Clinical Communications

Effect of specific IgE on eliciting dose in children with cow's milk allergy

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Clinical Implications

- Children with suspected cow's milk allergy with higher specific IgE required lower eliciting doses during oral food challenge, according to threshold distribution curves. Even in the same class, the threshold was significantly lower in children kept on an exclusive elimination diet.

Food allergy (FA) is an increasingly prevalent global public health issue.[1] Oral food challenge (OFC) is the criterion standard for FA diagnosis.[2] OFC is also used to calculate the threshold dose that children can safely consume, providing important information for risk management of children with FA. Because OFC may sometimes cause serious reactions, such as fatal anaphylaxis,[3] ensuring the safety and efficacy of the challenge is essential. Threshold-dose-distribution models created using clinical OFC study data provide valuable information regarding quantitative risk assessment (estimating the probability of allergic reactions)[4]; however, the reacting ingested dose, that is, the eliciting dose (ED), varies for each patient with FA.

Allergen-specific IgE (sIgE), an important indicator of FA, reflects the degree of sensitization. Furthermore, the predicted probability curves of positive outcomes in OFC based on sIgE levels facilitate suspected FA diagnosis.[5] However, little is known regarding whether the ED in OFC varies as a function of individual sIgE levels. Here, we demonstrated that EDs of children in the higher ImmunoCAP sIgE class (ImmunoCAP class) were significantly lower than those of children in the lower ImmunoCAP class by analyzing the threshold distribution curves.

We enrolled children with confirmed cow's milk allergy who were tested using an open milk challenge between October 2013 and March 2020 at the National Center for Child Health and Development, Tokyo, Japan. Among them, we analyzed OFCs that used only fresh milk as the challenge and included positive test results based on the presence of objective symptoms or negative test results based on a previously diagnosed milk allergy (see Figure E1 in this article's Online Repository at www.jaci-inpractice.org); subjective symptoms, such as oral itching, nausea, abdominal pain, and pruritus, were not considered. All participants provided written informed consent. This study was approved by the Ethics Review Committee of the National Center for Child Health and Development (no. 2019-003).

We conducted OFCs using a dosing method with 3 to 5 graded steps (see this article's Online Repository at www.jaci-inpracticeonline.org). Interval-censoring survival analysis was performed. Weibull, log-normal, and log-logistic distribution models were used to estimate the threshold-dose distribution and EDs. JMP 15 (SAS Institute Inc, Cary, NC) was used for statistical analysis. sIgE was measured using ImmunoCAP (Thermo Fisher, Uppsala, Sweden). Stratification analysis was performed to classify sIgE levels into various ImmunoCAP classes.

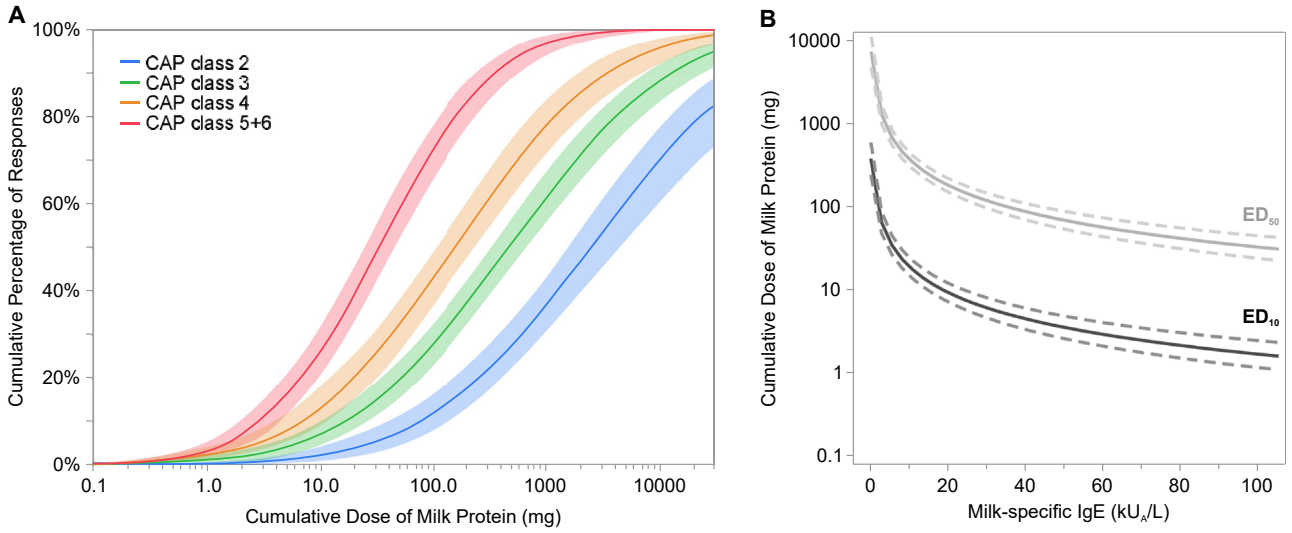


FIGURE 1. (**A**) Log-normal threshold distribution curves of milk-allergic children showing objective symptoms during OFC based on ImmunoCAP classes. ImmunoCAP classes: 2, blue; 3, green; 4, brown; 5 + 6, red. Soft-colored area: 95% CI. Thresholds are presented as milligram protein. (**B**) Relationship between milk sIgE and ED by different positive probability (10% and 50%); dashed line: 95% CI.

Table E1 in this article's Online Repository at www.jaci-inpractice.org lists the study population characteristics. All challenges were stratified into ImmunoCAP classes (0-6); however, because the number of children in ImmunoCAP class 0 and 1 was too small to create models, and further, to ensure that only confirmed sensitized subjects were analyzed, these were excluded. The total number of ImmunoCAP class 5 and 6 results was 170; in all, 779 challenges were divided into 4 approximately equal groups for analysis. The overall median age was 4 years (35.7% females); the sexes among groups did not differ, and the median age of each group was 3 to 5 years. The median sIgE level in the study population was 12.6 kU_A/L. Objective symptoms appeared in 64.2% of all challenges. We compared cumulative EDs in different models (Weibull, log-normal, and log-logistic distribution models,[6] data not shown). Data were analyzed using log-normal distribution, because this was the best-fit model based on Akaike information criteria.

Each threshold-dose-distribution curve significantly differed depending on the ImmunoCAP class (Figure 1, *A*). Most EDs were significantly different between all classes. Because we demonstrated that higher sIgE levels indicated a response at a lower dose, we plotted a curve showing the relationship between sIgE and ED_{10}/ED_{50} to give examples of clinically useful percentages by bivariate survival analysis (Figure 1, *B*). Furthermore, we analyzed whether the distribution curves changed with age, total IgE, and degree of dietary restrictions (Table I). Although significant differences were not observed among age groups (>4 or <4), the threshold dose tended to be high among individuals showing high total IgE (>500 kU/L, the median of this population), even in the same class. In the group with exclusive elimination, threshold doses were significantly lowered. In short, daily ingestion within the range termed as safe may reduce the risk of severe reaction upon accidental ingestion.

Medical practitioners categorize a child with a very low threshold as a "severe FA patient," and an inverse relationship between sIgE levels and the threshold dose is often observed. However, there are no clear criteria regarding the severity of FA itself, and limited reports show the association between sIgE and ED. Therefore, we evaluated this relationship, especially for milk (the most common cause of fatal anaphylaxis in Japan), and observed that the threshold distribution was significantly correlated with different ImmunoCAP classes. We then plotted curves that predict ED_{10} and ED_{50} on the basis of milk sIgE levels. These findings may provide useful information to determine the safe challenge doses in clinical practice.

This study has a few limitations. First, the threshold challenge data were collected at an allergy referral center; therefore, the patient population may be skewed toward being more sensitive.[7] Moreover, this was a retrospective study that used data of challenges conducted in clinical practice; further validation of the estimated threshold in a prospective large-scale multicenter study is warranted. Second, open rather than blinded protocols were used for OFCs. However, individual ED data obtained from open OFCs are also valuable.[8] This study provided valid information by including only objective symptoms as positive challenges to exclude uncertainty factors. Third, symptom severity was not evaluated in this study. Because many reports have shown a limited association between the threshold dose and appearance of serious symptoms when analyzing a mass population,[9] verification of safety using this method might be difficult.

In conclusion, EDs of children in the higher ImmunoCAP sIgE class were significantly lower than those of children in

TABLE I. Prediction models for the cumulative ED for cow's milk protein in different populations (age, total IgE level, and degree of elimination)

Fresh milk challenges	Age < 4 y	Age ≥ 4 y	Total IgE < 500	Total IgE ≥ 500	Exclusive elimination	Partial elimination
(n = 779)	(n = 386)	(n = 393)	(n = 399)	(n = 380)	(n = 456)	(n = 323)
ImmunoCAP class 2 (n = 174)	n = 96	n = 78	n = 138	n = 36	n = 83	n = 91
ED_{10}, 95% CI	54.7 (23.3-128)	143 (61.3-333)	60.4 (30.0-122)	348 (120-1,005)	61.8 (28.2-136)	117 (47.0-289)
ED_{50}, 95% CI	1,900 (915-3,944)	3,254 (1,633-6,486)	1,886 (1,068-3,331)	5,561 (2,021-15,304)	1,314 (692-2,497)	4,304 (1,966-9,423)
ImmunoCAP class 3 (n = 268)	n = 152	n = 116	n = 179	n = 89	n = 153	n = 115
ED_{10}, 95% CI	16.5 (8.39-32.4)	18.3 (9.17-36.5)	13.2 (7.32-23.7)	36.6 (16.7-79.9)	14.7 (8.47-25.4)	30.2 (13.8-66.4)
ED_{50}, 95% CI	513 (313-840)	424 (260-692)	339 (223-515)	836 (471-1,483)	246 (166-362)	1,041 (588-1,857)
ED_{90}, 95% CI	15,947 (6,517-39,024)	9,827 (4,407-21,910)	8,732 (4,245-17,962)	19,124 (7,044-51,917)	4,113 (2,135-7,926)	35,832 (12,666-101,368)
ImmunoCAP class 4 (n = 167)	n = 73	n = 94	n = 66	n = 101	n = 104	n = 63
ED_{10}, 95% CI	8.52 (3.41-21.3)	6.53 (3.23-13.2)	4.96 (2.23-11.0)	10.7 (5.02-22.7)	6.13 (3.24-11.6)	12.5 (4.61-34.1)
ED_{50}, 95% CI	237 (123-459)	115 (70.6-189)	76.4 (44.2-132)	262 (152-454)	97.6 (62.2-153)	338 (167-681)
ED_{90}, 95% CI	6,611 (2,162-20,219)	2,041 (947-4,398)	1,177 (518-2,678)	6,457 (2,541-16,412)	1,555 (759-3,184)	9,086 (2,790-29,593)
ImmunoCAP class 5 + 6 (n = 170)	n = 65	n = 105	n = 16	n = 154	n = 116	n = 54
ED_{10}, 95% CI	1.64 (0.71-3.82)	4.54 (2.89-7.13)	2.46 (0.84-7.22)	3.26 (2.10-5.06)	4.05 (2.69-6.09)	2.23 (0.86-5.75)
ED_{50}, 95% CI	29.2 (16.1-52.8)	35.8 (25.8-49.7)	16.0 (7.42-34.5)	35.9 (26.2-49.4)	27.0 (20.1-36.2)	48.7 (24.8-95.7)
ED_{90}, 95% CI	517 (206-1,297)	283 (174-459)	104 (34.0-318)	397 (245-642)	180 (116-281)	1,066 (387-2,942)

the lower ImmunoCAP class. Even in the same ImmunoCAP class, the threshold was significantly lower when children were kept on exclusive elimination. This model of inverse correlation between sIgE and threshold dose might aid in the development of safer management approaches in pediatric FA clinical practice.

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METHODS

Subjects and OFCs

This study included 1615 challenges for children younger than 15 years who were tested using an open milk challenge between October 2013 and March 2020 at the National Center for Child Health and Development and whose milk sIgE levels had been measured in the year before the challenges (Figure E1). To promote study homogeneity, only challenges with fresh milk were analyzed. Children who had been receiving treatment with systemic antihistamines, β2-agonists, systemic steroids, or immunosuppressive agents were excluded from this study. When multiple OFCs were performed on a single child during the study period, a positive challenge was selected to describe the ED more accurately. When there were still multiple applicable OFCs, the earliest was selected. OFCs were performed with a dosing method using 3 to 5 preplanned graded steps at intervals of 20 to 40 minutes, which were conducted according to the Japanese Food Allergy Guidelines[E1] and are commonly used as a standardized method in Japan. Generally, the total food amount for a child was determined by referring to already known safe or expected tolerated doses and was administered by increasing the initial dose by several-fold. All tests were carried out using the open challenge method. The definition of a positive test result was based on the presence of objective symptoms; purely subjective symptoms, such as oral itching, nausea, abdominal pain, and pruritus, were not considered. To include children with confirmed milk allergies, this study included children who had a positive milk challenge, as well as those whose challenge results were negative but who had been diagnosed with milk allergy by a physician (ie, children whose past milk challenges were positive or children who had a history of obvious symptoms due to milk ingestion). Finally, we analyzed 779 challenges for 779 children.

Identification of EDs and statistical analysis

EDp is the ED of total allergen protein that is predicted to induce a response in p% of the allergic population. For example, ED_{01} and ED_{05} are the doses at which only 1% and 5%, respectively, of an allergic population would react with objective symptoms. We performed interval-censoring survival analyses. Weibull, log-normal, and log-logistic distribution models were used to estimate the threshold-dose distribution and EDs. Some children who had tested negative in the OFC had been previously diagnosed with milk allergy but were negative at the scheduled challenging dose, which meant that their ED was right censored at the highest dose they were given. Data were treated as left censored if allergic reactions were induced at the first ingestion. To analyze the relationship between milk-specific IgE and ED, we performed a bivariate survival analysis. Differences in sex among all groups were tested using the Fisher exact test. Akaike information criterion was used for the comparison of goodness of fit. JMP 15 (SAS Institute Inc) was used for statistical analysis.

Cow's milk–specific serum IgE measurement

sIgE levels were measured using ImmunoCAP (Thermo Fisher). ImmunoCAP classification was used for the stratification of sIgE in this study because it is globally used, clinicians are familiar with it, and it is generally unbiased. Milk sIgE less than 0.35 kU_A/L is rated as ImmunoCAP class 0; greater than or equal to 0.35 to less than 0.7 kU_A/L as class 1; greater than or equal to 0.7 to less than 3.5 kU_A/L as class 2; greater than or equal to 3.5 to less than 17.5 kU_A/L as class 3; greater than or equal to 17.5 to less than 50 kU_A/L as class 4; greater than or equal to 50 to less than 100 kU_A/L as class 5; and greater than or equal to 100 kU_A/L as class 6. Finally, we generated the dose threshold distribution curves on the basis of milk sIgE for positively predicting ED_{10} and ED_{50}.

Ethical statements

All the subjects and their families provided written informed consent before the commencement of the OFCs. The current study was approved by the Ethics Review Committee of the National Center for Child Health and Development (no. 2019-003), Tokyo, Japan.

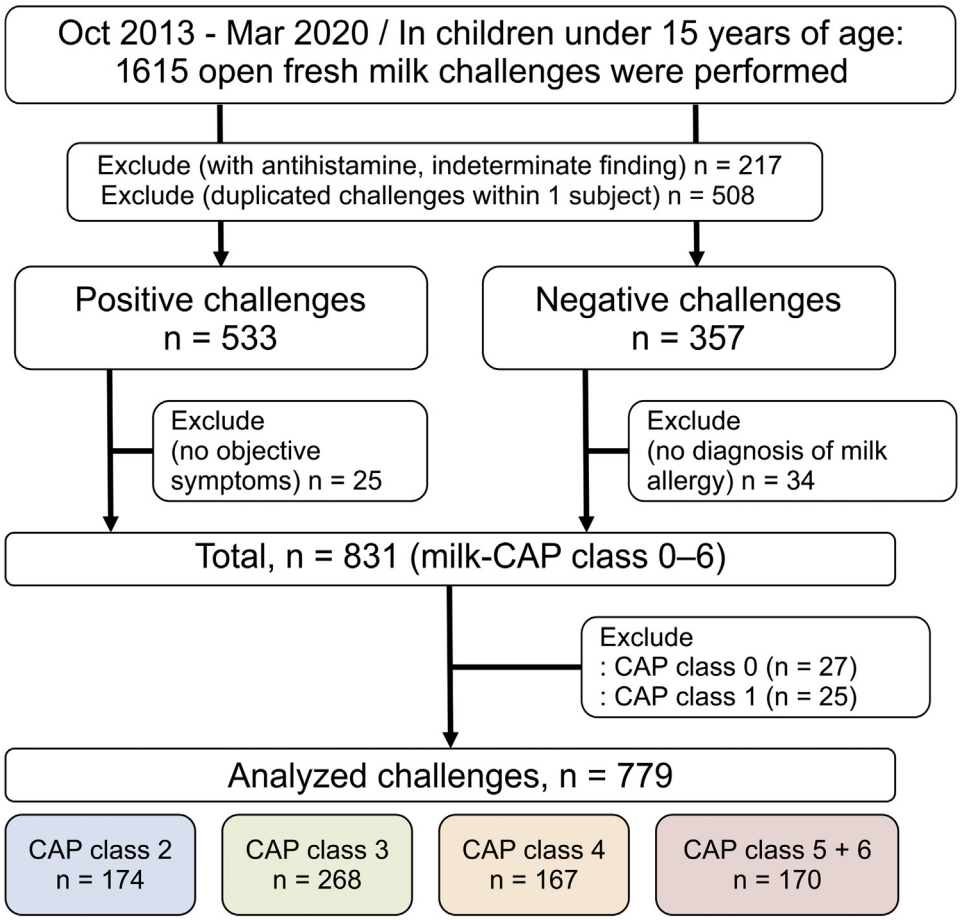


FIGURE E1. Flowchart describing the study participants and OFC outcomes.

TABLE E1. Characterization of the study population

Fresh milk challenges	Total (n = 779)	CAP class 2 (n = 174)	CAP class 3 (n = 268)	CAP class 4 (n = 167)	CAP class 5 + 6 (n = 170)
Age (y), median (range)	4 (0-15)	3 (0-15)	3 (0-15)	5 (0-14)	4 (0-14)
Sex: female, n (%)	278 (35.7)	60 (34.5)	103 (38.4)	50 (29.9)	65 (38.2)
Total IgE (kU/L), median (IQR)	502 (179-1,131)	166 (73.5-422)	326 (136-650)	672 (365-1,215)	1,482 (872-2,573)
Milk-specific IgE (kU_A/L), median (IQR)	12.6 (4.18-43.7)	1.93 (1.28-2.72)	8.37 (5.37-11.9)	30.6 (23.8-37.3)	94.2 (68.5-100)
Positive OFC, n (%)*	500 (64.2)	80 (46.0)	168 (62.7)	118 (70.7)	134 (78.8)
Negative challenge ≥ 100 mL of milk, n (%)	45 (5.78)	26 (14.9)	16 (5.97)	3 (1.80)	0 (0)
Epinephrine intramuscular injection, n (%)	10 (1.28)	1 (0.57)	2 (0.75)	3 (1.80)	4 (2.35)
Left censored, n (%)	70 (8.99)	12 (6.90)	28 (10.5)	19 (11.4)	11 (6.47)
Right censored, n (%)	279 (35.8)	94 (54.0)	100 (37.3)	49 (29.3)	36 (21.2)

IQR, Interquartile range.
*Only objective signs were included in this study (subjective symptoms, such as oral itching, nausea, abdominal pain, and pruritus, were excluded).

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