

Baseline specific IgE levels are useful to predict safety of oral immunotherapy in egg-allergic children

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Clinical & Experimental Allergy

Summary

Background Oral immunotherapy (OIT) is a promising treatment for food allergy but dose-related reactions are common.

Objective To evaluate safety of egg-OIT. To identify predictors of dose-related reactions.

Methods Fifty children aged 5–18 underwent egg-OIT after confirming IgE-mediated egg allergy by double-blind placebo-controlled challenge (DBPCFC). All dose-related reactions over a median period of 18 months on-OIT (range: 12–28) were registered. Children were retrospectively divided into three subgroups: (1) children who stopped reacting to OIT-doses over time (RR, *Resolved Reactions*); (2) children with ongoing dose-related reactions over the whole period on-OIT (PR, *Persistent Reactions*); (3) children who discontinued OIT within induction phase due to frequent reactions not improved by protocol re-adaptation and medication (ED, *Early Discontinuation*). Baseline clinical/immunological parameters associated with subgroups were investigated.

Results Reactions occurred in 7.6% of doses. Adrenaline was required in 26% of children. The three subgroups corresponded to three different safety phenotypes: (1) twenty-four children (48%, RR) experienced infrequent and mainly mild reactions that resolved over time. None required adrenaline; (2) seventeen children (34%, PR) experienced more frequent and severe ongoing reactions over time; (3) nine children (18%, ED) discontinued OIT due to very frequent and mainly moderate reactions. Early discontinuation was associated with underlying asthma, higher specific IgE (sIgE) and lower threshold at DBPCFC. In contrast, lower sIgE and less severe reactions at DBPCFC were associated with subgroup RR. sIgE showed excellent performance in predicting belonging to subgroup RR. Levels below the optimal cut-off (ovomucoid-sIgE 8.85 kU/L) indicated 77% probability of belonging to subgroup RR, whereas levels above it indicated 95% probability of early discontinuation or ongoing reactions over time.

Conclusions and Clinical Relevance Egg-OIT involves substantial risks. However, baseline parameters, particularly sIgE, may help identify children in whom the procedure is more likely to be safe. Egg-OIT safety needs improvement in children with more severe and persistent egg allergy.

Keywords egg allergy, oral immunotherapy, reactions, safety, specific IgE

Submitted 23 August 2013; revised 14 October 2013; accepted 6 November 2013

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Cite this as: M. Vazquez-Ortiz, M. Alvaro, M. Piquer, O. Dominguez, A. Machinena, M. A. Martín-Mateos and A. M. Plaza, *Clinical & Experimental Allergy*, 2014 (44) 130–141.

Introduction

Egg allergy affects 1.6–8.9% of children in developed societies [1, 2]. Hypersensitivity reactions to egg can be severe. Strict avoidance and prescription of rescue medication is the only accepted management [3]. However, reactions on accidental exposure occur, as egg is a common ingredient in many foods [4], and avoidance involves negative nutritional, social and psychological

consequences that impair quality of life [3]. Most egg-allergic children will outgrow egg allergy. In fact, up to 66% of egg-allergic children have developed tolerance 5 years after diagnosis [5]. Nonetheless, many children followed at referral centres retain egg allergy into adolescence [6, 7].

Thus, growing interest exists in developing therapeutic strategies that modify clinical reactivity in persistently egg-allergic children, such as oral immunotherapy (OIT)

[8]. Egg-OIT has shown promising results in inducing desensitization, that is, increasing the threshold that elicits reactions while on therapy, with efficacy rates between 55% and 100% [9–17]. However, safety is a concern, as adverse events affect 50–100% of children [9–17]. In most studies a subset of patients ranging from 12.5% to 36% need OIT discontinuation at early stages due to relevant reactions [9, 10, 17, 18]. Accordingly, some authors exclude asthmatic children or those with a past history of anaphylaxis or severe reactions to minimize adverse events [9, 11].

Reports about individual cases suggest that egg-OIT-related reactions may be more frequent in children with high specific IgE (sIgE) levels [14] or in those who react to low doses at challenges [17]. However, as one of the main goals of OIT is preventing potentially life-threatening reactions on accidental exposures [8], the greatest benefit from OIT might be for these children who react to low doses, experience more severe reactions, and have a more persistent food allergy. Thus, more precise evidence is needed about egg-OIT safety in this high risk group [8].

Therefore, we aimed to evaluate efficacy and safety of egg-OIT in a wide sample of 50 egg-allergic children from a tertiary referral centre, without excluding patients on account of allergy severity, sIgE levels, or underlying (well-controlled) allergic comorbidities. Additionally, we sought to identify risk factors for reactions to egg-OIT doses. First, we aimed to characterize those children who required early discontinuation. Secondly, we sought to characterize the subgroup of patients in which egg-OIT was acceptably safe, as well as to establish a sIgE cut-off to predict belonging to this subgroup. A comparable control group of egg-allergic children was recruited to assess the incidence of accidental reactions and natural tolerance development over an equivalent period of time.

Methods

Study design

Non-randomised, controlled, parallel group intervention study.

Patients

Inclusion criteria were (1) children aged 5–18 years, plus (2) IgE-mediated egg allergy confirmed by double-blind placebo-controlled food challenge (DBPCFC) at inclusion, plus (3) sIgE or skin prick test (SPT) to egg white (EW), ovalbumin (OVA), or ovomucoid (OVM) above 0.35 kU/L and 3 mm, respectively. Exclusion criteria were severe or uncontrolled asthma [19], severe atopic dermatitis according to an objective SCORAD

index above 40, cardiovascular diseases, or neuropsychiatric impairment.

Baseline parameters were recorded as potential risk factors for OIT-related reactions: (1) clinical parameters (age, gender, current allergic comorbidities – multiple food allergies, rhinitis, atopic dermatitis, asthma and its severity [19] –, threshold dose, and severity of reaction at baseline DBPCFC); (2) immunological parameters, performed within the month prior to inclusion (sIgE and SPT to EW, OVA and OVM – ImmunoCAP (ThermoFisher, Uppsala, Sweden) and ALK extracts (Madrid, Spain), respectively).

Children were divided into two groups, based on parents' choice: (1) active group (OIT, $n = 50$) or (2) control group ($n = 32$), in which children continued on egg avoidance diet for 12 months.

Patients were recruited consecutively from December 2009 until April 2011 at Sant Joan de Déu Hospital, Barcelona, Spain. The study was approved by the hospital's ethics committee (reference number: PIC-62-11). Written informed consent was obtained in all the cases.

Procedures

a) DBPCFC

Egg allergy was confirmed at inclusion by DBPCFC. First, DBPCFC to boiled EW (at 90°C for 10 min) was performed. Starting dose was 0.12 g EW protein, equivalent to 1/32 EW [20]. Doses were doubled consecutively (0.24 g, 0.48 g, 0.96 g, 1.9 g) with hourly intervals until objective IgE-mediated manifestations appeared [4]. Cumulative dose was 3.7 g EW protein, equivalent to one egg. If boiled EW was tolerated, raw egg DBPCFC was performed the following day. Consecutive doses were 0.48 g, 0.96 g, 1.9 g EW protein (cumulative dose: 3.4 g). Vegetable puree and chocolate drink were used for masking, respectively.

Reaction severity was evaluated according to 'Grading of Food-Induced Anaphylaxis According to Severity of Clinical Symptoms' by Sampson [21]. Criteria for anaphylaxis were evaluated according to EAACI position paper, that is, multisystem involvement including cardiovascular, lower respiratory tract, or persistent gastrointestinal manifestations [22].

b) Oral immunotherapy protocol

Dose schedule. The protocol proposed by the Spanish Society of Paediatric Clinical Immunology and Allergy was followed. Pasteurized liquid EW containing 8.3 g of protein per 100 mL was used (Clara Guillen, Valencia, Spain), whose allergenicity is equivalent to raw EW [23]. Induction phase involved 16 weeks and started with a 2-day in-hospital rush phase, in which increas-

ing doses were given hourly (first day – 1/100 dilution with water: 0.1 mL (0.083 mg EW protein), 0.2 mL, 0.3 mL, 0.5 mL, 0.7 mL and 1 mL; second day – 1/10 dilution: 0.2 mL, 0.3 mL, 0.5 mL, 1 mL, 1.5 mL and 2 mL; third day: 0.4 mL undiluted). Patients were then discharged home and continued having the last tolerated dose once daily throughout the week. Weekly increases were given at the outpatient clinic, as follows: 0.5 mL, 0.7 mL, 1 mL, 1.3 mL, 2 mL, 2.5 mL, 3.2 mL, 4 mL, 5 mL, 8 mL, 11 mL, 15 mL, 22 mL, 30 mL, and finally, one raw egg (60 g of the fresh product). If dose-related reactions occurred, the protocol was re-adapted as follows: doses were reduced one step if grade 2 and two steps if grades 3–4 for 1 week. Then, the scheduled increase was attempted. In maintenance phase, children continued having one raw egg (or their maximal tolerated dose) twice weekly uninterruptedly.

Medication. Patients with asthma on preventive treatment continued it during OIT. No other premedication was given. Families were instructed in recognizing and treating reactions according to EAACI Anaphylaxis Guideline [22]. Antihistamines were indicated for rhinoconjunctivitis or skin symptoms, inhaled salbutamol for mild cough/wheeze, and adrenaline for moderate/severe anaphylaxis. Families were advised to avoid potential cofactors (exercise, fasting, non-steroidal anti-inflammatory drugs) or minimize their impact (e.g. dose reduction during infections).

Study outcomes

Efficacy

Active group. Desensitization was evaluated by open challenge to one raw egg (3.8 g raw EW protein or, if not reached, to the highest tolerated dose) at induction phase end and 12 months after OIT start. Complete desensitization was defined as clinical unresponsiveness to one raw egg, whereas partial desensitization as increase in reaction threshold not reaching one raw egg.

Control group. Double-blind placebo-controlled food challenge was repeated at 12 months according to the above-mentioned methodology. Accordingly, control children were classified as persistent or resolved egg allergic.

Safety

Active group

Descriptive data. All allergic reactions occurring within 2 h after OIT doses, as well as delayed gastrointestinal and/or skin symptoms suggesting a non-IgE-mediated mechanism [3], were recorded in each patient's study

notebook. Severity was assessed according to 'Sampson's Grading' [21] by the study co-ordinator. The following subjective symptoms were recorded when children's activities were markedly impaired: mouth itchiness as grade 1 reaction, abdominal pain as grade 2 if mild and as grade 3 if moderate/severe. The type of symptoms observed, multisystem involvement and treatments required were evaluated. Patients on the process of receiving regular raw egg doses as OIT ('on-OIT') were evaluated weekly during induction and maintenance phase for safety data collection. All the cases on-OIT were evaluated for the last time in April 2012 ('final evaluation') after being on-OIT for at least 12 months.

Definition of subgroups of patients under egg-OIT. As known, there are no validated criteria to determine when OIT can be considered safe. In recent work from our group, we observed two different clinical phenotypes in a series of 81 children given milk-OIT over 25 months. In one subgroup, children stopped reacting over time on-OIT and reactions were infrequent and mainly mild. In this subgroup, the procedure was considered safe. In contrast, the other subgroup experienced more frequent and mainly moderate reactions that persisted over the entire period on-OIT [24]. We also observed both in our milk-OIT and egg-OIT patients in maintenance phase that the longest 'reactions-free' interval between two consecutive dose-related reactions was 2 months. According to these observations, we retrospectively classified patients undergoing egg-OIT at the time of the final evaluation into three subgroups: (1) children who had stopped reacting to egg-OIT doses over time, being free of reactions for at least the last 4 months on-OIT (named subgroup RR, *Resolved Reactions*); (2) children who had experienced ongoing dose-related reactions over the whole period on-OIT, including the last 4 months on-OIT (named subgroup PR, *Persistent Reactions*); (3) Finally, we defined a third subgroup of children who required egg-OIT discontinuation within induction phase due to frequent reactions not improved by the above-mentioned protocol re-adaptation and medication (named subgroup ED, *Early Discontinuation*). This patients' classification was used for downstream analyses.

Control group

Control patients were evaluated every 3 months to register accidental reactions occurring over the study period. All the controls were also evaluated for the last time for safety data collection in April 2012.

Statistics

Intention to treat analysis was applied. Descriptive data were analysed using percentages for qualitative variables

and median and interquartile range (IQR) for quantitative variables, which were not normally distributed. Univariate comparative analyses of clinical/immunological parameters, safety, and efficacy data were performed between groups and subgroups. Univariate and, subsequently, multivariate logistic regression was used to assess independent risk factors for belonging to subgroup RR. The diagnostic performance of sIgE to predict belonging to subgroup RR was studied by receiver operating characteristic curve analysis. The Youden's index, the indicator that combines the highest positive and negative predictive values [25], was used to select the optimal cut-off. Additionally, a supplementary assessment of baseline risk factors for OIT-related reactions independent of subgroups' classification was performed, in which risk factors for reactions severity, frequency, and persistence over time on-OIT were evaluated (see additional information in online repository). PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) was used. A two-tailed $P < 0.05$ was considered significant.

Results

Study population

In the entire sample ($n = 82$), median age was 8.3 years (IQR: 6.5–10.4), median EW-sIgE was 6.44 kU/L (IQR: 2.16–16.6) and median EW-SPT 8.5 mm (IQR: 6.5–10.5). 60.2% of patients had asthma, 65.1% rhinitis, 73.5% multiple food allergies, and 63.3% atopic derma-

titis [mild in 49 cases (SCORAD < 15), moderate in three cases (SCORAD 15–40)]. Forty-eight children (58%) experienced anaphylaxis at baseline DBPCFC (multisystem reactions including persistent gastrointestinal symptoms in 28 children and lower respiratory tract involvement in 20 children).

In active group, 13551 egg-OIT doses were administered. Median time in induction phase was 4.5 months (IQR: 3.7–6), and median time in maintenance phase was 11 months (IQR: 4.5–16). The median period on-OIT for safety data collection was 18 months (IQR: 13–25). The control group was also observed for a median period of 18 months to register accidental reactions (IQR: 13–21.7, $P = 0.26$). There was no loss to follow-up.

Efficacy

Active group. At the end of induction phase, 40 children (80%) had achieved complete desensitization, one child (2%) partial desensitization, and nine cases (18%) had discontinued OIT due to frequent dose-related reactions not improved by medication and protocol re-adaptation (subgroup ED). Subsequently, 12 patients required dose reduction due to dose-related reactions.

At 12 months, 28 patients (54%) achieved complete desensitization, whereas 4 (8%) partial desensitization (unresponsiveness to 0.8 g to 1.7 g raw EW protein). Nine more patients discontinued OIT in maintenance phase due to ongoing dose-related reactions (total discontinuation: 18 children -36%; Fig. 1).

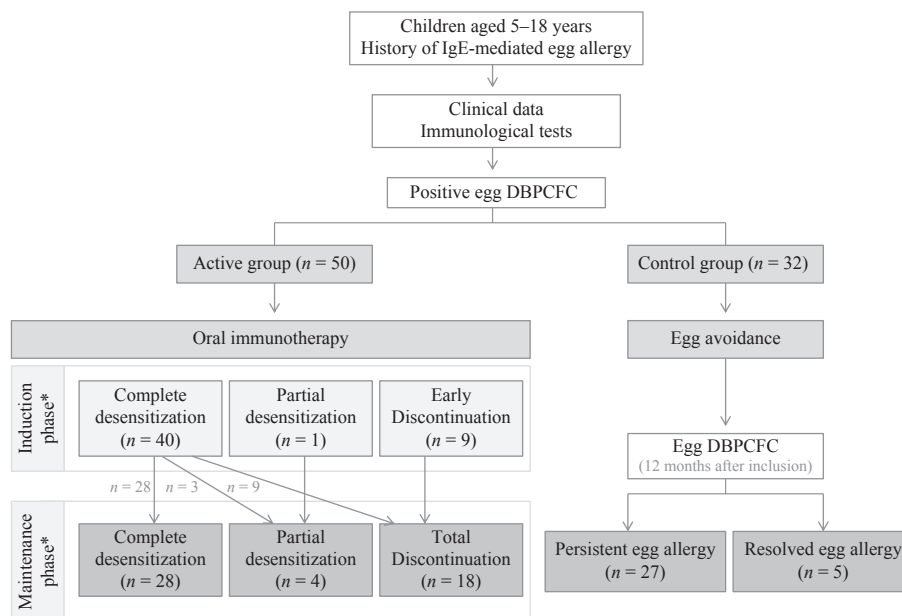


Fig. 1. Study flow chart and efficacy results. *Desensitization in the active group was assessed by open challenge at the end of induction phase and at 12 months. Complete desensitization was defined as clinical unresponsiveness to one raw egg, whereas partial desensitization as increase in the threshold dose not reaching one raw egg. Abbreviations: DBPCFC, double-blind placebo-controlled food challenge.

Control group. Five children (15.6%) passed the DBPCFC 12 months after inclusion and 27 (84.4%) still reacted. At 12 months, complete desensitization in active group was more frequent than natural tolerance development in control group ($P < 0.001$). No differences were found in baseline clinical/immunological parameters between active and control groups (Table 1).

Safety

Active group

Frequency, symptoms, severity of reactions, and treatments required. Forty-five children (90%) had dose-related reactions. One thousand and twenty-four reactions were detected, corresponding to 7.6% of OIT doses (Table 2). The highest incidence of reactions occurred in induction phase, with the lowest and the highest doses (9.7% of OIT doses below 83 mg EW protein-associated reactions, whereas 8.3% of OIT doses between 1.25 g EW protein- and one raw egg-associated reactions). Symptoms distribution was as follows: vomiting: 19.7% of all reactions, lower respiratory tract involvement: 18.8%, abdominal pain: 15.7%, mouth itchiness: 13.7%, urticaria: 13.1%, rhinoconjunctivitis: 7.7%, angio-oedema: 6.7%, hoarseness: 2.1%, diarrhoea: 1.5%, atopic dermatitis flare: 0.7%, dysphagia: 0.5%. Delayed reactions represented a total of 4.3% of all reactions (3.8% gastrointestinal plus 0.5% skin symptoms). Reactions severity distribution was as follows: grade 1: 30%, grade 2: 31.2%, grade 3: 15.9%, and grade 4: 22.9%. Grade 4 reactions were experienced by 58% of children on-OIT, consisting of mild cough/wheeze in 78% of occasions. One patient experienced a grade 5 reaction within induction phase, leading to discontinuation. Multisystem reactions represented a total of 11.5% of all dose-related reactions. When comparing induction and maintenance phases, no differences were found regarding frequency (Table 2), severity, and type of reactions (data not shown). Eighteen adrenaline injections were required, in 13 children. No adrenaline was needed during maintenance phase. Salbutamol was given in 159 occasions and antihistamines in 475. Of note, changing from daily OIT doses in induction phase to twice-weekly doses in maintenance phase exacerbated reactions in 11 cases. These patients were advised to receive daily doses again, and after 3 months, doses were stretched progressively.

Subgroups of patients under egg-OIT. Dose-related reactions resolved over time in 24 patients ('subgroup RR', 48%). The median time required to stop reacting to OIT doses in subgroup RR was 7.6 months (IQR: 1.5–10). In contrast, 17 children suffered ongoing reactions over the whole period on-OIT ('subgroup PR', 34%). At the time of the final evaluation, children in subgroup RR had been

free of reactions for a median period of 9 months (IQR: 6.6–16.4) vs. 1.1 months (IQR: 0.5–2.3) in subgroup PR, $P < 0.001$. Children who discontinued OIT in maintenance phase due to ongoing reactions were included in subgroup PR. Subgroups PR and RR were on-OIT for equivalent time periods (median: 16 months (IQR: 12.2–21.4) vs. 18.4 (IQR: 13.6–22.6), $P = 0.244$). In contrast, children in subgroup ED ($n = 9$, 18%) were on-OIT for a median period of 0.8 months (IQR: 0.2–3.4).

Children in subgroup PR had more frequent and severe reactions than children in subgroup RR (reactions/dose: 10.9% vs. 3.3%; grade 3–4: 43% vs. 17% of total reactions; $P < 0.001$). No adrenaline was required in subgroup RR ($P < 0.001$) (Table 2). Children in subgroup ED had the highest frequency and severity of reactions (reactions/dose: 22.5%; grade 3–4: 65.3% of total reactions, $P < 0.001$ for comparisons with subgroups RR and PR). Of note, 58.9% of reactions in subgroup ED consisted of gastrointestinal symptoms, which had a delayed onset in two children. These reactions could not be sufficiently controlled with antihistamines.

Oral immunotherapy was more effective in subgroup RR, as all these patients achieved complete desensitization. In contrast, in subgroup PR, only 4 of 17 children achieved complete desensitization at 12 months ($P < 0.001$).

Thus, these three subgroups represented three different efficacy and safety phenotypes during OIT, which associated different reactions frequency and severity along with different time required to resolve dose-related reactions over time.

Risk factors for dose-related reactions during egg-OIT

Factors associated with early OIT discontinuation (subgroup ED). Children in subgroup ED had higher sIgE than children who could progress into OIT (subgroups PR plus RR). All the children in subgroup ED had asthma and, particularly, more severe forms. All the ED cases had reacted at DBPCFC to doses equal to or below 1/4 cooked EW ($P = 0.021$; Table 1). Due to the limited number of individuals, further analysis to determine predictors of early OIT discontinuation provided no statistically significant results.

Factors associated with subgroup RR. Children in subgroup RR, in comparison with children in subgroups PR plus ED, had lower sIgE to EW, OVA, and OVM; higher total IgE levels; and less severe reactions at baseline DBPCFC ($P < 0.05$) (Table 1). Consistent results were found in the univariate logistic regression analysis (Table S1 in online repository). These parameters found significant were included into the multivariate regression model, which revealed lower OVM-sIgE levels as the independent protective factor for belonging to sub-

Table 1. Baseline clinical and immunological parameters of the three clinically different subgroups of patients under egg oral immunotherapy (active group): children who stop reacting to egg oral immunotherapy (OIT) doses over time (subgroup resolved reactions, RR), children with ongoing reactions over the whole period on-OIT [subgroup persistent reactions (PR)] and children requiring early discontinuation (subgroup ED). Comparative analysis between subgroup RR and non-RR (PR plus ED), as well as between subgroup ED and non-ED (RR plus PR)

Baseline parameters	Total active group and subgroups (egg oral immunotherapy)					P value		
	Control group (n = 32)	Active group (n = 50)	Subgroup RR (n = 24)	Subgroup PR (n = 17)	Subgroup ED (n = 9)	RR vs. non-RR	ED vs. non-ED	Active vs. control
Age (years, mean, SD)	8.3 (5.5–11.3)	8.3 (7–9.9)	8.5 (7.2–10.1)	8.4 (6.5–9.1)	7.8 (7.3–10.3)	0.33 [~]	0.77 [~]	0.65 [~]
Gender (male, %)	53.1	46	50	47.1	33.3	0.78 [†]	0.32 [†]	0.48 [†]
Atopic dermatitis (%)	62.5	66	62.5	70.6	77.8	0.55 [†]	0.40 [†]	0.70 [†]
Multiple food allergies (%)	81.2	72	70.8	70.6	77.8	1 [†]	0.51 [†]	0.30 [†]
Rhinitis (%)	65.6	64	54.2	64.7	88.9	0.24 [†]	0.09 [†]	0.79 [†]
Asthma ¹ (%)								
No asthma	43.7	36	45.8	41.2	0	0.14 [#]	0.007 [#]	0.64 [#]
Infrequent episodic	31.2	24	25	23.5	22.2			
Frequent episodic	18.8	30	29.2	23.5	44.4			
Persistent moderate	6.3	10	0	11.8	33.3			
Threshold dose at DBPCFC (g egg white protein; %)								
0.12 g (cooked)	0	7.8	4.2	17.6	0	0.42 [#]	0.28 [#]	0.10 [#]
0.24 g (cooked)	9.4	13.7	8.3	17.6	22.2			
0.48 g (cooked)	15.6	29.4	25	23.5	55.6			
0.96 g (cooked)	15.6	15.7	20.8	5.9	22.2			
1.9 g (cooked)	25	21.6	25	29.4	0			
0.48 g (raw)	21.9	7.8	12.5	0	0			
0.96 g (raw)	9.4	0	0	0	0			
1.9 g (raw)	3.1	3.9	4.2	5.9	0			
Reaction severity at DBPCFC ² (%)								
Grade 1	18.8	10	12.5	5.9	0	0.04 [#]	0.26 [#]	0.66 [#]
Grade 2	18.8	22	33.3	11.8	11.1			
Grade 3	40.6	42	25	52.9	77.8			
Grade 4	21.9	26	29.2	29.4	11.1			
Anaphylaxis at DBPCFC ³	56.3	60	45.8	64.7	88.9	0.08 [†]	0.052 [†]	0.69 [†]
Immunological tests, median (IQR)								
Total IgE (kU/L)	409 (197–1076)	511 (186–996)	329 (120–626)	644 (331–1258)	815 (527–1166)	0.014 [*]	0.167 [*]	0.89 [*]
Egg white-sIgE (kU/L)	8.59 (2.5–13.5)	5.54 (1.84–24)	1.9 (0.83–4.39)	14.6 (4.46–25.85)	74.2 (30.9–164.5)	< 0.001 [*]	< 0.001 [*]	0.84 [*]
OVA-sIgE (kU/L)	5.3 (1.14)	3.7 (1.23–12.1)	1.6 (0.72–2.96)	8.1 (3.27–17.2)	39.6 (19.9–90.3)	< 0.001 [*]	< 0.001 [*]	0.90 [*]
OVM-sIgE (kU/L)	5.36 (1–11.88)	5.68 (0.96–21.8)	1.85 (0.66–5.74)	10.7 (2.63–25.6)	36.5 (31.6–60.2)	< 0.001 [*]	< 0.001 [*]	0.50 [*]
Egg white-SPT (mm)	8 (7–13)	8 (6–10.5)	8.5 (6–11.5)	8 (6–8.5)	10.5 (7–11.5)	0.65 [*]	0.18 [*]	0.88 [*]
OVA-SPT (mm)	8 (6–11)	8 (6.5–10.5)	8.5 (7–10)	7 (6–8)	9 (7.5–14.5)	0.54 [*]	0.08 [*]	0.76 [*]
OVM-SPT (mm)	7.5 (6–12)	8 (6.5–10)	8.5 (6–10)	8 (6.5–9)	8 (6.5–9.5)	0.61 [*]	0.91 [*]	0.77 [*]

Tests used: [†]Student's t-test, [#]Fisher exact test, ^{*}chi-squared test and ^{*}Mann-Whitney U-test.

¹According to GEMA Spanish Asthma Guideline [19].

²According to Sampson's anaphylaxis grading [21].

³According to EAACI Anaphylaxis position paper [22].

DBPCFC, double-blind placebo-controlled food challenge; IQR, interquartile range; SD, standard deviation; sIgE, specific IgE; SPT, skin prick test; OVM, ovomucoid.

Table 2. Safety data in the entire sample under egg oral immunotherapy (active group), as well as in the three clinically different subgroups: children who stop reacting to egg oral immunotherapy (OIT) doses over time (subgroup resolved reactions, RR), children with ongoing reactions over the whole period on-OIT (subgroup persistent reactions, PR) and children requiring early discontinuation (subgroup ED)

Safety data	Total active group (n = 50)	Subgroup RR (n = 24)	Subgroup PR (n = 17)	Subgroup ED (n = 9)
Doses given (number, %*)				
Total	13551	7306 (53.9)	5808 (42.9)	423 (3.1)
Induction phase	6514	3006 (46.1)	3085 (47.4)	423 (6.5)
Maintenance phase	7037	4300 (61.1)	2737 (38.9)	0
Reactions (number, %†)				
Total	1024	241 (23.5)	688 (67.2)	95 (9.3)
Induction phase	503	107 (21.3)	301 (59.8)	95 (18.9)
Maintenance phase	521	134 (25.7)	387 (74.3)	Not applicable
Reactions/dose per child (median, IQR)				
Total	4.8% (1.6–13)	2.1% (0.8–4.6)	8.6% (4.8–13.2)	53.8% (33.3–66.6)
Induction phase	4.9% (2–10.3)	2.4% (0.6–5)	6.7% (4.7–10.8)	53.8% (33.3–66.6)
Maintenance phase	2.6% (0.9–11.6)	1.2% (0.4–3)	13.3% (10–16.9)	Not applicable
Reactions severity‡ distribution (%)				
Grade 1	30	45.6	25.3	24.2
Grade 2	31.2	37.8	31.8	10.5
Grade 3	15.9	9.1	13.4	51.6
Grade 4	22.9	7.9	29.5	13.7
Reactions type distribution (%)				
Skin	20.5	20.7	20.6	19
Gastrointestinal	37.2	28	37.5	57.9
Mouth itchiness	13.7	37.8	6	8.4
Rhinoconjunctivitis	7.7	8.3	7.6	7.4
Wheeze/cough	18.8	5	25.2	7.4
Hoarseness	2.1	0.2	3.1	0
Subjective symptoms (%)				
Total	27.7	50.5	23.5	7.4
Induction phase	17.5	28.5	19.1	7.4
Maintenance phase	37.5	68	27	Not applicable
Treatments (Number of doses)				
Adrenaline	18	0	13	5
Salbutamol	159	10	142	7
Antihistamine	475	104	290	81

*The denominator of percentages is the number of doses given in the total active group.

†The denominator of percentages is the number of reactions in the total active group.

‡According to Sampson's anaphylaxis grading [21]. Abbreviations: IQR: interquartile range.

group RR. Each increase in OVM-sIgE in 1 kU/L involved 0.81-times reduced probability of belonging to subgroup RR (odds ratio: 0.81, 95% CI: 0.69–0.94, $P = 0.005$). Inversely, each increase in OVM-sIgE in 1 kU/L involved 1.23-times increased probability of belonging to subgroups PR or ED ($1/0.81 = 1.23$).

Identification of a sIgE cut-off to predict belonging to subgroup RR. EW-sIgE showed excellent diagnostic performance in predicting belonging to subgroup RR, followed by OVA-sIgE and OVM-sIgE (area under curve: 0.907, 0.896, and 0.857, respectively, $P < 0.001$; Fig. 2). OVA-sIgE cut-off 6.49 kU/L obtained the highest Youden's index, followed by OVM-sIgE cut-off 8.85 kU/L. Levels below these OVA-sIgE and OVM-sIgE cut-offs indicated 79% and 77% probability of belonging to subgroup

RR, respectively, whereas levels above them indicated 95% probability of belonging to subgroups PR or ED (Table 3).

Supplementary analyses. Briefly, having grade 4 reactions at baseline DBPCFC was identified as the independent risk factor for experiencing grade 4 dose-related reactions on-OIT. Higher sIgE levels and more severe reactions at baseline challenge correlated with reactions frequency and they were independent risk factors for experiencing more persistent dose-related reactions over time on-OIT (see additional information).

Control group

Accidental reactions occurred in five children. Six reactions were grade 1–2, whereas one reaction consisted of

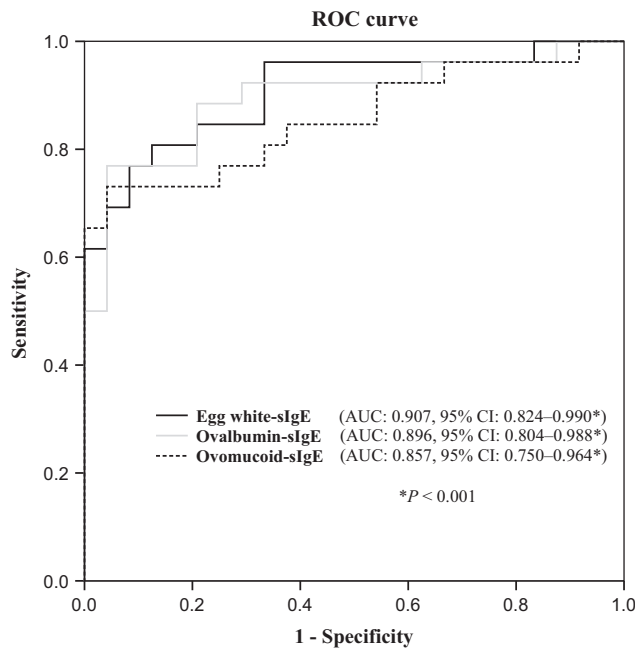


Fig. 2. Receiver operating characteristic (ROC) curve analysis showing how well specific IgE (sIgE) to egg white, ovalbumin, and ovomucoid can predict – before starting oral immunotherapy (OIT) – that a child will belong to subgroup resolved reactions (RR) (patients who stop reacting to egg-OIT doses over time) as opposed to subgroups persistent reactions (PR) (children with ongoing reactions over the whole period on-OIT) and ED (children requiring early OIT discontinuation due to frequent reactions not improved by medication or protocol re-adaptation). Egg white-sIgE showed excellent performance (area under curve – AUC: 0.907), whereas OVA-sIgE and OVM-sIgE showed good performance (AUC: 896 and 0.857). Optimal cut-offs are presented in Table 3. Abbreviations: CI: confidence interval.

self-limiting mild cough (grade 4). No adrenaline was required.

Factors associated with natural tolerance development

Tolerance to boiled egg at baseline DBPCFC as well as lower EW- and OVA-IgE levels were associated with raw egg tolerance development in the following 12 months (Table 4). Indeed, 45% of children who tolerated boiled egg at baseline DBPCFC developed raw egg tolerance in the following year. Among control children with baseline OVM-sIgE below 8.85 kU/L, 24% (five of 21) developed tolerance and none experienced accidental reactions over the study period.

Discussion

Oral immunotherapy is a promising experimental treatment for food allergy. Limited evidence exists about egg-OIT safety in large series which include severe cases, as well as about predictors of dose-related reactions. We provide a thorough description of egg-OIT safety in 50

Table 3. Diagnostic performance of specific IgE (sIgE) to egg white (EW), ovalbumin (OVA), and ovomucoid (OVM) in predicting belonging to subgroup RR (patients who stop reacting to egg-OIT doses over time) as opposed to subgroups PR (children with ongoing reactions over the whole period on-OIT) and ED (children requiring early OIT discontinuation due to frequent reactions not improved by medication or protocol re-adaptation). Based on receiver operating characteristics curve analysis

Predictive properties	Diagnostic test		
	EW-sIgE	OVA-sIgE	OVM-sIgE
Area under ROC curve (95% CI)	0.907 (0.824–0.99)	0.896 (0.804–0.988)	0.857 (0.75–0.964)
<i>P</i> value	< 0.001	< 0.001	< 0.001
Optimal cut-off ¹	9.41 kU/L	6.49 kU/L	8.85 kU/L
Sensitivity, specificity (%)	76.9, 91.7	77, 95.8	73.1, 95.8
Youden's index	0.686	0.727	0.689
NPV, PPV (%)	91, 78.6	95.2, 79.3	95, 76.7
PLR, NLR	9.27, 0.25	18.3, 0.24	17.4, 0.28

¹Optimal cut-off defined as the value with highest Youden's index, that is, the cut-off that combines the highest positive and negative predictive values [25].

PPV, positive predictive value; NPV, negative predictive value; PLR, positive likelihood ratio; NLR, negative likelihood ratio; CI, confidence interval; OIT, oral immunotherapy; PR, persistent reactions; RR, resolved reactions.

patients, being the largest series reported to date [9–18]. Of note, 60% of our patients on-OIT had underlying asthma and 61% had experienced anaphylaxis at baseline DBPCFC. Median time on-OIT was 18 month, which is longer than most previous series [9, 13–16]. Additionally, we provide for the first time statistical evidence of the usefulness of baseline clinical/immunological parameters in predicting the safety of egg-OIT.

In the present study, complete desensitization in OIT group was more frequent than natural tolerance development in control group, as in previous studies [9, 15, 16]. However, reactions to OIT doses were significantly more frequent than accidental reactions in controls. Indeed, allergic reactions occurred in 7.6% of OIT doses, and the frequency of reactions was consistent throughout induction and maintenance phases, as reactions resolved over time only in 24 children. In contrast, in most egg-OIT series, dose-related reactions occurred less frequently [15, 17], they decreased in maintenance phase [10, 11, 14, 16, 17], and were mainly mild [10, 16, 17]. In our OIT group, although 61.3% of reactions were mild (grade 1–2), 13 children required adrenaline, which is a higher rate than in previous reports [13, 16, 17]. In addition, one grade 5 reaction occurred. Both facts highlight the potential risks of OIT. Gastrointestinal symptoms were the most frequent type of reactions, as previously reported [17], and delayed reactions suggesting a cell-mediated mechanism were detected.

Table 4. Baseline clinical and immunological parameters in egg-allergic children on avoidance diet (control group). Comparative analysis between resolved egg-allergic children ($n = 5$), who developed spontaneous tolerance in 12 months' follow-up, and persistent egg-allergic children ($n = 32$), who did not

Baseline parameters	Resolved egg allergic ($n = 5$)	Persistent egg allergic ($n = 27$)	<i>P</i> value
Age (years, mean, SD)	9.5 (2)	8.6 (3.7)	0.58 [~]
Gender (male, %)	80	48	0.34 [†]
Atopic dermatitis (%)	100	80.8	0.13 [†]
Multiple food allergies (%)	60	85.2	0.23 [†]
Rhinitis (%)	60	66.7	1 [†]
Asthma ¹ (%)			1 [#]
No asthma	60	37	
Infrequent episodic	20	33.3	
Frequent episodic	20	18.5	
Persistent moderate	0	7.4	
Threshold dose at DBPCFC (g egg white protein, %)			
0.12 g (cooked)	0	0	0.006 [#]
0.24 g (cooked)	0	11.1	
0.48 g (cooked)	0	18.5	
0.96 g (cooked)	0	18.5	
1.9 g (cooked)	0	29.6	
0.48 g (raw)	60	14.8	
0.96 g (raw)	40	3.7	
1.9 g (raw)	0	3.7	
Reaction severity at DBPCFC ² (%)			
Grade 1	40	15.4	0.72 [#]
Grade 2	20	19.2	
Grade 3	40	42.3	
Grade 4	0	23.1	
Anaphylaxis at DBPCFC ³	20	63	0.14 [†]
Immunological tests, median (IQR)			
Total IgE (kU/L)	185 (105–727)	536 (240–1119)	0.14 [*]
Egg white-sIgE (kU/L)	2.5 (1.56–4.54)	11.1 (3.76–14.1)	0.046 [*]
OVA-sIgE (kU/L)	1.12 (1–1.93)	6.66 (2.81–11.6)	0.019 [*]
OVM-sIgE (kU/L)	0.98 (0.27–4.65)	6.26 (1.37–12.27)	0.053 [*]
Egg white-SPT (mm)	9 (7.5–12)	10 (7–13)	0.89 [*]
OVA-SPT (mm)	6.5 (–8.5)	8.5 (6–11.5)	0.35 [*]
OVM-SPT (mm)	7 (5.5–10)	7.5 (6–12.5)	0.48 [*]

Tests used: [~] Student's *t*-test, [#]Fisher exact test, [†]chi-squared test, and ^{*} Mann–Whitney *U*-test.

¹According to GEMA Spanish Asthma Guideline [19].

²According to Sampson's anaphylaxis grading [21].

³According to EAACI Anaphylaxis position paper [22].

DBPCFC, double-blind placebo-controlled food challenge; IQR, interquartile range; SD, standard deviation; sIgE, specific IgE; SPT, skin prick test; OVM, ovomucoid; OVA, ovalbumin.

The available treatments were insufficiently effective for them. Thus, globally, our safety results are less optimistic than in previous studies.

The reasons for this poorer outcome are difficult to determine, because significant methodological differences hinder direct comparisons among studies. For instance, selection criteria [9, 11], OIT protocols, and reaction severity classifications [9–14, 16, 17] are heterogeneous. However, some strategies proposed by other authors might be safer, such as using premedication [14, 17], cooked egg for OIT doses [9, 13], more progressive dose increases [9, 14, 15], lower doses for maintenance [9, 11], and/or spacing out doses progressively when starting maintenance phase [10].

Although OIT-related reactions were relatively frequent in our study, they were heterogeneously distributed among children. Indeed, reactions persistence over time was associated with increased reactions frequency and severity, which reflects the findings of our milk-OIT study [24]. Accordingly, three different safety and efficacy phenotypes were identified during egg-OIT based on reactions persistence or resolution over time. Those 24 children who stopped reacting over time (subgroup RR, 48%) had mainly mild and infrequent reactions. All of them had reached complete desensitization. Thus, OIT might be considered acceptably safe and effective in these children. In contrast, 17 children experienced ongoing, more frequent and severe reactions, which did not decrease over time, showing that desensitization was not robust in them (subgroup PR, 34%). Finally, nine children had to discontinue OIT within induction phase due to mainly moderate and very frequent reactions (subgroup ED, 18%).

Importantly, we have shown that baseline parameters are useful to predict the safety of egg-OIT. Children in subgroup ED had the highest sIgE levels, they reacted to lower cooked egg doses at baseline DBPCFC, and they had more severe forms of asthma. Most previous series reported cases who discontinued OIT within early stages [9, 10, 16–18], and we have characterized this challenging subset of patients. Higher sIgE levels and more severe reactions at baseline DBPCFC also helped to predict the severity, frequency, and persistence over time of OIT-related reactions. In contrast, lower baseline sIgE levels and less severe reactions at baseline DBPCFC were associated with subgroup RR. Levels below the proposed cut-off (OVM-sIgE: 8.85 kU/L) indicated 77% probability of belonging to subgroup RR, that is, of having infrequent and mainly mild dose-related reactions which resolve over time. Instead, sIgE levels above this cut-off associated 95% probability of needing early discontinuation or having ongoing reactions over time. Thus, our results can inform the process of risk stratification and patient selection in determining suitable candidates for egg-OIT and giving parents accurate information about the risks involved.

Of note, our results suggest that OIT was less safe in those children at higher risk of persistent egg allergy and more severe accidental reactions to low egg doses. Indeed, higher sIgE, more severe reactions, and underlying asthma have been associated with egg allergy persistence [5, 7, 26]. Likewise, higher EW-sIgE levels indicate an increased risk of moderate/severe accidental reactions [4], and asthmatic children are at higher risk of more severe reactions to foods [27]. Therefore, further research is needed to improve egg-OIT safety in these 'high-risk' patients. Future strategies might include the use of hypoallergenic molecules [28, 29], the sublingual route [30], or co-treatment with omalizumab [31].

Conversely, egg-OIT was safer in those children with less severe egg allergy and higher probability of developing tolerance without intervention [7, 26]. In fact, among controls with OVM-sIgE below 8.85 kU/L, 24% developed tolerance just in 12 months and none experienced accidental reactions, which suggests that OIT might provide limited benefit in these patients. In a series from an American tertiary centre, 56% of patients who remained egg allergic at age 5 years developed tolerance by age 18 [7]. As our protocol was successful in 48% of cases, egg-OIT might provide no additional benefit vs. no intervention in the long term. Hence, it remains arguable whether desensitizing mainly those children with milder and likely transient egg allergy would be justified. Diet liberalization following OIT might improve nutrition and quality of life years before tolerance occurs. However, the procedure involves high costs as well as intense family and medical commitment. Unfortunately, data about cost-effectiveness and quality of life following OIT are lacking, which may help elucidate to which extent OIT would be beneficial in these 'low-risk' patients. Additionally, tolerance to boiled egg at baseline DBPCFC is associated with raw egg tolerance development in the following 12 months. Accordingly, OIT seems less useful in patients who tolerate boiled egg.

Finally, we acknowledge some limitations in our study. First, children were not randomized to OIT or avoidance diet for practical reasons, but baseline characteristics in active and control groups were comparable. Secondly, subjective symptoms were included when child's activities were markedly affected. As they are common in allergic reactions to foods, and especially gastrointestinal symptoms in egg allergy [17], overlooking them seemed us inappropriate in a study focused on safety. Nonetheless, the true incidence of subjective symptoms can only be estimated through a blinded placebo-controlled trial [9]. The starting dose in our DBPCFC protocol was higher than the dose proposed by EAACI [32]. However, only 4 of 82 children reacted to

this first dose, suggesting that this fact might have had a limited impact. Of note, children's time period on-OIT was not homogeneous, which might have influenced their classification into subgroups RR or PR. Nonetheless, the median 'reactions-free' period was markedly different between subgroups RR and PR despite equivalent median periods on-OIT. Additionally, the survival analysis (see additional information), which is the most appropriate approach to evaluate risk factors for reactions persistence when the time on the study is heterogeneous, provided concordant results. Lastly, it is arguable whether OIT can be considered acceptably safe in all the children in subgroup RR. A consensus on to which extent OIT-related reactions are acceptable is needed.

In conclusion, egg-OIT involves a substantial risk of dose-related reactions. However, baseline parameters, particularly sIgE levels, are useful to identify those patients in whom the procedure is more likely to be safe. OVM-sIgE below 8.85 kU/L associates 77% probability of belonging to subgroup RR, that is, having infrequent and mainly mild dose-related reactions which resolve over time on-OIT. In contrast, higher sIgE levels, lower reactions threshold, and underlying asthma are associated with early OIT discontinuation. Thus, further research is needed to improve OIT safety in 'high-risk' cases.

Acknowledgements

The authors would like to thank the paediatric allergists (Dr Rosa Jiménez-Feijóo, Dr M. Teresa Giner, Dr Jaime Lozano, Dr Marcia Dias, Dr M. Beatriz Garcia and Dr Mar Folque) and allergy nurses (Silvia Del Valle, Mónica Llevot, M. Teresa De la Rosa, Manuela Olivé and Antje Muizelaar) at Sant Joan de Déu Hospital for their excellent clinical support. The authors thank Dr Emily Derrick, specialty registrar in paediatric allergy at Imperial College NHS Trust (London), as well as Dr Katharina Blümchen, consultant paediatric allergist at Charité Hospital (Berlin) and Mentor for Dr Vazquez-Ortiz within the EAACI JMA Mentorship Programme, for their valuable comments on this work.

Conflict of interests

This study was part of a research project supported by the Research Grant 2010 from the Spanish Society of Paediatric Allergy and Clinical Immunology (SEICAP) and the Research Prize 2011 from the Institute for Studies on Egg (Spain). Funding from the mentioned institutions was used for immunological studies not presented in this manuscript. The authors declare no other conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Kaplan–Meier estimate of survival, which represents the percentage of children who still suffer from dose-related reactions at each time interval while on oral immunotherapy (OIT).

Figure S2. Kaplan–Meier estimate of survival, showing the percentage of children who still suffer from dose-related reactions at each time interval while on-OIT,

stratified by reactions severity at baseline double-blind placebo-controlled food challenge (DBPCFC) [21]

Table S1. Univariate logistic regression analysis, showing baseline risk factors for belonging to subgroup RR (children who stop reacting to egg-OIT doses over time) as opposed to subgroups PR (children with ongoing reactions over the whole period on-OIT) and ED (children requiring early OIT discontinuation due to frequent reactions not improved by medication or protocol re-adaptation).