits (0.22 g of egg protein per biscuit). A 4-step protocol with 30 minutes intervals was used (0.11, 0.22, 0.44, and 1.1 g of BE protein). Patients were divided into 2 groups: BE-tolerant (positive EHE-OFC, negative

BE-OFC) and non-BE-tolerant (positive EHE-OFC, positive BE-OFC). The BE-tolerant group was advised to eat baked goods up to 1.1 g of egg protein (5 biscuits) daily and to introduce food containing minimal amount of egg protein. Monthly telephone calls were done to assess home tolerance. Non-tolerant patients were advised to follow a strict egg avoidance diet. Data for demographics, adverse events

at OFC, and immunologic parameters were collected. Comparison between groups was done using the non-parametric Mann-Whitney *U* test. Receiver operating characteristic (ROC) curves were performed to evaluate cutoff values in order to predict tolerance. Written informed consent was signed. The study was approved by our hospital's ethics committee.

Sixty patients were included, and 4 had an inconclusive OFC because they refused to take the biscuits. Fifty-six children completed the BE-OFC; 64.3% (36/56) were tolerant and 35.7% (20/56) were non-tolerant. Median age was 38.5 months (IQR, 30-51) with a mean SPT and sIgE of EW 9.6 mm (SD, 6.3) and 8.6 KU/L (SD, 15) and OVO 8.7 mm (SD, 4.7) and 8.5 KU/L (SD, 14.4), respectively (Table 1). From the non-tolerant group, at OFC, 70% presented urticaria, 60% gastrointestinal (GI) symptoms, and 20% rhinoconjunctivitis. Anaphylaxis was diagnosed in 45% of patients, only one compromising lower respiratory tract. Statistical significance between the BE-tolerant group vs the non-BE-tolerant group was obtained for EW, yolk, OVA, OVO-sIgE and yolk, and OVA SPT (Table 2). ROC analysis suggests that yolk, EW, OVA, and OVO SPT are not good predictors of BE allergy with an area under the curve (AUC) of less than 0.7, as well as for yolk and OVA-sIgE. However, EW and OVO-sIgE tend to be good predictors with an AUC of 0.731 and 0.756, respectively (Figure 1). Eligible cutoff values were chosen with 80% sensitivity for EW-sIgE ≥ 3.38 KU/L and OVO-sIgE ≥ 3.51 KU/L with 63.9% and 72.2% specificity, respectively (confidence interval, IC 95%). Positive predictive value (PPV) was 55.2% for EW-sIgE and 61.5% for OVO-sIgE. Although the results show 80% sensitivity, specificity is poor and this PPV is not enough to describe which children will be allergic to BE. Nevertheless, negative predictive value (NPV) was 85.2% for EW-sIgE and 84.7% for OVO-sIgE, allowing a better selection of the tolerant patients. Given the poor results in the AUC for SPTs, yolk-sIgE, and OVA-sIgE, we did not set any cutoff value.

In monthly telephone calls, mild and infrequent adverse events related to BE ingestion were registered: perioral erythema and mild abdominal pain. Families experienced a positive response during follow-up by increasing variety on the children's diet and safely introducing egg traces or baked goods containing <1.1 g of egg protein. The percentage of patients non-tolerant to BE goods in our cohort is bigger than previously reported; this could be explained by the study taking place in a single tertiary reference center acting as a confounding bias. There is evidence that most egg-allergic patients can safely tolerate BE products, but no objective parameters can assess this condition in children. OFC remains to be the gold standard, but performing it in everyday clinical practice to confirm tolerance is time-consuming and not exempt from adverse events. In fact, 45% (9/20) of our cohort had anaphylaxis at BE-OFC, supporting the need for safer and more practical tolerance biomarkers. It is important to find tools that can minimize the risk of adverse reactions but at the same time aid in broadening the child's diet in a controlled fashion. Few studies in the literature refer cutoff values for egg components SPT or sIgE that can help to predict tolerance to BE.⁸ Calvani et al⁷ included a review of 9 BE studies, in

which mostly had poor results in SPT cutoff values as well as in our data. For EW-sIgE and/or OVO-sIgE, there is a wide variability range: 10-26.2 KU/L and 6-50 KU/L, respectively. In our data, these cutoff values are considerably lower with a poor PPV but a non-negligible NPV. Regarding sensibility and specificity, both parameters tend to be good predictors with an AUC higher than 0.7 and 0.75.

In conclusion, 64.3% of our egg-allergic children under 6 years were tolerant to 1.1 gr of baked egg protein, allowing the inclusion of baked egg goods and food that can contain minimal amount of egg protein in their diet. EW-sIgE and OVO-sIgE tend to be good markers to predict tolerance with a high NPV.

These patients are part of a prospective long-term study to assess whether the introduction of baked foods into children's diet results in immunomodulation or desensitization, a current hot-topic debate.

CONFLICT OF INTEREST

The authors have no conflict of interest in relation to this work.

AUTHOR CONTRIBUTIONS

All authors contributed to the study and the preparation of the manuscript.

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REFERENCES

- Leonard SA, Sampson HA, Sicherer SH, et al. Dietary baked egg accelerates resolution of egg allergy in children. *J Allergy Clin Immunol*. 2012;130:473-480.
- Vazquez M, Álvaro M, Piquer M, et al. Baseline specific IgE levels are useful to predict safety of oral immunotherapy in egg allergic children. *Clin Exp Allergy*. 2014;44(1):130-141.
- Upton J, Nowak-Węgrzyn A. The impact of baked egg and baked milk diets on IgE and non-IgE-mediated allergy. *Clin Rev Allergy Immunol*. 2018;55(2):118-138.

4. Bird JA, Clark A, Dougherty I, et al. Baked egg oral immunotherapy desensitizes baked egg allergic children to lightly cooked egg. *J Allergy Clin Immunol Pract*. 2018;1-3.
5. Capucilli P, Cianferoni A, Fiedler J, et al. Differences in egg and milk food challenge outcomes based on tolerance to the baked form. *Ann Allergy Asthma Immunol*. 2018;121(5):580-587.
6. Leonard SA, Caubet JC, Kim JS, Groetch M, Nowak-Węgrzyn A. Baked milk- and egg-containing diet in the management of milk and egg allergy. *J Allergy Clin Immunol Pract*. 2015;3:13-23.
7. Calvani M, Arasi S, Bianchi A, et al. Is it possible to make a diagnosis of raw, heated, and baked egg allergy in children using cutoffs? A systematic review. *Pediatr Allergy Immunol*. 2015;26(6):509-521.
8. Sopo SM, Greco M, Cuomo B, et al. Matrix effect on baked egg tolerance in children with IgE-mediated hen's egg allergy. *Pediatr Allergy Immunol*. 2016;27(5):465-470.
9. Robinson ML, Lanser BJ. The role of baked egg and milk in the diets of allergic children. *Immunol Allergy Clin N Am*. 2018;65-76.