

ORIGINAL ARTICLE

Predicting the outcome of oral food challenges with hen's egg through skin test end-point titration

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Summary

Background Oral food challenge (OFC) is the diagnostic 'gold standard' of food allergies but it is laborious and time consuming. Attempts to predict a positive OFC through specific IgE assays or conventional skin tests so far gave suboptimal results.

Objective To test whether skin test with titration curves predict with enough confidence the outcome of an oral food challenge.

Methods Children ($n = 47$; mean age 6.2 ± 4.2 years) with suspected and diagnosed allergic reactions to hen's egg (HE) were examined through clinical history, physical examination, oral food challenge, conventional and end-point titrated skin tests with HE white extract and determination of serum specific IgE against HE white. Predictive decision points for a positive outcome of food challenges were calculated through receiver operating characteristic (ROC) analysis for HE white using IgE concentration, weal size and end-point titration (EPT).

Results OFC was positive (Sampson's score ≥ 3) in 20/47 children (42.5%). The area under the ROC curve obtained with the EPT method was significantly bigger than the one obtained by measuring IgE-specific antibodies (0.99 vs. 0.83, $P < 0.05$) and weal size (0.99 vs. 0.88, $P < 0.05$). The extract's dilution that successfully discriminated a positive from a negative OFC (sensitivity 95%, specificity 100%) was 1 : 256, corresponding to a concentration of 5.9 $\mu\text{g/mL}$ of ovotransferrin, 22.2 $\mu\text{g/mL}$ of ovalbumin, and 1.4 $\mu\text{g/mL}$ of lysozyme.

Conclusion EPT is a promising approach to optimize the use of skin prick tests and to predict the outcome of OFC with HE in children. Further studies are needed to test whether this encouraging finding can be extended to other populations and food allergens.

Keywords end-point titration, food-allergy, hen's egg, IgE, oral food challenge, prediction, ROC analysis, skin tests

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Introduction

Estimates of the prevalence of IgE-mediated allergic reactions to foods range from 2% to 5% and rates of sensitisation are even higher [1]. Upon ingestion, food allergenic molecules cross-link specific IgE antibodies bound on mast cells and basophils, trigger the release of pro-inflammatory mediators, which in turn cause local and/or systemic reactions leading to overt allergic symptoms [2]. This phenomenon requires the sequential intervention of multiple components, each one essential to full clinical manifestations. Such complexity explains why only oral food challenge (OFC), i.e. the controlled attempt to reproduce the whole chain of events of food allergic

reactions, is considered the diagnostic 'gold standard' of food allergies [3]. However, standardized OFC are laborious, time-consuming, require patient collaboration and safety measures [3]. Attempts have been made to eliminate the need to perform it by preliminary quantitative determination of specific IgE antibodies against food allergens, at least in a subset of patients who are likely to experience clinical reactions [4]. Therefore, encouraging results initially obtained with this approach were only partially reproduced [5, 6] and, so far, IgE testing is not considered by international guidelines as a substitute of OFC [7].

Skin prick tests (SPT) evaluate not only IgE antibodies *per se*, but also their capacity to trigger mast cell

degranulation and a local reaction. Therefore, this *in vivo* test should better predict a positive oral challenge test, when compared with *in vitro* determination of specific IgE antibodies. By contrast, using standard cut-off points for positivity (weal reaction ≥ 3 mm), SPT has a good sensitivity, but a quite low specificity in predicting a positive oral food provocation test [8]. In the attempt to improve SPT performance, higher and more specific cut-off points for weal diameter positivity have been identified through receiver operating characteristic (ROC) analyses [9, 10]. However, the decision points corresponding to a 99% positive predictive value (PPV) were so high [e.g. 17.8 mm for whisked white and yolk of hen's egg (HE)] that only a few or even none (in the case of cow's milk) of the examined patients could achieve them [10]. The decision points corresponding to a 95% PPV were only slightly lower (13 mm for HE) [10].

The most likely explanation of such a frustrating performance is that measuring weal size for a single allergen dose is not the best grading system for SPT [11]. More precise grading systems of allergen sensitivity have been based since the 1970s on mid-point [12] or end-point [13] titrations. So far, this method has not been applied to food allergy. We hypothesized that titration curves as a grading system would have improved the performance of SPT in predicting a positive OFC. We examined the predictive value of SPT end point titration using an HE white extract in the diagnosis of IgE-mediated egg allergy in comparison with the use of standard SPT and specific IgE tests.

Methods

Patients

The study population consisted of 47 children (27 boys) consecutively attending two paediatric allergology clinics during the period March 2005–June 2007, due to suspected IgE-mediated HE allergy ($n = 39$) based on history of allergic reactions after ingestion (25/47) and/or contact with HE (14/47) and previous SPT/IgE positive for HE. We also tested patients with HE allergy ($n = 8$) previously diagnosed on the basis of a recent (< 1 year) positive OFC or anaphylactic episode (i.e. a severe allergic reaction that is rapid in onset and may cause death) due to HE ingestion [14]. All the patients, at the time of the consultation, were following a HE elimination diet.

Molecular characterization of the hen's egg white extract

Quantitation was performed through SDS-PAGE. Purified egg proteins [ovalbumin (OVA), lysozyme and ovotransferrin] (Sigma-Aldrich Fine Chemicals, Milan, Italy) were suspended in sample buffer (0.25 M Tris-HCl, pH 6.8, 7.5% glycerol, 2% SDS and 5% β -mercaptoethanol) at a final concentration of 1 mg/mL and run in parallel with a pre-

stained molecular weight standard marker solution (Bio-Rad, Richmond, CA, USA). For quantitative analysis of protein bands a gel scanner (Sharp JX-330, Pharmacia Biotech, Milan, Italy) and the Image Master™ 1D Software were used.

Standard skin prick tests

SPT were performed with 1 mm-tipped lancets (Dome-Hollister-Stier, Warwickshire, UK) using a commercial HE white extract (Stallergènes SA, Antony, France) and fresh HE white [15]. Histamine (1 mg/mL) and a glycerol-saline solution were used as positive and negative standards, respectively. Results were expressed as the mean weal diameter in millimetres; weal reactions with a diameter of 3 mm or more were considered positive [15–17]. SPTs were performed by two trained investigators, whose inter-observer reproducibility was satisfactory [18].

Skin prick tests end-point titration

Twelve serial twofold dilutions of the HE white extract (Stallergènes) were prepared in sterile saline solution immediately before the test as described previously [19, 20]. The even dilutions (1 : 1, 1 : 4, 1 : 16, 1 : 64, 1 : 256, 1 : 1024, 1 : 4096) were used for prick tests placed at 4 cm intervals on the volar surface of both forearms for each subject. The mean weal diameter was calculated after 30 min, which is a time necessary to obtain the maximum reaction at very low allergen concentrations [19, 20]. The highest dilution inducing a mean weal diameter of 3 mm or more was considered the 'observed endpoint dilution'. Values were plotted on a semi-logarithmic scale and a more precise measure of the end-point was estimated by logarithmic interpolation ('interpolated end-point dilution').

Oral food challenge

When a diagnosis of IgE-mediated egg allergy was suspected, an open OFC with fresh raw HE (whisked egg white and yolk) was carried out in allergy clinics. The use of OFC in the outpatient clinics, in substitution of a DBPCFC test, is accepted by European guidelines [3]. OFC tests have been adopted in other studies targeted to compare the results of skin test and/or IgE assays to those of oral provocation tests with the offending food [10, 21–23]. Children were admitted to our day-clinic in the morning in a fasting state; administration of antihistamines, if any, was stopped at least 7 days before provocation. Increasing volumes of 0.1, 0.3, 1, 3, 10, 30 mL were orally administered at 15-min intervals until the highest dose was reached or positive reaction appeared. An OFC test was regarded as positive when an immediate reaction involved at least two target organ among the following: skin (any

of generalized pruritus, flushing, urticaria, angio-oedema), gastrointestinal tract (repetitive vomiting), respiratory tract (rhinorrhoea, or marked congestion, or sensation of throat pruritus or tightness), cardiovascular (tachycardia = increase > 15 beats/min) or neurological system (change in activity level plus anxiety); this definition corresponds to a grade 3 according to Sampson's criteria for food anaphylaxis (Table 3) [24]. A score 1 or 2, which corresponds to very mild symptoms, has been considered as negative.

Determination of serum immunoglobulin E antibodies to hen's egg

Allergen-specific IgE for egg white and yolk were measured through a capture immunoassay (CARLA, Radim, Pomezia, Italy) [25]. The performance of this test is comparable to that of the Immuno-CAP system; its lower and upper detection thresholds are 0.15 and 200 IU/mL, respectively [26].

Statistical analysis

Comparisons between OFC positive (Sampson's score ≥ 3) vs. OFC negative were examined by χ^2 tests for categorical variables (or Fisher exact test when appropriate) and Student's *t*-test for continuous ones. Mean comparison of more than two classes are made by ANOVA with Bonferroni correction. A *P*-value < 0.05 was considered significant. Two by two tables were used to calculate sensitivity, specificity, PPV and negative predictive value (NPV). Data are best fitted by logarithmic models $Y = b_0 + b_1 \times \ln(x)$ to estimate dilution by interpolation. We plotted ROC curves for positive OFC in relation to SPT and IgE assay. The greater the area is under the ROC curve, the better is the performance of the diagnostic test. The optimal cut-off point is examined from the ROC curve with equal weight of sensitivity and specificity. Statistical analysis was performed using SPSS for Windows (version 13.0. SPSS, Chicago, IL, USA) and Stata 9.

Results

Patient characteristics

The characteristics of the 47 patients (27 males) who entered the study are shown in Tables 1 and 2. In keeping with the study inclusion criteria, all the participants had a positive weal reaction (≥ 3 mm) to SPT performed with the undiluted HE white extract; in two patients, however, SPT with fresh HE white did not elicit a positive weal. The mean age of the patients was 6.2 ± 4.2 years (range 1.1–15.7 years). Atopic dermatitis (AD) was the most frequent diagnosis (31/47, 66%), while gastrointestinal symptoms, urticaria, angio-oedema and anaphylaxis were reported by

Table 1. Characteristics of patients with or without a positive oral provocation test

	All (<i>n</i> = 47)		OPT positive (<i>n</i> = 20)		OPT negative (<i>n</i> = 27)	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Male gender	27	57.4	11	55.0	16	59.3
Age (years)	6.2 ± 4.2		$7.9 \pm 4.5^{\circ}$		$4.86 \pm 3.6^{\circ}$	
≤ 2 years	10	21.3	3	15.0	7	25.9
Disease						
Atopic dermatitis	31	66.0	15	75.0	16	59.3
Asthma, AR	25	53.2	17	85.0	8	29.6
Gastrointestinal	14	29.8	8	40.0	6	22.2
UA	11	23.4	6	30.0	5	18.5
Anaphylaxis	9	19.1	6	30.0	3	11.1
SPT ≥ 3 mm						
Fresh hen's egg white*	44	93.6	20	100	24	88.9
Extract hen's egg white	47	100	20	100	27	100
IgE-Ab to hen's egg white ^{††}	20	64.5	15	88.2	5	35.7

*One missing value and two SPT = 0 mm among OPT negative children.

[†]children with at > 0.35 kU/L IgE-Ab.

^{††}Three and 13 missing values among OPT positive and negative children, respectively.

[°]*P* < 0.05 (positive vs. negative).

Age = mean $\pm$ SD.

AR, allergic rhinitis; Ab, antibody; OPT, oral provocation test; SPT, skin prick test; UA, urticaria angio-oedema; SD, standard deviation.

14 (30%), 11 (23%) and 9 (19%) patients, respectively. Concomitant allergic rhino-conjunctivitis and/or asthma were reported by 25/47 (53%) patients and asthma was the only reported pathology in three (6%) patients.

Oral food challenge

All the patients underwent an open OFC with raw whole HE. A positive reaction confirmed the diagnosis of HE allergy in 20/47 (42.5%) children; reactivity corresponded in 10 of these children to a Sampson's score of 3, and in other 10 to a Sampson's score of 4. The eliciting dose triggering objective symptoms was < 1, 1, 3, 10 and 30 mL in 2, 5, 4, 4 and 5 children, respectively. No child had an extremely severe reaction (Sampson's score 5). In 27 children the test was classified as negative: among them, mild reactions with a Sampson's score of 1 and 2 were observed in nine and three children, respectively; no reactions were observed in the remaining 15 children. In keeping with the negative outcome of their OFC, all these 27 children regularly ingested HE-containing food at home during the weeks following their negative OFC, but none of them reported any adverse reaction (average follow-up duration = 9.9 ± 9 months). Finally, children with a positive OFC were significantly older than those with a negative OFC (*P* < 0.02); they reported also more frequent allergic rhino-conjunctivitis and/or asthma (*P* < 0.0002) (Table 1).

Table 2. Characteristics of the patients with a positive OFC

Code	Sex	Age (months)	Disease	End-point (observed dilution)	OFC symptoms	Sampson's score	HE total dose (mL)	HE trigger dose (mL)
1	F	127	A, AR, AD	1 : 4096	O, C, R, NC, T	3	0.1	0.1
3	F	78	A, AD	1 : 4096	AP, D, W	4	44.4	30.0
5	F	119	A, AR, GI, UA, Ana	1 : 1024	NC, C, O, T, AP, V, D	4	44.4	30.0
10	F	129	AR, AD	1 : 1024	O, T, N, V, AL	3	1.4	1.0
12	M	26	A, AD, GI, UA	1 : 4096	UA, C, NC, S, W	4	1.4	1.0
13	M	29	A, AD	1 : 1024	UA, N, AL, W	4	14.0	10.0
14	F	56	A, AD, GI, UA, Ana	1 : 4096	UA, C, AP, AL, V, W	4	1.4	1.0
15	M	55	A, AD, GI, Ana	1 : 4096	C, NC, N, V, AL	3	0.4	0.3
17	F	118	A, AD	1 : 64	O, NC, AP	3	44.4	30.0
18	F	136	A, AD	1 : 4096	O, C, NC, N, AP, V	3	4.4	3.0
23	F	24	GI	1 : 256	UA(g), C, AL, AP, D	4	14.4	10.0
25	M	117	A, AD	1 : 256	UA(g), O, C, NC, AP, T, BC	4	14.4	10.0
27	M	127	A, AR, AD, Ana	1 : 4096	C, NC, S, V, A, Ta, Hy	4	4.4	3.0
29	M	110	A, AD	1 : 1024	O, C, UA, N, AP, Sw, AL	3	44.4	30.0
32	F	148	A	1 : 4096	O, C, UA, W	4	4.4	3.0
33	M	22	GI, UA	1 : 4096	UA(g), N, V	3	4.4	3.0
41	M	83	AR, AD, GI	1 : 256	UA, AP, V	3	14.4	10.0
42	M	186	A, AR, AD, GI, UA	1 : 1024	O, UA(g), C, NC, AL, A	3	1.4	1.0
45	M	188	A, AR, AD, Ana	1 : 256	O, C, NC, AP, AL, W	4	44.4	30.0
46	M	21	UA, Ana	1 : 256	C, NC, A, BC	4	1.4	1.0

A, asthma; AD, atopic dermatitis; Ana, anaphylaxis; AR, allergic rhinitis; GI, gastrointestinal symptoms; UA, urticaria angio-oedema; C, conjunctivitis; UA, localized urticaria/angio-oedema; UA(g), generalized urticaria/angio-oedema; BC, 'barky' cough; H, hoarseness; NC, nasal congestion; R, rhinorrhoea; S, sneezing; W, wheezing; AP, abdominal cramp or pain; D, diarrhoea; N, nausea; O, oral pruritus; Sw, difficulty swallowing; T, throat pruritus or tightness; V, repetitive vomiting; A, anxiety; AL, change in activity level; Ta, tachycardia; Hy, hypotension; HE, hen's egg; OFC, oral food challenge.

Table 3. Grading of food-induced anaphylaxis according to severity of clinical symptoms

Grade	Skin	GI tract	Respiratory tract	Cardiovascular	Neurological
1	Localized pruritus, flushing, urticaria, angio-oedema	Oral pruritus, oral 'tingling', mild lip swelling			
2	Generalized pruritus, flushing, urticaria, angio-oedema	Any of the above, nausea and/or emesis x's 1	Nasal congestion and/or sneezing		Change in activity level
3	Any of the above	Any of the above plus repetitive vomiting	Rhinorrhoea, marked congestion, sensation of throat pruritus or tightness	Tachycardia (increase > 15 beats/min)	Change in activity level plus anxiety
4	Any of the above	Any of the above plus diarrhoea	Any of the above, hoarseness, 'barky' cough, difficulty swallowing, dyspnoea, wheezing, cyanosis	Any of the above, dysrhythmia and/or mild hypotension	'Light headedness', feeling of 'pending doom'
5	Any of the above	Any of the above, loss of bowel control	Any of the above, respiratory arrest	Severe bradycardia and/or hypotension or cardiac arrest	Loss of consciousness

All symptoms are not mandatory. The severity score should be based on the organ system most affected, e.g., if grade 3 respiratory symptoms are present but only grade 1 GI symptoms, then the anaphylaxis severity score would be 'grade 3'. Boldface symptoms are absolute indications for the use of epinephrine; use of epinephrine with other symptoms will depend on patient's history [24].

GI, gastrointestinal.

Hen's egg white extract

The electrophoretic patterns of purified HE white proteins and prick test reagent (HE white extract) were rather clear (Fig. 1). The most important HE white allergens (ovotrans-

ferrin, OVA and lysozyme) were well separated and identifiable; by contrast, ovomucoid, because of its high content of sugars, could not be well evaluated by gel electrophoresis. The elaboration of gel by densitometry demonstrated that the concentration of each allergenic

molecule in the HE white extract was about 1/10 of the corresponding concentration in fresh HE white (extract vs. fresh HE white: ovotransferrin, 1.5 vs. 13.9 mg/mL; OVA, 5.7 vs. 59.9 mg/mL; lysozyme, 0.35 vs. 3.9 mg/mL).

Skin test and immunoglobulin E assays in relation to oral food challenge

Skin reactivity to undiluted fresh HE white or extract were examined in relation to OFC outcome (Figs 2a and b). Weal

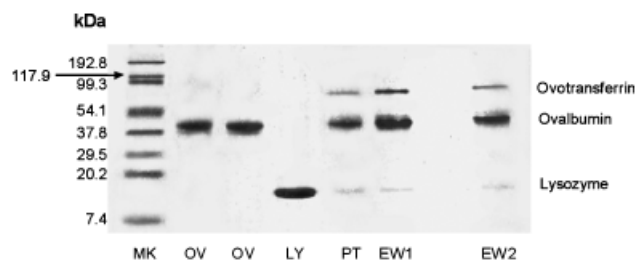


Fig. 1. SDS-PAGE of Prick Test Reagent (hen's egg white extract, Stallergenes) and purified proteins from egg white. MK, molecular weight standard solution; OV, ovalbumin (5 µg); LY, lysozyme (5 µg); PT, prick test reagent (1 µL after 1 : 4 dilution, w : w in sample buffer); EW1, egg white (3 µL after 1 : 50 dilution, w : w, in sample buffer); EW2, egg white (3 µL after 1 : 100 dilution, w : w, in sample buffer).

reactions to the undiluted fresh HE were larger among children with a positive OFC (mean diameter ± SD: 11.5 ± 3.8 mm), intermediate (6.6 ± 2.7 mm) among children with a mild reaction to OFC and minimal (5.1 ± 2.6 mm) among children with no reaction to OFC (Fig. 2a). A similar trend was observed when skin reactivity to the undiluted HE white extract was taken into consideration (Sampson's score 3–4: 7.8 ± 1.9 mm; score 1–2: 5.6 ± 1.8 mm; score 0: 4.2 ± 1.0 mm) (Fig. 2b). However, with both the fresh HE white and the HE white extract the values obtained in children with a negative OFC (grade 0–2) widely overlapped those obtained in children with a positive OFC (Figs 2a and b).

Serum samples from 31 patients (17 males; age 1.4–15.7 years; mean age, 6.4 years) were examined. IgE antibodies against HE white were detected (>0.35 kU/L) in 20/31 children (64%); in 13 of these 20 children, IgE-Ab values were higher than 3.5 kU/L. The frequency of subjects with IgE-Ab against HE white was significantly higher in children with a positive OFC than in those with a negative OFC [15/17 (88%) vs. 5/14 (36%); $P < 0.01$]. The values of IgE-Ab levels obtained in children with a negative OFC (grade 0–2) widely overlapped values obtained in children with a positive OFC (Sampson's score 3–4:

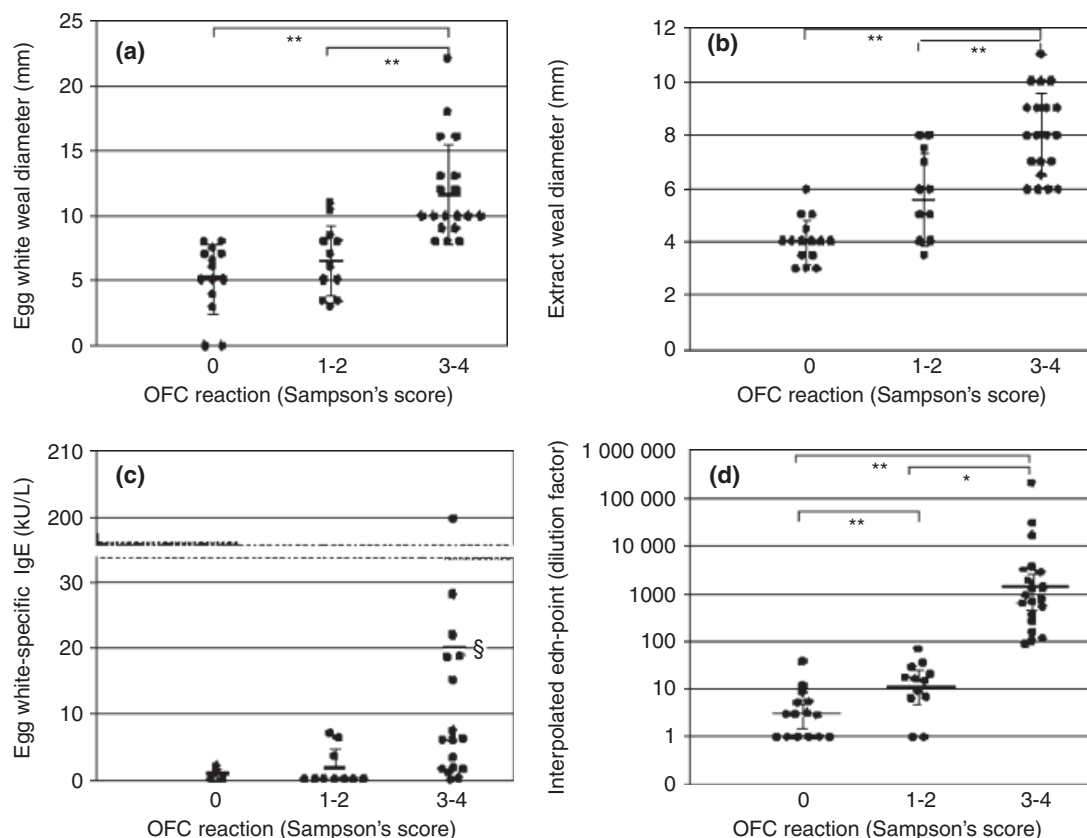


Fig. 2. Relationship of skin prick test reactivity with oral food challenge. Weal diameter elicited by undiluted (a) hen's egg (HE) white fresh or (b) HE white extract in patients with no, mild or severe reactivity to an oral food challenge with raw HE (see "Methods" for definitions) with mean and standard deviation. (c) Egg white-IgE-specific level related to oral food challenge (OFC) severity, $\bar{x} = 47.2$ kU/L SD. (d) The interpolated dilution of the HE white extract eliciting a reaction of 3 mm is reported in patients with no, low or high reactivity to OFC with HE, with the geometric mean and 95% CI. * $P < 0.01$; ** $P < 0.001$.

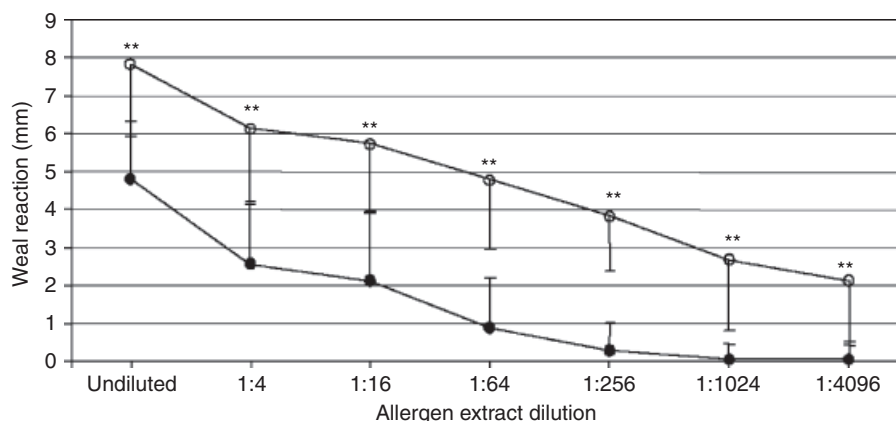


Fig. 3. Dose-response to hen's egg white extract by titrated skin prick tests. Mean and standard deviation of weal reactions to undiluted and scalar fourfold dilutions of a hen's egg white extract in children with a positive (open circles) or negative (closed circles) oral food challenge. ** $P < 0.001$.

19.8 ± 47.2 kU/L; score 1–2: 1.7 ± 2.8 kU/L; score 0: 0.75 ± 0.96 kU/L (Fig. 2c).

End-point titration

Participants with a positive OFC on average had larger weal reactions than those with a negative OFC at each of the examined dilution (Fig. 3). The *interpolated* end-point dilutions ranged from 1:1 to 1:176 408. The average *interpolated* end-point dilution was significantly higher among children with a positive OFC (geometric mean and 95% CI: 1141.1; 455.1–2860.8), than in those with a score 1–2 to OFC (10.8; 4.7–24.5), (Kolmogorov–Smirnov test, $P < 0.01$) and in those with a score 0 to OFC (2.9; 1.6–5.4) ($P < 0.001$). Interestingly, no overlap was observed between the end-point titration (EPT) values of children with no or mild reaction to OFC and those of children with positive reactions to OFC (Fig. 2d).

Receiver operating characteristic analysis and prediction of a positive oral food challenge

The power of skin test EPT and of routine skin test, with undiluted HE white fresh preparation or with undiluted HE white extract, and by measuring IgE against HE white to discriminate as positive a child with a positive OFC was calculated by ROC analysis and the results compared (Fig. 4). The area under the curve obtained with the *interpolated* EPT method was significantly bigger than the one obtained by using for the SPT the undiluted HE white extract (0.99 vs. 0.88, $P < 0.05$) (Fig. 4a), or the fresh HE white (0.99 vs. 0.35, $P < 0.000$) or by measuring IgE-specific antibodies (0.99 vs. 0.83, $P < 0.05$) (Fig. 4b).

From a practical point of view, the *observed* extract's dilution that could best discriminate a positive from a negative OFC was 1:256. On the basis of the gel-electrophoresis measurements (Fig. 1) a 1:256 dilution corresponds to a concentration of 5.9 $\mu\text{g/mL}$ of ovotransferrin,

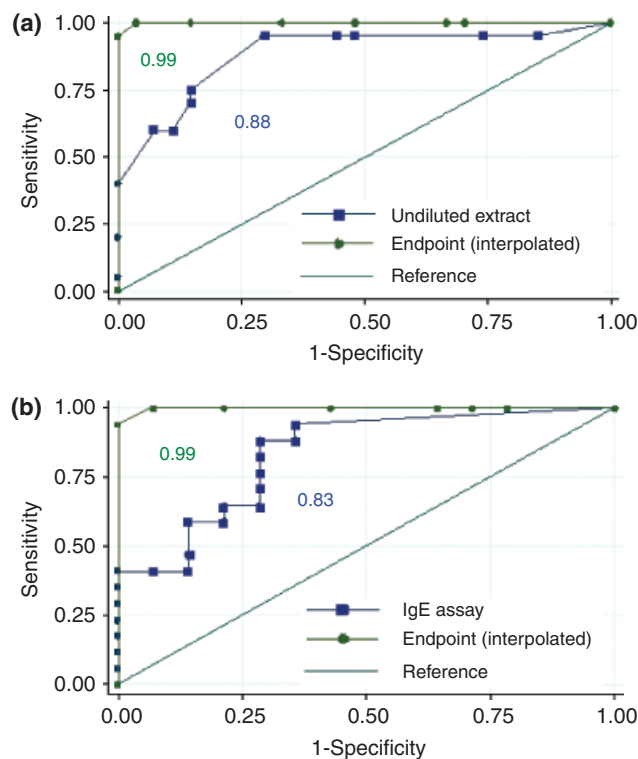


Fig. 4. Receiver operating characteristic (ROC) analysis of a positive oral food challenge (OFC) in relation to skin prick tests (SPT) and IgE assay. ROC curves of (a) weal size (mm) elicited by undiluted hen's egg (HE) white extract SPT (■, area under the curve = 0.88) and by *interpolated* end-point titration (●, area under the curve = 0.99) (47 patients examined; $P < 0.05$), (b) concentration of specific IgE against HE white (kU/L) (■, area under the curve = 0.83) and *interpolated* end-point titration (●, area under the curve = 0.99) (31 patients examined; $P < 0.05$), in relation to a positive OFC.

22.2 $\mu\text{g/mL}$ of OVA and 1.4 $\mu\text{g/mL}$ of lysozyme. A 1:256 extract dilution was an excellent predictors of a positive OFC, and its sensitivity, specificity, PPV and NPV, efficiency and likelihood ratios of a positive and negative outcome were significantly higher than those obtained by

Table 4. Best prediction (%) of a positive oral provocation test with hen's egg white skin prick tests (undiluted and titrated) and IgE assays

	Best cut-off point*	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Likelihood ratio (positive test) (%)	Likelihood ratio (negative test) (%)	Efficiency (%)
Fresh hen's white egg (SPT, undiluted)	9 mm	80.0	92.3	88.9	85.7	10.4	0.22	92.6
Hen's egg white extract								
SPT, undiluted	6 mm	95.0	70.4	100	95.0	3.2	0.07	80.1
SPT, end-point titration (measured dilution)	1 : 256	95.0	100	100	96.4	∞	0.05	97.9
IgE immunoassay, hen's egg white [†]	7.4 kU/L	41.2	100	100	58.3	∞	0.58	71.0

*Established with ROC analysis.

[†]*n* = 31.

ROC, receiver operating characteristic; SPT, skin prick test.

measuring the weal diameter elicited by undiluted fresh HE white or the HE white extract, and by IgE testing against HE white (Table 4). Finally, the performance of a 1 : 256 dilution in discriminating a positive from a negative OFC was similar among patients older or younger than 2 years of age, or among patients with or without AD (not shown).

Discussion

We found that SPTs, made quantitative through EPT, can predict the outcome of an oral provocation test in children suspected to be allergic to egg. ROC analysis confirmed that this diagnostic approach was significantly more efficient than the 'conventional' SPT, i.e. the simple measure of weal diameters induced by an undiluted extract or by fresh HE white. Measuring skin sensitivity through EPT is an old and rather neglected diagnostic procedure; nevertheless, to our knowledge, this is the first time that such methodology is used to measure skin sensitivity against food allergens and used to predict the outcome of OFC tests.

The evidence that EPT had a better performance in predicting OFC outcomes, compared with conventional SPT, can be easily explained from a methodological perspective. Conventional skin tests have a reduced quantitative discrimination power of skin sensitivity, as their quantitative result can be expressed only through the dimensions of a weal and flare reaction. The diameter of a positive weal reaction ranges from the generally accepted threshold of 3 mm up to a maximum of 12–15 mm, with only very few patients arriving up to 20–25 mm [9, 10, 27]. This offers a discrimination power, between maximum and minimum, which is rarely higher than a factor of 8 (25/3). By contrast, the discrimination power of EPT is in the order of several thousands (dilutions ranging from 1 : 1 up to 1 : 4096 or more). In our series, for example, 9/47 children still reacted positively to an extract dilution of 1 : 4096. Therefore, skin test EPT was in our study more powerful than conventional skin tests in

discriminating weak from strong sensitizations. Intuitively, EPT (i.e. the application of growing concentrations of a food allergen to the skin surface) is less dissimilar from an OFC test (i.e. the ingestion of growing amounts of the same food allergen) than conventional skin tests.

On the other hand, the evidence that EPT had a better performance in predicting OFC outcomes, compared with an IgE assay is also not surprising. Oral food challenges are *in vivo* assays whose outcome is the result of complex interplays among several biological components, including food allergens and their digestion, IgE and other allergen-specific antibodies, degranulating cells, the target tissue, etc. Immunoenzymatic IgE assays can be highly precise in measuring serum IgE concentration but, in contrast to SPT, they do not involve any other biological component among those facilitating or inhibiting an allergic reaction to the offending food.

The methodological and the biological plausibility of our results do not guarantee their reproducibility. Actually, we examined only 47 patients with a quite broad age and disease range; moreover, our analysis was restricted to one allergenic food only. An important question is therefore whether our results can be extrapolated to other settings, populations, and food allergens. For example, our patient series did not include any child with a positive OFC and a SPT weal to undiluted HE extract of 1 or 2 mm [9, 16]. We do not know whether the inclusion of this category of patients with low SPT reactivity and high clinical sensitivity would have affected the interpretation of our data. By contrast, the performance of the different test examined to predict OFC were substantially similar among children younger or older than 2 years of age, or among children with or without atopic eczema (not shown). All these considerations suggest that the conclusions drawn from our results must be balanced. Actually, different concentrations of HE allergens may be identified as the best ones in predicting the outcome of an OFC in different populations of children with suspected IgE-mediated HE allergy; therefore each centre will probably have to validate their own data in view of different

methodologies for SPT and extract potency being used. To facilitate a comparison of future studies with ours, we have measured the concentration of single HE proteins in our commercial extract (the results are already given in the Fig. 1).

We believe that EPT-SPT can be a realistic diagnostic choice in a clinical setting. Indeed, just a couple of minutes are required to prepare the HE extract dilutions; moreover, data interpolation can be pragmatically substituted by expressing the test result as the actual dilution producing a weal reaction of 3 mm or more. In addition, EPT does not substitute, but integrates conventional prick tests in routine clinical practice. In our opinion, conventional skin tests with extracts and fresh food can be used as a first diagnostic step to identify sensitization in a 'qualitative' way (positive, negative). Then, EPT can be used as a second step to quantify the intensity of sensitization to the allergens eliciting a positive weal reaction during conventional skin tests and to predict the outcome of an OFC test. Further studies on population samples enriched in patients with anaphylaxis are necessary to test the interesting hypothesis that the EPT-SPT can be used to specifically predict anaphylactic reactions.

In conclusions, EPT allowed us to optimize the use of SPTs and to predict the outcome of OFC in a group of children with suspected and diagnosed HE allergy. Further studies in large population samples are needed to test whether this encouraging conclusion and this novel diagnostic approach can be extended to other settings, patient populations and food allergens.

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