

Validity of specific IgE antibodies in children with egg allergy

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Summary

Background The demonstration of specific IgE antibodies to egg supports the existence of allergy to this food, but a correct diagnosis can only be obtained after a challenge test. Several studies have assessed different cut-off points in the level of these antibodies as predictors of clinical reactivity.

Objective Validation of the specific IgE antibodies measured by the CAP System Fluorescence enzyme immunoassay (FEIA) technique in the diagnosis of egg allergy in children under 2 years of age.

Methods A prospective study of 81 children with suspected egg allergy was performed. Specific IgE antibodies was quantified for egg white, egg yolk, ovalbumin and ovomucoid. The diagnostic challenge test was carried out following the previously established criteria. The validity of the specific IgE antibodies was analysed using children with a negative diagnostic challenge test as control group.

Results The prevalence of egg allergy in the group studied was 79% and egg white was the allergen that showed the greatest diagnostic efficacy. The sensitivity and positive predictive value of the prick test and of the CAP to egg white were excellent and the specificity and the negative predictive value had lower values. A level of ≥ 0.35 KU_A/L for specific IgE antibodies to egg white predicted the existence of reaction in 94% of the cases.

Conclusions Quantification of the specific IgE antibodies to egg white is useful in the diagnosis of egg allergy. In children under 2 years of age with a background of immediate hypersensitivity after egg ingestion and presence of specific IgE antibodies to egg white of ≥ 0.35 KU_A/L, diagnostic challenge test is not necessary to establish the diagnosis of allergy to this food.

Keywords: egg hypersensitivity, CAP system FEIA, skin prick test, predictive values, challenge test.

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Introduction

Egg allergy generally appears in infants, coinciding with the first intake of the food, and disappears in the first years of life in most of the children. Its prevalence is not well known in the general population; however, it is more frequent in children with allergic diseases, especially with

atopic dermatitis, and can affect more than 50% of the children whose types are more severe. The IgE-mediated immunological reaction is the most frequent and best known form of the food allergy.

The diagnosis of this disease is based on the patient's clinical history and demonstration of the specific IgE antibodies to the food, although it can only be verified after the challenge test. It is very important to confirm this diagnosis because the only way to treat this allergy is by implementing an egg-excluding diet; however, eggs are a

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highly nutritious source of protein and their consumption is very widespread all over the world. In general, studies coincide on the high sensitivity and poor specificity of the prick tests, while the quantification of the specific IgE antibodies in serum has greater specificity than the prick tests and less sensitivity [1–4].

The CAP System FEIA has been used in the quantification of these antibodies for the last several years. Although they are assessed in arbitrary units, this system can detect a wide range of quantities, ranging from 0.35 to 100 KU_A/L. Several authors have studied the association between specific IgE levels to inhalants and foods and the presence of allergic respiratory disease [5] or clinical reactivity with food [6], respectively. Another study has analysed the relationship between the severity of the reactions suffered by a group of latex-allergic patients and the levels of specific IgE antibodies to this compound [7]. All of them support the idea that quantifying specific IgE antibodies in serum is useful because the presence of high levels seems to be equivalent not only to the existence of a greater probability of suffering an allergic disease but also, if such is the case, of this being more severe.

The principal objective of our work was to validate the CAP System FEIA technique in the diagnosis of egg allergy with four different egg allergens in children under 2 years of age in order to establish a cut-off point that would clearly separate allergic from tolerant subjects and would avoid the need to perform challenge tests in the future. In addition, we have studied the association between specific IgE levels to egg and the clinical response to two forms of egg white, that is cooked and raw, to verify if children with a milder form of the disease present lower specific IgE levels.

Methods

Study population

This is a prospective study which included children under 2 years of age with a background of reactions suggestive of immediate hypersensitivity after egg ingestion and who had come to our unit for the first time.

It was considered that there was an allergic reaction when the clinical picture had an acute presentation of some of the following symptoms: (i) mucocutaneous, including morbilliform pruriginous rash, urticaria and/or angioedema; (ii) digestive, such as vomiting, nausea, diarrhoea and/or abdominal pain; and (iii) respiratory, affecting the upper respiratory tract (hydrorrhoea, sneezing, nasal itching and/or congestion) or lower respiratory tract (dyspnoea, cough and/or wheezing). The time period from egg ingestion to symptom development should not be greater than 2 h.

A total of 81 children, whose characteristics are shown in

Table 1. Clinical characteristics of the patients

	Mean (range)	n	%
Age at visit to doctor (months)	16 (11–24)		
Age first reaction to egg (months)	12 (6–20)		
Age first intake of egg (months)	10 (6–14)		
No. of reactions (mean/patient)	2.3 (1–7)		
Gender			
Male		49	60.5
Female		32	39.5
Family background of atopy		62	79.5
Atopic dermatitis		35	43
Bronchial asthma		46	57
Symptoms reported			
Cutaneous		72	89
Digestive		36	44
Respiratory		10	12
Two organs or more		30	37
Latency period of intake symptoms			
< 30 min		64	83
30–60 min		7	9
> 60 min		6	8
Previous tolerance to cooked egg yolk		61	76

Table 1, were included in the study over a 4-year period (1993–97).

Laboratory studies

CAP System FEIA Serum samples from all patients were analysed for total and antigen-specific IgE antibodies to egg white, yolk, ovalbumin (OVA) and ovomucoid (OVM). The method used was Pharmacia CAP System FEIA, used according to the manufacturer's instructions.

Skin prick tests Skin prick tests were performed in all children with glycerinated food extracts of raw egg white (10 mg/mL) prepared in our laboratory, OVA (10 mg/mL) and OVM (10 mg/mL) from Sigma (St Louis, MO, USA), and commercial extract of yolk (1/20 w/v, Dome Laboratories, Stallergenes DHS, Barcelona, Spain). Saline solution and histamine hydrochloride at 1% were applied by the prick technique as negative and positive controls. Food allergens eliciting a mean wheal diameter of 3 mm or greater than the negative control were considered positive.

Challenge tests Open controlled challenge test with egg white was performed in the Allergy Unit, under the supervision of an allergologist and with previous authorization of the parents.

Specific criteria for the diagnostic challenge test were

previously established in the work design. The test should be done in all of the children, except for when the following situations exist: (i) children with a history of severe anaphylaxis; (ii) children who have each and every one of the following characteristics: (a) a history of urticaria and/or angioedema after ingestion of egg, (b) time interval under 2 hours between intake and symptoms, (c) more than one episode having similar characteristics, (d) last episode in the 3 months prior to the doctor's visit, and (e) positive prick test and specific IgE antibodies ≥ 0.35 KU_A/L to any of the allergens tested. However, the diagnostic challenge test was performed in all of those children who reported perioral urticaria as the only clinical symptom, even if they had all of the other previously mentioned criteria.

The challenge was performed with cooked (10 min of boiling) and raw egg whites. The first day, cooked egg white amounts corresponding to one-eighth and one-quarter of the egg, separated by an interval of 90 min, were offered. On the second day, half an egg white was administered. Afterwards, the test continued with raw egg white, in similar amounts, on 3 different days. In some patients who reported a reaction after ingestion of the yolk, a diagnostic challenge test was performed with this fraction, following a similar regime as that done with the egg white.

When the diagnostic challenge test was positive and also when the test was not indicated according to the criteria established, the children were diagnosed as being allergic to egg. Those patients who tolerated the amount of egg white corresponding to one-half of a chicken egg were labeled as tolerant, and made up our control group.

Statistics

We performed a prospective study to validate diagnostic methods in egg allergy. Sensitivity, specificity, efficacy, positive and negative predictive values and the positive and negative likelihood ratios were calculated for the prick tests and the CAP System FEIA for the allergens of the egg white, egg yolk, OVA and OVM. An alpha error of 0.05% was accepted as the level of statistical significance. The ROC (receiver operating characteristics) curves were analysed with the Graphroc program (Veli Kairisto, Turku, Finland) [8]. The Mann-Whitney non-parametric test was used to compare the medians of the specific IgE between allergic and tolerant patients and between allergic patients who tolerated or did not tolerate cooked egg white, using the SPSS 7.5 software for this test.

Results

The diagnostic challenge test was carried out in 56 children (69%). The test was initiated with cooked egg white in 45 children, 18 of whom presented symptoms of immediate

hypersensitivity and 27 of whom tolerated the maximum amount administered. In the 27 patients, the diagnostic challenge test was continued with raw egg white, which was positive in 15 and negative in 12 of them. The diagnostic challenge test was begun with the raw fraction in 10 patients with no previous challenge with cooked egg with the result that five of them reacted and the other five tolerated it. In summary, 18 patients had a positive diagnostic challenge test with cooked egg white, 20 reacted to raw egg white, and 17 tolerated the amount corresponding to half an uncooked egg. One child reacted to the cooked egg yolk. Twenty-five children in whom no diagnostic challenge test had been performed were diagnosed with egg allergy, 20 of them because they fulfilled all of the points specified in section B of the diagnostic challenge test indications and the remaining five because severe anaphylaxis symptoms were reported.

The symptoms which occurred after diagnostic challenge test were cutaneous in 37 of the 39 children with positive tests (95%), this being the only clinical manifestation in 24 of them (62%). The cutaneous symptoms consisted of urticaria in 28 children, oral allergy syndrome in four, erythema in four and angioedema in two (one of them with urticaria). Vomiting was observed in 11 (28%) of the cases, one of which also had diarrhoea. Respiratory symptoms, which consist only of rhinitis in many cases, were observed in eight (21%) children. Thirty-three per cent of the children suffered cutaneous symptoms associated with digestive and/or respiratory ones. The symptoms appeared during the first half hour in 84% of the diagnostic challenge test, between 30 and 60 min in three children and after 2 h in three others. The symptoms that occurred after the challenge tests were easily controlled in most of the children. Eleven (28%) did not require treatment, and only antihistamines were administered in 18 (46%). Ten patients required other drugs, such as adrenaline and corticosteroids.

Table 2 shows the performance characteristics of the skin prick tests. The sensitivities were excellent, especially for egg white and OVA, and the positive predictive values were $>90\%$ for all allergens tested. The specificities are good for some allergens, egg yolk and OVM, and the negative predictive values are very low, except for egg white and OVA. The best likelihood ratio was negative for the egg white and OVA.

The specific IgE results are quite similar to skin prick tests (Table 3). When the 0.35 KU_A/L level is used, the sensitivity is very good for egg white, but not for other allergens, and the positive predictive values are $\geq 94\%$ for all allergens. Specificity for yolk is very good, and good for OVA and OVM. Negative predictive value is poor or very poor. The best likelihood ratios were negative for the egg white and positive for the yolk.

Table 2. Performance characteristics of skin prick tests at the cut-off level of 3 mm

Parameter evaluated	Allergen			
	Egg white	Yolk	OVA	OVM
Sensitivity (%)	97 (93–100)	78 (68–88)	95 (90–100)	78 (68–88)
Specificity (%)	71 (49–93)	88 (73–100)	65 (42–88)	82 (64–100)
PPV (%)	93 (86–98)	96 (91–100)	91 (84–98)	94 (88–100)
NPV (%)	86 (68–100)	52 (34–70)	79 (58–100)	50 (31–69)
PLR	3.3	6.6	2.7	4.4
NLR	22.6	4	13.8	3.8
Efficacy (%)	91	80	89	79

The data that appear between parenthesis correspond to the confidence interval at 95%. OVA = ovalbumin; OVM = ovomucoid; PPV = positive predictive value; NPV = negative predictive value; PLR = positive likelihood ratio; NLR = negative likelihood ratio.

The results of both prick test and the specific IgE to egg white showed a high concordance between both tests (Table 4). When both were positive, the probability of being allergic was very high. When both were negative, all of the children tolerated the food. In the discordant cases, that is, positive prick test and negative CAP or negative prick test and positive CAP, about two-thirds of the children were allergic.

The analysis of the ROC curve makes it possible to determine the optimal decision point (ODP). This point was 0.43 kU_A/L for egg white. It was not possible to establish a better cut-off point than 0.35 kU_A/L for the other allergen tests, that is, egg yolk, OVA and OVM, because specific IgE levels cannot be quantified below this point with the CAP System FEIA technique.

The median specific serum IgE concentrations to egg white, yolk, OVA and OVM were significantly higher in allergic patients than in the control group: egg white 2.01 kU_A/L vs. < 0.35 kU_A/L ($P < 0.001$); yolk 0.529 kU_A/L vs.

< 0.35 kU_A/L ($P < 0.001$); OVA 1.625 kU_A/L vs. < 0.35 kU_A/L ($P < 0.001$); OVM 1.68 kU_A/L vs. < 0.35 kU_A/L ($P < 0.002$). The median serum IgE levels in the allergic group were 41 IU/L (range 3–597), and 39 in the control group (range 5–355).

We have also compared the specific IgE levels in children who had a positive diagnostic challenge test to cooked egg white with those who had tolerated this form of egg but who had reacted to the uncooked egg white. The median of specific IgE levels to egg white in the first group was 2.82 kU_A/L vs. 0.78 kU_A/L in the second group ($P < 0.05$); yolk 0.75 kU_A/L vs. 0.36 kU_A/L ($P > 0.05$); OVA 1.73 kU_A/L vs. 0.43 kU_A/L ($P < 0.05$); OVM 1.83 kU_A/L vs. 0.18 kU_A/L ($P < 0.05$).

Discussion

The diagnosis of food allergy is indicated by clinical history. Demonstration of food-specific IgE antibodies,

Table 3. Performance characteristics of CAP System FEIA at 0.35kU_A/L

Parameter evaluated	Allergen			
	Egg white	Yolk	OVA	OVM
Sensitivity (%)	91(84–98)	63(51–75)	72(61–83)	73(62–84)
Specificity (%)	77(57–97)	93(80–100)	83(62–100)	82(59–100)
PPV (%)	94(88–100)	98(94–100)	96(90–100)	96(90–100)
NPV (%)	68(47–89)	37(22–52)	36(19–53)	35(16–54)
PLR	3.9	9	4.3	4
NLR	8.2	2.5	3	3
Efficacy (%)	88	68	74	74

The data that appear between parenthesis correspond to the confidence interval at 95%. OVA = ovalbumin; OVM = ovomucoid; PPV = positive predictive value; NPV = negative predictive value; PLR = positive likelihood ratio; NLR = negative likelihood ratio.

Table 4. The analysis of both prick test and CAP System FEIA for egg white at 0.35 KU_A/L

Prick/CAP result	Allergics (n)	Tolerants (n)
Prick (+) and CAP (+)	56	3
Prick (+) and CAP (–)	6	2
Prick (–) and CAP (+)	2	1
Prick (–) and CAP (–)	0	11

whether on the skin or in serum, supports the existence of allergy to it; however, a definite diagnosis can only be established after the challenge test, since, in general, both the skin tests as well as the presence of specific IgE antibodies in serum present good sensitivity and negative predictive value, but the specificity and the positive predictive value are lower [3,9,10].

In recent years, some studies have related the specific IgE levels in serum, determined with the CAP system FEIA technique, and the presence of respiratory symptoms in patients sensitized to inhalant allergens or who have clinical reactivity in relationship to foods. Pastorello *et al.* [5] found that levels of 8.4 KU_A/L for mites or 10.7 KU_A/L for pollens could be useful to separate patients with respiratory manifestations due to these allergens from other sensitized asymptomatic patients. Sampson *et al.* [6] have established cut-off points in the specific IgE serum levels for different foods that predict clinical reactivity after ingestion in 90% and 95% of the group studied.

When initiating this study, our principal objective was to establish a cut-off point in the specific IgE levels to egg that would make it possible to differentiate allergic patients from tolerant ones. The study was limited to children under 2 years of age because at this age most of the allergic children have already manifested the first symptoms and because their evolution at this point is similar. As is well known, children who are allergic to egg frequently become sensitized to other allergens, especially inhalants, and they experience increases, which can sometimes be very significant, in their total serum IgE levels, these being aspects that could interfere in the specific IgE levels to egg at a later age.

The results of the skin tests and the specific serum IgE to egg were quite similar. As a whole, the egg white allergen had the greatest diagnostic efficacy with both methods, although the result with OVA in the prick test was similar. If we consider the predictive values of the prick tests and the CAP, considering 0.35 KU_A/L as the cut-off point, it can be observed that the positivity of either one is accompanied by clinical reactivity in more than 90% of the children for all of the allergen tests. However, the

negative predictive value was good for the prick test with egg white and OVA, decreased to 68% for the CAP to egg white and was poor for the prick and CAP with other allergens. Thus, in our group, in which the allergy prevalence was 79%, the positivity of either the Prick or CAP diagnostic test for any allergen could have been sufficient to establish an accuracy diagnosis in more than 90% of the children with the positive test.

The analysis of the specific IgE levels with the ROC curves makes it possible to establish the ODP, which is the value in which the sensitivity and specificity reach equal weight. The cut-off point for egg white was 0.43 KU_A/L and for the rest of the allergens 0.35 KU_A/L, the detection limit level of the CAP technique. At this point, the sensitivity and specificity were greater than 80% for the egg white; however, the sensitivity for the remaining allergens did not reach 80% even when the lowest detection level of the technique was used.

However, the ODP does not always coincide with the best cut-off level used in our clinical practice as this depends on the relative importance of establishing a false diagnosis compared with the risk of subjecting the patient to the challenge test. When the value was changed from a range of 0.35 to 0.43 KU_A/L in the diagnosis of egg allergy, the positive predictive value was hardly modified and the negative predictive value only decreased minimally. The latter could not be improved above 68%.

This cut-off point is quite different from that found by Sampson *et al.* [6] In their ODP study for egg white, the cut-off point was 3.4 KU_A/L, with a sensitivity of 82% and a specificity of 84%. A CAP value equal to or greater than 2 KU_A/L was accompanied by a positive challenge test in more than 90% of the cases and ≥ 6 KU_A/L in more than 95% of them. In addition, when the value was under 0.6 KU_A/L, tolerance was assured in more than 90%. This discordance could be due to the different populations analysed. In the first place, the age of the group was older, 5.2 years compared with 16 months in our study, and in the second place, the selection criteria were very different. We included children with a history of allergic reaction after ingestion of egg white, whereas, in the above mentioned work, Sampson *et al.* dealt with a group of 'highly atopic' patients, all of whom had atopic dermatitis. Both characteristics, age and especially the high degree of atopy, could condition the higher levels of total serum IgE and specific IgE to egg white in the group studied by Sampson in relation to our study. In fact, the median total serum IgE levels in the group of that author was 3000 IU/L (range of 100 to more than 40 000 IU/L) while this was 40 IU/L (range 3–597) in our study. Regarding the specific IgE levels to egg white, Sampson's work reported a median value of 9.8 KU_A/L in allergic children vs. 1.0 KU_A/L in the control group. In our study, the median value in the

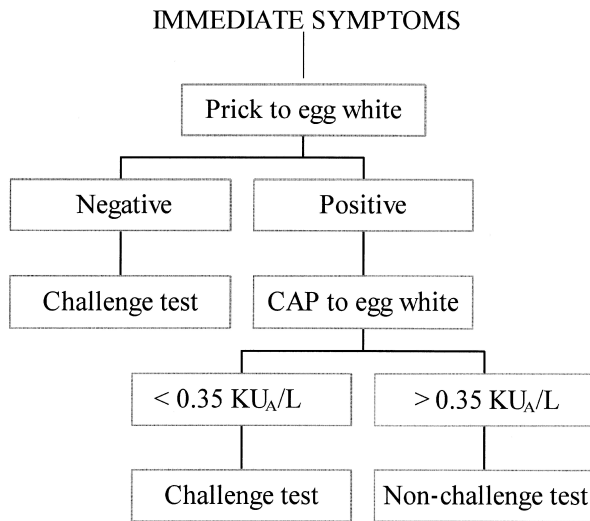


Fig. 1. Egg allergy diagnostic algorithm.

allergic children was 2.01 KU_A/L vs. < 0.35 KU_A/L in tolerant children.

The CAP System FEIA technique, used in the determination of the specific IgE antibodies, makes it possible to quantify a wide range of values, ranging from 0.35 KU_A/L to more than 100 KU_A/L [11]. As has been demonstrated by Pastorello with inhalants and by Sampson with food, the highest values have been associated with a greater likelihood of finding clinical symptoms in relationship to the allergen. In latex-allergic patients, these values have also been associated with more severe symptoms, which mean a higher 'degree of allergy'. In one study performed in children who were allergic to egg, Urisu *et al.* [12] observed that tolerance to heated egg white was associated with lower levels of specific IgE to egg white and OVM, determined by RAST. In our group, we demonstrated that children who were allergic to egg and who tolerated cooked egg white had significantly lower specific IgE antibodies levels to egg white, OVA and OVM than those allergic patients who reacted to cooked egg white, supporting the idea that the quantification of the specific IgE antibodies to an allergen provides us with information on the intensity of the sensitization.

In summary, the quantification of the specific IgE antibodies to the egg white with the CAP System FEIA technique has been shown to be useful in the diagnosis of allergy to this food in children under 2 years of age. A specific IgE antibodies level to this allergen ≥ 0.35 KU_A/L predicted clinical reactivity in more than 90% of the children in our study and the highest levels were associated with clinical reactivity to the less allergenic forms of the food. Thus, we consider that the diagnostic challenge test is

not necessary to establish the diagnosis of allergy to this food in children under 2 years of age who have a background of immediate hypersensitivity reaction after the intake of egg and the presence of specific IgE antibodies to egg white ≥ 0.35 KU_A/L. As a final conclusion to this study, we propose the diagnostic algorithm which is shown in Fig. 1.

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