

BRIEF COMMUNICATION

Total serum IgE level influences oral food challenge tests for IgE-mediated food allergies

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Keywords

food allergy; oral food challenge test; specific IgE; total IgE.

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The oral food challenge test (OFC) is the most reliable method to diagnose food allergies. However, to avoid serious allergic reactions in OFCs, less invasive methods have been evaluated. Sampson investigated the relationship between OFC results and food-specific IgE antibody levels and developed food allergy probability curves (1). Probability curves enable predictions of food-specific IgE antibodies to facilitate more accessible examinations (2–4); nevertheless, threshold levels differ for various populations (5).

Several factors affect OFC results, including heat treatment of the challenge foods, exercise, and patient age (1, 2). Age is considered a critical confounding factor affecting OFC results (2), although the precise reason remains unknown. Furthermore, some studies have indicated that total IgE levels could influence the results of an OFC (6–8); therefore, we

Abstract

Probability curves predicting oral food challenge test (OFC) results based on specific IgE levels are widely used to prevent serious allergic reactions. Although several confounding factors are known to affect probability curves, the main factors that affect OFC outcomes are currently unclear. We hypothesized that an increased total IgE level would reduce allergic reactivity. Medical records of 337 and 266 patients who underwent OFCs for 3.5 g boiled hen's egg white and 3.1 ml raw cow's milk, respectively, were examined retrospectively. We subdivided the patients into three groups based on total IgE levels and age by percentile (<25th, 25–75th, and >75th percentiles), and logistic regression analyses were performed on each group. Patients with higher total IgE levels were significantly less responsive. In addition, age did not significantly affect the OFC results. Therefore, total IgE levels should be taken into account when predicting OFC results based on food-specific IgE levels.

aimed to determine whether total IgE levels might be a more suitable confounding factor to be controlled than age.

Methods

Study population

The medical records of 337 patients (boys, 221; girls, 116; median age, 3.57 years; range, 1.01–8.94 years) and 266 patients (boys, 177; girls, 89; median age, 4.36 years; range, 1.04–8.81 years) who received an OFC for boiled hen's egg white and cow's milk, respectively, at the Division of Allergy, National Center for Child Health and Development of Japan between April 2010 and September 2013 were examined retrospectively (Table S1).

Study procedures and measurements

After informed consent was obtained from the participants' parents, the OFC was performed with boiled egg white (0.5,

Abbreviations

OFC, oral food challenge; ROC, receiver operator characteristic; SD, standard deviation.

1, and 2 g at 30-min intervals) or raw cow's milk (0.1, 1, and 2 ml at 30-min intervals). Oral food challenge methods are described in the Supporting Information associated with this article. The total IgE levels and food-specific (egg white, ovomucoid, and milk) levels in the serum were measured within 6 months before the OFC (median, 75 and 86 days for egg white and milk, respectively) using a fluorescent enzyme immunoassay and ImmunoCAP® (Thermo Scientific Inc.; Uppsala, Sweden), respectively. We excluded 21 and 15 patients from the egg white and milk groups, respectively, because of lack of objective symptoms (Fig. S1). This study was approved by the Institutional Ethics Committee.

Statistical analysis

Because the total and specific serum IgE levels follow a lognormal distribution, (Fig. S2), the nonparametric Kolmogorov–Smirnov method was used for statistical analyses. The correlation between the total IgE levels and age was assessed by Spearman's rank correlation coefficient. Receiver operator characteristic (ROC) analyses were performed to compare the sensitivity and specificity between egg white and ovomucoid responses for evaluation of their diagnostic ability, and ROC analysis was also used to compare specific IgE levels alone and the ratio of specific to total IgE levels or specific IgE to age in predicting the OFC results. Binary logistic regression analyses were performed to determine the association between the food-specific IgE levels and positive OFC results and to fit probability curves; multivariate logistic regression analyses were performed to determine the significance of the effect of total serum IgE level and age. The Kruskal–Wallis test was used to analyze the difference in the amount of consumed foods required to trigger an allergic reaction between each group. The statistical analyses were conducted with SPSS 17.0 for Windows (SPSS Inc.; Chicago, IL, USA), and $P < 0.05$ was regarded as significant.

Results

A significantly positive correlation was observed between the total serum IgE level and patient age (Fig. 1). The area under the ROC curve for ovomucoid-specific IgE concentration (79.3%) was higher than that for egg white-specific IgE concentration (71.6%). Thus, only the probability curves for ovomucoid- and milk-specific levels were analyzed. The area under the curve for the ratio of specific IgE to total IgE levels was also slightly higher than that for the ratio of specific IgE to age or specific IgE alone (Fig. S3); however, these differences were small. In addition, the patients were subdivided into three groups according to total serum IgE level or age: (1) low group (<25th percentile), (2) moderate group (25–75th percentile), and (3) high group (>75th percentile). Patients with higher total serum IgE levels were significantly less responsive in the OFC for the same allergen-specific IgE levels compared to the low and moderate groups (Fig. 2A,B). Alternatively, age did not have a significant effect on the OFC results (Fig. 2C,D). No significant differences between the amounts of consumed foods required to trigger an allergic reaction were found among the three groups (Table S2).

Discussion

The precise reason for the influence of age on OFC results remains unclear, although immature digestive functions and intestinal macromolecular absorption in young infants (9) and active eczematous symptoms (10) have been proposed. However, in our study, the symptoms of atopic dermatitis were almost in remission from prior treatment (Table S1). Our results demonstrated that the variation in OFC results is mainly due to differences in total serum IgE levels. In fact, nonspecific or low-affinity IgE suppresses specific IgE-mediated activation of basophils *in vitro* (11), and total serum IgE levels reportedly affect the clinical severity or reactivity of

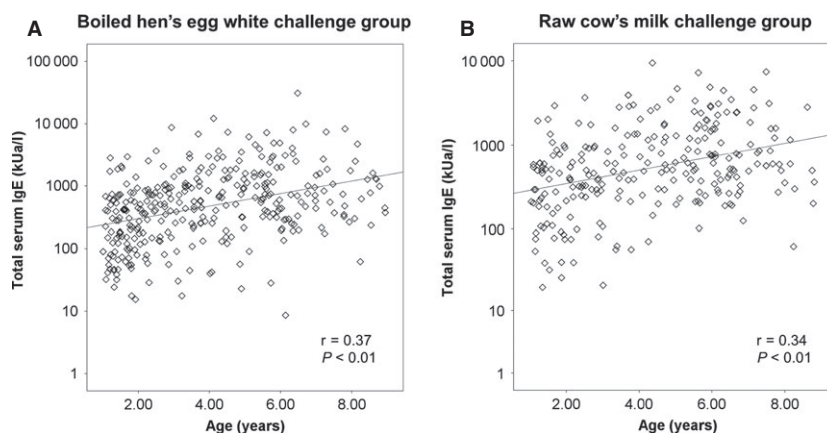


Figure 1 Scatter plots of total serum IgE level vs patient age in the boiled egg white challenge group (A) and raw cow's milk challenge group (B). A significant positive correlation between the total serum IgE level and patient age was observed in both the boiled

egg white challenge group ($n = 337$) and the raw cow's milk challenge group ($n = 266$) (Spearman's rank correlation coefficient; $P < 0.01$ and $P < 0.01$, respectively).

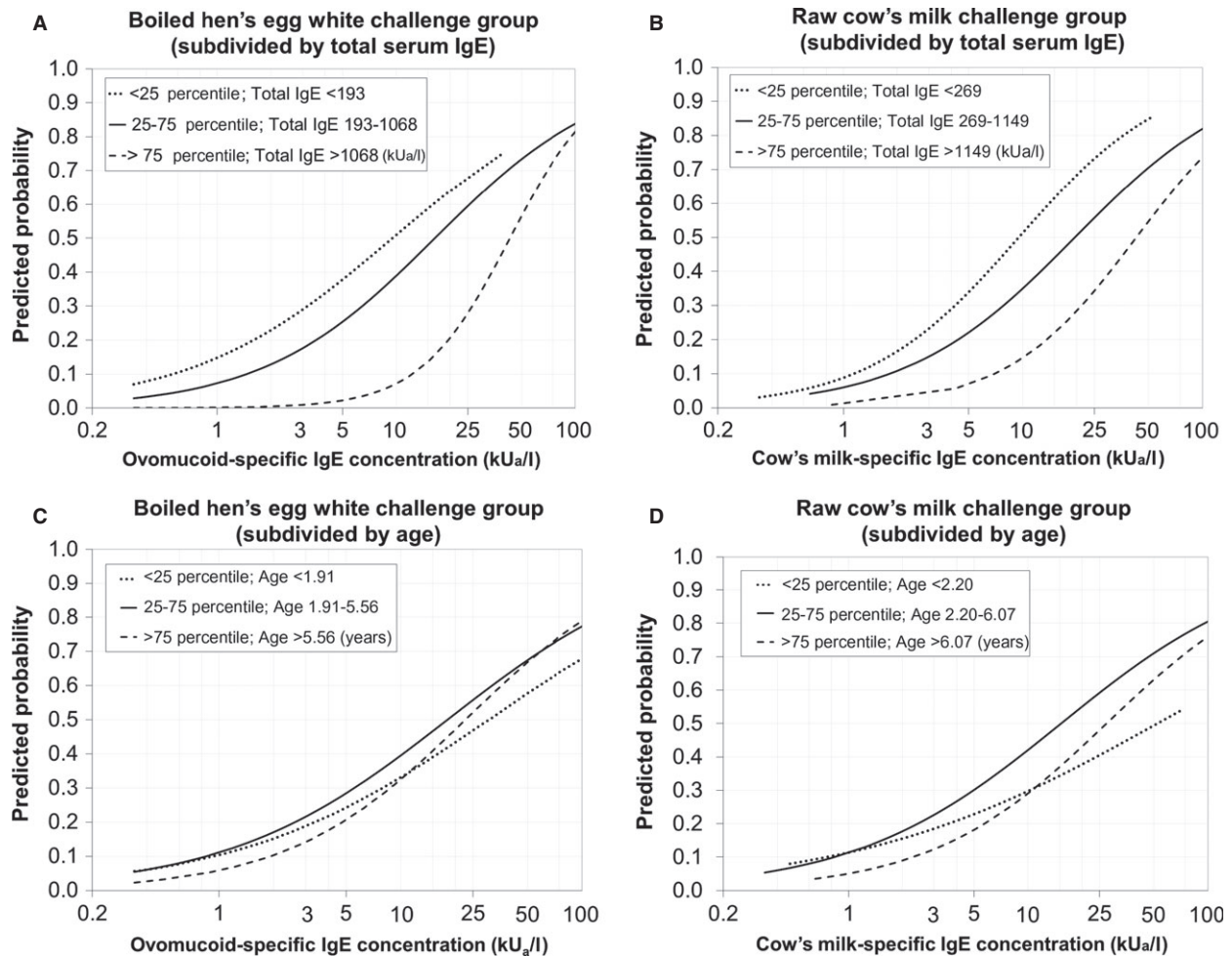


Figure 2 Fitted predicted probability curves of oral food challenge test (OFC) results in relation to the food-specific serum IgE concentration according to total serum IgE level or age group. Patients were subdivided into three groups according to total serum IgE lev-

els or age: (1) low group (<25th percentile), (2) moderate group (25–75th percentile), and (3) high group (>75th percentile). Ovomucoid- (A and C; $n = 316$) and cow's milk-specific serum IgE concentrations (B and D; $n = 251$) were measured.

patients with a milk (6), β -lactam (7), or Hymenoptera venom (8) allergy. Most previous studies indicate that older patients show increased total IgE levels (12), and the present study confirmed this. One reason for the effect of age on OFC results might be the correlation between age and total IgE levels as confounders. Conversely, a previous study showed that the ratio of specific IgE/total IgE offers no advantage for diagnosing symptomatic food allergies (13); using a similar method, our results confirmed this lack of a clear advantage. However, as previously described, as the total IgE level followed a lognormal distribution, but the specific IgE level did not, it is clear that the correlations between specific IgE and total IgE levels are not linear; therefore, the calculation of the ratio might be too simple to accurately reflect the true relationship between the two factors. Furthermore, stratified analysis by total IgE levels was identified as a potentially appropriate approach suitable for user-friendly analysis.

One of the limitations of our study is the open challenge design. We attempted to exclude the effects of subjective symptoms as much as possible by limiting the age of patients to 1–8 years and excluded a small number of undeterminable cases only reporting subjective symptoms. No correlation was observed between the average number of excluded cases and age. Another limitation to the study is that the maximal dose was relatively low and was fixed. Therefore, as the dose might have been lower in older patients, we tried to minimize this effect by reducing the subjects' maximum age (1, 2). In addition, our study was target toward evaluation in infants and young children (1–8 years), and as only a portion of patients were examined for aeroallergen sensitization (data not shown), it was difficult to investigate the effect of aeroallergens.

Our results clearly demonstrate that in addition to age, the total IgE level should be considered for predicting OFC results from food-specific IgE titers. Other factors, including

affinity of the food-specific IgE antibodies (11, 14, 15) and allergen-specific IgG1 and IgG4 as blocking antibodies, should also be evaluated for the prediction of IgE-mediated food allergies.

Author contributions

K. Horimukai, I. Nomura, and K. Matsumoto were involved in study conception and design. K. Horimukai, Y. Tsumura, M. Narita, and Y. Ohya were responsible for data acquisition. K. Horimukai, K. Hayashi, and K. Matsumoto performed the statistical analysis and data interpretation. K. Horimukai and K. Matsumoto drafted the manuscript. K. Horimukai, I. Nomura, M. Narita, Y. Ohya, H. Saito, and K. Matsumoto revised the manuscript critically for important intellectual content. All authors read and approved the manuscript.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Supporting Information

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

Figure S1. Percentage of positive, negative, and undeterminable cases in the boiled egg white challenge group (A; $n = 337$) and raw cow's milk challenge group (B; $n = 266$) for each age group.

Figure S2. Histograms showing the log distribution of total (A and C) and specific (B and D) serum IgE levels in the boiled egg white (A and B; $n = 337$) and cow's milk (C and D; $n = 266$) challenge groups, analyzed with the nonparametric Kolmogorov–Smirnov method.

Figure S3. Receiver operator characteristic (ROC) curves for assessing the predictive ability of OFC results using the ratio (e.g., specific IgE to total IgE or specific IgE to age) compared with specific IgE alone.

Table S1. Characteristics and clinical features of the patients.

Table S2. Correlation between total IgE levels and the amount of antigen required to start triggering an allergic reaction.

Method S1. Details of the oral food challenge test.

Supplementary Figures

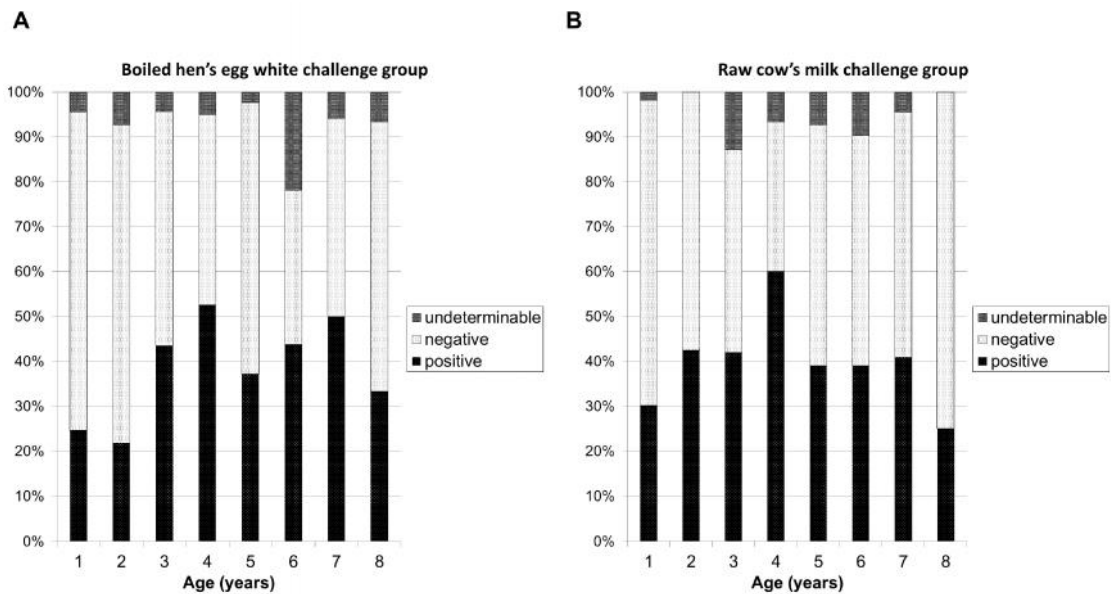


Figure S1. Percentage of positive, negative, and undeterminable cases in the boiled egg white challenge group (A; n = 337) and raw cow's milk challenge group (B; n = 266) for each age group. There was no significant difference in the ratio of the number of undeterminable cases (i.e., excluded cases) in each age group by Spearman's rank correlation coefficient ($P > 0.05$).

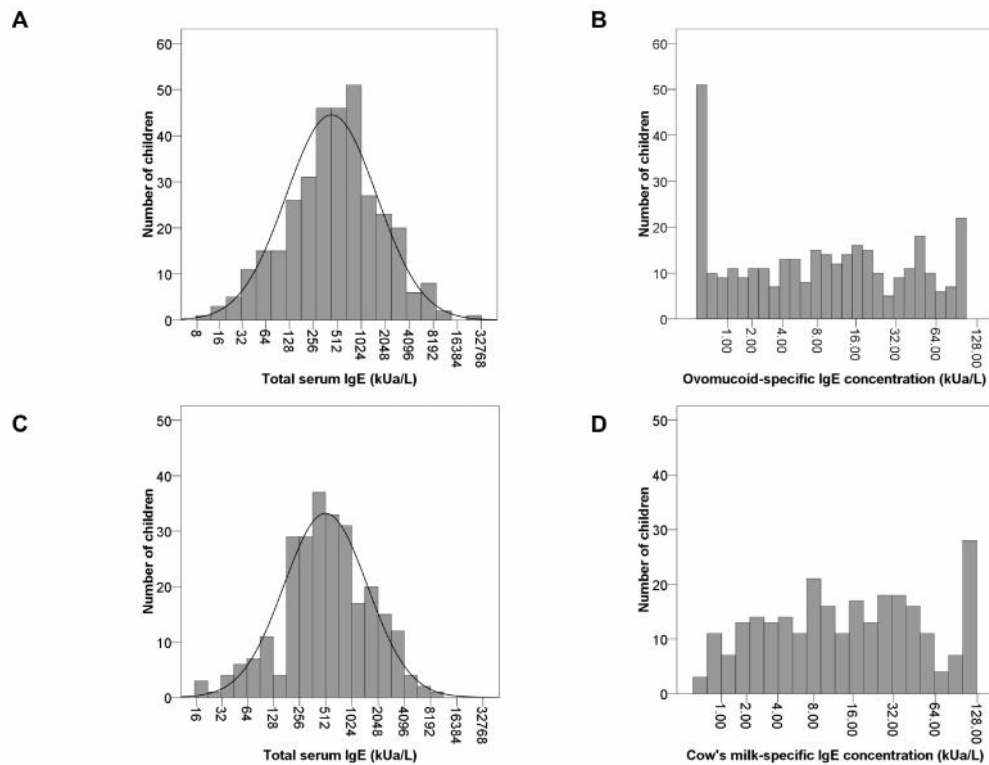


Figure S2. Histograms showing the log distribution of total (A and C) and specific (B and D) serum IgE levels in the boiled egg white (A and B; n = 337) and cow's milk (C and D; n = 266) challenge groups, analyzed with the nonparametric Kolmogorov–Smirnov method. The total serum IgE levels showed a lognormal distribution, but specific IgE levels did not.

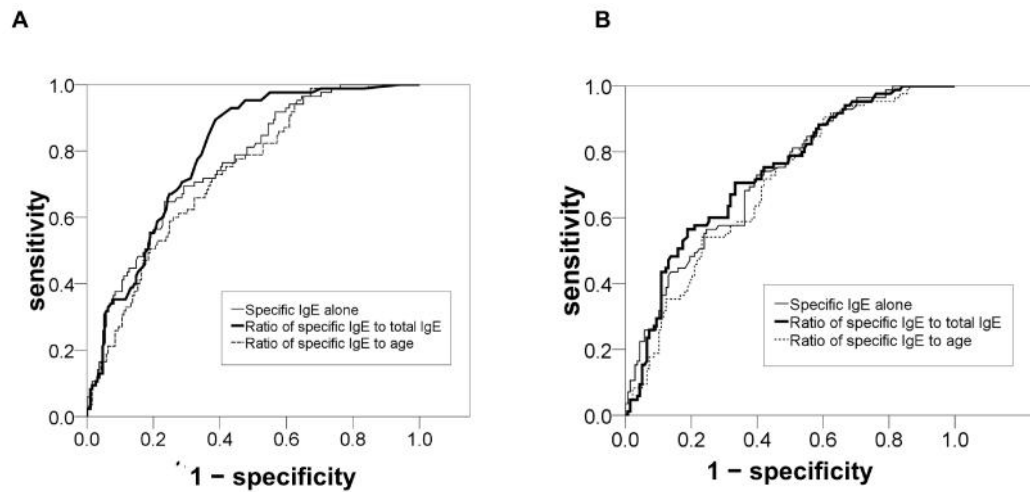


Figure S3. Receiver operator characteristic (ROC) curves for assessing the predictive ability of OFC results using the ratio (e.g., specific IgE to total IgE or specific IgE to age) compared with specific IgE alone. We recruited only patients with specific IgE levels ranging from 0.35 to 99.9 kU/L to calculate an accurate ratio (A, boiled hen's egg white group, 274 cases; B, raw cow's milk group, 223 cases). A slight increase in area under the curve was observed only in the ratio of specific IgE to total IgE levels compared to specific IgE levels alone; however, the differences were small.

Supporting Table S1 Characteristics and clinical features of the patients

	Boiled hen's white egg	Raw cow's milk
No. of subjects	337	266
Sex, girls, n (%)	116 (34.4)	89 (33.5)
Age (y), median	3.57	4.36
Previous adverse reactions related to challenged foods, n (%)	180 (53.4)	199 (74.8)
Previous and/or current medical history		
Atopic dermatitis, n (%)	297 (88.1)	239 (89.8)
Current skin symptoms just before OFCs*		
Present, n (%)	42 (12.5)	44 (16.5)
Absent, n (%)	295 (87.5)	222 (83.5)
Bronchial asthma, n (%)	123 (36.5)	116 (43.1)
Current controller drugs for asthma, n (%)	102 (30.3)	108 (40.6)
Total serum IgE level (kUa/L), median	505.0	513.5
Egg white-specific IgE level (kUa/L), median	20.1	-
Ovomucoid-specific IgE level (kUa/L), median	8.57	-
Milk-specific IgE level (kUa/L), median	-	13.3
OFC results		
Positive, n (%)	111 (32.9)	107 (40.2)
Negative, n (%)	205 (60.8)	144 (54.1)
Undeterminable, n (%)	21 (6.2)	15 (5.6)

* The symptoms of atopic dermatitis were in remission with prior anti-inflammatory treatment, and almost all of the current symptoms were mild.

Supporting Table S2 Correlation between total IgE levels and the amount of antigen required to start triggering an allergic reaction

A. Boiled hen's egg white group

Amount of boiled hen's egg white to start triggering an allergic reaction (n)						Specific IgE to ovomucoid	
(g)		0.5	0.6–1.5	1.6–3.5	Total	(g) mean ± SD	(kUa/L) mean ± SD
Total IgE	<193	2	7	11	20	2.36 ± 1.23	7.35 ± 9.04
levels	193–1068	3	21	35	59	2.48 ± 1.12	32.4 ± 27.9
(kUa/L)	>1068	2	7	23	32	2.72 ± 1.04	68.0 ± 32.0
Total		7	35	69	111	2.53 ± 1.11	38.2 ± 34.1

B. Raw cow's milk group

Amount of raw cow's milk to start triggering an allergic reaction (n)						Specific IgE to cow's milk	
(mL)		0.1	0.2–1.1	1.2–3.1	Total	(mL) mean ± SD	(kUa/L) mean ± SD
Total IgE levels (kUa/L)	<269	2	7	14	23	2.21 ± 1.17	15.7 ± 14.8
	269–1149	4	18	30	52	2.11 ± 1.20	34.6 ± 29.9
	>1149	4	10	18	32	2.07 ± 1.19	77.4 ± 31.9
Total		10	35	62	107	2.12 ± 1.19	43.3 ± 36.4

SD: Standard deviation.

The patients were subdivided into three groups according to their total serum IgE levels: (1) low group (<25th percentile); (2) moderate group (25–75th percentile); and (3) high group (>75th percentile). No significant difference was found in the amount of challenged foods required to start triggering an allergic reaction among the three groups by the Kruskal-Wallis test (boiled hen's egg white, $P = 0.608$; raw cow's milk, $P = 0.872$) or by ANOVA (boiled hen's egg white, $P = 0.417$; raw cow's milk, $P = 0.887$).

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Details of the Oral Food Challenge Test

Patients who consulted the Division of Allergy, National Center for Child Health and Development in Japan between April 2010 and September 2013 were enrolled in the study. Oral food challenge tests (OFCs) were performed according to the Japanese Pediatric Guideline for Food Allergy [JPGFA; first edition 2005 (S1), revised edition 2012 (S2)]. In brief, patients ingested increasing amounts (at 30-min intervals) of boiled hen's egg white (0.5, 1, and 2 g; total 3.5 g) or raw cow's milk (0.1, 1, and 2 mL; total 3.1 mL). Results were evaluated using the criteria proposed in the JPGFA. Although this OFC was not conducted in a blinded manner, OFCs based on this protocol have been performed at nearly 100 hospitals (almost all hospitals perform OFCs for children) in Japan, and the protocol has been validated in a subsequent review article (S3).

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