

# Threshold dose of cow's milk in sensitization to casein higher than those of casein and $\beta$ -lactoglobulin in children with cow's milk allergy

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## Abstract

**Background:** Recent treatment for food allergies involves the intake of allergy-causing foods at doses lower than the threshold dose determined by the oral food challenge (OFC). For a more successful treatment, it is necessary to identify a biomarker to establish safer doses of allergens in foods consumed at home.

**Objective:** In this study, we aim to investigate whether the pattern of sensitization to cow's milk (CM) is related to the threshold dose of CM.

**Methods:** Fifty patients with sensitization to casein (casein-specific IgE titer  $\geq 0.7$  UA/ml) and who have undergone the CM OFC test from July 2013 to July 2015 were enrolled. They were examined for the presence or absence of sensitization to  $\beta$ -lactoglobulin (BLG) (BLG-specific IgE  $\geq 0.7$  UA/ml). They were divided into two groups, namely, the only-casein-specific IgE-positive (C) group, and both casein- and BLG-specific IgE-positive (C + B) group.

**Results:** The C group had 26 patients and the C + B group had 24. Both the CM- and casein-specific IgE titers were higher in the C + B group than in the C group. The positivity rates determined from OFC test results were 53.8 and 87.5%, and the threshold doses of CM were 88.7 and 31.1 ml in the C and C + B groups, respectively. In patients with low casein-specific IgE titers ( $\leq 10$  UA/ml), the C + B group showed a significantly lower threshold dose of CM than the C group.

**Conclusion:** Our results suggest that children with CM allergy sensitized to casein alone have a higher threshold dose than those sensitized to both casein and BLG.

**Key words:** Food allergy, Cow's milk, Casein,  $\beta$ -lactoglobulin, Oral food challenge, Sensitization

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## Introduction

Cow's milk (CM) allergy (CMA) is commonly encountered in pediatric practice. The prevalence of CMA is approximately 2 to 3% of the general population.<sup>1,2</sup> Although 70–80% of affected children outgrow this sensitivity by the age of 3, the remaining 20–30% do not.<sup>3–4</sup>

CM consists of casein and whey. Casein comprises approximately 80% of CM protein. Whey makes up 20% of CM protein, with BLG and  $\alpha$ -lactalbumin (ALA) being the most abundant components.

Several studies have aimed to identify the correlation between clinical CMA and component-specific IgE antibodies in children with CMA. Some studies have shown that casein is a major allergen in CM; however, a report showed that BLG and casein are major allergens that caused sensitization in Thai children.<sup>5</sup> Sensitization to multiple CM allergens is also involved in the persistence of CMA.<sup>6</sup> A study in Japan showed that the predictors of persistent CMA are the history of anaphylaxis and high CM- and casein-specific IgE titers.<sup>7</sup>

The intake of baked CM in children accelerates the development of tolerance to regular CM compared with strict dietary avoidance. Casein-specific IgE titer is useful for predicting reactivity to baked CM, although casein- and BLG-specific IgE titers are related to persistent allergy to regular CM.<sup>8,9</sup> As recent treatment for food allergy, even for foods that demonstrate confirmed positive results of an OFC test, patients tend to be instructed to take in lower amounts of such foods determined by referring to thresholds and symptoms.<sup>10</sup> Therefore, we investigated whether the threshold dose could be inferred by casein and BLG sensitization.

## Methods

We conducted a retrospective study. Clinical information was collected from medical charts. We explained the possible symptoms induced by open OFC to the guardians both orally and in writing and obtained their written informed consent. This study was conducted in accordance with the Declaration of Helsinki and Ethical Guidelines for Clinical Research.

### Diagnosis of CMA by OFC test

We define CMA as having a convincing history of acute reaction after CM protein intake, sensitization to CM allergens (CM-specific IgE  $\geq$  0.7 UA/ml), or positive results in a previous CM OFC test. The OFC test was conducted to diagnose and confirm CMA and to evaluate the tolerance to CM.

### Subjects

Fifty-six patients with IgE-mediated reaction to CM and who underwent an open OFC test at the Division of Pediatric Allergy National Hospital Organization, Nagara Medical Center from July 2013 to July 2015 were recruited in this study.

### Determination of threshold dose and dose of CM intake at home

In this study, we defined the elicit dose as the threshold dose and the tolerated dose as the dose of CM intake at home. The open OFC test of CM was performed in accordance with the Japanese Guideline for Food Allergy 2017 throughout the study period.<sup>10</sup> Anaphylaxis was defined in accordance with National Institute of Allergy and Infectious Disease.<sup>11</sup> Raw CM was administered in increasing doses (typically 5–7 doses from 0.1, 0.2, or 0.5, to 1, 2, 5, 25, 50, and 107 ml) every 20 min. The maximum challenge dose was 3–200 ml. The challenge dose in the OFC depends on the dose of CM

that is ingested daily before the OFC test. The threshold dose was determined as the lowest dose of CM eliciting an objective allergic reaction.

On the basis of symptoms and the threshold dose in the OFC test, we determined the appropriate dose of regular CM intake at home. If the test showed negative results, the patients need to repeat taking CM at the threshold dose about twice a week at least over 1 year. Specifically, depending on their symptoms, patients started consuming CM at home from about one-fourth to one-tenth of the threshold dose. When anaphylaxis was induced with a small amount of CM, we instructed the guardians to immediately stop feeding CM. We instructed the guardians to administer antihistamines and oral corticosteroids when adverse reactions occurred and to see a doctor when moderate or severe symptoms occurred.<sup>10</sup> Depending on symptoms induced by the OFC,  $\beta$ 2 stimulant inhalation and adrenaline auto-injectors were also prescribed. The intake dose of CM was confirmed at the next outpatient visit, which is the maximum dose at which the patients could take CM without symptoms at home.

### Statistical analysis

The values are presented here as [median interquartile range (IQR)]. We used the t- and chi-square tests to compare continuous variables and the Mann–Whitney U test to compare non continuous variables between the groups. Statistical significance was set at  $P < 0.05$ . Statistical analyses were conducted using R-2.6-2. statistical software.

The institutional ethics committee of National Hospital Organization, Nagara National Hospital, Nagara approved the study.

## Results

### Patient characteristics

There were 56 patients sensitized to CM (CM-specific IgE titer  $\geq$  0.7 UA/ml), among which 26 were sensitized to casein only, 24 to both casein and BLG, 4 to BLG only, and 2 to neither casein nor BLG (**Figure 1**). The characteristics of all the patients enrolled in this study are shown in **Table 1**. Twenty-six were classified into the C group and 24 into the C + B group. The median ages at the time of the OFC test of the C and C + B groups were 34.5 and 44.5 months, respectively. The total-IgE-, CM-, casein- and BLG-specific IgE titers were significantly higher in the C + B group than in the C group.

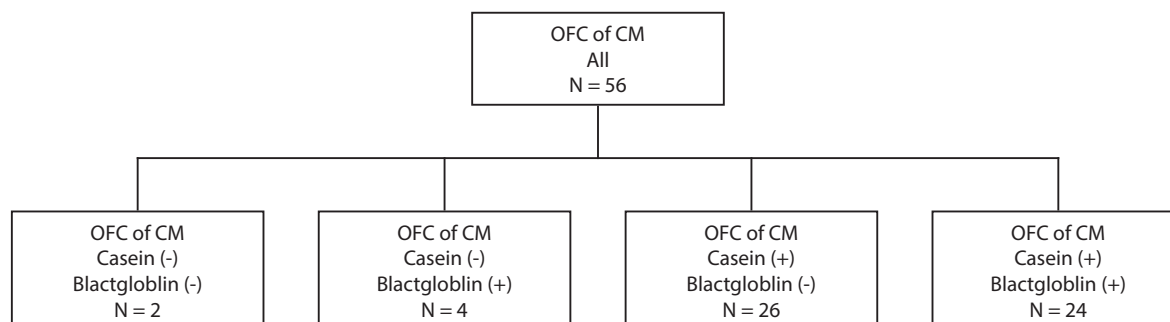


Figure 1. Among the 69 patients who underwent the OFC test of CM from July 2013 to July 2015, 56 were sensitized to CM.

**Table 1 . Characteristics of the study subjects.**

	C group	C + B group	P value
Number of patients	26	24	
Age of onset (month)	6.00 (6.00-7.00)	6.00 (5.75-7.25)	0.905
CM specific IgE (kU <sub>A</sub> /L) of onset	10.9 (2.50-19.0)	38.1 (16.5-68.7)	< 0.01
Age at OFC (month)	34.5 (18.3-70.0)	44.5 (31-67.3)	0.44
Total IgE (kU <sub>A</sub> /L)	256 (129-469)	570 (353-2143)	< 0.01
CM specific IgE (kU <sub>A</sub> /L)	3.99 (2.46-5.78)	30.3 (16.0-60.7)	< 0.01
Casein specific IgE (kU <sub>A</sub> /L)	4.18 (1.86-6.36)	24.3 (7.81-49.4)	< 0.01
BLG specific IgE (kU <sub>A</sub> /L)	0.17 (0.10-0.38)	3.65 (1.09-6.37)	< 0.01
Previous history of Immediate reaction to CM	17 (65.3%)	19 (79.1%)	0.27
Previous history of anaphylaxis to CM	3 (11.5%)	5 (20.8%)	0.37
Bronchial asthma, current	7 (26.9%)	4 (16.7%)	0.38
Atopic Dermatitis, current	10 (38.5%)	13 (54.2%)	0.26

C group: sensitized to casein only

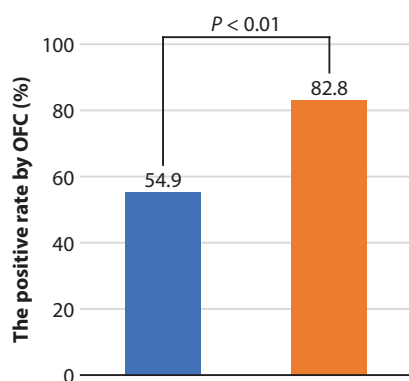
C + B group: sensitized to both casein and BLG

Values are reported as median with 25% to 75% interquartile ranges provided in parentheses or n (%).

P-values were determined using Mann-Whitney or chi-square tests, as appropriate.

### OFC test results

The positivity rates determined by the OFC test in the C and the C + B groups were 54.9% (14/26) and 82.8% (21/24), respectively. Skin, respiratory, and gastrointestinal symptoms appeared during the OFC test in 26 patients (n = 10, C group; n = 16, C + B group), 18 patients (n = 9, C group; n = 9, C + B group), and 7 patients (n = 3, C group; n = 4, C + B group), respectively. Skin symptoms were the most common presentations. When only skin symptoms were induced, we treated them with antihistamine. Some patients had two or more-symptoms, however those symptoms were mild, for example,



	C group	C + B group	p
The threshold dose Median (range)	88.7 ml (0.3 ml-200 ml)	31.1 ml (3.6 ml-200 ml)	0.113
The dose of CM intake at home Median (range)	88.0 ml (0 ml-200 ml)	5.0 ml (0 ml-200 ml)	0.066

**Figure 2. Positivity rate determined by OFC test of CM, threshold dose and dose of CM intake at home in C and C + B groups.**

P-values were determined using the chi-square test, as appropriate.

intermittent cough and mild abdominal pain. No patients with two or more symptoms had respiratory compromise (eg, dyspnea, wheeze-bronchospasm, stridor, reduced PEF, hypoxemia) nor persistent gastrointestinal symptoms (eg, crampy abdominal pain, vomiting).

The median doses of CM at which the threshold dose was reached in the CM OFC test were 88.7 ml in the C group and 31.1 ml in the C + B group. The median doses of CM intake at home were 88.1 ml in the C group and 5.0 ml in the C + B group (Figure 2, Table 2).

**Table 2. Reactions and required treatments in patients with positive results after an OFC**

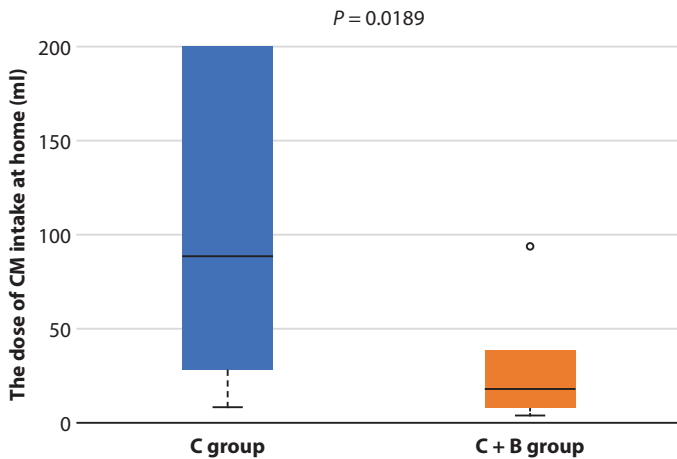
	C group	C + B group	P value
reactions	14	21	
Skin	10 (71.4%)	16 (76.2%)	1
Gastrointestinal	3 (21.4%)	4 (19.0%)	1
Respiratory	9 (64.3%)	9 (42.9%)	0.56
Shock	0 (0%)	0 (0%)	1
Treatments			
Antihistamine (p.o. <sup>†</sup> , i.m. <sup>‡</sup> or i.v. <sup>§</sup> )	10 (71.4%)	15 (71.4%)	1
$\beta$ 2 stimulant inhalation	8 (57.1%)	5 (23.8%)	0.21
Steroid (i.v)	5 (35.7%)	5 (23.8%)	0.72
Adrenaline	0 (0%)	0 (%)	1

Allergic reactions provoked by the cow's milk provocation test (C group : n = 32 , C + B group : n = 29 )

p.o.<sup>†</sup>, per os; i.m.<sup>‡</sup>, intramuscular; i.v.<sup>§</sup>, intravenous

Values are reported as n (%).

P-values were determined using chi-square tests, as appropriate.



**Figure 3. Threshold doses of CM (casein-specific IgE  $\leq$  10 UA/ml) in C and C + B groups. The threshold dose of CM in the C-group was 88.8 ml (8.0–200 ml) and that in the C + B group was 18.0 ml (3.6–93.8 ml) [median (range)].**

*P*-values were determined using the Mann–Whitney U test.

#### Casein-specific IgE titer (UA/ml) and threshold dose of CM

We compared the threshold dose of CM against the casein-specific IgE titer between the C and the C + B groups. There was no correlation between the casein-specific IgE titer and the threshold dose in the C and C + B groups ( $r = 0.21$   $p = 0.32$  in C group, and  $r = -0.28$   $p = 0.17$  in C + B group); however, for the patients with low casein-specific IgE titers ( $\leq$  10 UA/ml), the C group showed significantly higher threshold doses of CM intake than in the C + B group (Figure 3). BLG-specific IgE titer was not correlated with the threshold dose of CM in the C + B group ( $r = 0.36$   $p = 0.08$ ), and the proportions of casein and BLG were also not correlated ( $r = 0.12$   $p = 0.91$ ).

#### Discussion

Among the patients with low casein-specific IgE titers ( $\leq$  10 UA/ml), those in the C + B group showed a significantly lower threshold dose of CM than those in the C group. This main finding suggests that children with CMA sensitized to casein alone have a higher threshold dose than those sensitized to both casein and BLG. Moreover; among the patients with low casein-specific IgE titers ( $\leq$  10 UA/ml), those in the C group showed significantly higher threshold doses of CM intake than those in the C + B group.

Regarding the pattern of sensitization to CM components, it has been reported that high titers of IgE to ALA, BLG and casein predict less successful CM oral immunotherapy,<sup>6</sup> suggesting that the distribution of multiple CM components could predict the outcome of CMA.

In southern China, ALA and BLG were the main allergenic components detected in CM-specific IgE-sensitized children with respiratory allergic diseases.<sup>12</sup> It was reported that the positivity rate for casein was higher than those for ALA and BLG in children with CMA in northern China;<sup>13</sup> therefore, in different regions, the pattern of CM protein sensitization differs. Previous studies have suggested that IgE titers to casein and BLG could be used as markers of reactive CMA,<sup>9</sup> which is in agreement with our present study.

Canonica et al. suggested that combining different serological markers improves predictions of the clinical response to immunotherapy.<sup>14</sup> We also noted that a combination of high titers of IgE to casein and BLG could be used as markers of low threshold doses of CM intake. This would help in the general treatment of CMA that is not immunotherapy.

Minimal avoidance is a management strategy for current food allergies. Depending on the symptoms and the threshold dose determined by the OFC test, we often determined the dose of intake at home to promote regular intake.<sup>9</sup>

Depending on their symptoms, we recommend that patients continue consuming CM at home from about one-fourth to one-tenth of the threshold dose determined by the OFC test. One limitation of this study is that there was a range of the starting doses of intake at home. Severe symptoms induced with very small amounts of CM indicated the complete withdrawal of CM from the diet. We consider incorporating a new scoring system (Anaphylaxis SCoring Aichi: ASCA) for a quantitative evaluation of the anaphylactic reaction that is observed in the OFC test.<sup>15</sup> There were also some other limitations of this study. One was that we do not have long-term follow-up data. However, we followed up the intake of CM twice a week at home in all of the patients at least over 1 year. The other was that we tested for two allergens, testing not a panel of CM allergens such as Bos D 1–10. Our investigation using a panel of CM allergens is underway.

The higher the threshold dose of CM, the higher the dose of CM intake at home, which is considered to correlate with effective desensitization to CMA. Some patients in the C + B group with high casein-specific IgE titers can take in CM at a certain dose. It is considered that such patients may have high titers of specific IgE to some other allergens, so their ratios of casein-specific IgE titers to total IgE titers may be low. However, we were unable to investigate sensitization to other antigens because of the small number of patients in this study.

Finally, we conclude that children sensitized to casein alone have a higher threshold dose than those sensitized to both casein and BLG.

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