Outcomes of Baked Milk and Egg Challenge in Cow's Milk and Hen's Egg Allergy: Can Tolerance Be Predicted with Allergen-Specific IgE and Prick-to-Prick Test?

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Keywords

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Abstract

Background: Adding baked food into the diets of patients with cow's milk allergy (MA) and hen's egg allergy (EA) has several benefits. **Objective:** We aimed to determine baked and unbaked food tolerance and evaluate the effectiveness of laboratory findings on the prediction of baked and unbaked food tolerance in patients with MA and EA. **Methods:** Clinical outcomes of the patients with MA and EA who had been exposed to oral food challenge with baked food were retrospectively analyzed. **Results:** Ninety-one patients were evaluated. The median age of the study group was 22 months. Forty-nine and 42 patients had IgE-mediated MA and EA, respectively. While all patients with EA tolerated baked egg, 24.5% patients with MA could not tolerate baked cow's milk (BM). In patients with MA, BM tolerance showed negative association with milk-specific IgE, skin prick test (SPT), and prick-to-prick test (PTP), and the PTP was the most significant parameter (sensitivity 83.8%, specificity 91.7% for PTP ≤ 7 mm). Negative association was seen between milk-specific IgE, SPT, PTP, and unbaked milk (UBM) tolerance, and PTP was the most significant parameter (sensitivity

100%, specificity 55% for PTP ≤ 4 mm). In patients with EA, at the end of 6 months of baked hen's egg (BE) consumption, scrambled egg tolerance showed negative association with egg white-specific IgE level, egg white SPT and PTP. Egg white PTP was the most significant parameter (sensitivity 82.4%, specificity 96.0% for PTP ≤ 5 mm). **Conclusion:** Specific-IgE, SPT, and PTP should be kept in mind as parameters that can be used to predict tolerance to BM and BE for patients with MA and EA.

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Introduction

Food allergy is an increasing public health issue in developed countries. Cow's milk and hen's egg are the most common allergens that cause food allergy in the young pediatric age group. Clinical studies have indicated that the prevalence of cow's milk allergy (MA) and hen's egg allergy (EA) appear to have increased during the last 2 decades [1–3]. Furthermore, recent evidence suggests that both allergies may persist into adolescence and even adulthood [4]. These conditions drive clinicians to seek different clinical approaches for the management of aller-

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gies instead of elimination diet and oral immunotherapy which may prove to be inconvenient. In this context, most patients with milk and egg allergy have been shown to tolerate baked products [5–8].

The main idea of introducing cow's milk or hen's egg in baked form is to reduce the allergenicity of proteins. Baking cow's milk or hen's egg with other components such as fats, sugar and wheat create a food matrix between epitopes which helps to reduce exposure of epitopes to the immune system. Besides, heat may denature conformational epitopes, making them no longer recognizable by the epitope-specific IgE [9, 10].

Several clinical studies have investigated the relationship between laboratory parameters and skin prick test (SPT) and baked cow's milk (BM) and baked hen's egg (BE) tolerance. Studies have also evaluated the impact of baked food consumption on immunological parameters and SPT [5, 7, 11]. Although some studies emphasize that baked products accelerate tolerance to the unbaked forms of allergens, there are also publications claiming otherwise [8, 12].

We primarily aimed to determine the baked product tolerance in patients with IgE-mediated MA and EA. The secondary objective of this study is assessment of the unbaked food tolerance after ingestion of baked products and evaluation of the effectiveness of laboratory findings on the prediction of baked and unbaked food tolerance in patients with IgE-mediated MA and EA.

Materials and Methods

A single-center, retrospective study was conducted between March 2015 and December 2017 in the Pediatric Immunology and Allergy Outpatient Clinic. Patients diagnosed with IgE-mediated MA and EA who had been exposed to oral food challenge (OFC) with baked products (BM or BE) were included in the study. Demographic characteristics and laboratory findings, SPT and prick-to-prick test (PTP) wheal sizes, and clinical outcomes of the patients were analyzed. This study was granted approval by the University Research Ethics Board.

Diagnosis of MA and EA

The diagnosis of MA and EA was established with directed history, physical examination and OFC or laboratory findings with positive skin-prick test.

In patients diagnosed with IgE-mediated type immune reaction, milk-specific IgE >5 kU/L and/or egg white-specific IgE >2 kU/L using immunoCAP (Phadia AB®, Uppsala, Sweden) with a positive skin-prick test (≥3 mm), which has a 95% predictive value, was accepted as diagnostic for MA and EA, respectively [13, 14]. OFC was performed in patients without any laboratory findings suggestive of IgE-mediated reaction type [15].

Table 1. Baked-egg muffin recipe

One cup of milk
2 tablespoons of sunflower/corn oil
1 egg
1 teaspoon of vanilla extract
1 1/2 cup of flour
1 teaspoon of baking powder
Bake at 180° C (370°F) for 40 min
Yield: 4 muffins or cupcakes with each cupcake/muffin containing 1.65 g egg protein
1.65 g egg protein; given in four increasing doses every 15 min, followed by a 2-h observation period

Skin testing (SPT and PTP) was performed according to the standard procedure by trained technicians in our clinic. Positive SPT with commercially available milk and egg white extracts (Alk-Abello®, Hørsholm, Denmark) and positive PTP with scrambled egg (SE) and fresh milk were defined as a wheal size at least 3 mm greater than the negative control.

Oral Food Challenge

OFCs were performed as graded open challenges in line with the recommendations of American Academy of Asthma, Allergy and Immunology and American College of Allergy, Asthma, and Immunology [16].

For baked egg challenge, cupcakes were prepared by parents according to our clinical recipe presented in Table 1. One cupcake was consumed as per standard procedure during the challenge.

Baked milk challenge was performed with cupcakes containing 1.3 g milk protein per cupcake as described in previous studies. One cupcake (1.3 g milk protein given in 4 increasing doses every 15 min followed by a 2-h observation period) was served in the baked milk challenge. Fermented milk (yogurt; FM) challenges were performed with increasing doses up to portions containing 8–10 g milk protein in line with previous studies [17–19].

Patients who tolerated BM or BE continued consuming cupcakes for 6 months. After 6 months, OFC with unbaked milk (UBM) and SE was performed for all patients who tolerated baked food. Oral challenges with UBM and SE were performed as described in previous studies [20]. After 3 months, patients with MA who could not tolerate UBM were re-evaluated with FM (yogurt) challenge to determine FM (yogurt) tolerance (Fig. 1).

Statistical Analysis

Categorical variables (gender, type of allergy, clinical symptoms) were assessed by means of the χ^2 test and expressed as number and percentage. The Kolmogorov-Smirnov and Shapiro-Wilk tests were performed to evaluate the normality of continuous variables. Non-parametric continuous variables (median [25–75 percentile]; age, eosinophils, eosinophil percentage, specific-IgE, SPT and PTP wheal sizes, percent decrease in specific-IgE levels, SPT

Fig. 1. Study design. BE, Baked hen's egg; FM, fermented milk; SE, scrambled egg; UBM, unbaked milk.

and PTP values) were analyzed with Mann-Whitney U test or Kruskal-Wallis test. Receiver operator characteristic (ROC) curve analysis was used to determine threshold values in the laboratory to establish SPT and PTP wheal sizes to differentiate food tolerance in patients with MA and EA. The relationship between SPT or specific-IgE and the food challenge outcome was analyzed by logistic regression. Probability curves were plotted according to outputs of the logistic regression. Specificity, sensitivity, positive predictive value and negative predictive value of laboratory parameters, SPT, and PTP were determined.

Data were analyzed with the Statistical Package for Social Sciences (SPSS) computer software (version 21.0; SPSS, Chicago, IL, USA) and MedCalc v12.5 software. A two-tailed p -value <0.05 was considered significant.

Results

Ninety-one patients with IgE-mediated MA and EA were evaluated. Among these, 38 (41.8%) were female. Median age of the study group was 22 (17–42) months. Forty-nine and 42 patients had MA and EA, respectively. Demographic characteristics and symptoms at the time of presentation are shown in Table 2.

Patients with MA

While 9 (75%) BM-reactive patients presented with anaphylaxis, 5 (55.5%) with BM tolerance, 2 (18.2%) with FM tolerance, and 1 (5.9%) with UBM tolerance had an anaphylactic reaction (Table 2). In patients with MA, 12 (24.5%) patients could not tolerate BM.

Six months after initiation of baked food, 17 (34.7%) patients with MA were able to tolerate UBM. Eleven patients (22.5%) became FM tolerant. Nine (18.3%) patients continued consuming BM. Age was greater among BM-reactive patients compared to other groups ($p = 0.001$). On the other hand, there was no difference between subgroups in terms of gender and presenting symptoms (Table 2; statistical data not shown in tables).

Laboratory Findings in MA

In the evaluation of laboratory findings of patients with MA, BM-reactive patients had higher milk-specific IgE (kU/L) levels, SPT wheal size, and PTP wheal size than the other groups (Table 3). At the end of the 6-month BM administration, milk-specific IgE and PTP wheal size were higher in BM-tolerant patients compared to FM- and UBM-tolerant patients. Furthermore, final SPT

Table 2. Demographic and clinical characteristics of patients with MA and EA

	MA group					EA group		
	BM-reactive (n = 12)	BM-tolerant (n = 9)	FM-tolerant (n = 11)	UBM-tolerant (n = 17)	total (n = 49)	BE-tolerant (n = 25)	SE-tolerant (n = 17)	total (n = 42)
Gender, female/male	4 (33.3)/ 8 (66.6)	2 (22.2)/ 7 (77.8)	5 (45.5)/ 6 (54.5)	8 (47.1)/ 9 (52.9)	19 (38.8)/ 30 (61.2)	11 (44.0)/ 14 (56)	8 (47.1)/ 9 (52.9)	19 (45.2)/ 23 (54.8)
Age, months	53 (33–61)*	20 (17.5–67)	15 (13–25)	17 (22–29)	24 (17–47.5)	20 (16.5–31.5)	33 (18.5–41.5)	22 (17–36.3)
Presenting symptoms								
Skin	3 (25)	3 (33.3)	9 (81.8)	9 (52.9)	23 (46.9)	19 (76)	13 (76.4)	32 (76.2)
Skin + GI	0 (0)	1 (11.1)	0 (0)	7 (41.2)	9 (18.4)	5 (20)	2 (11.8)	7 (16.7)
Anaphylaxis	9 (75)	5 (55.6)	2 (18.2)	1 (5.9)	17 (34.7)	1 (4)	2 (11.8)	3 (7.1)

Data are presented as n (%) or median (25–75th percentile). GI, gastrointestinal; BM, baked milk; SE, scrambled hen's egg; FM, fermented milk; UBM, unbaked milk. * $p = 0.001$.

Table 3. Laboratory findings of patients with cow's milk allergy

Parameter	BM-reactive (n = 12)	BM-tolerant (n = 9)	FM-tolerant (n = 11)	UBM-tolerant (n = 17)	<i>p</i> value
Initial eosinophils, cells/ μ L	400 (200–675)	400 (300–800)	400 (200–700)	400 (250–600)	0.881
Initial eosinophil percentage	3.3 (2–7.2)	5.7 (3.2–6.4)	2.9 (1.9–5–8)	4.4 (2.5–7.5)	0.631
Initial IgE, IU/mL	135.5 (77.8–453.8)	182 (44–259.5)	107 (51–222)	66 (41.5–187)	0.550
Initial milk-specific IgE, kU/L	95.9 (54.9–100)*	38.6 (15.8–100)	5 (3.2–10.3)	3.2 (1.6–7.2)	<0.0001*
Initial SPT wheal size, mm	7 (5–8)*	5 (3.5–7.5)	4 (4–5)	4 (3–4)	<0.0001*
Initial PTP wheal size, mm	9.5 (8.3–11)*	8 (7–9.5)	6 (5–7)	4 (4–5.5)	<0.0001*
Final milk-specific IgE, kU/L	–	40 (8.9–82.9)*	1.6 (1.2–3.9)	0.9 (0.7–1.7)	<0.0001*
Final SPT wheal size, mm	–	4 (3–5.5)	3 (3–4)	1 (0–3)*	<0.0001*
Final PTP wheal size, mm	–	7 (4.5–8)*	4 (3–5)	3 (1–4)	<0.0001*
Decrease in milk-specific IgE, %	–	28.9 (0–45.8)	57.9 (21.9–61.8)	68.9 (59.1–81.3)*	0.039*
Decrease in SPT wheal size, %	–	20 (0–30.9)	20 (0–25)	66.7 (25–100)*	0.005*
Decrease in PTP wheal size, %	–	27.3 (0–36.7)	16.7 (0–50)	50 (20–80)	0.883

Data are presented as median (25–75th percentile). BE, baked hen's egg; FM, fermented milk; UBM, unbaked milk. * statistically significant parameters.

wheal size was lower in UBM-tolerant patients. In UBM-tolerant patients, decreases in milk-specific IgE (68.9% [59.1–81.3]) and SPT wheal size (66.7% [25–100]) were greater than those in other subgroups (Table 3).

Logistic regression analysis was used in patients with IgE type MA to assess the relationship between initial laboratory findings and BM tolerance. BM tolerance showed a negative association with milk-specific IgE, SPT wheal size, and PTP wheal size. PTP was found to be the most significant parameter to predict BM tolerance (Table 4). Initial milk-specific IgE levels ≤ 39.1 kU/L, SPT wheal size ≤ 5 mm, and PTP wheal size ≤ 7 mm were almost identical with regard to best prediction of tolerance to BM by ROC analysis (Table 4). Sensitivity and specificity of initial PTP

wheal size ≤ 7 mm were found to be 83.8% (95% CI 67.9–93.8%) and 91.7% (95% CI 61.5–99.7%), respectively (Table 5). Probability curve of the development of BM tolerance was calculated as determined by the wheal sizes in PTP (Fig. 2a).

At the end of 6 months of BM consumption, a negative association was seen between milk-specific IgE, SPT wheal size, PTP wheal size, and UBM tolerance. Moreover, percent decrease in milk-specific IgE, SPT wheal size and PTP wheal size showed a positive association with UBM tolerance. PTP test was found to be the most significant parameter to predict UBM tolerance (Table 6). Probability curve of the development of tolerance to UBM was calculated as determined by the wheal sizes in

Table 4. Logistic regression and ROC analysis of initial laboratory parameters for prediction of BM tolerance in patients with MA

Parameter	Univariate regression model		Multivariate regression model		ROC analysis	
	OR (95% CI lower–upper)	<i>p</i> value	OR (95% CI lower–upper)	<i>p</i> value	AUC (95% CI lower–upper)	<i>p</i> value
Initial milk-specific IgE, kU/L	0.964 (0.944–0.983)	0.001			0.874 (0.775–0.973)	<0.0001
Initial SPT wheal size, mm	0.463 (0.288–0.745)	0.001			0.828 (0.684–0.971)	0.001
Initial PTP wheal size, mm	0.418 (0.250–0.699)	0.001	0.527(0.316–0.880)	0.014	0.910 (0.830–0.990)	<0.0001

Table 5. Diagnostic value of initial laboratory parameters for prediction of BM tolerance in patients with MA

Parameter	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Initial milk-specific IgE ≤39.1 kU/L	81.1 (64.8–92.0)	91.7 (61.5–99.8)	96.8 (82.0–99.5)	61.1 (44.1–75.8)
Initial SPT wheal size ≤5 mm	78.9 (62.7–90.5)	63.6 (30.8–89.1)	88.2 (77.1–94.3)	46.7 (29.0–65.2)
Initial PTP wheal size ≤7 mm	83.8 (67.9–93.8)	91.7 (61.5–99.7)	96.9 (82.5–99.5)	64.71 (46.4–79.6)

Table 6. Logistic regression and ROC analysis of laboratory parameters at 6 months of BM consumption for prediction of UBM in patients with MA

Parameter	Univariate regression model		Multivariate regression model		ROC analysis	
	OR (95% CI lower–upper)	<i>p</i> value	OR (95% CI lower–upper)	<i>p</i> value	AUC (95% CI lower–upper)	<i>p</i> value
Final milk-specific IgE (kU/L)	0.721 (0.482–0.978)	0.037			0.837 (0.707–0.967)	<0.0001
Final SPT wheal size (mm)	0.133 (0.230–0.768)	0.024			0.853 (0.735–0.971)	<0.0001
Final PTP wheal size (mm)	0.280 (0.104–0.756)	0.012	0.078 (0.07–0.856)	0.037	0.899 (0.799–0.998)	<0.0001
Decrease in milk-specific IgE (%)	149.1 (4.4–5,051.312)	0.015			0.806 (0.657–0.955)	0.002
Decrease in SPT wheal size (%)	126.644 (4.717–3,400.053)	0.024			0.806 (0.651–0.961)	0.002
Decrease in PTP wheal size (%)	22.031 (1.570–309.189)	0.022			0.712 (0.540–0.884)	0.028

PTP, measured at 6 months of BM consumption (Fig. 2b). At 6 months, milk-specific IgE levels ≤2.75 kU/L, SPT wheal size ≤3 mm, PTP wheal size ≤4 mm, and decrease in milk-specific IgE ≥43%, in SPT wheal size ≥22.5% and in PTP wheal size (%) ≥18.3% were almost identical with regard to best prediction of tolerance to BM by ROC analysis (Table 6). Sensitivity and specificity of PTP wheal size measured as ≤4 mm at 6 months of BM consumption were calculated as 100.0% (95% CI 80.5–100%) and 55.0% (95% CI 31.5–76.9%), respectively (Table 7).

Patients with EA

There were 3 patients diagnosed with EA who presented with anaphylaxis. While 2 (66.6%) of them could consume SE, 1 (33.3%) of them tolerated baked eggs (BEs; Table 2).

All patients with EA tolerated baked eggs. At the end of 6 months of baked food administration, 17 (40.5%) patients were able to tolerate SEs. There was no significant difference between food tolerance and gender, age, and presenting symptoms (Table 2; statistical data not shown in tables).

Laboratory Findings in EA

In the assessment of laboratory findings of the EA group, initial eosinophils, egg white-specific IgE levels, egg white SPT wheal size, and egg white PTP wheal size were significantly higher in the BE-tolerant group compared to the SE-tolerant group (Table 8). At the end of the 6-month BE administration, final egg white-specific IgE levels, egg white SPT wheal size, and egg white PTP wheal size were lower in SE-tolerant patients than those in BE-

Table 7. Diagnostic value of laboratory parameters at 6 months of BM consumption for prediction of UBM tolerance in patients with MA

Parameter	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Final milk-specific IgE ≤ 2.75 kU/L	94.1 (71.3–99.9)	65.0 (40.8–84.6)	69.6 (55.4–80.8)	92.9 (65.4–98.9)
Final SPT wheal size ≤ 3 mm	94.1 (71.3–99.9)	55 (31.5–76.9)	64 (51.9–74.5)	91.7 (61.2–98.7)
Final PTP wheal size ≤ 4 mm	100.0 (80.5–100)	55.0 (31.5–76.9)	45.9 (29.5–63.1)	65.4 (53.8–75.4)
Decrease in milk-specific IgE $\geq 43\%$	94.1 (71.3–99.9)	60.0 (36.1–80.9)	66.7 (53.6–77.6)	92.3 (63.4–98.8)
Decrease in SPT wheal size $\geq 22.5\%$	82.4 (56.6–96.2)	55 (31.5–76.9)	60.9 (47.7–72.6)	78.6 (54.9–91.7)
Decrease in PTP wheal size $\geq 18.3\%$	82.4 (56.6–96.2)	55.0 (31.5–76.9)	60.9 (47.7–72.6)	78.6 (54.9–91.7)

Fig. 2. Logistic relationship between the probability of developing: tolerance to baked milk (BM) and wheal size in the prick-to-prick test (PTP), measured at the time of cow's milk allergy diagnosis (a); tolerance to unbaked milk (UBM) and wheal size in the PTP, mea-

sured at 6 months of BM consumption (b); tolerance to scrambled egg (SE) and wheal size in the egg white PTP, measured at 6 months of baked egg consumption (c).

tolerant patients. Unlike the decrease in egg white-specific IgE, the decrease in egg white SPT wheal size and egg white PTP wheal size was higher in the SE-tolerant group (Table 8).

At the end of 6 months of baked egg consumption, SE tolerance showed a negative association with egg white-specific IgE level, egg white SPT wheal size and egg white PTP wheal size. Furthermore, SE tolerance had a positive

Table 8. Laboratory findings of patients with hen's egg allergy

Parameter	BE tolerant (<i>n</i> = 25)	SE tolerant (<i>n</i> = 17)	<i>p</i> value
Initial eosinophils, cells/ μ L	500 (300–700)	200 (100–650)	0.049
Initial eosinophils, %	5.1 (2.4–6)	2.1 (1.6–2.1)	0.056
Initial IgE, IU/mL	141 (44.5–293)	82 (47–111)	0.130
Initial egg white-specific IgE, kU/L	30.7 (10.8–44.5)	4.6 (1.9–13.3)	<0.0001
Initial egg white SPT wheal size, mm	5 (4–7)	4 (3–5)	0.006
Initial egg white PTP wheal size, mm	9 (9–11.5)	7 (4–9)	0.001
Final egg white-specific IgE, kU/L	9.4 (2.8–27.2)	1.4 (0.7–2.2)	<0.0001
Final egg white SPT wheal size, mm	5 (4–5)	2 (0.5–3)	<0.0001
Final egg white PTP wheal size, mm	7 (6.5–9.5)	3 (2–4)	<0.0001
Decrease in egg white-specific IgE, %	50.2 (28.5–64.3)	67 (38.8–84.2)	0.144
Decrease in egg white SPT wheal size, %	20 (0–33)	40 (27.1–57.5)	0.003
Decrease in egg white PTP wheal size, %	22.2 (10.5–36.4)	50 (35–66.1)	<0.0001

Data are presented as median (25–75 percentile). BE, baked hen's egg; SE, scrambled egg.

Table 9. Logistic regression and ROC analysis of laboratory parameters at 6 months of BE consumption for prediction of SE tolerance in patients with EA

Parameter	Univariate regression model		Multivariate regression model		ROC analysis	
	OR (95% CI lower–upper)	<i>p</i> value	OR (95% CI lower–upper)	<i>p</i> value	AUC (95% CI lower–upper)	<i>p</i> value
Final egg white-specific IgE (kU/L)	0.818 (0.691–0.968)	0.020			0.891 (0.784–0.997)	<0.0001
Final egg white SPT wheal size (mm)	0.442 (0.263–0.742)	0.002			0.853 (0.717–0.988)	<0.0001
Final egg white PTP wheal size (mm)	0.435 (0.237–0.707)	<0.0001	0.445 (0.257–0.770)	0.004	0.921 (0.822–1.000)	<0.0001
Decrease in egg white-specific IgE (%)	8.739 (0.676–113.015)	0.097			0.619 (0.433–0.805)	0.224
Decrease in egg white SPT wheal size (%)	17.493 (1.480–206.716)	0.023			0.766 (0.612–0.920)	0.006
Decrease in egg white PTP wheal size (%)	2,265.244 (14.626–350,846.849)	0.003			0.829 (0.689–0.969)	0.001

association with percent decrease in egg white SPT wheal size and egg white PTP wheal size. Egg white PTP wheal size was found to be the most significant parameter to predict SE tolerance (Table 9). Probability curve of the development of tolerance to unbaked egg was calculated as determined by the wheal size in the egg white PTP, measured at 6 months of baked egg consumption (Fig. 2c). Sensitivity and specificity of egg white PTP wheal size measured as ≤ 5 mm at 6 months of baked egg consumption were detected as 82.4% (95% CI 56.6–96.2%) and 96.0% (95% CI 79.7–99.9%), respectively (Table 10).

Discussion

In this study, tolerance to baked food was assessed in patients with IgE-mediated MA and EA. In addition, specific-IgE levels, SPT wheal size and PTP wheal size were

analyzed in terms of predicting tolerance to BM and BE. We found a negative association between SPT wheal size, PTP wheal size, specific-IgE levels, and baked food tolerance. However, we could not establish an adequate cut-off value for SPT wheal size, PTP wheal size and specific-IgE levels to predict tolerance to BM and BE. Also, after 6 months of baked food consumption, laboratory findings and prick test results did not help to properly identify UBM and SE tolerance.

Studies have shown that 73–83% of children with MA can safely consume BM [10, 21, 22]. Consistently, we found the same rate of BM tolerance in children with MA. Surprisingly, all patients with EA were able to tolerate BE, and this finding was different from previous studies that reported the rate of BE tolerance as 64–83% [23, 24]. This may be related to consumption of cupcakes containing a lower amount of eggs compared to the baked products in other studies. We introduced 1.65 g egg protein for chal-

Table 10. Diagnostic value of laboratory parameters at 6 months of baked egg consumption for prediction of SE tolerance in patients with EA

Parameters	Sensitivity, %	Specificity, %	PPV, %	NPV, %
Final egg white-specific IgE ≤ 4.15 kU/L	88.2 (63.6–98.5)	68 (46.5–85.1)	65.2 (50.8–77.3)	89.5 (69.2–87.9)
Final egg white SPT wheal size ≤ 3 mm	94.1 (71.3–99.9)	80.0 (59.3–93.2)	76.2 (59.2–87.6)	95.2 (74.7–99.3)
Final egg white PTP wheal size ≤ 5 mm	82.4 (56.6–96.2)	96.0 (79.7–99.9)	93.3 (66.9–98.9)	88.9 (74.1–95.7)
Decrease in egg white SPT wheal size $\geq 22.5\%$	93.8 (69.8–99.8)	60 (38.7–78.8)	60.0 (47.7–71.1)	93.8 (68.6–99.0)
Decrease in egg white PTP wheal size $\geq 23.6\%$	94.1 (71.3–99.9)	56.0 (34.9–75.6)	59.3 (47.9–69.7)	93.3 (66.9–98.9)

lenge while other studies used a third of whole egg per muffin, that is, 2.2–2.6 g of egg protein [23–27]. Additionally, while cupcakes or muffins were baked for 20–30 min in other studies, we baked the cupcakes for 40 min [26, 27]. We speculate that a longer baking process may cause further denaturation of conformational epitopes, which allows a higher rate of tolerance to baked eggs.

Although cupcakes contained low-dose egg protein and were baked for a long duration, the rate of raw egg tolerance was about 40% at the end of 6 months of baked food consumption, as reported in previous studies [28, 29]. This finding suggests that BE with a lower dose of egg protein and a longer baking duration does not result in any difference in tolerance to SEs.

In the literature, while some studies declare that ingestion of BM and BE increases resolution of the allergy and accelerates the resolution process, others have reported no effect of baked food consumption on the allergy status [8, 18, 29, 30]. In our retrospective study, there was no control group and we could not evaluate the effect of baked food on the acceleration of resolution. Sicherer et al. [2] reported that EA resolved in 49.3% of patients with EA at a median age of 72 months in a study on the natural course of the disease. In another study concerning the same topic, resolution of MA was seen in 52.6% of patients with MA at median age of 66 months [1]. The similar feature of all these studies is the median age of study groups, which is older than 4 years. It is well known that especially after 4 years of age, there is a significant decrease in milk allergy. However, the median age of our study is about 24 months. In other words, the median age of our study group was under 4 years old age, and we found resolution of allergy in 40% of patients. In the comparison of age status, our findings underline that consumption of baked products has a positive effect on accelerated resolution of cow's milk and EA.

As determined in previous studies, baked food tolerance showed a negative association with SPT wheal size, PTP wheal size and specific-IgE levels in the present

study [21, 22, 27]. At 6 months of baked food consumption, decreases were noted in SPT and PTP wheal sizes as well as specific-IgE levels in the entire study group, a finding that contradicts with the results of previous studies [18, 25]. Most of those studies analyzed SPT and specific-IgE levels to create probability curves in patients with cow's milk and EA [21, 31–33]. In our study however, initial and final PTP values were found to be most significant parameters to predict baked and UBM tolerance in patients with MA, respectively. We found that patients diagnosed with MA who had an initial PTP wheal size ≤ 5 mm had a 97.4% probability of tolerance to BM. At the end of 6 months of BM consumption, patients with PTP wheal size ≤ 2 mm had an 87.9% probability of tolerance to UBM. At the end of 6 months of BE consumption, an 83.6% probability of tolerance to SE was detected in patients with EA and PTP wheal size ≤ 3 mm.

Our study has several limitations. First, we did not analyze immunological parameters such as ovomucoid-specific IgE, casein-specific IgE, and specific IgG4, which may be superior to other parameters in predicting the outcome of challenge. Second, the low-number subgroup limited our conclusions of the analysis parameters. Third, retrospective analysis of variables makes it difficult to declare the proper application of protocol. Lastly, absence of a control group limited us in terms of better understanding the relationship between the parameters and challenges. Additionally, not all patients involved in the study were diagnosed by OFC, which is the gold standard for the diagnosis of food allergy, and other potentially concomitant allergic conditions were not evaluated.

The present study shows that baked food might have a positive effect on the acceleration of allergy resolution in children with IgE-mediated cow's milk and EA. We showed that decreasing the amount of egg protein in baked food and increasing the cooking time may result in an improved tolerance to BEs. The PTP should be kept in

mind as a parameter that can be used to predict tolerance to baked milk and egg for patients with MA and EA, respectively. Prospective, case-control studies performed with different age groups are warranted to better analyze the value of laboratory parameters and skin tests in the prediction of baked food tolerance and to evaluate the impact of baked food consumption on the resolution of allergies.

Statement of Ethics

This study was granted approval by the Dokuz Eylul University Research Ethics Board.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.A., N.U., O.K., and O.A. analyzed the data. S.S.K. analyzed data and drafted the manuscript. All authors approved the final manuscript.

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