



Component resolved diagnostic study of cow's milk allergy in infants and young children in northern China

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ARTICLE INFO

Keywords:

Cow's milk allergy
Infants and young children
Specific IgE antibodies
Component resolved diagnostics (CRD)
Indirect-ELISA

ABSTRACT

Background: Increasing dairy consumption in China has been accompanied by rising incidence of milk allergy. Here we analyzed profiles of specific immunoglobulin E (sIgE) against cow's milk proteins, and assessed their value for milk allergy diagnosis among infants and young children from northern China.

Methods: Sera collected from 48 patients with milk allergy and 27 negative control subjects was analyzed by enzyme-linked immunosorbent assay to measure sIgE to α -lactalbumin (Bos d 4), β -lactoglobulin (Bos d 5), α -casein (Bos d 9), β -casein (Bos d 11), and κ -casein (Bos d 12).

Results: Among milk-allergic individuals, most were sensitized to at least one milk protein; about half were sensitized to Bos d 5, Bos d 9, Bos d 11 and Bos d 12, respectively, while few had positive serum sIgE against Bos d 4. Bos d 12 sIgE had the largest area under curve (AUC) (0.878; 95% CI, 0.800–0.957) and thus showed the best diagnostic performance in discriminating between milk-allergic and non-milk allergic patients, with a sensitivity of 92.6% and specificity of 72.9% using a statistically optimal cut-off value (OD_{450nm} , 0.191). The combinations of Bos d 5 + Bos d 12 showed an AUC of 0.926, which was larger than for any individual components.

Conclusions: Our results revealed inter-individual variation in the sensitization to different milk allergen component. Bos d 12 sIgE showed best performance in diagnosing milk allergy. Milk allergy diagnostic accuracy was further improved using combinations of milk allergen components by application of ROC curves based on logistic regression.

1. Introduction

Milk is one of the most common causes of allergic reactions to food in infants and young children [1,2]. Up to 7.5% children have a milk allergy, making this the predominant food allergy in children under 3 years old [3]. While most children outgrow this allergy by adulthood, a minority may suffer from persistent milk allergy, which is a lifelong inconvenience [4]. Allergic responses to milk are most commonly mediated by immunoglobulin E (IgE), and usually occur within a few minutes to an hour, after ingestion of even a small amount of milk. Symptoms vary in severity. Most patients experience mild response, including urticaria, vomiting, cough, and dizziness, but in rare cases patients may suffer life-threatening allergic reactions, such as anaphylactic shock [5,6]. Milk contains a variety of proteins that can mainly be divided into two fractions: lactoserum (whey proteins; 20%) and coagulum (caseins; 80%) [7]. In theory, any protein in natural milk has potential allergenic activity, however, it is generally believed that the major milk allergens are Bos d 4, Bos d 5, and Bos d 8 (Bos d 11, Bos d 9,

and Bos d 12) [8,9]. Rather than being confined to a single component, sensitivities to different milk allergens show great variability [10,11]. Moreover, there is considerable cross reactivity between cow's milk and other related products, such as goat's milk, sheep's milk, mare's milk, and cow's meat [12]. Current milk allergy management primarily involves avoiding milk and milk products until the allergy becomes resolved [13]. There have also been efforts to reduce the allergenic properties of milk proteins, for example, by inactivation of allergen epitopes and denaturation of the native proteins [14,15]. Moreover, progress has been made in milk allergy prevention and treatment via immunotherapy, including oral immunotherapy and sublingual immunotherapy [16–18].

Routine diagnostic modalities in vitro for milk allergy, include measurement of milk sIgE levels, skin prick testing (SPT), and specific patch testing (APT) based on crude milk extract, which exhibit limited specificity [19]. Component-resolved diagnostics (CRD) shows promise for improving diagnostic accuracy, and has recently been introduced into routine clinical practice [20]. In 2016, the European Academy of

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Allergy and Clinical Immunology (EAACI) released CRD guidelines that comprehensively described CRD applications and prospects [21].

The interpretation of IgE sensitization test results is substantially influenced by patient and environmental factors [22]. The tendency to develop or exhibit IgE antibodies may vary among individuals of different ages or from different ethnic backgrounds [23,24]. Moreover, reaction patterns vary considerably in relation to a patient's geographical location [25–27].

Scarce information is available regarding the milk allergen component recognition patterns among Chinese children. Here we performed the first analysis of the profiles of sIgE to cow's milk proteins in infants and young children from the northern area of China. Additionally, we evaluated the clinical performance of allergen components for milk allergy diagnosis in this patient population.

2. Materials and methods

2.1. Patients

A total of 93 infants and young children from the northern area of China were consecutively enrolled through the Tianjin Children's Hospital from April to October of 2017. Eligible individuals were 0.1–8 years of age. All study participants underwent an evaluation that included clinical history, physical examination, and measurement of serum milk sIgE levels. Milk allergy was diagnosed based on a convincing history of acute allergic reactions after intake of cow's milk, including objective symptoms, such as urticaria, vomiting, nasal congestion, etc., as well as on positive serum sIgE antibodies (milk-sIgE > 0.35 kUA/L, measured by ImmunoCAP). No oral food challenges were performed. After an allergist reviewed the cases, 18 of the recruited subjects were excluded due to an unclear history of milk allergy, leaving 75 infants and young children for analysis.

We investigated the participants' clinical presentations of hypersensitivity reactions to cow's milk. The symptoms were categorized into three grades based on clinical response severity: symptom grade I, affecting isolated skin or gastrointestinal systems; symptom grade II, affecting two systems or organs; and symptom grade III, affecting multiple systems, including the skin and respiratory and digestive organs. Informed consent was obtained prior to patient inclusion, and this study was approved by the Ethics Committees of Tianjin Medical University (TMUHMEC2017008).

2.2. Serological analysis

We applied a widely used indirect enzyme-linked immunosorbent assay (Indirect-ELISA) to determine sIgE antibodies against milk components. Briefly, commercially available milk protein components were purchased from Sigma-Aldrich Co and were dissolved and diluted to 20 µg/mL using 0.05 M carbonate buffer (pH 9.6). Next, ELISA plates were coated with these protein diluents at 125 µL/well and incubated overnight at 4 °C. Subsequently, the coated plates were washed three times (5 min each) with PBST containing 0.1% (v/v) Tween 20 on a shaking platform. The plates were then blocked with 250 µL/well 2% (v/v) polyvinyl alcohol (PVA) in PBST overnight at 4 °C, followed by an additional three washes with PBST. Next, 100-µL serum samples were diluted 10 times with PBST, added to the 96-well ELISA plates, and then incubated for 2 h at 37 °C. Nonspecific IgE antibodies were washed away. Then horseradish peroxidase-labelled secondary anti-human IgE antibodies produced in goat (diluted 1:1000 in PBST; Sigma-Aldrich Co.) were added at 100 µL/well, followed by a 1-h incubation at 37 °C. The plates were washed six times with PBST (5 min each) on a shaking platform, and the substrate 3,5',5'-tetramethylbenzidine (TMB) was added for colour development. This reaction was stopped by addition of 2 M sulfuric acid. Finally, the absorbance was measured at 450 nm using an Epoch microplate reader (Bio Tek, VT, USA).

Following the established indirect ELISA method, we analyzed non-

milk allergic serums in parallel as negative controls. We calculated the mean value and standard deviation (SD) of the sample's optical density at 450 nm (OD₄₅₀). Since the specific IgE cutoff can differ with each allergen [28], we have determined the cutoff values of Bos d 4, Bos d 5, Bos d 9, Bos d 11 and Bos d 12, respectively by adding 2 SD to the mean optical density value of 27 non-cow's milk allergy controls. The levels of sIgE to milk components were expressed as OD₄₅₀ values.

2.3. Statistical analysis

Between-group differences were analyzed using the independent *t*-test for parametric data, and the Mann-Whitney *U* test for nonparametric data. The Kolmogorov-Smirnov test was used to assess normality. Spearman's rank correlation was used to evaluate the associations. Two-tailed *P* values were reported, with *P* values of < 0.05 considered statistically significant. Receiver operating characteristics (ROC) curves were generated to assess the diagnostic value of different tests, with the calculation of sensitivity, specificity, and predictive values (PPV, NPV) using statistically optimal cut-off points. All statistical analyses were performed using GraphPad Prism Version 5.0 Software and SPSS Version 23.0 Software.

3. Results

3.1. Patient characteristics

According to the diagnostic criteria, 48 of the 75 subjects included in this study were diagnosed with cow's milk allergy (CMA). The remaining 27 participants were non-allergic to cow's milk (Non-CMA) and served as negative controls. The Non-CMA group comprised 5 subjects who were milk-sensitized but tolerant (positive serum milk-sIgE level, but no clinical reactions), 6 atopic individuals (allergy to foods other than milk), and 16 nonatopic healthy volunteers (no convincing history of clinical reaction, and negative serum milk-sIgE level). Supporting Information Table S presented the demographic and clinical characteristics of the study subjects.

3.2. Milk allergen component-specific IgE antibodies in CMA and Non-CMA groups

The CMA and Non-CMA groups showed significant differences in milk allergen components sIgE levels (Fig. 1). Compared to the non-CMA group, the CMA group had significantly higher mean levels of sIgE antibodies against Bos d 4 (OD₄₅₀ 0.264 vs 0.186), Bos d 5 (0.215 vs 0.160), Bos d 9 (0.310 vs 0.219), Bos d 11 (0.200 vs 0.147), and Bos d 12 (0.219 vs 0.150). Additionally, the level of sIgE to Bos d 11 was significantly correlated with the levels of sIgE to Bos d 9 ($r_s = 0.438$, $P = 0.002$), Bos d 12 ($r_s = 0.363$, $P = 0.011$), and Bos d 5 ($r_s = 0.293$, $P = 0.043$).

3.3. Profiles of sIgE antibodies against milk proteins

Our data showed inter-individual variation in the sensitization to different milk allergen components (Supporting Information Table S). The cut-off values were generated as described above. Fig. 2 shows the proportions of patients in the CMA group who had serum sIgE against milk allergen components. Among patients with IgE-mediated milk allergy, 50.0% (24/48) had measurable sIgE to Bos d 5, 41.7% (20/48) to Bos d 9, 56.3% (27/48) to Bos d 11, 50.0% (24/48) to Bos d 12, and only 22.9% (11/48) to Bos d 4. Thus, among the five tested proteins, Bos d 4 showed the weakest allergenicity.

3.4. Frequency of sensitization to milk allergen components

Fig. 3 shows the frequency of sensitization to milk allergen components. Most patients with IgE-mediated milk allergy were sensitized

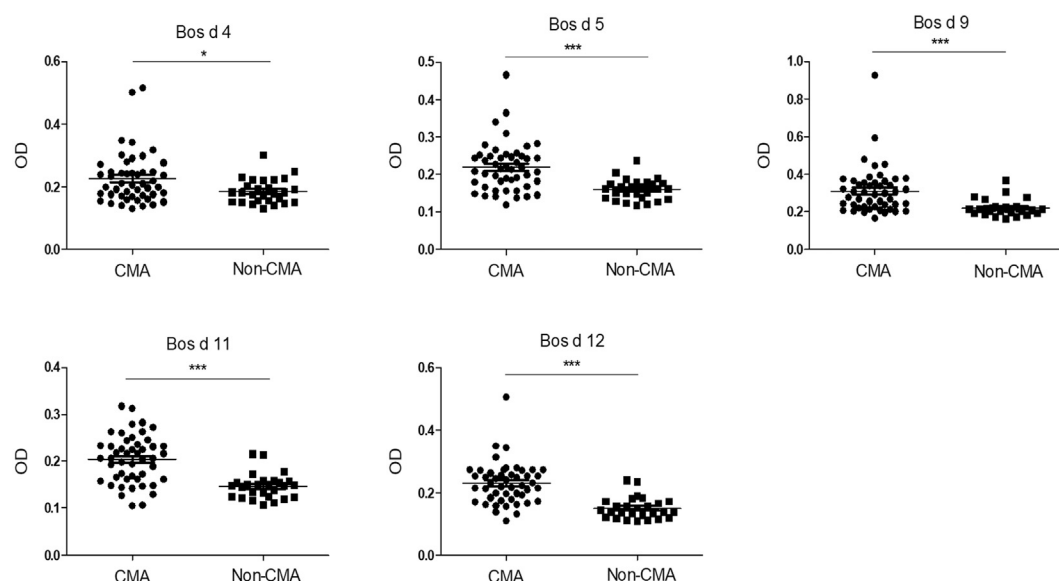


Fig. 1. Comparison of serum sIgE against milk allergen components between CMA and non-CMA groups. * $p < 0.05$, *** $p < 0.001$. OD, optical density.

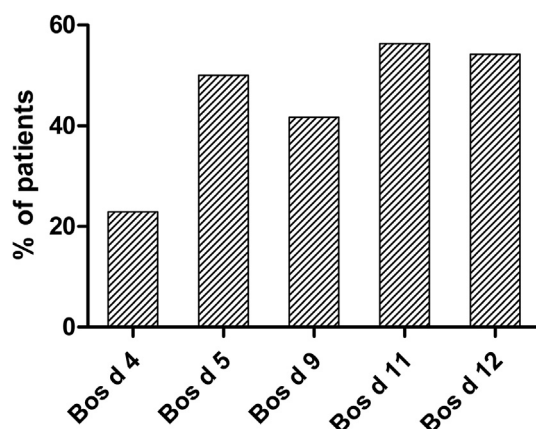


Fig. 2. Proportions of CMA patients with reactive sIgE antibodies against milk allergen components.

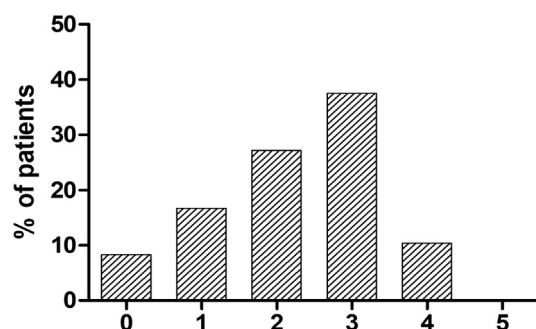


Fig. 3. Comparison of single component allergy and multiple components allergy in CMA patients. 0–5, the number of components detected among the five components.

to several components. The frequency of sIgE reactivity to two or more proteins (36/48, 75%) was much higher than that to a single protein (8/48, 16.7%). Most patients were sensitive to three of the five tested milk protein components. Four patients in the CMA group were not sensitized to any of the five tested proteins. It is possible that these four patients were only sensitized to minor allergens, such as bovine serum albumin, immunoglobulins, or lactoferrin.

3.5. Association between sensitization to components and symptoms

Among the 48 milk-allergic patients, 17 (35.4%) presented isolated cutaneous or gastrointestinal symptoms; 19 (39.6%) reported symptoms in both skin and digestive organs; and 12 (25%) had severe symptoms involving multiple systems, including the skin and gastrointestinal and respiratory organs. Analysis did not reveal any significant association between symptom severity and IgE sensitization test results. We also divided the 48 milk-allergic patients into two groups based on their sensitivity to casein proteins. Higher symptom severity tended to be more common among the 36 patients showing casein protein sensitization compared to the patients who were solely sensitized to whey proteins, with symptom grade I in 8 vs 9 patients; symptom grade II in 16 vs 3 patients; and symptom grade III in 12 vs 0 patients, respectively.

3.6. Diagnostic performance of sIgE reactivities to milk allergen components

We conducted ROC analysis to evaluate the diagnostic performance of IgE reactivity to each milk component. We compared calculations of the area under the curve (AUC), sensitivity, specificity, and predictive values (PPV, NPV) of the different components. Specific immunoglobulin E to Bos d 12 had the largest AUC (0.878; 95% CI, 0.800–0.957) and thus exhibited the best diagnostic performance in discriminating between the CMA and non-CMA groups. Using sIgE to Bos d 12 yielded a sensitivity of 92.6% and specificity of 72.9% when using the statistically optimal cut-off value determined by ROC analysis. Additionally, we found that sIgE to Bos d 11, Bos d 9, and Bos d 5 had comparable AUC values, ranging from 0.796 to 0.833, while Bos d 4 had a poor AUC value of 0.656 (Table 1, Fig. 4).

3.7. Diagnostic performance of sIgE reactivities to combinations of milk allergen components

We next assessed the diagnostic performance of sIgE responses to combinations of milk allergen components using a ROC curve established by logistic regression. Our results showed that the AUC for different combinations of the components was larger than that for the five components individually. The diagnostic accuracy was highest for the two-protein combination of Bos d 5 + Bos d 12, which showed an AUC of 0.926 (Table 2, Fig. 5).

Table 1
Diagnostic performance of sIgE reactivities to milk allergen components.

	Bos d 4	Bos d 5	Bos d 9	Bos d 11	Bos d 12
AUC	0.656	0.810	0.796	0.833	0.878
(95% CI)	(0.532–0.780)	(0.714–0.960)	(0.693–0.898)	(0.741–0.925)	(0.800–0.957)
Optimal cut-off (OD ₄₅₀)	0.232	0.181	0.226	0.160	0.191
Sensitivity (%)	92.6	85.2	81.5	85.2	92.6
Specificity (%)	43.8	72.9	75.0	79.2	72.9
PPV (%)	91.3	89.7	86.0	90.5	94.6
NPV (%)	48.1	63.9	65.6	69.7	65.8

AUC: the area under curve; CI: confidence interval; PPV: positive predictive value; NPV: negative predictive value.

Cut-offs (OD₄₅₀) for the five components assay were based on ROC curves.

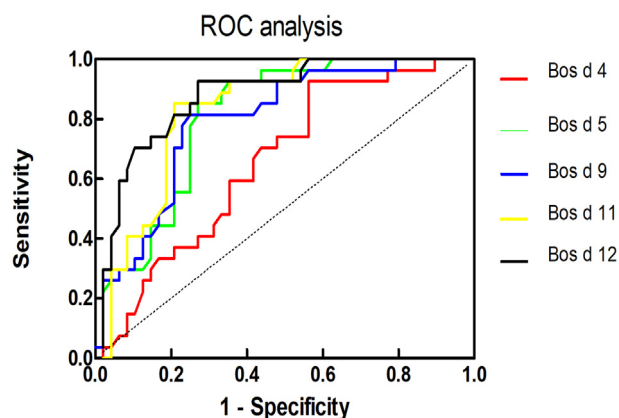


Fig. 4. Clinical values of five components in diagnosing CMA.

Table 2
Diagnostic performance of sIgE reactivities to combinations of milk allergen components.

Combined determinations of components	AUC (95%-CI)
Bos d 4 + Bos d 5	0.836(0.743–0.930)
Bos d 4 + Bos d 9	0.856(0.769–0.942)
Bos d 4 + Bos d 11	0.867(0.782–0.951)
Bos d 4 + Bos d 12	0.881(0.803–0.959)
Bos d 5 + Bos d 9	0.894(0.818–0.971)
Bos d 5 + Bos d 11	0.883(0.809–0.958)
Bos d 5 + Bos d 12	0.926(0.859–0.993)
Bos d 9 + Bos d 11	0.858(0.773–0.943)
Bos d 9 + Bos d 12	0.909(0.836–0.982)
Bos d 11 + Bos d 12	0.911(0.844–0.978)
Bos d 9 + Bos d 11 + Bos d 12	0.915(0.848–0.982)

AUC: the area under curve; CI: confidence interval.

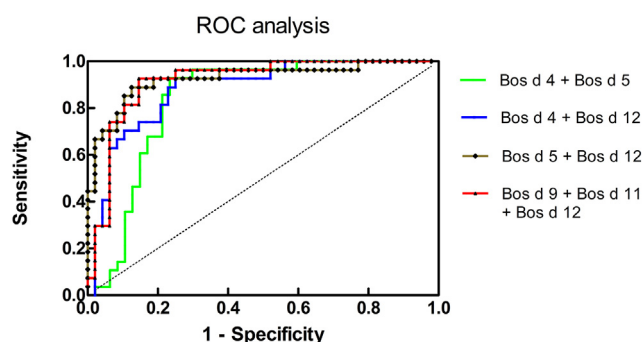


Fig. 5. ROC curves for evaluating diagnostic values of sIgE reactivities to combinations of milk allergen components.

4. Discussion

Here we analyzed profiles of sIgE against cow's milk proteins and further assessed their value in clinical diagnosis among infants and young children from northern China. Our results showed higher levels of sIgE antibodies against cow's milk proteins among individuals who were allergic to cow's milk than among participants without cow's milk allergy. Moreover, we found high inter-individual variation in sIgE profiles, and demonstrated the high diagnostic performance of the combination of sIgE against Bos d 5 + Bos d 12. It is possible that the inter-individual variations in IgE reactivity could be due to some patients having co-existing IgG antibodies that would compete for epitopes on milk allergens and potentially block the IgE from binding since the ratio between cow's milk proteins-specific plasma IgE and IgG4 in children with CMA is approximately 1:1000 [29]. Different studies report wide variation in the prevalence of IgE responses to certain milk proteins. These differences are likely related to the selection of study subjects according to different diagnostic criteria and the determination of cut-off values based on different statistical criteria [30,31]. Our present results suggested that about half of the milk-allergic patients were sensitized to Bos d 5, Bos d 9, Bos d 11, and/or Bos d 12, while few patients had positive serum sIgE against Bos d 4. Similarly, Shek et al. [32] tested the five major milk proteins, and found that Bos d 4 had the weakest allergenicity and antigenicity, with only 36.2% of patients with IgE-mediated reactions exhibiting sensitivity to this protein. Additionally, previous studies demonstrate a high frequency of sIgE response to caseins in patients with milk allergy, and highlight Bos d 9 as the most important allergen of the casein fraction [33,34]. However, our present data did not show that Bos d 9 was more allergenic compared to Bos d 11 and Bos d 12. We demonstrated that most patients with IgE-mediated milk allergy were sensitized to several milk proteins, which was in agreement with previous studies [30,35]. In our study, most patients were sensitive to three of the tested milk proteins. Four milk-allergic patients were not sensitized to any of the five major allergens, and may have instead been allergic to only minor allergens. In a previous report, 51%, 19%, and 21% of patients had detectable sIgE to bovine serum albumin, immunoglobulins, and lactoferrin, respectively [36].

Food challenge test is the gold standard for food allergy diagnosis; however, the procedure can cause severe allergic reactions in patients, and is time-consuming, laborious, and difficult to implement in clinical practice [3,37,38]. Allergen component-resolved diagnostic testing (CRD) is a new diagnostic method for clinical food allergy. Rather than relying on a mixture of crude allergen extracts, CRD measures sIgE against individual allergen that can cause anaphylaxis. In clinical practice, this method enables improved discrimination between clinically allergic patients versus sensitized but tolerant patients, and reduces the number of oral provocation tests required. Moreover, CRD can also determine the severity of anaphylaxis in specific cases and can predict the development of food tolerance [20,38].

It is well established that diagnostic performance of tests was assessed using the area under curve (AUC) from receiver operating

characteristic (ROC) analysis [39,40]. Here we evaluated the diagnostic performance of sIgE reactivities to milk allergen components, and found that Bos d 12 had the best accuracy for diagnosing milk allergy in infants and young children in northern China, with an AUC of 0.878, and yielding a sensitivity of 92.6% and specificity of 72.9% using a statistically optimal cut-off value (OD_{450} , 0.191) determined by the ROC curve. Prior studies indicate that high levels of sIgE to casein are correlated with prolonged milk allergy, and predict clinical allergy to baked milk [41,42]. We identified a trend toward patients with positive sIgE against casein fractions more commonly exhibiting higher symptom severity. This information combined with our present findings suggests that measurement of sIgE to Bos d 12 could be useful not only for diagnosing milk allergy in children, but also for predicting sIgE reactivities to baked milk and the prognosis of milk allergy. Some studies show that CRD provides increased specificity but decreased sensitivity compared with traditional testing methods [43,44]. Thus, to further improve the diagnostic efficiency, we used ROC analyses by logistic regression to assess the performance of sIgE responses to combinations of milk allergen components rather than to a single protein. We found that the AUC for different combinations of protein components was larger than that for the five components individually. Specifically, we found the highest diagnostic accuracy using the combination of Bos d 5 + Bos d 12, with an AUC of 0.926.

Our study had several limitations. Notably, we did not diagnose milk allergy in the recruited subjects by performing double-blind placebo-controlled food challenges (DBPCFC), which was mainly because parents of the infants and children refused to accept the challenge. However, diagnosis was based on a convincing history of anaphylaxis following intake of the suspected food allergen [45], together with a positive SPT and/or sIgE > 0.35 kU_A/L [3,46]. Secondly, the concentration of sIgE to milk components was measured using ELISA rather than ImmunoCAP (Phadia). However, the application of ELISA showing good sensitivity is a validated and frequently used method for allergy research and allergy diagnosis [47,48]. Another limitation was that the sera samples were obtained from patients showing relatively low milk-sIgE levels, which may explain the different IgE recognition patterns between this and previous studies. Differences in sex distribution and symptoms between the CMA and non-CMA groups also contributed to variability of the IgE test results. Thus the present results must be validated before they can be applied to clinical practice.

Individuals from different ethnic backgrounds may have different tendencies to develop or demonstrate specific IgE results [23]. More importantly, CRD has unveiled that these sensitization profiles might show geographical differences with clear distinct clinical outcomes [25,49]. As a consequence, milk component testing results may vary considerably in relation to a patient's ethnic backgrounds or geographical location. In this study, we tried to explore the application of milk component testing (CRD) in infants and young children in northern China. Our results on component resolved diagnostic study of cow's milk allergy will help clinicians diagnose milk allergy in infants and young children in northern China.

In conclusion, this study is the first to report the profiles of sIgE to cow's milk proteins in children of northern China, and to evaluate the clinical performance of measuring allergen components for diagnosing milk allergy in this population. Our findings confirmed that most patients with milk allergy were sensitized to several milk proteins, and that the patients showed substantial inter-individual variation of sensitization to the tested milk allergen components. Our results indicate that sIgE to Bos d 12 had the best accuracy in diagnosing milk allergy in infants and young children in northern China. Moreover, the diagnostic accuracy for milk allergy was further improved using combinations of milk allergen components by application of ROC based on logistic regression. In particular, the combination of Bos d 5 and Bos d 12 sIgE seems to be suitable for screening infants and young children with milk allergy in northern China and may be considered a candidate diagnostic model.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2018.05.027>.

Acknowledgements

This study was funded by the National Natural Science Foundation of China (# 81772259).

Disclosure statement

All authors have declared they have no conflict of interest.

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Supporting Information Table S

Clinical characteristics of the study subjects

Subject group	Subject no.	Age (years)/ Gender (M/F)	CAP to milk (kUa/L)	ELISA to milk components (OD ₄₅₀)					Symptoms with milk/severity
				Bos d 4	Bos d 5	Bos d 9	Bos d 11	Bos d 12	
Cow's milk allergy	1	3.0/F	0.37	0.175	0.232	0.375	0.206	0.209	U, D/II
	2	0.2/M	0.80	0.138	0.218	0.306	0.279	0.505	U, V/II
	3	0.9/M	0.54	0.209	0.246	0.591	0.225	0.215	P/I
	4	3.0/F	1.60	0.172	0.282	0.373	0.245	0.344	U, AP, D, NC/III
	5	2.0/F	0.70	0.218	0.309	0.354	0.260	0.217	V, LE/II
	6	0.5/M	0.90	0.210	0.226	0.443	0.272	0.156	U, V, NC/III
	7	2.0/F	1.44	0.233	0.244	0.395	0.244	0.213	F, D/II
	8	1.0/M	0.67	0.139	0.167	0.226	0.129	0.132	U/I
	9	8.0/F	0.56	0.195	0.137	0.289	0.161	0.178	F/I
	10	1.0/M	3.40	0.237	0.185	0.478	0.262	0.349	U, V, C, OS/III
	11	0.9/M	1.20	0.180	0.155	0.237	0.217	0.229	U, AP, C/III
	12	1.0/M	1.30	0.160	0.196	0.362	0.312	0.262	U, W/II
	13	1.0/F	2.00	0.243	0.179	0.266	0.189	0.280	U/I
	14	2.0/F	3.53	0.151	0.465	0.235	0.231	0.253	P, D/II
	15	0.1/M	0.83	0.158	0.182	0.298	0.217	0.252	U, V/II
	16	3.0/F	0.82	0.170	0.198	0.364	0.158	0.271	F, LE/II
	17	4.0/F	0.55	0.175	0.142	0.321	0.162	0.171	U, N/II
	18	4.0/M	6.43	0.142	0.148	0.340	0.192	0.174	P, D, LE, OS/III
	19	2.0/M	0.89	0.167	0.118	0.376	0.165	0.248	U, V/II
	20	2.0/M	1.50	0.153	0.145	0.244	0.216	0.273	U/I
	21	1.0/M	0.68	0.177	0.208	0.194	0.218	0.273	U/I
	22	0.1/M	1.60	0.146	0.275	0.303	0.161	0.137	F/I
	23	3.0/F	1.50	0.247	0.249	0.354	0.173	0.274	U, V, LE/III
	24	0.4/M	10.10	0.129	0.243	0.243	0.106	0.110	P/I
	25	1.0/F	2.90	0.242	0.202	0.337	0.233	0.230	U/I
	26	5.0/F	0.43	0.184	0.189	0.319	0.206	0.244	U, V, N, W/III
	27	2.0/F	0.43	0.199	0.257	0.277	0.174	0.219	F/I
	28	2.0/M	16.69	0.348	0.213	0.308	0.236	0.248	U, AP/II
	29	1.5/M	2.90	0.191	0.242	0.924	0.282	0.163	U, V, AP, NC/III
	30	0.8/F	0.36	0.205	0.248	0.344	0.226	0.222	U/I

	31	2.0/F	0.68	0.198	0.237	0.201	0.198	0.158	P, OI/II
	32	4.0/M	0.61	0.515	0.244	0.220	0.168	0.172	U/I
	33	4.0/M	0.58	0.298	0.215	0.203	0.225	0.183	U, V, LE/III
	34	1.0/F	5.10	0.300	0.140	0.385	0.250	0.252	U, W/II
	35	3.0/F	9.68	0.246	0.253	0.336	0.232	0.198	V/I
	36	3.0/F	0.35	0.342	0.252	0.227	0.317	0.313	U, V, W, OS/III
	37	0.6/M	2.40	0.239	0.339	0.212	0.232	0.254	F, N, C, NC/III
	38	1.0/F	4.59	0.190	0.266	0.212	0.127	0.214	U/I
	39	3.0/F	0.82	0.276	0.278	0.200	0.144	0.192	P, N/II
	40	1.5/M	1.50	0.270	0.364	0.207	0.263	0.274	U/I
	41	3.0/F	1.90	0.156	0.182	0.451	0.205	0.167	E, NC/II
	42	1.0/M	3.69	0.316	0.156	0.254	0.194	0.197	N/I
	43	1.0/F	0.44	0.244	0.140	0.255	0.148	0.196	U, V/II
	44	0.5/M	2.40	0.278	0.192	0.265	0.148	0.257	U, NC/II
	45	0.5/F	1.70	0.500	0.207	0.206	0.147	0.240	U, OI, C, OS/III
	46	2.0/M	0.68	0.239	0.222	0.238	0.204	0.270	V, LE/II
	47	2.0/M	0.63	0.290	0.165	0.195	0.142	0.279	U, W/II
	48	1.0/M	0.76	0.241	0.155	0.165	0.105	0.163	F/I
Cow's milk tolerance	49	0.1/F	1.11	0.231	0.168	0.204	0.148	0.143	
	50	2.0/F	1.20	0.301	0.189	0.191	0.135	0.116	
	51	2.8/M	0.43	0.222	0.162	0.368	0.159	0.234	
	52	0.5/F	1.46	0.176	0.162	0.226	0.214	0.134	
	53	3.0/M	0.39	0.181	0.153	0.214	0.125	0.172	
Atopic	54	1.2/F	<0.35	0.155	0.162	0.204	0.134	0.128	
	55	0.9/F	<0.35	0.183	0.204	0.172	0.151	0.135	
	56	1.2/F	<0.35	0.173	0.179	0.218	0.155	0.121	
	57	0.4/M	<0.35	0.183	0.180	0.211	0.173	0.112	
	58	1.3/F	<0.35	0.226	0.168	0.215	0.216	0.110	
	59	2.9/F	<0.35	0.195	0.177	0.191	0.122	0.118	
Nonatopic	60	3.1/F	<0.35	0.144	0.138	0.162	0.145	0.158	
	61	0.8/M	<0.35	0.145	0.137	0.215	0.124	0.138	
	62	3.0/F	<0.35	0.162	0.133	0.223	0.150	0.185	
	63	1.1/F	<0.35	0.151	0.129	0.224	0.155	0.157	
	64	0.3/M	<0.35	0.130	0.127	0.265	0.117	0.165	
	65	0.7/F	<0.35	0.139	0.117	0.280	0.159	0.172	
	66	0.9/F	<0.35	0.156	0.121	0.278	0.177	0.111	
	67	3.0/F	<0.35	0.151	0.124	0.174	0.158	0.152	
	68	3.1/M	<0.35	0.222	0.237	0.202	0.151	0.240	
	69	0.4/F	<0.35	0.194	0.174	0.196	0.151	0.158	
	70	0.6/F	<0.35	0.249	0.188	0.184	0.148	0.138	
	71	1.0/M	<0.35	0.203	0.151	0.207	0.125	0.182	

72	6.3/F	<0.35	0.220	0.148	0.181	0.120	0.137
73	0.9/M	<0.35	0.152	0.153	0.213	0.107	0.120
74	2.3/M	<0.35	0.192	0.168	0.306	0.138	0.190
75	0.2/F	<0.35	0.177	0.174	0.212	0.112	0.133

M: male; F: female; U: urticaria; P: pruritus; F: flushing; V: vomiting; D: diarrhea; OI: oral itching; AP: abdominal pain; N: Nausea; LE: laryngeal edema; NC: nasal congestion; C: coughing; W: wheezing; OS: other symptoms.