

Prediction of tolerance on the basis of quantification of egg white-specific IgE antibodies in children with egg allergy

Teresa Boyano-Martínez, MD, Carmen García-Ara, PhD, José María Díaz-Pena, PhD, and Manuel Martín-Esteban, PhD *Madrid, Spain*

Background: It is thought that the natural evolution of food allergy has a good tolerance prognosis. However, there are few follow-up studies that determine the exact probability of tolerance to a given food or that analyze prognostic factors that can help us to understand the evolution of a child who begins life with a food allergy.

Objective: We sought to determine the likelihood that children younger than 2 years of age with allergy to egg would eventually have tolerance to it and to analyze several prognostic predictors using egg white-specific IgE level as the main variable.

Methods: We performed a prospective study of 58 children younger than 2 years of age with egg allergy, who were studied periodically until tolerance developed or until the end of the study. During the follow-up period, open challenge tests were carried out according to previously established criteria to verify tolerance to egg. Factors such as egg white-specific IgE level, serum total IgE level, symptoms after egg ingestion, size of skin prick test reactions to egg white, atopic dermatitis, and sex were analyzed as prognostic markers. Kaplan-Meier survival curves were used to calculate cumulative tolerance probability. Predictor influence and relative prognostic importance were estimated with the Cox proportional regression model.

Results: The median time from the appearance of the first symptoms to tolerance was 35 months. Cumulative tolerance probability was 16% at 12 months of follow-up, 28% at 24 months, 52% at 36 months, 57% at 48 months, and 66% at 60 months. The relative weight of prognostic factors, expressed as the hazard ratio, was 50.95 for symptoms and 3.74 for the size of skin prick test reactions, with both being independent effects. The hazard ratio was 1.173 for every 0.1-unit decrease in the concentration log (decimal logarithm) of specific IgE level, with this effect being associated with tolerance only in children with cutaneous symptoms.

Conclusions: Half of the children younger than 2 years of age with egg allergy will tolerate the food at 35 months of follow-up, and the proportion could be 66% after 5 years. At that age, the main predictors were the symptoms experienced after egg ingestion, followed by the size of skin prick test reactions. In addition, the specific IgE antibody level is an important prognostic marker in children who only had cutaneous symptoms. (*J Allergy Clin Immunol* 2002;110:304-9.)

Key words: Egg white-specific IgE antibodies, egg allergy, prognostic factors, natural history

Food allergy is most frequently seen in children, and it is thought that they outgrow this allergy with age. The hen's egg is one of the foods that most commonly causes this problem worldwide, with its prevalence having been calculated at 1.6% in children aged 2.5 years,¹ and it is estimated that this reaction exists in more than 35% of children with atopic dermatitis.²

Several authors have analyzed the follow-up of children with egg allergy,³⁻⁹ although it is difficult to compare their results. Even though the studies are prospective and the diagnosis is based on challenge tests with the food, there are 2 factors that differ greatly in each follow-up study and that greatly influence the proportion of children who achieve tolerance: the length of follow-up and the age of the children when the follow-up is initiated.

The prognostic factors that condition the evolution to tolerance of children allergic to food are little known. In general, different authors have considered that there is a relationship between the kind of food and symptom severity after ingestion and the likelihood of outgrowing the problem.³⁻⁶ Other factors, such as sIgE (specific IgE) antibody level^{4,7,8} and age at diagnosis,⁶ have also been associated with prognosis of food allergy, although these findings have not been found in all studies.^{5,10}

Our study was designed to examine the evolution of a group of children allergic to hen's egg who were given that diagnosis before 24 months of age, and the study had 2 principal objectives: (1) to understand the likelihood of tolerance at different moments of evolution and (2) to analyze the relationship between tolerance and several factors present at the initial evaluation by using the sIgE antibody level to egg white as the principal variable.

METHODS

Study population

This is a prospective follow-up study of 58 children, 36 boys and 22 girls with a mean age of 16 months (range, 11-24 months), who were given a diagnosis of egg allergy in our outpatient allergy unit according to previously established criteria.¹¹ Briefly, children evaluated in our allergy clinic for immediate hypersensitivity reactions after the ingestion of an egg were included in the study. Inclusion was performed consecutively. At the time of the first visit, skin prick tests and total and sIgE determination were performed, and the diagnosis was confirmed by means of food challenge tests. However, those children with a history of severe anaphylaxis and those in whom it was considered that the probability of clinical reactivity at this time was very high¹¹ because they fulfilled a series of previously established requirements were excluded from the challenge test.

From Servicio de Alergia Infantil, Hospital Universitario La Paz, Madrid.
Received for publication December 14, 2001; revised March 18, 2002;
accepted for publication •••.

Reprint requests: M^a Teresa Boyano Martínez, C/Ginzo de Limia 55, 9^oC,
28034 Madrid, Spain.

© 2002 Mosby, Inc. All rights reserved.

0091-6749/2002 \$35.00 + 0 1/87/126081

doi:10.1067/mai.2002.126081

After diagnosis, the children's parents were advised to exclude egg from the children's diets, and periodic revisions were carried out every 6 months until the age of 3 years and afterward annually until tolerance was achieved. We asked the patients or their parents about possible reactions as a result of accidental ingestion of egg, and we performed new skin prick tests and measurements of serum total IgE and sIgE antibodies at each visit. Challenge tests with the food were done during the follow-up period to verify whether the allergy persisted or, on the contrary, whether tolerance had been achieved, taking the following criteria into account.

First, for children in whom a diagnostic challenge test had been performed at the time of the first visit, if the only symptoms observed with the challenge test were local urticaria, a new challenge test was performed 1 year later. If the symptoms were generalized urticaria, angioedema, or symptoms affecting the digestive or lower respiratory airways, the new test was performed 2 years later, and if the child had experienced an anaphylactic reaction (2 or more organs affected), the test was carried out at 3 years.

Second, for children in whom no diagnostic challenge test had been performed at the time of the first visit, the same time periods were established by using the last reaction reported in the history taken at the first visit as a reference.

During follow-up, the same criteria were taken into account to perform the challenge tests: in other words, the use of symptoms and the time period from the last challenge test or, when this had not been performed, the last allergic reaction experienced and clearly related to egg ingestion.

Two exceptions were established. The challenge test was not performed if the child reported some allergic reaction clearly related to egg ingestion within the 3 months before the date of the follow-up visit, and the challenge test was always performed when the skin prick test responses were negative, although this indication was not in agreement with previous criteria.

Laboratory studies

CAP System FEIA. Serum samples from all patients were analyzed for total and antigen-sIgE antibodies to egg white, yolk, ovalbumin, and ovomucoid. The Pharmacia CAP system FEIA method was 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, ovalbumin (10 mg/mL) and ovomucoid (10 mg/mL) from Sigma, and commercial extract of yolk (1:20 wt/vol) from Dome Laboratories. Saline solution and histamine hydrochloride at 1% were applied by means of the prick technique as negative and positive controls. Food allergens eliciting a mean wheal diameter of 3 mm or greater than that produced by the negative control were considered positive.

Challenge tests. Open controlled challenge tests with egg white were performed in the allergy unit under the supervision of an allergologist and with prior authorization of the parents. The challenge was performed with cooked (10 minutes of boiling) and raw egg whites. Cooked egg white amounts corresponding to one eighth and one fourth of the egg, separated by an interval of 90 minutes, were offered on the first day. On the second day, half an egg white was administered. Afterward, the test was continued with raw egg white in similar amounts on 3 different days. The challenge test was begun directly with raw egg white when a child had tolerated the cooked egg white in another previously performed challenge test. Those patients who tolerated the amount of raw egg white corresponding to one half of a hen's egg were labeled as tolerant. On the contrary, those children who presented topical symptoms of immediate hypersensitivity, observable by those performing medical supervision, in a period of less than 2 hours after intake were considered to be allergic to the food.

Several prognostic factors were analyzed. All of them were collected at the initial evaluation (ie, at the time of the diagnosis). We assessed egg white sIgE antibodies as the principal variable and other factors, such as symptoms, serum total IgE levels, size of skin prick test reactions, atopic dermatitis, and sex, as covariables. The symptoms observed after eating egg were classified as the dichotomous variable: on the one hand, only the cutaneous symptoms and, on the other hand, the rest, including associated symptoms or only respiratory or digestive symptoms. At the time of the first visit, diagnostic challenge tests were carried out on 35 children, and the symptoms experienced after challenge tests were assessed. For the remaining 23 children, we took the symptoms reported in the history into account. Twenty-seven (47%) patients only experienced cutaneous symptoms, and 31 (53%) experienced other types of symptoms.

Statistical analysis

The median and interquartile ranges were used as summary measures because of the asymmetric distribution of egg white sIgE antibodies and total IgE levels. The concentration of egg white sIgE antibodies was stratified according to sex, atopic dermatitis, size of skin prick test reactions, serum total IgE levels, and symptoms after challenge tests. For 2-group comparisons, we used the nonparametric Mann-Whitney test. Allergic disease survival was defined as that beginning on the date of the first allergic reaction after egg ingestion to the date of tolerance or last follow-up. The Kaplan-Meier method was used to determine the relationship between the cumulative probability of egg tolerance and the predictors analyzed (egg white sIgE antibody level, sex, atopic dermatitis, size of skin prick test reactions, symptoms after challenge tests, and serum total IgE levels). Statistically significant differences were verified by means of the Mantel log-rank test. The relative importance of multiple prognostic factors on allergic disease survival was estimated with the aid of the Cox proportional regression model. Interaction effects between egg white sIgE antibody levels and other variables were assessed. For the final model, the assumption of proportional hazards was confirmed both graphically and by introducing the interaction of egg white sIgE antibody level with the log of time as a time-dependent covariable.¹² The statistical calculations were carried out with the SPSS 9.0 statistical program.

RESULTS

The follow-up time ranged from 7 to 86 months, with a median of 32 months. The median serum total IgE level was 43.5 KU_A/L (range, 3-597 KU_A/L). The median skin prick test reaction size with egg white, as assessed on the basis of mean wheal diameter, was 6 mm (range, 2.5-16.5 mm). Twenty-nine (50%) children had atopic dermatitis.

A total of 94 challenge tests, 35 at the onset of the study to establish the diagnosis and the remaining 59 during follow-up, were performed. The result was positive in 60 (35 at diagnosis and 25 follow-up) and negative in 34 (corresponding to the 34 children who reached tolerance). The symptoms found were exclusively cutaneous in 27 (73%) of the 37 challenge tests performed in the tolerant subjects, and in 7 (30%) of the 23 tests performed in the nontolerant subjects. During the follow-up period, allergic reactions caused by involuntary transgressions, especially among the nontolerant subjects, were frequent. In these children 47 reactions were counted in clear relationship with egg (35 oral transgressions, 10 contact transgressions, and 2 inhalation transgressions), and there were another 7 probable reactions.

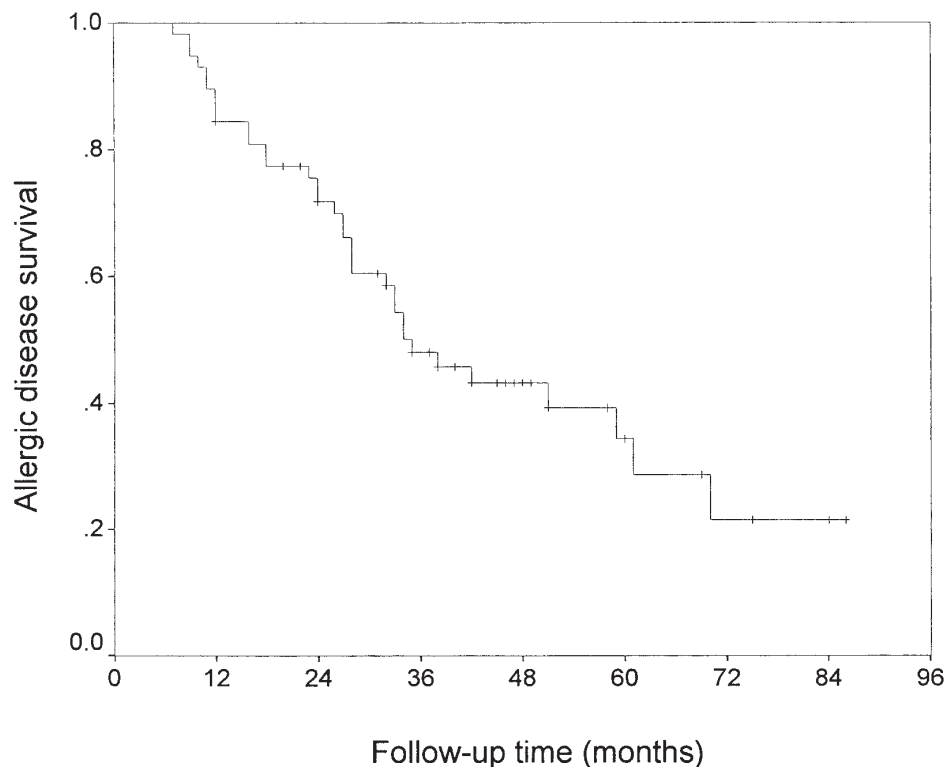


FIG 1. Cumulative egg allergy survival probability.

TABLE I. Egg white-specific IgE antibodies level in serum categorized by patients' characteristics

	N	Median	25th-75th Percentile	P value
Total	58	1.98	0.65-5.27	
Sex				.320
Male	36	2.68	0.60-7.22	
Female	22	1.52	0.68-3.71	
Atopic dermatitis				.602
With	29	1.82	0.45-6.40	
Without	29	2.15	0.94-4.80	
Symptoms				.019
Cutaneous only	27	1.52	0.45-2.75	
Others	31	2.90	1.21-11.5	
Wheal size				.007
<6 mm	27	1.46	0.45-2.85	
≥6 mm	31	2.90	1.23-11.5	
Total IgE (KU _A /L)				.000
<43.5	29	1.02	0.45-2.45	
≥43.5	29	4.91	1.77-14.5	

Global tolerance to egg

During the follow-up period, 34 (59%) children achieved tolerance to egg. Median survival time, defined as the time that cumulative survival probability includes 50% of the population, was 35 months. Twelve-month cumulative allergic disease survival probability was 84%, followed by 72% at 24 months, 48% at 36 months, 43% at 48 months, and 34% at 60 months (Fig 1).

Description of egg white sIgE antibodies

Egg white sIgE antibody level in serum ranged from class 0 (<0.35 KU_A/L) to 46.7 KU_A/L, with a median value of 1.98 KU_A/L (25th and 75th percentiles were 0.65 and 5.27 KU_A/L, respectively). The frequency distribution was not skewed, and this variable was introduced into the analysis as log (decimal logarithm of CAP).

Egg white sIgE antibody level and distribution of results by the patients' characteristics are shown in Table I. Concentrations of sIgE antibodies did not differ significantly among subgroups defined by sex and presence of atopic dermatitis. Cutaneous symptoms, smaller size of skin prick test reactions, and lower levels of serum total IgE indicated lower concentrations of egg white sIgE antibodies than other symptoms, larger size of skin prick test reactions, and higher levels of total IgE, respectively, and these differences reached statistical significance.

Analysis of predictive value

Univariate analysis showed that cumulative allergic disease survival was related to symptoms, wheal size,

Among the tolerant subjects, there were 14 certain (9 caused by intake and 5 by contact) and 2 probable reactions. A total of 25 (71%) of the 35 oral transgressions reported by the nontolerant subjects and 8 (89%) of the 9 transgressions reported by the tolerant subjects were exclusively cutaneous reactions.

TABLE II. Predictors of egg tolerance according to the univariate analysis

Variable	No. of patients	No. of events	Survival (mo)				Hazard ratio	95% CI	P value
			12	24	36	48			
Sex									
Male	36	21	83	62	48	45	1.38	0.69-2.78	.3598
Female	22	13	86	86	49	42	1		
Atopic dermatitis									
No	29	21	72	58	36	32	1.86	0.93-3.72	.0734
Yes	29	13	97	86	61	55			
Symptoms									
Cutaneous	27	21	74	50	29	24	3.27	1.62-6.60	.0004
Others	31	13	94	90	63	59	1		
Wheal size									
<6 mm	27	20	78	54	28	21	2.73	1.36-5.51	.0032
≥6 mm	31	14	90	87	65	61	1		
Total IgE (KU _A /L)									
<43.5	29	18	79	61	42	35	1.34	0.68-2.64	.3865
≥43.5	29	16	90	83	55	50	1		
Egg white IgE									
<1.98	29	22	79	58	23	23	3.10	1.50-6.39	.0012
>1.98	29	12	90	86	74	65	1		

and egg white sIgE antibodies (Table II). Atopic dermatitis approached a statistical level of significance. The analysis of allergic disease survival by egg white IgE antibodies was influenced by categorizing patients according to their antibody concentration. The 50th percentile of the frequency distribution (1.98 KU_A/L) was used as a cut-off value. The tolerance rate increased in indirect proportion to egg white IgE antibody concentration: 76% (22/29) for egg white sIgE antibody level of less than 1.98 KU_A/L and 41% (12/29) for sIgE levels of greater than 1.98 KU_A/L. The median time from the first reaction after eating egg to tolerance was 27 months in children in the low-level group and 59 months among those in the high-level group. Forty-eight month cumulative allergic disease survival probability was 23% in the low-level group and 65% in the high-level group ($P = .0012$, Fig 2).

Stratification for other variables did not change the predictor value of egg white sIgE antibodies. For many categories, the best results were recorded for children with the lowest sIgE levels. For others, such as female sex, other symptoms, or skin prick test reaction size of less than 6 mm, small numbers might have prevented the study from having sufficient statistical power to attain significance at a 95% CI level, or an interaction effect could be present.

The multivariate model results are shown in Table III. In the final model the egg white sIgE level was considered a continuous variable, thereby avoiding the use of arbitrary cut-off points. An interaction effect was observed between the variable symptoms and egg white sIgE level. The likelihood of tolerance increased by 1173 for every 0.1-unit decrease in the concentration log of sIgE level in children with cutaneous symptoms; no effect of sIgE level could be demonstrated in children with other symptoms. The likelihood of tolerance increased strongly in children with cutaneous symptoms

versus children with other symptoms (hazard ratio of 50.95); in addition, the likelihood increased by 3.74 in those with small size of skin prick test reactions versus those with larger reactions. Both variables revealed themselves to be independent predictive factors. These effects were controlled by sex, atopic dermatitis, and serum total IgE level.

Graphic analysis did not show any violation of the proportional hazards assumption, and the interaction between exposure and time was not significant. The latter results imply that the egg white sIgE antibody concentration did not lose its predictor value with time for the follow-up period analyzed.

DISCUSSION

The prognosis of food allergy is considered good because it is thought that most children will outgrow the problem as they become older. However, there are few long-term follow-up studies that show tolerance data at different moments of evolution or that analyze prognostic factors (ie, factors that help us to predict the future of a child allergic to a given food).

Tolerance probability and time to achieve it

In the case of egg allergy, there are several studies that analyze the evolution of allergic children regarding different foods,^{5,6,9} and because egg is one of the most frequently responsible foods for this problem, results on its prognosis can be found. In addition, there are some other articles that study the follow-up of allergy to this food individually³ or preferentially.⁴

In 1982, Ford and Taylor³ analyzed the evolution of 25 children with a median age of 17 months with egg allergy. After a period of 2 to 2.5 years, they observed that 11 (44%) children tolerated the food. Other studies found that between 31% and 51% of children allergic to

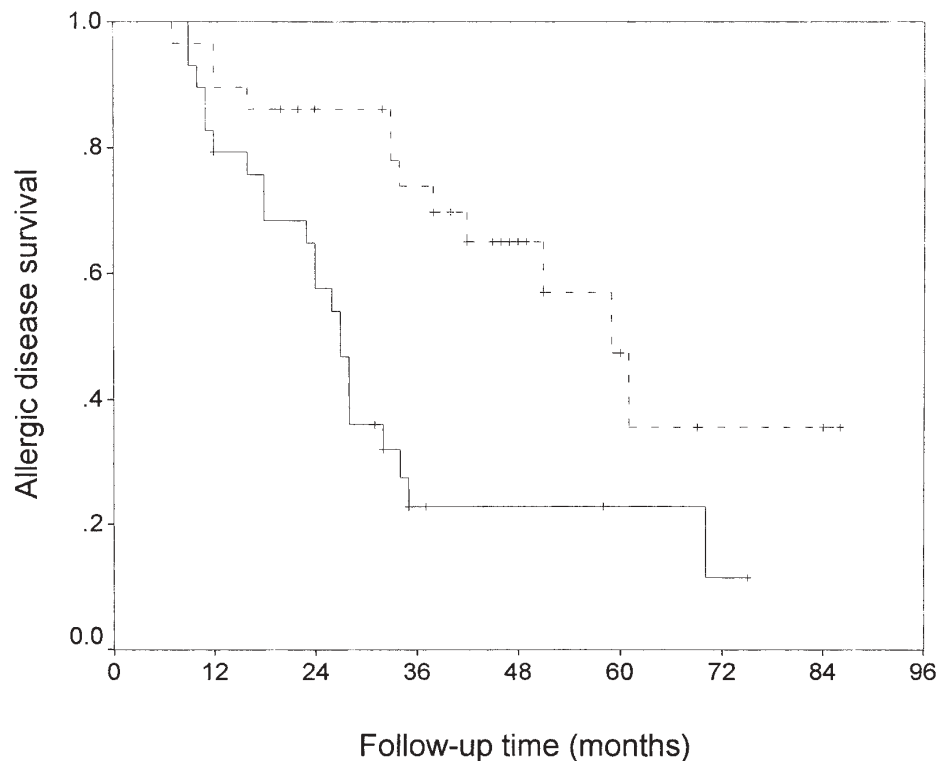


FIG 2. Sixty-month cumulative egg allergy survival probability on the basis of egg white sIgE level. *Solid line*, Less than 1.98 KU_A/L; *dashed line*, greater than 1.98 KU_A/L.

TABLE III. Predictors of allergic disease survival in egg allergy according to the multivariate analysis

Variable	Hazard ratio	95% CI	P value
Symptoms			
Others symptoms	50.95	6.94-374	<.001
Cutaneous	1		
Egg white skin prick test reaction size			
<6 mm	3.74	1.60-8.74	.002
≥6 mm	1		
Egg white-specific IgE level (only for cutaneous symptoms) for every 0.1 U log (CAP)*	1.17	1.05-1.30	.002

*Log (CAP), Logarithm of specific IgE concentration.

analysis technique that takes these differences into account is used.

In this study 58 children allergic to egg were followed up during a time period of between 7 and 86 months, initiating the follow-up at a moment close to when the symptoms began (all the children were <2 years of age). Thirty-four (59%) achieved tolerance to egg. According to the results of the Kaplan-Meier curves, cumulative tolerance probability was 50% at 35 months of follow-up. This means that half of the children allergic to egg will tolerate the food when they are 4 to 4.5 years old. The best period of tolerance was the third year of follow-up because the tolerance probability during this time accounted for 24%, a proportion that nearly corresponded to 28% in the first 2 years. The decrease in the survival curve was slower after the third year, there being a lower likelihood of tolerance.

Analysis of prognostic factors

The prognostic factors for the development of tolerance in food allergy are poorly understood. Some authors have reported that cow's milk and egg allergy are more easily outgrown than allergy to fish or peanut^{4-6,10} and that symptoms experienced after ingestion of the food^{3,5} are associated with evolution; as these become more serious, the likelihood of tolerance decreases. Recently, Chatchatee et al¹³ have found sIgE to linear epitopes of casein in children older than 9 years of age with persistent allergy to cow's milk proteins that was not present in

egg overcame the problem.^{4-6,9} However, it is difficult to compare the results of the previous studies because some of their conditions are quite different. On the one hand, the age when the follow-up of the children was initiated varies greatly in terms of both between-study and within-study age, and the age range is great if we consider that egg allergy generally begins around 12 months of age and that most children outgrow the problem in the first years. Thus the likelihood of tolerance can vary with the age at which the follow-up is initiated. On the other hand, the length of follow-up time varies greatly, between 1 and 15 years. This fact makes it impossible to compare the results if no statistical

any children less than 3 years of age. Other factors, such as the level of sIgE antibodies to food, seem to be related to prognosis in some studies^{4,7,8} but not in others.¹⁰ Finally, no association has been found between the size of the cutaneous skin prick test reaction or serum total IgE level and tolerance of the food.^{3,5,10}

Those studies that have analyzed the prognostic factors have generally been carried out, as has already been mentioned, in children who are allergic to different foods, and the age at which the follow-up was initiated varies greatly among them. The factors used to analyze prognosis, such as the size of the cutaneous test reactions or the level of sIgE antibodies, are those that children show when the follow-up is initiated and correspond to different moments in the disease evolution. On the other hand, the analysis has not considered the follow-up time periods of children who continue with their allergy. These facts make it difficult to compare the findings.

In our study we have used Cox regression to analyze the prognostic factors. This is a statistical multivariate survival method that permits us to calculate the weight of each variable introduced in the analysis. According to the results of the analysis, children who had only experienced cutaneous symptoms had the best prognosis; in addition, those with smaller skin prick test reactions had better prognosis. The sIgE antibody level, which was analyzed as the principal variable, was associated with prognosis only in children who had cutaneous symptoms but not in children with other symptoms. We could not demonstrate any association between sex, atopic dermatitis, or serum total IgE level and tolerance.

Thus according to the results obtained in the multivariate analysis, symptoms and size of the skin prick test reaction are the most important predictive variables for children with sIgE antibody levels of less than 0.35 KU_A/L. In children with cutaneous symptoms, as the sIgE antibody concentration increases, the likelihood of tolerance attributed to this marker decreases. In children with sIgE levels of greater than 2 KU_A/L, the negative influence of the antibodies becomes as important as the influence of the size of the skin prick test reaction, and levels of greater than 3.5 KU_A/L reduce tolerance probability 5-fold.

One fact to keep in mind when interpreting our results is the possible overestimation of the importance of symptoms evoked by egg ingestion in predicting the time until tolerance develops. In other words, children with milder cutaneous symptoms were challenged earlier and potentially more frequently than children with respiratory symptoms. However, there are data that lead us to believe that this bias might not be so important. First, the fre-

quent corroboration with clinical reactivity is maintained among the nontolerant subjects by the large number of reactions caused by accidental or voluntary transgressions with egg and by the challenge tests performed. Second, the lower frequency of exclusively cutaneous reactions experienced by the nontolerant subject supports the effect discussed. Third, because this variable conditions the periodicity of the challenge tests only initially, the following periods could vary depending on the symptoms presented in the final challenge test performed, so that the children who initially waited 2 or 3 years might only need to wait 1 year for the new challenge test.

In summary, our work shows that half of children less than 2 years of age with egg allergy will tolerate the food at 35 months of follow-up, and this proportion could be 66% after 5 years. In the children younger than 2 years of age, the main predictor was the symptoms experienced after egg ingestion, followed by the size of skin prick test reactions. In addition, the sIgE antibody level was an important prognostic marker in those children who only experienced cutaneous symptoms.

REFERENCES

1. Eggesbo M, Botten G, Halvorsen R, Magnus P. The prevalence of allergy to egg: a population-based study in young children. *Allergy* 2001;56:403-11.
2. Oehling A, Resano A, Sanz ML, Fernández Benítez M. Importance of food allergy in atopic dermatitis. *Allergy* 1998;53 (suppl 46):139-42.
3. Ford RPK, Taylor B. Natural history of egg hypersensitivity. *Arch Dis Child* 1982;57:649-52.
4. Dannaeus A, Inganäs M. A follow-up study of children with food allergy. Clinical course in relation to serum IgE- and IgG-antibody levels to milk, egg and fish. *Clin Allergy* 1981;11:533-9.
5. Sampson HA, Scanlon SM. Natural history of food hypersensitivity in children with atopic dermatitis. *J Pediatr* 1989;115:23-7.
6. Bock SA. The natural history of food sensitivity. *J Allergy Clin Immunol* 1982;69:173-7.
7. García Ara MC, Boyano Martínez T, Martín Esteban M, Martín Muñoz F, Díaz Pena JM, Ojeda Casas JA. Therapeutic approach to and prognosis of food allergy. *Allergol Immunopathol* 1996;24(suppl 1):31-5.
8. James JM, Sampson HA. Immunologic changes associated with the development of tolerance in children with cow milk allergy. *J Pediatr* 1992;121:371-7.
9. Boyano Martínez MT, Martín Esteban M, Pascual C, Ojeda JA. Food allergy in children. II. Prognostic factors and long-term development. *An Esp Pediatr* 1987;26:241-5.
10. Eggleston P. Prospective studies in the natural history of food allergy. *Ann Allergy* 1987;59:179-81.
11. Boyano Martínez T, García-Ara C, Díaz Pena JM, Muñoz FM, García Sánchez G, Esteban MM. Validity of specific IgE antibodies in children with egg allergy. *Clin Exp Allergy* 2001;31:1464-9.
12. Kleinbaum DG. Survival analysis: a self-learning text. 1st ed. New York: Springer-Verlag; 1996.
13. Chatchatee P, Järvinen K-M, Bardina L, Beyer K, Sampson HA. Identification of IgE- and IgG-binding epitopes on α_{s1} -casein: differences in patients with persistent and transient cow's milk allergy. *J Allergy Clin Immunol* 2001;107:379-83.