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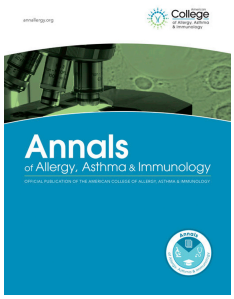

Utility of fresh egg skin prick test and egg-yolk specific IgE for outgrowth

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Utility of fresh egg skin prick test and egg-yolk specific IgE for outgrowth

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Running title: Predicting outgrowth of egg allergy

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Egg allergy (EA)

Skin prick test (SPT)

Specific immunoglobulin E (sIgE)

Oral food challenge (OFC)

Fresh egg (FE)

Egg yolk (EY)

Egg white (EW)

Optimal decision points for outgrowth (ODP^{fo})

Positive predictive value (PPV)

Negative predictive value (NPV)

Positive likelihood ratio (LR+)

Negative likelihood ratio (LR-)

Receiver-operating characteristic (ROC)

Areas under the ROC curve (AUC)

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Background : In children <2 years old, studies evaluating the value of skin prick tests (SPT) and specific immunoglobulin E (sIgE) results to predict persistence or resolution of egg allergy (EA) are limited. Additionally, the value of egg yolk (EY) sIgE and fresh egg (FE) SPT has not been well characterized.

Objective: We investigated the optimal decision points for outgrowing (ODP fo) allergy with both SPT and sIgE tests for egg allergen preparations.

Methods: SPTs for FE, egg white (EW), EY, sIgEs for EW and EY and oral food challenges (OFC) were performed in children with suspected EA. Reactive patients strictly avoided all dietary egg. After one year, EA was re-evaluated with repeat OFC, SPTs, and sIgEs.

Results: Eighty-one children, median age 7 months (range 2-24 months) were enrolled. Four children with a history of anaphylaxis and 60/77 children with a positive challenge underwent egg elimination. The 1 year follow-up challenge test was performed on 59 children. Twenty-seven reacted to egg. No persistent patient had a follow-up SPT for FE ≤4mm ($p<0.001$; 100% PPV, 56% NPV for outgrowth). The diameters of the initial SPT for FE decreased 50% or more in half of the patients who outgrew EA. The ODP fo for follow-up sIgE for EY and EW were ≤2.1 kU/L (86.2% PPV) and ≤4.0 kU/L (84.6% PPV), respectively.

Conclusion: A diameter of SPT for FE ≤4mm and sIgE values of ≤2.1 kU/L for EY and ≤4.0 kU/L for EW have good PPV for outgrowth of EA under two years of age.

Introduction

Egg allergy (EA) is one of the most common food allergies in infants and young children and usually begins before the age of two.[1] As approximately 50% of children with EA become clinically tolerant, that is they outgrow the allergy by the third year of life, it is important that follow up occur at regular intervals during this time period.[1] Therefore once diagnosed, skin prick test (SPT) and specific immunoglobulin E (sIgE) levels are assessed in sequential follow-up visits to evaluate for potential allergy outgrowth.

Oral food challenge (OFC) is accepted as the gold standard to diagnose an IgE mediated food allergy.[2-3] However, OFCs are time-consuming, expensive and have inherent risk including a severe allergic reaction. Thus, clinicians would benefit from more robust clinical data regarding cut-off SPT and sIgE values to predict which patients are optimal candidates for OFC.

Previous studies aimed to identify risk cut-offs for sIgE or SPT in children under two years of age, but these cut-off values reflect the likelihood of failing an OFC, rather than passing the OFC.[2] A few retrospective studies have aimed to find cut-offs to predict allergy persistence.[3-5] However these retrospective studies investigating cut-off values have some limitations, including enrollment of children with wide age ranges.

SPT represents one of the primary diagnostic tools in clinical practice. Although it can be performed both with commercial allergen extracts and fresh foods, the standard application is with commercial extracts. Fresh food extracts have been reported to be more effective in detecting sensitization than commercial extracts.[6] Fresh egg (FE) has been used for SPT in some studies, particularly those looking to identify cut-off values in the diagnosis of raw or baked EA.[7-9] To the best of our knowledge there is no published study evaluating the accuracy

of SPT using FE for predicting outgrowth of allergy in the follow-up period of egg-allergic children.

Since most of the allergenic egg proteins are found in egg white (EW), the primary diagnostic tools for EA have been based on EW testing.[1] The value of egg yolk (EY) sIgE has not been well characterized, but some studies suggest that a minority of allergenic protein can be identified in yolk.[10,11] Sensitisation to those allergens in children diagnosed with EA have been demonstrated.[12,13] In a recent study using component-resolved diagnostic technology, Gal d 5, an EY allergen, was observed to be strongly associated with persistent EA.[14] Therefore, performing EY sIgE alongside EW sIgE could improve the specificity of the diagnostic workup and aid in the decision when to rechallenge egg-allergic children.

Ultimately, in children <2 years old, studies evaluating the value of SPT and sIgE levels to predict persistence or resolution of EA are limited. The aim of the present study, performed in children <2 years of age with IgE-mediated EA, was to evaluate the optimal decision points for outgrowth (ODP^{fo}) with SPTs using FE, EW and EY, and sIgE levels for EW and EY, to predict persistence of allergy or outgrowth after a one-year period of strict egg avoidance.

Methods

Study group

Participants were recruited prospectively from the Pediatric Allergy Clinics of Kocaeli University Medical Faculty from 2015 to 2017. Children between the ages of two and 24 months who were referred because of a history of reaction to egg were included.

A positive clinical history of IgE-mediated EA was defined as skin reactions (urticaria, angioedema, atopic dermatitis flare), digestive symptoms (vomiting), or respiratory

symptoms (rhinitis, cough, dyspnea) occurring in the two hours after egg intake. Patients with concomitant serious disease, unstable asthma and multiple sensitizations, other than to milk and egg, were excluded in order to minimize the number of confounding variables in the population of interest.

The study was approved by the Ethical Committee of the Medical Faculty, and written informed consent was obtained from the parents of participating patients.

Study design

Initial allergy evaluation with sIgEs (EW and EY) and SPTs for EW, EY, and FE were performed. After initial evaluation, all forms of egg were eliminated from the diet of participants. The patients with atopic dermatitis were treated with the moisturizing agents and topical steroids. A week later, participants underwent baseline OFCs, the methodology of which is described in detail below. But, in the patients with atopic dermatitis OFCs were deferred until gaining remission with the elimination diet, moisturizer and topical steroids and performed one week after remission. Egg consumption (raw, boiled, cooked and baked) was strictly avoided in patients who failed the baseline OFC. The patients were followed up clinically at 3-month intervals. After one year, EA was re-evaluated with repeat OFC, SPTs, and sIgEs.

Oral egg challenges

Challenges were performed in an open manner with gradual feeding with an age-appropriate serving of up to one boiled egg (boiled for 10 minutes), which contained approximately 6.1 grams of protein. OFC doses were given every 15 minutes under physician supervision in the Pediatric Allergy Unit in accordance with published guidelines and were terminated at the first signs of clinical reactivity.[15] The OFC was considered to be positive when there were skin reactions (urticaria, angioedema, or atopic dermatitis flare), gastrointestinal (vomiting),

or respiratory (rhinoconjunctivitis, bronchospasm, cough, or dyspnea) manifestations within two hours of egg ingestion. For atopic dermatitis an increase of at least 10 scoring atopic dermatitis (SCORAD) points after egg exposure was considered a flare.[16] If a patient developed signs or symptoms indicative of anaphylaxis during the challenge, the severity of anaphylaxis was graded as mild, moderate, or severe.[17] In cases of negative OFCs, regular dietary egg re-introduction was undertaken (one whole boiled egg/day for at least seven consecutive days after OFC).

Children with positive laboratory findings did not undergo OFC if they had a history of anaphylaxis within the last 12 months which was associated with egg ingestion.

Specific IgE tests

Serological sIgE concentrations for EW and EY were assessed using UniCAP (Phadia UniCAP; Pharmacia Diagnostics, Uppsala, Sweden). The lower and upper limits of detection are 0.35 kU/L and 100 kU/L, respectively.

Skin prick test for commercial extracts and fresh foods

SPTs were performed with EW and EY (ALK-Abelló, Horsholm, Denmark) with a negative (saline) and positive (histamine) control. The size of the skin test response was calculated as the mean of the longest diameter and the longest orthogonal measured at 15 minutes. The SPT for FE was performed in the same way using a whisked whole egg. The same brand of medium size eggs, which included only one egg yolk, were used.

Statistical analyses

Statistical analyses were conducted using IBM SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA) and MedCalc 14.10.2 (MedCalc Software bvba, Ostend, Belgium). Kolmogorov-Smirnov tests were used to assess the normality of data distribution. Continuous variables were expressed as mean ± standard deviation, median (25th to 75th percentiles) and

categorical variables were expressed as counts (percentages). The relationship between sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and the ODPs for sIgE and SPTs were determined by analysis with the receiver-operating characteristic (ROC) curve. Correlation between the SPT diameters for EW and FE was evaluated with Spearman correlation coefficient. The Yates and Fisher chi-squared test was used for comparison between groups. The Mann-Whitney nonparametric test was used to compare continuous variables between two groups. Wilcoxon signed rank test was used to compare the initial and last SPT and sIgE values of the persistent patients and the patients who outgrew egg allergy. A two-sided p value <0.05 was considered statistically significant.

Results

The main characteristics of the patients with persistent EA and the patients who outgrew EA with regard to age, symptoms and family history are shown in Table 1.

Initial challenge at diagnosis

Eighty-one children (63% male), median age seven months (range 2-24 months), underwent an initial evaluation. Sixty initial challenges out of seventy-seven (77.9%) were assessed as positive (Figure 1). For patients with positive challenges, symptoms included atopic dermatitis flare (n=39), urticaria (n=8), both atopic dermatitis flare and urticaria (n=7), or vomiting (n=6) within two hours of egg ingestion. No patient had anaphylaxis. The challenge was not performed in four infants because of a history of a life-threatening anaphylactic reaction to egg, contraindicating an OFC. These four patients were considered to have a positive initial OFC. All 64 children strictly avoided all dietary egg.

SPT and sIgE characteristics during the initial challenge

The median (25th to 75th percentiles) initial sIgE levels for EW were 4.0 kU/L (1.92-10.37) and 10 kU/L (4.3-62.6) and the median (25th to 75th percentiles) initial sIgE levels for EY were 0.88 kU/L (0.4-3) and 3.0 kU/L (0.75-16.8) in the patients who outgrew EA and in the persistent patients, respectively (p=0.022 and p=0.017).

The median (25th to 75th percentiles) initial SPT measurements for EW were 3 mm (3-5) and 5 mm (4-7) and the median (25th to 75th percentiles) initial SPT levels for EY were 0 mm (0-3) and 3 mm (0-5) in the patients who outgrew EA and in the persistent patients, respectively (p=0.007 and p=0.039).

The median (25th to 75th percentiles) initial SPT measurement for FE were 10 mm (7-16.5) and 11 mm (9-16) in the patients who outgrew EA and in the persistent patients, respectively (p=0.380).

Challenge after 1 year of avoidance

Five patients were lost to follow-up. The one year follow up challenge test was performed on the remaining 59 children. Twenty-seven (46%) reacted to egg. For patients with positive challenges, symptoms included atopic dermatitis flare (n=17), urticaria (n=3) and anaphylaxis (n=3) within two hours of egg ingestion. Urticaria accompanied by atopic dermatitis flare developed in four children.

Thirty-two children (54%) outgrew their allergy and passed an OFC one year after initial diagnostic challenge. The mean (±SD) ages of patients with persistent allergy and those who outgrew their allergy were 20.38±10.10 and 18.03±7.91 months, respectively. Age at symptom onset and at initial evaluation was not statistically different when comparing the patients with persistent EA and those who outgrew EA (Table 1).

Performance of SPT and sIgE obtained during the last challenge in predicting outgrowing EA

The performance of the last SPTs and sIgE assays, indicating EA persistence or outgrowth, compared with the last OFC is shown in table 2. Analysis of the ROC curve characteristics showed that ODP^{fo} values for SPTs for EW, EY and FE were ≤3 mm, ≤3 mm and ≤11 mm, with PPV of 78.3%, 58.7% and 78.8%, respectively. An SPT diameter for FE ≤4mm had 100% PPV and 56% NPV for outgrowth (Table 2). No patient with persistent EA had an SPT for FE ≤4mm (Table 3).

The ODP^{fo} values for the last assays of EW and EY sIgEs were 4 kU/L and 2.1 kU/L (PPV, 84.6% and 86.2%), respectively (Table 2). Areas under the ROC curve (AUC) are given in table 2 and figure 2.

The proportions of patients with the last SPTs and sIgE levels below these ODP^{fo} values were higher among patients who outgrew EA than those with persistent allergy (Table 3). The initial parameters of the patients with persistent allergy were higher at diagnosis and remained higher in the follow-up period (Table 4).

The diameters of SPT for FE during initial evaluation decreased significantly during the last evaluation in the patients who outgrew EA (11.3mm versus 6.7mm, $p<0.001$) whereas it was unchanged for those with persistent EA (12.8 vs 14.5 mm, $p=0.385$; see Table 4). Fifty percent or more reduction in initial SPT diameters for FE was found during the last evaluation in 50% of the patients who outgrew EA, while this proportion was only 12% of the persistent patients ($p=0.004$). We also observed a reduction of ≥50% of the levels of initial EW and EY sIgE in half of the patients who acquired tolerance ($p=0.015$ and $p=0.032$, respectively).

Discussion

We investigated the optimal criteria for SPTs with EW, EY and FE and sIgE levels for EW and EY to predict persistence or outgrowth of allergy in children <2 years with IgE-mediated EA after a period of strict egg avoidance. The present study adds to the literature in several aspects. Firstly, EA commonly occurs in the first two years of life and resolves by the third year in up to 50% of patients.[1] However, only a few studies have investigated laboratory parameters predictive of outgrowth in children <2 years with EA.[1,3,18-20] For this reason, our findings in the first few years of life are useful to guide clinicians through ODP cut-off values for SPT and sIgE which predict outgrowth reliably and help optimal patient selection for OFC. Secondly, although FE was used for SPT in some studies with the aim of identifying cut-offs for the diagnosis EA, to the best of our knowledge there is no published study evaluating the accuracy of SPT using FE for outgrowth in the follow-up period of children with EA.[5,7-8] Lastly, the value of EY sIgE in the evaluation of EA outgrowth was investigated, as this has not been well characterised previously.

Shek *et al*. reported that in children under four years of age, a 50% decrease in sIgE in any 12 month period was associated with a 52% probability of developing tolerance.[5] We observed a reduction of ≥50% in SPT diameters for FE when comparing initial and the last evaluation after a median follow-up of 13 months in half of the patients who outgrew their EA. The initial levels of EW and EY sIgE were also decreased ≥50% in half of our patients who acquired tolerance, supporting the findings of Shek *et al*.

We found that an SPT for FE diameter of ≤4mm had 100% specificity and an NPV of 56% for outgrowing EA. When the threshold value was increased to ≤11mm, a PPV of 78.8%, an NPV of 76.2% and LR+ of 2.76 were obtained. It has been suggested that children with ≤50% likelihood of reacting to a food are suitable candidates for OFC in the follow-up

period of a known food allergy, so that an SPT for FE ≤4mm may be of use in selecting patients who have the best risk/benefit ratio of having a negative OFC.[15] A follow-up FE SPT had AUC >0.8 indicating that it had good power in predicting the outcome of the challenge in children < 2 years with heated EA.

The NPV of fresh food SPTs was reported to be higher than that of commercial extracts in previous studies which evaluated diagnostic performance.[6,21] Rance *et al* established that the NPV of fresh EW SPT was 100% versus 28% of commercial EW extract.[6] Calvani *et al* showed that at a cut-off point of 3 mm, fresh milk had the greatest NPV with a value of 98%.[21] Consistent with these reports, we found a higher NPV with SPT using FE at the level of ODP^{fo} than with SPT using commercial EW (76.2% versus 58.1%, respectively).

In clinical practice, SPT and sIgE are used for following the course of EA to assess whether outgrowth has occurred. Hence, studies typically focus on the use of SPT and sIgE to predict the persistence or resolution of EA.[20] A few studies have reported cut-offs for persistence in children under two years old (Table 5).[3,18,19] Montesinos *et al*. showed that EW sIgE levels of 1.52, 1.35, and 2.59 kUA/L, predicted clinical reactivity (PPV >95%) at the ages of 25-36, 37-48 and 49-60 months, respectively.[19] Dieguez *et al*. reported that SPT for EW ≥7 mm and sIgE ≥1.5 kUA/L at follow-up had 90% PPV for persistent EA, with a LR+ of 6.7 and 5.5, respectively.[3] In an earlier prospective study, SPT for EW <6 mm at follow-up increased the likelihood of tolerance by 3.74.[1] The same study reported that for every 0.1 unit decrease in sIgE, the likelihood of tolerance increased by 1.17.[1] We found that the ODP^{fo} for follow-up sIgE for EW was ≤4 kU/L, with an LR+ of 4.17 and a PPV of 84.6%, and the AUC was >0.8. The ODP^{fo} of follow-up SPT for EW was ≤3mm with a PPV of 78%, NPV of 58% and LR+ of 2.67. However, the AUC of EW SPT approached 0.7, suggested a modest to moderate association.

There is little evidence in the literature for the value of EY sIgE in the evaluation of allergic response to egg. In a recent study using component-resolved diagnostic technology, Gal d 5, an EY allergen, was observed to be strongly associated with persistent allergy ($p<0.01$).[14] In another study, Dieguez *et al*. reported that EY sIgE ≥ 1 kUA/L at follow-up had 95% PPV for persistent EA, with a LR+ of 13.7.[3] The AUC was 0.74. In our study, we found a follow-up sIgE for EY ≤2.1 kU/L and a follow-up sIgE for EW ≤4 kU/L had an LR+ of 4.91 and 4.17 and a PPV of 86.2% and 84.6%, respectively. The accuracy of EY sIgE was found to be close to the accuracy of EW. Having a follow-up EY sIgE ≤2.1 kU/L was almost five times more frequent in the patients who outgrew EA than in the persistent patients. Only four patients (16%) had an EY sIgE level of ≤2.1 kU/L in the persistent group. The AUC was between 0.8 and 0.9, indicating that a follow-up EY sIgE had high accuracy for predicting the outcome of the challenge in children <2 years with heated EA. On the basis of these findings, it is suggested that sIgE for EY could be useful for monitoring outgrowth, in addition to other well-established diagnostic markers. Having a higher level of sIgE for both EW and EY would seem to be a more clinically useful tool, thus avoiding unnecessary OFCs.

There are some limitations of the present study. First, we had a high number of reactions involving atopic dermatitis. The assessment of OFC in patients with atopic dermatitis can be challenging. Therefore, the patients with atopic dermatitis were first treated with topical agents and an elimination diet. OFC was performed after remission and an increase of at least 10 SCORAD points over the basal point after allergen exposure was considered a positive challenge in accordance with published guidelines.[16] Secondly, we did not use any component-resolved diagnostic technology. Thirdly, we recommended children avoid all types of egg during the study period, including baked egg, even if this may have been tolerated and has been shown to help expedite outgrowth of EA. This recommendation

was made because the consumption of baked eggs could have been a confounding factor in the outgrowth data of the study population.

During the follow-up period of the children <2 years of age with EA, an SPT for FE ≤4mm, sIgE for EY ≤2.1 kU/L and sIgE for EW ≤4.0 kU/L, have high accuracy in selecting the patients who have outgrown their allergy and should be offered an OFC. Our findings suggest that SPT for FE and sIgE for EY are important follow-up parameters for outgrowth, in addition to sIgE and SPT for EW. Given that EA is one of the most prevalent food allergies globally and that resolution often occurs in the first few years of life, inexpensive and easily available tests such as SPT with FE may aid in selecting patients to undergo OFC, improve the safety profile of OFCs, and contribute to cost savings.

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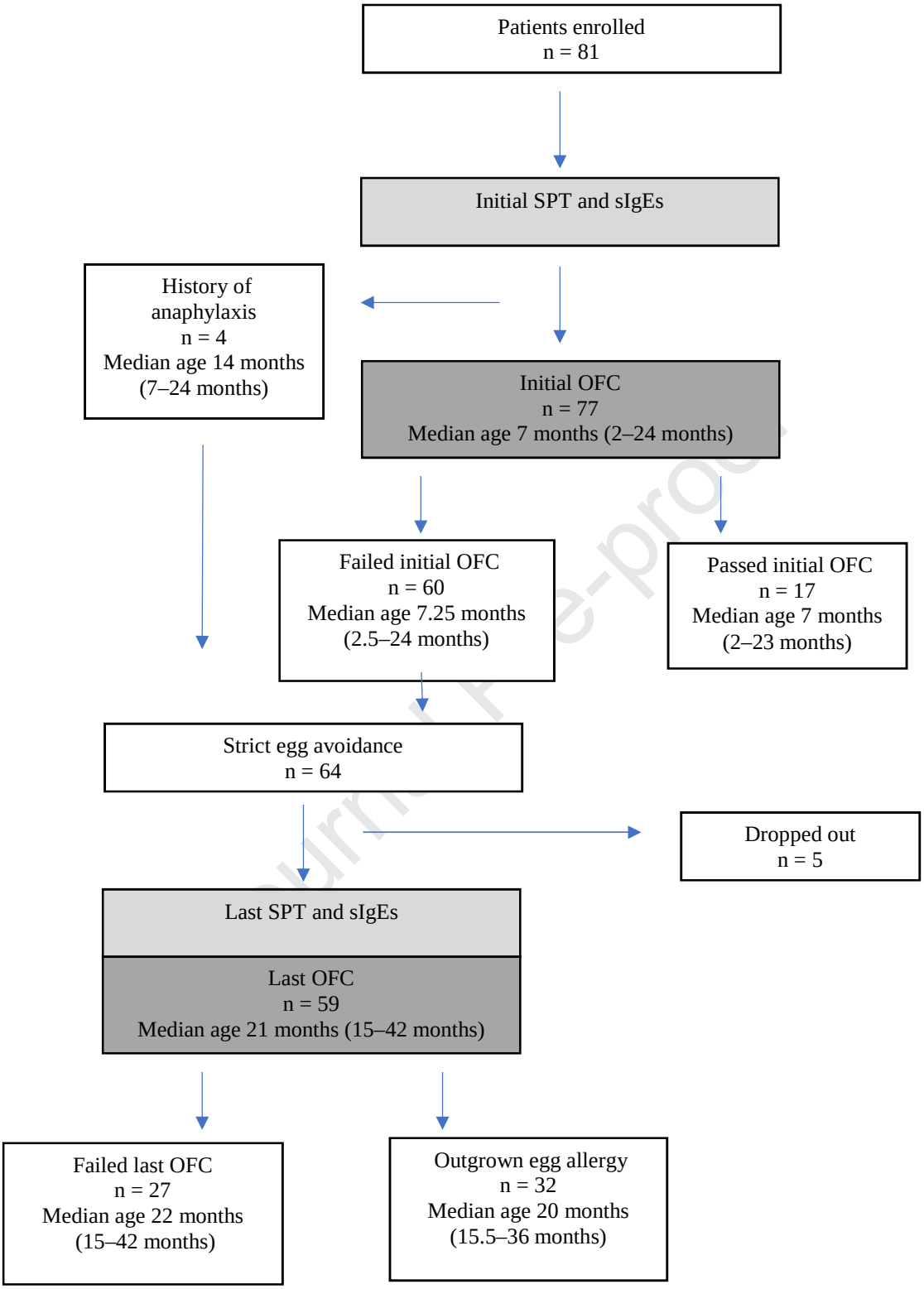
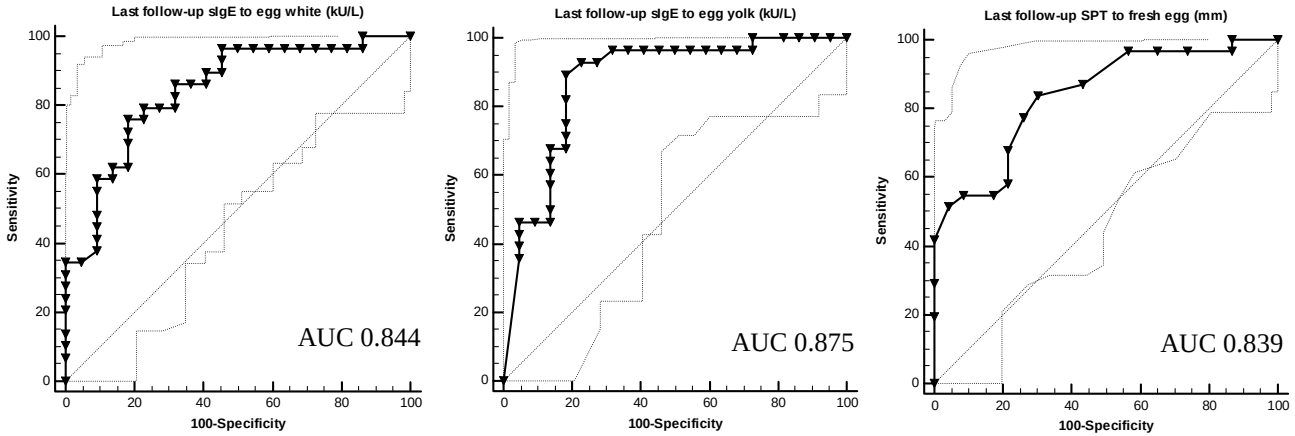

Patients enrolled
n = 81
Initial SPT and sIgEs
History of anaphylaxis
n = 4
Median age 14 months
(7–24 months)
Initial OFC
n = 77
Median age 7 months (2–24 months)
Failed initial OFC
n = 60
Median age 7.25 months
(2.5–24 months)
Passed initial OFC
n = 17
Median age 7 months
(2–23 months)
Strict egg avoidance
n = 64
Dropped out
n = 5
Last SPT and sIgEs
Last OFC
n = 59
Median age 21 months (15–42 months)
Failed last OFC
n = 27
Median age 22 months
(15–42 months)
Outgrown egg allergy
n = 32
Median age 20 months
(15.5–36 months)


Last follow-up sIgE to egg white (kU/L)
Sensitivity
100-Specificity
AUC 0.844
Last follow-up sIgE to egg yolk (kU/L)
Sensitivity
100-Specificity
AUC 0.875
Last follow-up SPT to fresh egg (mm)
Sensitivity
100-Specificity
AUC 0.839

A B C

Figure legends

FIGURE 1

Study design and enrollment.

OFC, oral food challenge; SPT, skin prick test; sIgE, specific immunoglobulin E

FIGURE 2

Performance characteristics of optimal cutoff values of egg white (A) and egg yolk (B) specific IgE and skin prick test for fresh egg (C) established by receiver operating characteristic curve analysis.

SPT, skin prick test; AUC, area under the curve

TABLE 1 Patient characteristics

		Patients with persistent egg allergy (n=27)	Patients who outgrew egg allergy (n=32)	p
Age at first evaluation (month) mean±SD[median (minimum-maximum)]		9.50±5.60 [7 (5.4-12)]	8.81±4.52 [7.5 (6-10)]	NS
Age at symptom onset (month) mean±SD[median (minimum-maximum)]		3.23±2.49 [2 (2-5)]	3.57±2.15[3 (2-6)]	NS
Male sex n (%)		16/27 (59%)	21/32 (66%)	NS
Family history n (%)		4/27 (15%)	6/32 (19%)	NS
Symptoms on admission n (%)				
	Atopic dermatitis	22/27 (81%)	24/32 (75%)	
	Urticaria	7/27 (26%)	8/32 (25%)	NS
	Anaphylaxis	3/27 (9%)	1/32 (3%)	NS
	Vomiting	3/27 (11%)	3/32 (9%)	NS
	Asthma	4/27 (15%)	6/32 (19%)	NS
	Rhinitis	3/27 (11%)	6/32 (19%)	NS

TABLE 2 Optimal decision points obtained during the initial and the last evaluation to predict outgrowth

	Initial evaluation								Last evaluation							
	Optimal decision points for outgrowth	SE‡ (%) [95% CI]	SP§ (%) [95% CI]	PPV¶ [95% CI]	NPV†† [95% CI]	LR+‡‡ [95% CI]	LR-§§ [95% CI]	AUC¶¶ [95% CI]	Optimal decision points for outgrowth	SE (%) [95% CI]	SP (%) [95% CI]	PPV [95% CI]	NPV [95% CI]	LR+ [95% CI]	LR- [95% CI]	AUC [95% CI]
SPT to commercial egg yolk extract (mm)	≤3	83.87 (66.3 – 94.5)	37.50 (18.8 – 59.4)	63.4 (55.1 – 71.0)	64.3 (40.9 – 82.4)	1.34 (0.9 – 1.9)	0.43 (0.2 – 1.1)	0.639 (0.498-0.764)	≤3	87.1 (70.2 – 96.4)	20.83 (7.1 – 42.2)	58.7 (52.6 – 64.5)	55.6 (27.3 – 80.6)	1.10 (0.9 – 1.4)	0,62 (0.2 – 2.1)	0.592 (0.451- 0.723)
SPT to commercial egg white extract (mm)	≤3	61.29 (42.2 – 78.2)	79.17 (57.8 – 92.9)	79.2 (62.4 – 89.7)	61.3 (49.3 – 72.1)	2.94 (1.3 – 6.7)	0.49 (0.3 – 0.8)	0.720 (0.583-0.833)	≤3	58.06 (39.1 – 75.5)	78.26 (56.3 – 92.5)	78.3 (61.1 – 89.2)	58.1 (46.5 – 68.8)	2.67 (1.2 – 6.1)	0,54 (0.3 – 0.9)	0.694 (0.554-0.812)
SPT to fresh egg (mm)	≤7	35.48 (19.2 – 54.6)	79.17 (57.8 – 9.2)	68.7 (46.9 – 84.6)	48.7 (40.5 – 57.0)	1.70 (0.7 – 4.2)	0.81 (0.6 – 1.1)	0.547 (0.407-0.682)	≤11	83.87 (66.3 – 94.5)	69.57 (47.1 – 86.8)	78.8 (66.3 – 87.5)	76.2 (57.8 – 88.2)	2.76 (1.5 – 5.2)	0.23 (0.10 – 0.5)	0.839 (0.713- 0.925)
									≤4	41.94 (24.5 – 60.9)	100 (85.2 – 100.0)	100	56.1 (48.6 – 63.3)		0.58 (0.4 – 0.8)	
Egg yolk sIgE (kU/L)	≤8.8	100 (88.4 – 100.0)	29.17 (12.6 – 51.1)	63.8 (57.7 – 69.5)	100	1.41 (1.1 – 1.8)	0	0.652 (0.510-0.777)	≤2.1	89.29 (71.8 – 97.7)	81.82 (59.7-94,8)	86.2 (71.8 – 93.9)	85.7 (66.9 – 94.7)	4.91 (2.0 – 12.0)	0.13 (0.04 – 0.4)	0.875 (0.751- 0.952)
Egg white sIgE (kU/L)	≤14.8	87.10 (70.2 – 96.4)	41.67 (22.1 – 63.4)	65.9 (57.3 – 73.5)	71.4 (47.2 – 87.5)	1.49 (1.0 – 2.1)	0.31 (0.1 – 0.9)	0.649 (0.509-0.773)	≤4	75.86 (56.5 – 89.7)	81.82 (59.7-94,8)	84.6 (68.9 – 93.2)	72.0 (56.7 – 83.5)	4.17 (1.7 – 10.4)	0.30 (0.2 – 0.6)	0.844 (0.715- 0.930)

‡ SE, sensitivity; § SP, specificity; ¶ PPV, positive predictive value; †† NPV, negative predictive value; ‡‡ LR+, positive likelihood ratio; §§ LR-, negative likelihood ratio; ¶ ¶ AUC, area undercurve

TABLE 3 Distribution of the persistent patients and the patients who outgrew egg allergy according to the optimal decision points for outgrowth obtained during the initial and the last evaluation

	Initial evaluation				Last evaluation			
	ODP[fo]	Patients who outgrew egg allergy (n=32)	Persistent patients (n=27)	p-Value	ODP[fo]	Patients who outgrew egg allergy (n=32)	Persistent patients (n=27)	p-Value
SPT to commercial egg yolk extract n (%)	≤3mm	27/32 (84.4)	17/27 (63)	0.060	≤3mm	28/32 (87.5)	22/27 (81.5)	0.719
SPT to commercial egg white extract n (%)	≤3mm	18/31 (58.1)	5/27 (18.5)	0.002	≤3mm	19/32 (59.4)	6/26 (23.1)	0.006
SPT to fresh egg n (%)	≤7mm	11/32 (34.4)	5/27 (18.5)	0.172	≤11mm	27/32 (84.4)	8/26 (30.8)	<0.001
					≤4	13/32 (40.6)	0/26 (0)	<0.001
Egg yolk sIgE n (%)	≤8.8kU/L	31/31 (100)	18/27 (66.7)	<0.001	≤2.1kU/L	26/29 (89.7)	4/25 (16)	0.001
Egg white sIgEn (%)	≤14.8kU/L	28/32 (87.5)	15/27 (55.6)	0.008	≤4kU/L	22/30 (73.3)	4/25 (16)	0.001

ODP[fo], optimal decision point for outgrowth; SPT, skin prick test; sIgE, specific immunoglobulin E.

TABLE 4 Initial and last SPT and sIgE characteristics of the persistent patients and the patients who outgrew egg allergy

	Patients who outgrew egg allergy n=32					Persistent patients n=27				
	Initial evaluation		Last evaluation		p-Value*	Initial evaluation		Last evaluation		p-Value*
	mean±SD	median (25th to 75th percentiles)	mean±SD	median (25th to 75th percentiles)		mean±SD	median (25th to 75th percentiles)	mean± SD	median (25th to 75th percentiles)	
SPT to commercial egg yolk extract (mm) (mean±SD)	1.5±3.2	0 (0-3)	0.7±1.5	0 (0-0)	0.259	2.5±2.7	3 (0-4.25)	1.4±1.9	0 (0-3)	0.039
SPT to commercial egg white extract (mm) (mean±SD)	3.8±3.6	3 (1.5-5)	2.9±2.9	3 (0-5)	0.169	4.9±1.9	4.5 (4-6.25)	4.9±3.2	4 (3.5-7)	0.807
SPT to fresh egg (mm) (mean ±SD)	10.9±5.1	10 (6-15)	6.6±5.2	6 (3-10)	0.001	13.8±6.0	11 (9-15.25)	14.5±6.1	13 (8.5-18)	0.295
Egg yolk sIgE (kU/L) (mean± SD)	1.9±2.4	0.84 (0.4-3)	1.3±2.7	0.63 (0-1.77)	0.017	15.2±28.2	3 (0.73-15.90)	20.5±31.8	7.15 (2.42-18.0)	0.236
Egg white sIgE (kU/L) (mean ±SD)	9.2±16.3	3.6 (1.5-9.1)	5.5±14.6	2 (0.45-4.55)	<0.001	28.8±37.9	8.75 (3.97-38.90)	30.9±34.7	15.15 (4.8-46.5)	0.747

SPT, skin prick test; sIgE, specific immunoglobulin E.

* Wilcoxon signed rank test was used.

TABLE 5 Studies conducted in children <2 years which aimed to find predictive values for development of tolerance or persistence of allergy using SPT and/or sIgE cut-off values.

Study (N)	Age	Cooking degree of egg administered in OFC[†]	Design	Follow-up period	Tolerance Diagnosis	Tolerance %	Statistical methodology	SPT[‡] (mm)	Statistical methodology	sIgE[§] (KU_A/L)

								Egg white	**Fresh egg**		**Egg white**	**Egg yolk**
Crespo 1994[18] N=40	1 year		Retrospective	2.5 years	OFC	38%		-		92% PPV[‡‡] to persistence	≥ 1.2	
Montesinos 2010[19] N=42	Mean 15.7 months (range 8–27.5 months)	Sequential administration of cooked egg white and raw egg white	Retrospective	15 to 118.6 months, with an average of 49 months	Open OFC	50%		-		96% PPV, to persistence 66% NPV[§§] (AUC[¶¶]0.85) 100% PPV to persistence 75% NPV (0.913) 100% PPV to persistence 75% NPV (0.889) 100% PPV to persistence 60% NPV (0.861)	≥1.37 (≤ 2 years) ≥0.36 ≥1.52 (25-36 months) ≥0.54 ≥1.35 (37-48 months) ≥0.36 2.59 (49-60 months) ≥0.96	
Dieguez 2009[3] N=157	Median 2.5 years (range 15months-16 years)	Sequential administration of cooked egg yolk, egg white, raw egg white, cooked whole egg	Retrospective	Not reported	DBPCFC[¶]	36.3%	90% PPV to persistence LR+ [‡‡‡] 6.7 (AUC 0.79)	≥7	-	90% PPV to persistence LR+ 5.5 (AUC 0.77)	≥1.5	
							96% PPV to persistence LR + 12.3	≥9		90% PPV to persistence LR+ 7.2 95% PPV to persistence LR+ 13.78		0.35 1.0
Dang TH 2018[14] N=451	Median age was 12 and 14 months at the	Raw	A subset of subjects was selected from	Until 4 years of age	OFC	48% at age 2 83% at age	64% PPV to persistence Sp 95%, LR+ 9.36 (AUC 0.83)	>11				

	time of SPT and OFC respectively		the HealthNuts cohort			4					
Boyano-Martinez 2002[1] N=59	<2years	Cooked (10 minutes of boiling) and raw egg white	Prospective	7 to 86 months, with a median of 32 months	Open OFC	59%	The likelihood (HR††) of tolerance increased by 3.74 (95% CI 1.60–8.74)	< 6	-	*The likelihood (HR) of tolerance increased by 1.17 (95% CI 1.05–1.30)	Every 0.1 unit decrease in sIgE
Present study N=59	Median 7 months (range 2-24 months)	Cooked (10 minutes of boiling) whole egg	Prospective	Median 13 months (range 12-18 months)	Open OFC	54%	78.3% PPV to outgrowth Sp 78.2%, NPV 58% LR+ 2.67 (AUC 0.694)	≤3		85% PPV to outgrowth LR+ 4.17 (AUC 0.844)	≤4
							78.8% PPV to outgrowth Sp 69%, NPV 76% LR+ 2.76 (AUC 0.839)		≤11	PPV 86% to outgrowth LR+ 4.91 (AUC 0.875)	≤2.1
							100% PPV to outgrowth Sp†† 100%, NPV 56%		≤4		

† OFC, Oral food challenge; ‡ SPT, skin prick test; § sIgE, specific immunoglobulin E; ¶ DBPCFC, double blind placebo-controlled food challenge; †† HR, hazard ratio; ‡‡ PPV, positive predictive value; §§NPV, negative predictive value; †† Sp, specificity; ‡ ‡ ‡ LR+, positive likelihood ratio; ¶ ¶ AUC, Area under the curve

*Only in children with cutaneous symptoms at diagnosis