International Immunopharmacology 61 (2018) 126–131

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

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

Junpu Li, Jiayi Zhang, Cuiya Qiong, Tiantian She, Ying Bian, Shuxiang Lin, Huiqiang Li*

School of Medical Laboratory, Tianjin Medical University, Tianjin, China
Department of Medical Laboratory, Tianjin Children's Hospital, Tianjin, China

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

* Corresponding author at: Department of Clinical Laboratory, School of Medical Laboratory, Tianjin Medical University, Tianjin 300203, China.
E-mail address: lhq@tmu.edu.cn (H. Li).

https://doi.org/10.1016/j.intimp.2018.05.027
Received 10 April 2018; Received in revised form 25 May 2018; Accepted 25 May 2018

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′,5,5′ - 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 (OD_{450}). 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 OD_{450} 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 (OD_{450} 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