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Serum levels of specific IgE to cow's milk and its components as predictors of anaphylaxis in Chinese children with cow's milk allergy

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Abstract

Background: Cow's milk allergy (CMA) is one of the most common food allergies in young children. As improved diagnostic tools, allergic tests are inconsistent and limited in predicting anaphylaxis.

Objective: To explore risk factors for anaphylaxis and to determine practical cut-offs for allergic tests in predicting anaphylaxis.

Methods: This is a prospective cohort study. Children with IgE-mediated CMA were enrolled and divided into three groups (Group 1: non-anaphylaxis; Group 2: GRADE I anaphylaxis; Group 3: GRADE II-IV anaphylaxis that warranted epinephrine). Prick-to-prick tests (PTPs) using fresh cow's milk (CM) were performed. Serum specific IgE (sIgE) against CM and its components, including casein, alpha-lactalbumin, beta-lactoglobulin, and bovine serum albumin were measured. The 90% and 95% positive predictive value (PPV) decision points for predicting anaphylaxis were determined. Potential predictors of anaphylaxis were evaluated in logistic regression models.

Results: This study included 134 CMA patients with a median age of 14.4 months. The sensitization rate to any CM component was 89%. Group 3 was more likely to be sensitized to multiple CM components and have higher sIgE levels. The 95% PPV diagnostic decision points of casein-sIgE in predicting anaphylaxis was 13.0 kUA/L. For GRADE II-IV anaphylaxis, casein-sIgE ≥ 54.9 kUA/L could provide a PPV of 88.9%. The elevated casein-sIgE level (OR 14.0, P = 0.025) and complicating respiratory allergic diseases (OR 4.8, P = 0.022) were independent risk factors for GRADE II-IV anaphylaxis.

Conclusion: High casein-sIgE levels are strongly associated with CM anaphylaxis. Detection of casein-sIgE may offer an additional value for the prediction of CM anaphylaxis.

Key words: casein, IgE-mediated cow's milk allergy, specific IgE, anaphylaxis, specificity, positive predictive value

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Introduction

Cow's milk allergy (CMA) is one of the most common and earliest food allergies in children and the primary cause of food-induced anaphylaxis.[1] According to the World Allergy Organization,[2] the estimated prevalence of CMA in children ranges from 1.9% to 4.9%. In China, the prevalence of CMA in infants is 0.8% to 3.5%.[3] The CMA mainly affects infants in their first year of life, potentially causing malnutrition and developmental disabilities.[4]

Based on the pathological mechanisms involved, CMA can be classified as immunoglobulin E (IgE) mediated, non-IgE mediated, and mixed mediated types.[5] The IgE-mediated CMA can involve multiple systems, including skin-mucosal tissue, gastrointestinal, respiratory, nervous, and cardiovascular systems, with symptoms ranging from mild localized skin symptoms to life-threatening anaphylactic shock.

For IgE-mediated CMA, a clear history of an acute-onset reaction coupled with a positive prick-to-prick test (PTP) or serum specific IgE (sIgE) test should usually be sufficient to confirm a diagnosis.[6] The Oral Food Challenge (OFC) is recommended for diagnosing CMA to minimize risks of anaphylaxis in PTP or sIgE false negative cases and prevent unnecessary treatment for false positive cases.[7]

Although the OFC test is the gold standard, PTPs, and sIgE tests have proved to be valuable ways to diagnose allergies and eliminate unnecessary risky tests for children.[8] The positive predictive values (PPV) provided by CM-sIgE and CM-PTP were excellent, but the proposed cut-offs varied greatly due to the difference in prevalence, calculation methods and types of allergens among studies.[9] Similarly, the cut-offs of sIgE for CM components were less reported with significant differences between studies.[8,10-12]

According to reports from the UK Fatal Anaphylaxis Registry, more than half of the food allergy-related deaths occurred in individuals with prior mild reactions.[13] It was necessary to explore risk factors for anaphylaxis and establish severity-stratified practical decision points to help with clinical decisions, while the performance of CM component-sIgE as an improved diagnostic tool needs to be evaluated.

The purpose of our study was to describe the clinical characteristics and to identify risk factors and cut-offs for CM-PTP and sIgE, as well as its components for predicting anaphylaxis and GRADE II-IV anaphylaxis.

Methods

Study design and participants

This was a single-center, prospective cohort study. Children with IgE-mediated CMA were sequentially enrolled in the Children's Hospital Affiliated to Capital Institute of Pediatrics from 2020 to 2022. The diagnosis of CMA was established when a patient reported a convincing history of acute (< 2 h) reaction after consuming CM or CM-containing food within the past 12 months, accompanied by a positive CM-PTP or CM-sIgE result. In cases where the patient's medical history and serum sIgE or PTP tests were inconsistent, or the reaction occurred more than 12 months previously, an OFC test was needed to verify the diagnosis. Clinical information was collected through medical records and supplemented by questionnaires.

Patients were categorized into three distinct groups based on the severity of their symptoms, as outlined by a clinical practice guideline for the emergency management of anaphylaxis published in 2022:[14]

- Group 1 (Non-anaphylaxis): The severity did not meet the diagnostic criteria of Group 2 or Group 3.
- Group 2 (GRADE I anaphylaxis): An acute-onset illness involving the skin-mucosal tissue, along with persistent gastrointestinal symptoms but no signs of cardiovascular or respiratory involvement.
- Group 3 (GRADE II-IV anaphylaxis): Any signs or symptoms of cardiovascular or respiratory system involvement warranted epinephrine.

Study protocols were approved by the ethical committee of hospital (SHERLLM2021011), and informed consent was obtained from all patients.

PTP and serum sIgE

Fresh cow's milk (3.1% of commercial pasteurized CM) was used to perform PTPs on the medial upper arm following the practical guide.[15] Histamine hydrochloride solution (10 mg/ml) and saline were used as positive and negative controls, respectively. Mean wheal diameters (MWD) ≥ 3 mm were considered positive in PTPs.[16] Serum sIgE against CM and its components including casein (Bos domesticus 8, Bos d 8), alpha-lactalbumin (ALA, Bos d 4), beta-lactoglobulin (BLG, Bos d 5), and bovine Serum Albumin (BSA, Bos d 6) were measured by the ImmunoCAP Phadiatop tests (Thermo Fisher Scientific, Uppsala, Sweden). Additionally, serum sIgE against other food allergens (egg white, wheat, sesame, peanut, soybean, shrimp, and crab) were tested. Serum sIgE ≥ 0.35 kUA/L was considered as sensitization. Serum sIgE or PTP values from the first visit were included in the analysis. For children diagnosed by the OFC test, the sIgE and PTP results that were detected at the same time as the OFC were used in the analysis.

Statistical analysis

Quantitative data were described as medians with quartiles, and categorical data as counts with percentages. Categorical data were compared using the Pearson chi-square test (or chi-square test with Yates' continuity, or Fisher exact test as appropriate), while the Mann-Whitney U test was used for quantitative data comparison. Variables with clinical significance and *P* values < 0.1 in the univariate analysis were included in the subsequent multivariate analysis to identify independent risk factors for CM anaphylaxis and GRADE II-IV anaphylaxis. The Spearman correlation coefficient between sIgE levels and PTP MWDs was calculated, and the correlation matrix was plotted. Receiver operating characteristic (ROC) curves of PTPs or sIgEs levels for the GRADE II-IV anaphylaxis diagnosis were drawn. The optimal cut-offs (based on the maximum sum of sensitivity and specificity) were identified. Additionally, diagnostic decision points based on 95% or 90% positive predictive value (PPV) were established. *P*-values of less than 0.05 were regarded as significant. All statistical analysis was performed with R software (version 4.3.0).

Results

Participants' characteristics

A total of 134 children with CMA were enrolled, diagnosed either through a convincing clinical history associated with positive CM-PTP or CM-sIgE (n = 97), or via OFC in ambiguous cases (n = 37). The baseline characteristics and allergic testing of the patients were summarized in **Table 1**. Among them, 72 (53.7%) were males with a median age of onset of 7 months. Most of them (91.4%) lived in cities, 78.6% had parents with high education levels, 75.4% had first-degree relatives with a history of allergic diseases, and 95.7% had a mixed feeding (formula feeding as a supplement to breastfeeding) within the first month after birth. There was no significant difference in sIgE positivity against other food allergens (egg white, wheat, sesame, peanut, soybean, shrimp, and crab) across the three groups.

As shown in **Table 2**, sixty-five (48.5%) children were classified as anaphylaxis based on symptoms at onset, of which 30 children in Group 3 had a severity of GRADE II to IV that warranted epinephrine. All patients had skin-mucous membrane involvement, and urticaria was the most common symptom. Patients in Group 3 (n = 30) all had respiratory compromise, of which 5 (16.7%) patients had cardiovascular involvement, and 2 (6.7%) patients experienced loss of consciousness. Eight (26.7%) patients received epinephrine, three (10%) of whom also received systemic steroid therapy. Among the remaining patients, five (16.7%) received systemic steroid therapy alone, two (6.7%) were administered antihistamines, one (3.3%) received aerosol therapy, and 13 (43.3%) received no treatment.

Performance of serum sIgE and PTP as diagnostic tools

Serum sIgE levels of four CM components were detected in 82 (61.2%) patients. The MWDs of CM-PTP and serum sIgE levels of CM and its components in Group III were significantly higher than those in the other two groups (**Table 1** and **Figure 1**). In four CM components, BLG (64.6%) and casein (56.1%) were the common components causing sensitization in CMA patients. The proportion of patients sensitized to at least one CM component was 89% in the overall population and 100% in Group 3. For patients in Group 3, 95.2% were sensitized to two or more CM components, and more than half (61.9%) were sensitized to all four CM components (**Figure 2A**). Nearly half of the patients in Group 3 reacted to baked CM products (e.g. muffins, biscuits, cakes, and yogurt melts), who had significantly higher casein-sIgE levels than non-responders (**Table 1** and **Figure 2B**).

For predicting GRADE II-IV anaphylaxis, casein-sIgE had the largest AUC (0.817) with the optimal cut-off value of 12.4 kUA/L, followed by CM-sIgE (AUC 0.812) with the optimal cut-off value of 3.7 kUA/L. The ROC curves in **Figure 3** displayed the natural properties of sIgEs and PTP as diagnostic tools, where casein-sIgE tended to provide high specificity (93.5% at the cut-off of 12.4 kUA/L) for optimal prediction efficiency, while CM-sIgE tended to provide high sensitivity (90.0% at the cut-off of 3.7 kUA/L) to optimize prediction efficiency. As shown in the correlation matrix (**Figure 3**), casein (ρ = 0.86) had the strongest correlation with CM in sIgE levels, followed by BLG (ρ = 0.75) and ALA (ρ = 0.72).

The 90% and 95% PPV diagnostic decision points of serum sIgE levels and PTP MWDs in predicting anaphylaxis were summarized in **Table 3**. Higher thresholds are needed for BSA, CM-sIgE, and CM-PTP to achieve a similar prediction level. Corresponding to a PPV of 95%, the cut-off points are 13.0, 47.9, and 28.3 kUA/L for casein, ALA, and BLG-sIgE, respectively. Allergic testings were less predictive for GRADE II to IV anaphylaxis. BLG-sIgE and CM-PTP could provide a PPV of over 95% with cut-off points of 50.4 kUA/L and 16.5 mm, respectively. Casein-sIgE could provide a PPV of 88.9% with a cut-off of 54.9 kUA/L. Using cut-off points of 14.4, 83, and 80.6 kUA/L for ALA, BSA, and CM-sIgE, respectively, only appropriately predicted GRADE II to IV anaphylaxis in 66.7-77.8% (PPV) of patients.

Table 1. Baseline characteristics, allergic testing, and results of univariate analysis.[a]

	All (n = 134)	Group 1 (n = 69)	Anaphylaxis		*P* value[b]	OR (95%CI)[b]	*P* value[c]	OR (95%CI)[c]
			Group 2 (n = 35)	Group 3 (n = 30)				
Gender, male	72 (53.7)	42 (60.9)	13 (37.1)	17 (56.7)	0.088	0.6 (0.3 - 1.1)	0.714	1.2 (0.5 - 2.6)
Age of onset (month)	7.0 (5.0, 10.0)	11.0 (8.0, 34.0)	6.8 (6.0, 15.0)	11.8 (7.5, 36.0)	0.032*	1.0 (0.9 - 1.0)	0.234	1.1 (1.0 - 1.2)
Age (month)	14.4 (8.4, 26.4)	17.0 (13.0, 57.0)	18.0 (12.0, 61.0)	67.4 (43.4, 132.0)	0.042*	1.5 (1.2 - 2.0)	< 0.001*	2.1 (1.5 - 2.8)
Urban Residence	95 (91.4)	45 (84.9)	26 (100.0)	24 (96.0)	0.042*	8.9 (1.1 - 73.9)	0.588	2.7 (0.3 - 22.8)
Well-educated[d] parents	81 (78.6)	41 (87.2)	23 (74.2)	17 (68.0)	0.051	0.4 (0.1 - 1.0)	0.136	0.5 (0.2 - 1.3)
Allergic history[e] of first-degree relatives	101 (75.4)	54 (78.3)	24 (68.6)	23 (76.7)	0.424	0.7 (0.3 - 1.6)	0.852	1.1 (0.4 - 2.9)
Maternal pregnancy								
Antibiotics	6 (4.7)	0 (0.0)	3 (8.8)	3 (10.3)	0.033*	Inf (NaN - Inf)	0.255	3.7 (0.7 - 19.4)
Probiotics	8 (6.5)	3 (4.8)	3 (8.8)	2 (7.1)	0.715	1.7 (0.4 - 7.6)	1.000	1.2 (0.2 - 6.1)
Nutritional supplements[f]	68 (54.8)	31 (50.0)	24 (70.6)	13 (46.4)	0.279	1.4 (0.7 - 2.8)	0.309	0.7 (0.3 - 1.5)
Active or passive smoking	33 (27.1)	12 (20.0)	13 (39.4)	8 (27.6)	0.085	2.1 (0.9 - 4.7)	0.941	1.0 (0.4 - 2.6)
Pets	19 (14.8)	9 (13.8)	4 (11.8)	6 (20.7)	0.747	1.2 (0.4 - 3.1)	0.478	1.7 (0.6 - 5.0)
Smoking	52 (40.6)	25 (38.5)	17 (50.0)	10 (34.5)	0.613	1.2 (0.6 - 2.4)	0.444	0.7 (0.3 - 1.7)
Caesarean section	49 (41.2)	27 (44.3)	11 (33.3)	11 (44.0)	0.483	0.8 (0.4 - 1.6)	0.747	1.2 (0.5 - 2.8)
Premature birth	1 (0.8)	0 (0.0)	0 (0.0)	1 (3.7)	0.480	Inf (NaN - Inf)	0.216	Inf (NaN - Inf)
Complementary feeding introduction ≥ 6 months of age	47 (38.2)	24 (37.5)	14 (43.8)	9 (33.3)	0.866	1.1 (0.5 - 2.2)	0.555	0.8 (0.3 - 1.9)
CM intake at onset								
< 10 ml	64 (52.0)	35 (56.5)	11 (35.5)	18 (60.0)	0.323	0.7 (0.3 - 1.4)	0.315	1.5 (0.7 - 3.5)
10~100 ml	45 (36.6)	23 (37.1)	14 (45.2)	8 (26.7)	0.906	1.0 (0.5 - 2.0)	0.195	0.6 (0.2 - 1.4)
> 100 ml	14 (11.4)	4 (6.5)	6 (19.4)	4 (13.3)	0.083	2.8 (0.8 - 9.6)	0.955	1.3 (0.4 - 4.4)
React to baked CM products[g]	39 (32.5)	18 (28.6)	7 (25.0)	14 (48.3)	0.334	1.5 (0.7 - 3.1)	0.037	2.5 (1.0 - 5.8)

Table 1. (Continued)

	All (n = 134)	Group 1 (n = 69)	Anaphylaxis		*P* value[b]	OR (95%CI)[b]	*P* value[c]	OR (95%CI)[c]
			Group 2 (n = 35)	Group 3 (n = 30)				
Comorbidity (allergic disease)								
Eczema or atopic dermatitis	96 (72.2)	47 (69.1)	25 (71.4)	24 (80.0)	0.420	1.4 (0.6 - 2.9)	0.277	1.7 (0.6 - 4.6)
Respiratory allergic diseases[h]	39 (29.1)	15 (21.7)	5 (14.3)	19 (63.3)	0.053	2.1 (1.0 - 4.5)	< 0.001*	7.3 (3.0 - 17.6)
CM-PTP MWD (mm)	7.5 (5.2, 11.0)	8.8 (7.5, 16.0)	10.5 (5.8, 15.5)	15.8 (10.2, 21.5)	0.108	1.13 (1.02 - 1.25)	< 0.001*	1.26 (1.12 - 1.42)
CM-sIgE (kUA/L)	3.1 (1.3, 17.8)	5.6 (2.1, 100.0)	5.6 (2.6, 100.0)	98.9 (30.2, 100.0)	0.002*	1.03 (1.01 - 1.05)	< 0.001*	1.03 (1.02 - 1.05)
Specific IgE ≥ 0.35 kUA/L								
Any CM component	73 (89.0)	30 (83.3)	22 (88.0)	21 (100.0)	0.270	2.9 (0.7 - 12.4)	0.144	Inf (NaN - Inf)
Two or more CM components	53 (64.6)	19 (52.8)	14 (56.0)	20 (95.2)	0.047*	2.5 (1.0 - 6.4)	0.001*	17.0 (2.1 - 134.6)
Three or more CM components	38 (46.3)	14 (38.9)	7 (28.0)	17 (81.0)	0.231	1.7 (0.7 - 4.2)	0.000*	8.1 (2.4 - 27.2)
Casein	46 (56.1)	18 (50.0)	10 (40.0)	18 (85.7)	0.325	1.6 (0.6 - 3.8)	0.002*	7.1 (1.9 - 26.5)
BLG	53 (64.6)	19 (52.8)	14 (56.0)	20 (95.2)	0.047*	2.5 (1.0 - 6.4)	0.001*	17.0 (2.1 - 134.6)
ALA	45 (54.9)	19 (52.8)	9 (36.0)	17 (81.0)	0.735	1.2 (0.5 - 2.8)	0.005*	5.0 (1.5 - 16.6)
BSA	44 (53.7)	14 (38.9)	14 (56.0)	16 (76.2)	0.018*	3.0 (1.2 - 7.3)	0.016*	3.8 (1.2 - 11.6)
Number of other food sensitization[i]	1.0 (0.0, 3.5)	2.0 (1.0, 8.0)	3.0 (1.0, 7.0)	5.0 (2.0, 8.0)	0.520	1.1 (0.9 - 1.3)	0.413	1.3 (0.9 - 1.6)

OR, odds ratio; CI, confidential interval; Inf, Infinity; NaN, not a number.
*$P < 0.05$;
[a]Quantitative data was described in a median (the first quartile, the third quartile) format, and categorical data were described in a count (percentage) format;
[b]P value and OR (95%CI): Group 1 compared to Group 2 and Group 3;
[c]P value and OR (95%CI): Group 3 compared to Group 1 and Group 2;
[d]The well-educated was defined as having a bachelor's degree or above;
[e]A history of allergic diseases was defined as a history of rhinitis, asthma, eczema, urticaria, food allergy, or drug allergy;
[f]Nutritional supplements included cod-liver oil, vitamins, docosahexaenoic acid (DHA), calcium supplements, and iron supplements;
[g]Extensively heated or baked CM products such as muffins, biscuits, cakes, and yogurt melts;
[h]Respiratory allergic diseases included asthma and allergic rhinitis;
[i]The sIgE levels indicated sensitization to other food, except cow's milk.

Table 2. Clinical symptoms and medical treatments of cow's milk allergy.

Number (%)	All (n = 134)	Group 1 (n = 69)	Group 2 (n = 35)	Group 3 (n = 30)
Skin-mucous membrane	134 (100.0)	69 (100.0)	35 (100.0)	30 (100.0)
Localized urticaria	50 (37.3)	34 (49.3)	7 (20.0)	9 (30.0)
Generalized urticaria	63 (47.0)	26 (37.7)	21 (60.0)	16 (53.3)
Localized pruritus or flushing	17 (12.7)	10 (14.5)	5 (14.3)	2 (6.7)
Generalized pruritus or flushing	2 (1.5)	1 (1.4)	1 (2.9)	0 (0.0)
Localized angioedema	36 (26.9)	12 (17.4)	11 (31.4)	13 (43.3)
Generalized angioedema	1 (0.8)	0 (0.0)	0 (0.0)	1 (3.3)
Nose itching	2 (1.5)	2 (2.9)	0 (0.0)	0 (0.0)
Eye itching	4 (3.0)	3 (4.3)	1 (2.9)	0 (0.0)
Gastrointestinal tract	46 (34.3)	7 (10.1)	29 (82.9)	10 (33.3)
Oral pruritus or oral tingling	2 (1.5)	2 (2.9)	0 (0.0)	0 (0.0)
Vomiting	40 (29.9)	5 (7.2)	13 (77.1)	8 (26.7)
Nausea	2 (1.5)	0 (0.0)	0 (0.0)	2 (6.7)
Diarrhea	5 (3.7)	0 (0.0)	5 (14.3)	0 (0.0)
Abdominal cramps	3 (2.2)	0 (0.0)	0 (0.0)	3 (10.0)
Respiratory Tract	33 (24.6)	1 (1.4)	2 (5.7)	30 (100.0)
Nasal congestion or sneezing	4 (3.0)	1 (1.4)	1 (2.9)	2 (6.7)
Rhinorrhea	3 (2.2)	0 (0.0)	2 (5.7)	1 (3.3)
Sensation of throat pruritus or tightness	5 (3.7)	0 (0.0)	0 (0.0)	5 (16.7)
Cough	8 (6.0)	0 (0.0)	0 (0.0)	8 (26.7)
Hoarseness	2 (1.5)	0 (0.0)	0 (0.0)	2 (6.7)
Wheeze bronchospasm	12 (9.0)	0 (0.0)	0 (0.0)	12 (40.0)
Dyspnea	15 (11.2)	0 (0.0)	0 (0.0)	15 (50.0)
Hypoxemia or cyanosis	8 (6.0)	0 (0.0)	0 (0.0)	8 (26.7)
Cardiovascular	5 (3.7)	0 (0.0)	0 (0.0)	5 (16.7)
Reduced BP	4 (3.0)	0 (0.0)	0 (0.0)	4 (13.3)
End-organ dysfunction	5 (3.7)	0 (0.0)	0 (0.0)	5 (16.7)
Neurological	24 (17.9)	0 (0.0)	14 (40.0)	10 (33.3)
Persistent crying or restlessness	19 (14.2)	0 (0.0)	12 (34.3)	7 (23.3)
Listless or hypersomnia	4 (3.0)	0 (0.0)	2 (5.7)	2 (6.7)
Loss of consciousness or confusion	2 (1.5)	0 (0.0)	0 (0.0)	2 (6.7)
Treatments				
Seek medical care	56 (41.8)	19 (27.5)	18 (51.4)	19 (63.3)
No treatment given	88 (65.7)	50 (72.5)	25 (71.4)	13 (43.3)
Epinephrine given	8 (6.0)	0 (0.0)	0 (0.0)	8 (26.7)
Antihistamines given	33 (24.6)	17 (24.6)	9 (25.7)	7 (23.3)
Intravenous/oral steroids given	11 (8.2)	0 (0.0)	3 (8.6)	8 (26.7)
Other treatments given[a]	5 (3.7)	2 (2.9)	0 (0.0)	3 (10.0)

[a]Other treatments included atomization, intravenous fluid therapy, or topical medication.

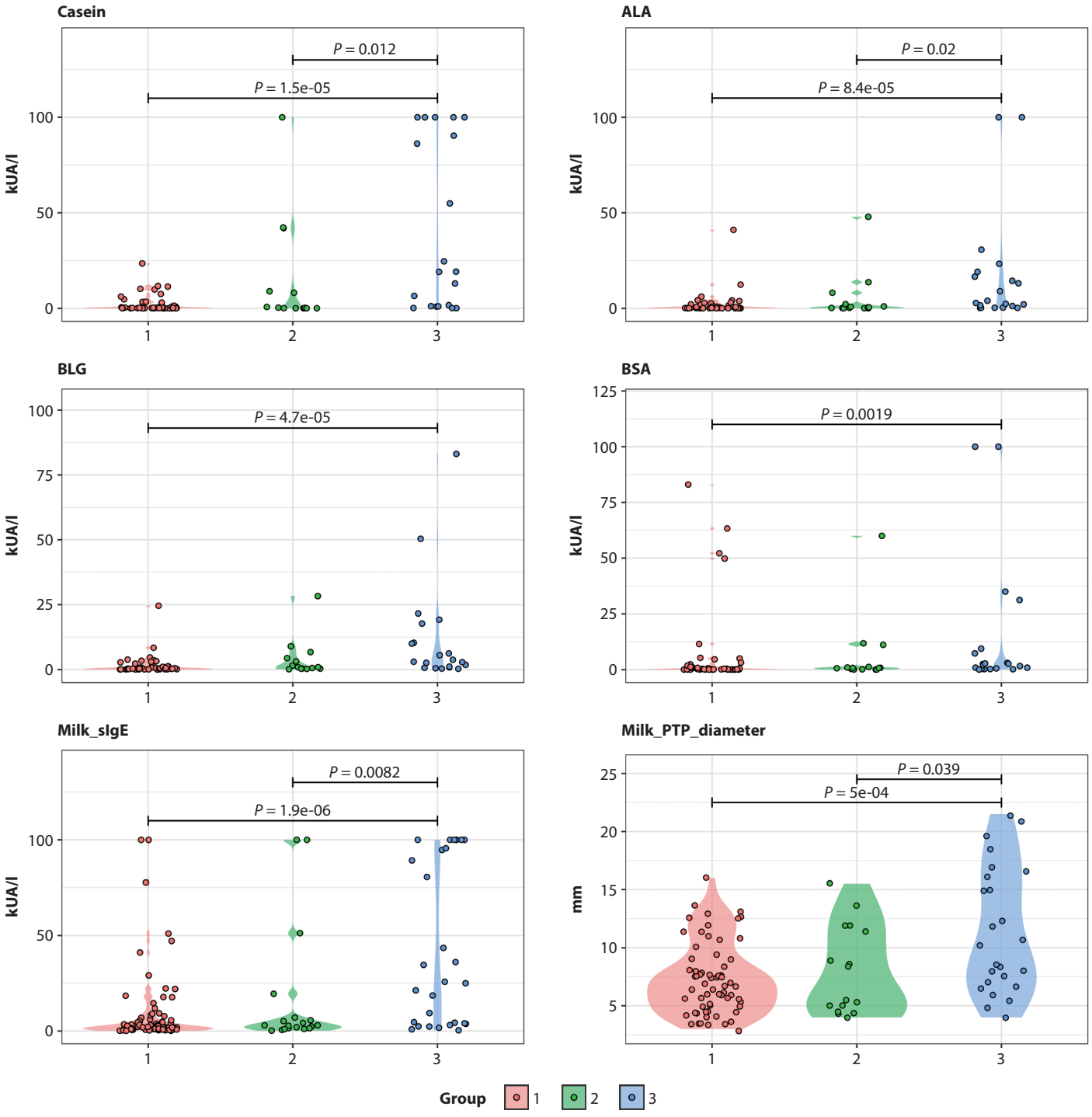

Figure 1. The sIgE levels and PTP diameters in different groups.

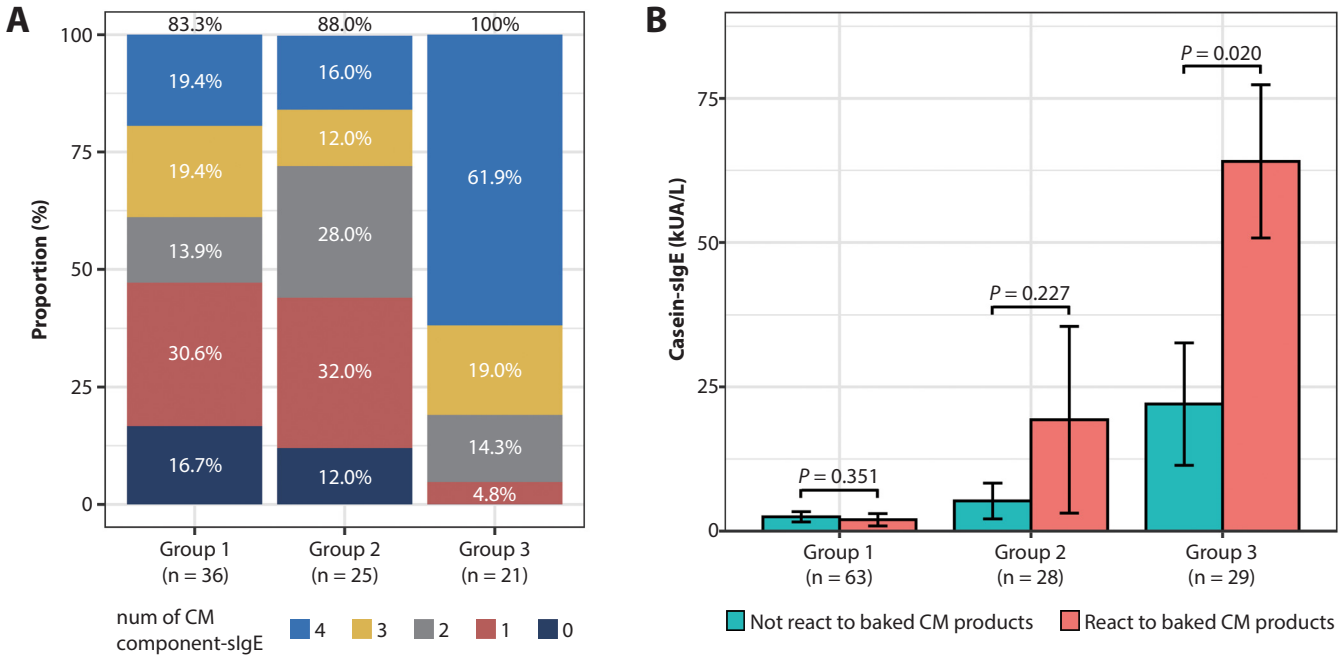

Figure 2. A) The number of positive sIgEs (≥ 0.35 kUA/L) of four CM components in different groups. The numbers above the bars indicated the percentages of patients with any positive CM component-sIgE, while the percentages in white indicated percentages of each part; B) Comparison of casein-sIgE levels in children with or without reaction to baked CM products in each group. *P* value: Mann-Whitney U test. The error bars indicated standard error.

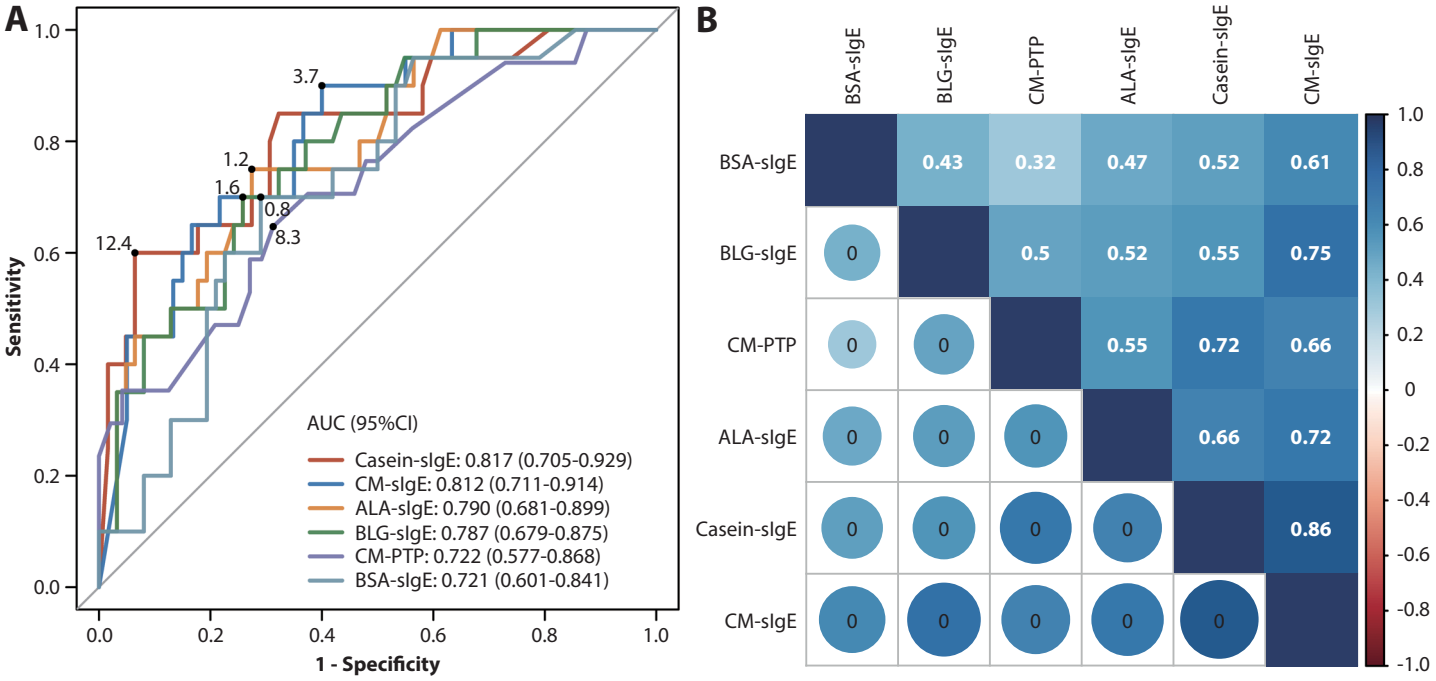

Figure 3. A) The ROC curves of sIgE levels and PTP MWDs in predicting anaphylaxis; B) The correlation matrix of sIgE levels and PTP MWDs. Spearman correlation coefficients were shown in the upper triangular matrix and *P*-values in the lower triangular matrix

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Table 3. The 90% and 95% PPV diagnostic decision points of allergic testing in predicting anaphylaxis and GRADE II to IV anaphylaxis.

		90% PPV diagnostic decision points							95% PPV diagnostic decision points						
		Cutoff	SEN	SPE	PPV	NPV	ACC	+LR	Cutoff	SEN	SPE	PPV	NPV	ACC	+LR
Anaphylaxis	Casein-sIgE	11.7 kUA/L	0.348	0.972	0.941	0.538	0.622	12.4	13.0 kUA/L	0.348	1.000	1.000	0.545	0.634	Inf
	ALA-sIgE	13.1 kUA/L	0.217	0.972	0.909	0.493	0.549	7.7	47.9 kUA/L	0.065	1.000	1.000	0.456	0.476	Inf
	BLG-sIgE	8.9 kUA/L	0.196	0.972	0.900	0.486	0.537	7.0	28.3 kUA/L	0.065	1.000	1.000	0.456	0.476	Inf
	BSA-sIgE	60 kUA/L	0.087	0.972	0.800	0.455	0.476	3.1	60 kUA/L	0.087	0.972	0.800	0.455	0.476	3.1
	CM-sIgE	51.2 kUA/L	0.254	0.985	0.941	0.580	0.628	16.9	51.2 kUA/L	0.254	0.985	0.941	0.580	0.628	16.9
	CM-PTP	15 mm	0.179	0.980	0.909	0.521	0.561	8.9	16.5 mm	0.107	1.000	1.000	0.505	0.533	Inf
GRADE II to IV anaphylaxis	Casein-sIgE	54.9 kUA/L	0.4	0.984	0.889	0.836	0.841	25.0	54.9 kUA/L	0.4	0.984	0.889	0.836	0.841	25.0
	ALA-sIgE	14.4 kUA/L	0.35	0.968	0.778	0.822	0.817	10.9	14.4 kUA/L	0.35	0.968	0.778	0.822	0.817	10.9
	BLG-sIgE	50.4 kUA/L	0.095	1.000	1.000	0.775	0.780	Inf	50.4 kUA/L	0.095	1.000	1.000	0.775	0.780	Inf
	BSA-sIgE	83 kUA/L	0.1	0.984	0.667	0.772	0.768	6.2	83 kUA/L	0.1	0.984	0.667	0.772	0.768	6.2
	CM-sIgE	80.6 kUA/L	0.379	0.96	0.733	0.842	0.829	9.5	80.6 kUA/L	0.379	0.96	0.733	0.842	0.829	9.5
	CM-PTP	16.5 mm	0.240	1.000	1.000	0.812	0.822	Inf	16.5mm	0.240	1.000	1.000	0.812	0.822	Inf

SEN, sensitivity; SPE, specificity; PPV, positive predictive value; NPV, negative predictive value; ACC, accuracy; +LR, positive likelihood ratio; Inf, infinity.

Risk factors of anaphylaxis

As shown in **Table 1**, univariate analysis showed that the age of onset, urban residence, and maternal antibiotic use during pregnancy are risk factors for anaphylaxis. Respiratory allergic diseases, elevated sIgE levels of CM and its components, and larger PTP MWDs were risk factors for anaphylaxis and GRADE II to IV anaphylaxis. The number of other food sensitizations tended to be higher in children with GRADE II to IV anaphylaxis with no significant difference.

Multivariate logistic regression revealed that casein-sIgE ≥ 13.0 kUA/L (OR 28.2, P = 0.002) was the independent risk factor of anaphylaxis, while casein-sIgE ≥ 54.9 kUA/L (OR 14.0, P = 0.025) and respiratory allergic diseases (OR 4.8, P = 0.022) were independent risk factors of GRADE II to IV anaphylaxis.

Discussion

Our study shows that 89% of CMA patients are sensitized to cow's milk components, and patients who have experienced GRADE II-IV anaphylaxis are more susceptible to sensitization by multiple CM components with higher sIgE levels. Among four CM components, BLG and casein were the most common allergens causing sensitization, as previously reported in a Thai cohort.[17] The efficiency of casein-sIgE in predicting anaphylaxis is better than that of traditional allergic tests. The 95% PPV diagnostic decision points proposed for casein-sIgE in predicting anaphylaxis was 13.0 kUA/L. For GRADE II-IV anaphylaxis, casein-sIgE could provide a PPV of 88.9% with a cut-off of 54.9 kUA/L. Casein-sIgE levels above the corresponding threshold were independent risk factors of anaphylaxis and GRADE II-IV anaphylaxis.

Casein is one of the major allergens that cause CMA.[5] Several studies have reported the importance of CM components in predicating CMA.[8,12,18] In the present study, the sIgE levels of casein are significantly elevated in patients who experienced GRADE II-IV anaphylaxis to CM. Of the four CM components tested, levels of casein-sIgE showed the strongest correlation with that of CM-sIgE. Consistent with previous studies, our study showed that casein-sIgE outperforms other CM components and traditional allergic tests in diagnosing anaphylaxis. The ROC curve of casein-sIgE had the largest area under the curve with remarkable specificity. A casein-sIgE level greater than 11.7 kUA/L was a predictor of anaphylaxis with a PPV of 94.1%, while levels greater than 54.9 kUA/L predicted GRADE II-IV anaphylaxis with a PPV of 88.9%, despite a low prevalence (22.4%). The casein-sIgE level exceeding the corresponding threshold has been found to be the independent risk factor for anaphylaxis and GRADE II-IV anaphylaxis. In practice, this means that patients having casein-sIgE greater than 11.7 kUA/L are not suitable for OFCs, and CM should be more strictly avoided. In a Japanese study, the reported cut-off point for casein-sIgE was 6.6 kUA/L, corresponding to 100% specificity.[12] Similarly, a Spanish study reported the cut-off point of 9 kUA/L corresponding to a 95% PPV.[8] The selection of clinical decision points in our study was predicated on the presence of anaphylaxis, besides, the included children were older, both of which contributed to the observed higher cut-off values.[8,18]

Cow's milk components were also useful for predicting children's reactions to baked CM products and for monitoring the natural course of CMA. Casein has the strongest immunogenicity, and was noted to be heat stable for up to 60 min of heating to 95°C.[19] Children with high levels of casein-IgE are less likely to tolerate baked CM products and have more difficulty acquiring natural tolerance.[20-22] In our cohort, Group 3 patients had the highest casein-sIgE level, and nearly half of them had reactions to baked CM products. In cases of casein sensitization, additional sensitization to BLG may further reduce the OFC threshold dose.[23] The BSA-sIgE sensitization may reflect cross-reactivity with beef.[24-26] However, our cohort did not report any allergies to beef, likely due to consumption of only well-cooked beef instead of raw beef. Detailed analysis of sIgE and IgG4-binding patterns of CM components may also predict response to CM oral immunotherapy.[27]

For GRADE II-IV anaphylaxis, preparation to promptly treat allergic reactions is essential and depends on patient education and the level of management in the healthcare facility.[28] An observational study included 512 infants with documented or possible allergy to milk or egg with a median follow-up of 36 months. They found that epinephrine was used in only 29.9% of all recorded severe allergic reactions that warranted epinephrine.[29] The reasons underlying undertreatment included failure to recognize the severity, unavailability of epinephrine, and fears of administering epinephrine. In our study, of the 30 patients with GRADE II-IV anaphylaxis, only eight (26.7%) were given epinephrine. These similar results reflect a lack of awareness of epinephrine administration and the importance of patient education.

Limitations of the study include recall bias due to retrospective reporting from parents and selection bias due to its singular execution in a tertiary hospital. Serum sIgE or PTP values at first visit rather than at onset were included in the analysis, and the older age of detection in Group 3 may be a confounding factor. Due to the limited number of samples, we did not group patients further by age, so age-stratified diagnostic decision points were unavailable. These results need to be further verified in prospective, multi-center studies with larger samples.

In conclusion, CMA patients who have experienced GRADE II-IV anaphylaxis tend to be sensitized to multiple CM components, the most common of which are BLG and casein. Casein-sIgE testing provides the greatest additional value in diagnosing CM anaphylaxis. The elevated casein-sIgE level is an independent risk factor for anaphylaxis. Additional casein-sIgE testing is recommended for children with CM-sIgE below the clinical decision point but with multiple risk factors of anaphylaxis, in order to prevent anaphylaxis and avoid risky OFCs.

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Conflict of Interests

The authors report no conflict of interests.

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Authors' contributions

- WQ, JC, HZ, and WZ: acquisition of data, analysis and interpretation of data, and drafting the article.
- LS and KG: conception and design, acquisition of data, revising the article critically for important intellectual content, and giving final approval of the version to be published.
- All authors contributed to the article and approved the submitted version.

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