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Charakterystyka manifestacji klinicznej alergii w korelacji ze składnikami sIgE mleka krowiego u dzieci – doniesienia wstępne

Characteristics of clinical manifestations of allergy in correlation with cow's milk-sIgE components in children – preliminary reports

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Streszczenie

Wprowadzenie i cel pracy: Mleko krowie jest jednym z pierwszych pokarmów w życiu człowieka. Stanowi również jedną z najczęstszych przyczyn alergii pokarmowych u niemowląt i małych dzieci oraz anafilaksji u dzieci. Standardem w diagnostyce alergii IgE-zależnej jest udokumentowanie obecności swoistych przeciwciał IgE. Dzięki diagnostyce komponentowej możliwe jest dokładne określenie stężenia przeciwciał specyficznych dla poszczególnych białek mleka krowiego. Celem pracy było określenie zależności między przeciwciałami IgE swoistymi dla składników mleka krowiego a rodzajem klinicznej manifestacji alergii. **Materiał i metody:** Do badania zakwalifikowano 18 dzieci z udokumentowaną IgE-zależną alergią na białko mleka krowiego. Od każdego z pacjentów pobrano 2 ml krwi do diagnostyki komponentowej za pomocą testu ALEX. **Wyniki:** Przeciwciałami najczęściej stwierdzanymi u dzieci z alergią na białka mleka krowiego były przeciwciała przeciwko kazeinie (Bos d 8). Nie stwierdzono statystycznie istotnych zależności między objawami klinicznymi a stężeniami poszczególnych składników. Zaobserwowano statystycznie istotną zależność między stężeniem α-laktoalbuminy (Bos d 4) a czasem trwania objawów klinicznych alergii na białko mleka krowiego. **Wnioski:** Wyniki badania własnego wskazują, że najczęściej występującym składnikiem białka mleka krowiego jest kazeina (Bos d 8). Związek między uczuleniem na inne składniki białka mleka krowiego a objawami klinicznymi alergii wymaga dalszych badań, które są obecnie prowadzone.

Słowa kluczowe: anafilaksja, przeciwciała IgE swoiste, alergia na białko mleka krowiego, atopowe zapalenie skóry, diagnostyka komponentow

Abstract

Background: Cow's milk, which is one of the first foods in human life, is also one of the most common causes of food allergy in infants and young children, as well as anaphylaxis in the youngest population. Thanks to component diagnostics, it is possible to accurately determine the concentrations of antibodies specific to individual milk proteins. The aim of the study was to determine the relationship between the cow's milk component-specific IgE and the type of clinical manifestation of allergy. **Materials and methods:** Eighteen children with documented IgE-mediated allergy to cow's milk proteins were enrolled in the study. Blood samples (2 mL) were collected from each patient for component diagnostics using the ALEX test. **Results:** The antibodies that were most frequently found among the children with cow's milk allergy were antibodies against casein (Bos d 8). There were no statistically significant relationships between the clinical symptoms and the levels of individual components. A statistically significant relationship was observed between the concentration of α-lactalbumin (Bos d 4) and the duration of clinical symptoms of allergy to cow's milk protein. **Conclusions:** The results of our study indicate that the most common component is casein (Bos d 8). The relationship between the sensitisation to other components and clinical symptoms requires further research, which is currently being conducted.

Keywords: anaphylaxis, specific IgE antibodies, cow's milk protein allergy, atopic dermatitis, component diagnostics

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INTRODUCTION

In the era of personalised medicine, and "tailor-made" therapies, diagnostics based on allergen components (component-resolved diagnostics, CRD) is becoming more and more important in allergology. The standard in the diagnosis of IgE-mediated allergy is to document the presence of specific IgE antibodies. Typically, two tests serve this purpose: skin prick tests (SPT) and the presence and level of allergen-specific IgE (asIgE) in the serum. However, significant differences between the results of SPT and asIgE in serum against the same source allergens have been reported in numerous publications. This is due to the fact that allergen extracts, i.e. mixtures of many proteins originating from one allergen source, e.g. milk, are used to perform SPT and to determine asIgE. Among these proteins, there are those that are part of the allergen as well as those surrounding the allergen, which do not have allergenic properties. There are about forty proteins in the composition of milk, with as many as thirty capable of causing allergies[1]. At the same time, the proteins that cause allergic reactions differ in their primary, secondary and tertiary structures. This fact results in the presence of IgE antibodies specific for each of these proteins. Consequently, their clinical significance may be completely different[2]. Also for this reason, there are many false positive results, which exposes patients to unnecessary restrictions related to the avoidance of allergens, primarily food allergens. The use of allergen extracts does not distinguish between primary sensitisation and cross-reaction.

Molecular diagnostics in allergology is used to demonstrate the presence and measure the concentration of IgE antibodies in relation to allergenic components currently referred to as allergen molecules. According to the CRD rules, each molecule represents a separate allergen. The source allergen is made up of many molecules. The name of the allergen molecule is formed from the Latin name, using the first three letters of the genus, followed by the first letter of the species name and the number indicating the order in which the component was discovered (e.g. one of the components of cow's milk protein – Bos d 2 – here, the name unusually comes from the name of the cow itself – *Bos domesticus*)[2–4].

Cow's milk is often the first food in human life; it is also one of the most common causes of food allergy in infants and small children, as well as one of the most predominant trigger of anaphylaxis in children, especially in the first year of life. Milk and its products are among the cheaper sources of proteins, fats, sugars, and micro- and macronutrients, and the best source of calcium. Milk also contains magnesium, zinc, bromine, manganese, iodine, and fluorine. However, cow's milk consists of about 30 proteins that can cause allergies. Thanks to the CRD, it is possible to precisely determine which molecules are the most harmful, which are resistant to temperature, and which are markers of persistent allergy. The components most often causing allergic reactions include bovine serum albumin (BSA) and lactalbumin, followed by casein[5–7].

α-lactalbumin (Bos d 4) – a partially thermostable protein, belongs to the group of major allergens. Sensitisation to this molecule may cause an allergic reaction after the consumption of raw milk, while the risk decreases after cooking[7,8].

β-lactoglobulin (Bos d 5) is a thermolabile protein. Allergic reactions occur mainly after eating raw milk, and may cause anaphylactic reactions.

Casein (Bos d 8) is the main milk allergen, extremely thermostable, associated with the risk of an allergic reaction after consuming milk in any form, as it is resistant to high temperatures and digestive enzymes. It can trigger many severe systemic allergic reactions. Monitoring the concentration of asIgE against Bos d 8 can be used to control the development of immunotolerance to cow's milk protein[7,8].

Bovine serum albumin (Bos d 6) is a weak milk allergen, but a strong beef allergen, and may cause allergic reactions to raw milk and beef. It is a thermolabile molecule[7,8].

IgE-dependent allergy to cow's milk protein (CMP) may cause symptoms involving the gastrointestinal tract, skin, respiratory system; it may also take the most severe systemic form – anaphylaxis. In addition to IgE-mediated allergy to cow's milk proteins, there is allergy to non-IgE-mediated cow's milk proteins such as eosinophilic esophagitis, eosinophilic gastroenteritis, proctocolitis, gastroesophageal reflux disease, infant colic, protein enterocolitis (food protein-induced enterocolitis syndrome, FPIES), etc.[7,9].

The aim of the study was to determine whether there is a relationship between the presence of sensitisation to particular cow's milk molecules and the type of cow's milk protein allergy (CMPA) manifestations.

MATERIALS AND METHODS

Patients

In the period from May 2021 to December 2022, children (1–10 years old) hospitalised at the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine in Warsaw were evaluated to select patients with allergy symptoms who were then subjected to molecular diagnostics. Out of 100 tests performed, a group of 18 children with cow's milk protein allergy was selected. At the time of enrolment in the study, the children were healthy, with no fever or symptoms of respiratory tract infection. Food allergies were diagnosed on the basis of clinical symptoms (convincing history of an allergic reaction, confirmed by a positive food challenge test) and serum specific IgE determinations. The study's inclusion and exclusion criteria are presented in Tab. 1.

A detailed history of symptoms from birth to hospitalisation was collected from the parents of children selected for

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Inclusion criteria	Exclusion criteria
Child's age 1–10 years	Specific immunotherapy
Documented IgE-mediated cow's milk allergy	Symptoms of acute respiratory infection
Specific IgE determined at least four weeks after anaphylactic reaction	Undocumented IgE-mediated infectious process
No symptoms of acute respiratory infection	No consent to participate in the study
Consent to participate in the study	

Tab. 1. Study inclusion and exclusion criteria

the study. Interviews were conducted with particular emphasis on the infancy period (regurgitation, colic, stools with pathological admixtures, weight gain, and the course of the food elimination and provocation test), skin changes, recurrent bronchial obstruction and wheezing, abdominal pain, and symptoms of acute anaphylactic reaction after consuming milk and milk products.

The research project received a positive opinion of the Bioethics Committee at the Military Medical Chamber on 13 May 2022 (consent No. 219/22).

The study was financed by a grant implemented as part of the statutory activity of the Military Institute of Medicine (No. 15/W/2022).

Legal representatives of the children included in the study gave written informed consent to their children's participation in the study, with the option of resignation without giving a reason at any stage of its duration.

Molecular allergy diagnostics

Molecular allergy diagnostics was performed using the new ALEX2 Allergy Explorer test according to the manufacturer's protocol (MacroArray Diagnostics, Wien, Austria). This multiplex array allows simultaneous and quantitative measurement of total IgE and specific IgE antibodies (sIgE) against 178 molecular allergens and 117 allergen extracts, forming a macroscopic matrix on a nitrocellulose membrane. In short, serum samples diluted 1:5 in the Sample Diluent, containing a cross-reactive carbohydrate determinants (CCD) inhibitor, were added to the membranes placed in the cartridges and incubated in a humid incubator chamber on the lab rocker at 8 rpm for 2 h at room temperature. After incubation, the membranes were washed three times and re-incubated with anti-human IgE detection antibody for 30 min. Next, the cartridges were washed five times and incubated with the Substrate Solution for 8 min., and the enzymatic reaction was stopped with the Stop Solution. Once completely dry, the membranes were scanned with the MADxImageXplorer, which measured the intensity of colour reaction for each allergen spot. The membrane images were analysed using the Raptor software and finally, a list of allergens and their extracts was generated. Total IgE antibodies are presented as kU/L and sIgE concentrations as IgE response units (kUA/L), and defined as: 1) negative or uncertain results (IgE <0.3 kUA/L), 2) low IgE level (0.3–1 kUA/L), 3) medium IgE level (1–5 kUA/L), 4) high-level positive (5–15 kUA/L) and very high positive (>15 kUA/L)[10].

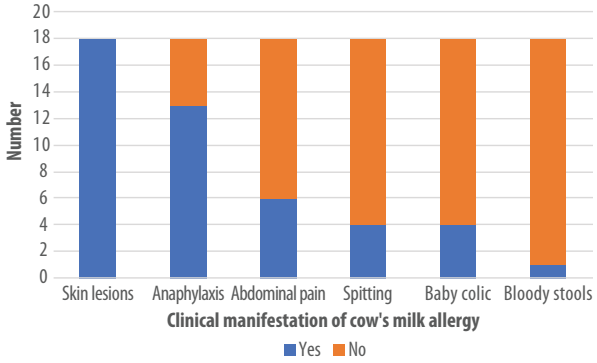

Fig. 1. Clinical manifestations of cow's milk protein allergy in the first year of life

Variable	*N* = 18
Mean age (± *SD*)	5.2 ± 4.6
Family history of allergies:	
• positive	14
• negative	4
Gender [females:males]	7:11
Elimination diet	17
Asthma	10
Atopic dermatitis	11
SD – standard deviation.	

Tab. 2. Clinical characteristics of the study group

The results were analysed statistically using the StatSoft software (STATISTICA 2014). The analyses were initially verified using the diagram of normal distribution (Smirnov and Liliefors test). The Student's *t*-test was used to evaluate variables with the normal distribution. For variables inconsistent with the normal distribution, the non-parametric test was used (Mann–Whitney *U* test). A correlation was calculated using Spearman's test (variables lacking the normal distribution) or Pearson's correlation factor (variables with the normal distribution). The *p*-value <0.05 was considered as a statistically significant.

RESULTS

Characteristics of the study group

Eighteen children with cow's milk protein allergy aged 1–10 years (mean age 5.2 ± 4.6) were included in the study, including 7 girls and 11 boys. Children aged 1–6 years accounted for 63% of the study group. At the time of enrolment, one child was not on an elimination diet; the rest were on a dairy-free diet. In all children, the clinical manifestations of allergy to cow's milk protein occurred in the first year of life. The most common symptoms at that time were skin changes. In contrast, bloody stools were present in only one child (Fig. 1).

Detailed characteristics of the study group are presented in Tab. 2.

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Allergen	Percentage of sensitised children	AsIgE concentration [mean ± *SD*]
Bos d 4	72.2%	7.3 ± 11.9 kU/L
Bos d 5	77.8%	7.8 ± 12.3 kU/L
Bos d 8	94.4%	13.9 ± 15.8 kU/L

Tab. 3. Percentages of children allergic to individual components in the study group and levels of specific IgE

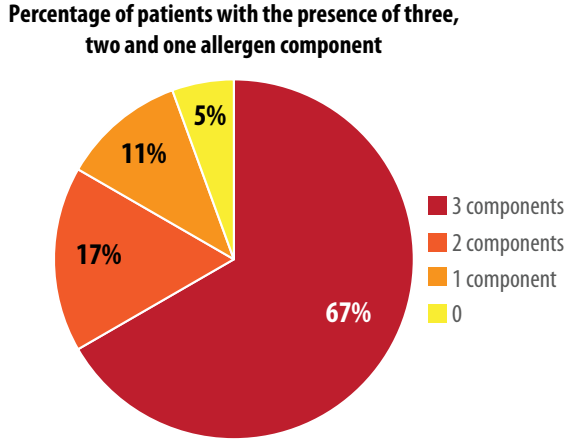

Fig. 2. Distribution of individual allergenic components of cow's milk protein in the study group

	Bos d 4	Bos d 5	Bos d 8
Correlation index (*p*)	0.49*	0.37	0.26
* Statistically significant.			

Tab. 4. Correlation between the duration of clinical symptoms and the concentration of antibodies against particular components of CMP

Cow's milk protein components

The average concentration of IgE antibodies directed against cow's milk protein was 13.2 ± 14.8 kU/L. The most common cow's milk protein allergen was casein (Bos d 8) (Tab. 3).

In the analysed group of children, the presence of three types of antibodies against allergenic components of cow's milk protein was observed in 12 children (Fig. 2).

Component diagnosis and clinical manifestations

A statistically significant relationship was observed between the concentration of asIgE against Bos d 4 protein and the duration of clinical symptoms of allergy to cow's milk protein. For the other components, this relationship was not significant (Tab. 4).

No significant differences were observed in the levels of antibodies against individual allergenic components of cow's milk protein in the group of children with asthma and in the group with atopic dermatitis (AD) (Tab. 5).

In the conducted analysis, no statistically significant differences were found between the allergenic components of cow's milk protein in the group of children with anaphylaxis in the past and those with gastrointestinal symptoms (Tab. 6).

DISCUSSION

For many years, there has been a heated debate in the literature regarding the actual prevalence of food allergy. The problem of overdiagnosis of food allergy and its clinical, dietary, and economic consequences is frequently emphasised[11,12]. Difficulties in conducting a reliable epidemiological study on food allergy are the result of diverse methodologies, differences in respondents' place of residence, and thus in their exposure to food, as well as the problem of varying definitions of allergy[13]. An additional problem is related to different clinical symptoms of food allergies. Symptoms of cow's milk allergy affect the gastrointestinal tract, respiratory tract, and skin; they can also involve multiple body organs and manifest in the form of anaphylactic shock[6]. The overestimation of the problem of food allergy also results from the possibility of finding positive asIgE in people who do not present symptoms of allergy. A large HealthNuts study of 3,000 infants in Australia documented allergy to peanut in 9% of children, to egg in

	Asthma (+)	Asthma (–)	*p*
Bos d 4 [kU/L]	7.9 ± 13.9	2.10 ± 2.24	0.26
Bos d 5 [kU/L]	9.7 ± 14.3	1.59 ± 1.16	0.13
Bos d 8 [kU/L]	18.2 ± 18.2	6.83 ± 9.59	0.13
Milk extract [kU/L]	18.1 ± 17.3	7.14 ± 8.43	0.12
	AD (+)	AD (–)	*p*
Bos d 4 [kU/L]	4.1 ± 6.8	7.18 ± 15.4	0.57
Bos d 5 [kU/L]	5.7 ± 8.6	6.73 ± 15.3	0.86
Bos d 8 [kU/L]	16.2 ± 16.2	8.38 ± 14.7	0.32
Milk extract [kU/L]	16.4 ± 14.7	8.31 ± 14.6	0.27
AD – atopic dermatitis.			

Tab. 5. Levels of antibodies against different components of cow's milk protein in a group of children with asthma and AD

	Anaphylaxis (+)	Anaphylaxis (–)	*p*
Bos d 4 [kU/L]	3.8 (0.10–4.5)	1.3 (1.03–32.4)	0.73
Bos d 5 [kU/L]	3.1 (0.67–2.8)	14.0 (0.23–27.6)	0.84
Bos d 8 [kU/L]	11.5 (1.07–11.9	17.4 (1.35–32.7)	0.62
Milk extract [kU/L]	11.6 (1.23–14.8)	13.2 (1.35–32.7)	0.69
	Gastrointestinal symptoms (+)	Gastrointestinal symptoms (–)	*p*
Bos d 4 [kU/L]	0.7 (0.1–1.3)	1.5 (0.5–5.3)	0.45
Bos d 5 [kU/L]	1.8 (0.1–27.6)	1.5 (0.4–2.9)	0.96
Bos d 8 [kU/L]	10.2 (1.57–32.7)	3.3 (0.8–20.3)	0.43
Milk extract [kU/L]	11.2 (1.0–32.4)	5.1 (1.25–19.9)	0.61

Tab. 6. Levels of antibodies against different components of cow's milk protein in a group of children with history of anaphylaxis and gastrointestinal symptoms

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17% of children, to sesame in 2.5% of children, while the oral challenge test was positive in only 3%, 9%, and 0.7% of infants tested, respectively[14].

The EuroPrevall European study found that cow's milk protein allergens are the most common cause of food allergy in children. It has been estimated that CMP allergy occurs in 0.54% of children under the age of two, and in Poland the proportion is 0.65%[15].

In the study conducted at the Department of Paediatrics, Paediatric Nephrology and Allergology of the Military Institute of Medicine in Warsaw, component diagnostics (ALEX) was performed in 100 children, of whom 18% were confirmed to have an IgE-dependent allergy to cow's milk allergy. It should be emphasised that only children with symptoms of allergy were included in the study. This result confirms that cow's milk allergy is one of the most common food allergies in children[6,13,15,16]. A similar result was obtained by American researchers who, out of 3,218 children with food allergy studied, diagnosed allergy to cow's milk in 657 children, which constituted 19.9%[17,18].

In the study by Majsiak and Buczyłko, asIgE against milk extract and its four molecules (α-lactalbumin, β-lactoglobulin, BSA, and casein) was determined in 2,120 patients with diagnosed allergies. AsIgE antibodies to CMP were detected in 98 of them, which accounted for 4.62% of all subjects. Analysing the results in terms of asIgE determined against individual milk allergen molecules, a total of 570 positive results against BSA (26.89%) were recorded, which was the largest percentage among allergies to cow's milk proteins. In contrast, in our study no antibodies against BSA were found, so the molecule was not included in the statistical calculations. Majsiak and Buczyłko showed 427 positive results against α-lactalbumin (20.14%), while in their own study the presence of these antibodies was found in 13/18 subjects (72.2%). The study by Majsiak and Buczyko showed 11.23% positive results for β-lactoglobulin versus 77.7% in our own study. Also, Majsiak and Buczyłko confirmed 64 positive results for casein (3.02%), whereas in our study, casein was the most common component of cow's milk (found in 94.4% of children with cow's milk protein allergy). Such large differences in the reported results may be due to the fact that adults were also included in the cited study[19].

Similar findings were published by Adamska et al. The authors documented the highest prevalence of asIgE against β-lactoglobulin – 72.2%, followed by α-lactalbumin – 66%, and casein – 50%[19,20]. Similar to the results of our study, no asIgE against BSA was found. Many authors emphasise the importance of casein, α-lactalbumin and β-lactoglobulin as sensitive and specific markers of cow's milk allergy, and point to the lack of correlation between the coexistence of asIgE to CMP and milk allergy at the same time[21]. The study by Adamska et al. was additionally extended to include an assessment of asIgE levels against cow's milk molecules in the absence of antibodies against milk extract. In this population, the results regarding antibodies to BSA were found to differ significantly. In this group of 64 children, the most positive results were obtained for β-lactalbumin – 65.62% (n = 42), followed by BSA – 56.25% (n = 36), α-lactalbumin – 45.31% (n = 29), and finally casein at 1.56% (n = 1).

One of the most common associated diseases in young children with cow's milk allergy is AD.

In our own study, the presence of IgE antibodies against all components of Bos d 4, Bos d 5, Bos d 8, and against the Bos d-milk extract was documented in children with food allergy manifested as AD. It should be noted that, according to the current approach, AD is a chronic inflammatory skin disease; therefore, an increased permeability of the epithelium is a gateway to the penetration of allergens. Therefore, many anti-food antibodies are found in patients with AD, and only a small percentage of them respond to these foods by exhibiting clinical symptoms. These patients require confirmation of allergy by oral challenge[22].

Similarly to AD, no significant differences were observed in the concentrations of individual allergenic components of cow's milk protein in the group of children with asthma (Tab. 4). Rarely, CMPA is manifested by isolated asthma. Recurrent obstruction and wheezing may be due to IgE-mediated cow's milk allergy in infants. Conversely, in older children, bronchospasm in response to cow's milk is one of the components of anaphylaxis[6,23,24].

CMPA can also manifest itself with gastrointestinal symptoms. The most common symptoms are regurgitation, vomiting, and diarrhoea, including stools with blood and mucus, as well as abdominal pain[8]. In our study, out of 18 children with cow's milk allergy, one child had bloody stools, four children reported colic, four children had regurgitation, and six children experienced abdominal pain (Fig. 1). There were also no statistically significant differences between the allergenic components of cow's milk protein in the group of children with gastrointestinal symptoms (Tab. 5). However, it should be emphasized that gastrointestinal symptoms (in the absence of other typical symptoms of IgE-mediated allergy) are rarely caused by IgE-mediated allergy. More commonly, they result from delayed type reactions[25].

Anaphylaxis is the clinical phenotype of food allergy that occurs most rapidly after food ingestion and can be directly life-threatening to the child. The mechanism of anaphylaxis is usually, but not always, IgE-mediated. In the population of Polish children, the food allergens most often causing anaphylactic reactions are cow's milk proteins[18]. Similarly in other European countries – a systematic review of 65 studies conducted in 41 European countries found that food anaphylaxis in children was caused predominantly by cow's milk proteins – 4%[26]. A UK study has documented a steadily increasing number of hospitalisations and deaths due to food anaphylaxis over the past two decades. Cow's milk protein allergen was responsible for 26% of school-aged children's deaths[26,27].

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In our own study, in a group of children allergic to CMP, a total of 13 children had a history of anaphylaxis, which is a great percentage in this population. It should be noted that the study was conducted only among hospitalised children, which probably contributed to such a high result. In children with a history of anaphylaxis, allergic to cow's milk protein, the highest concentration of asIgE antibodies against the Bos d 8 component was determined, on average 11.5 kU/L, followed by 3.8 kU/L against Bos d 4, and 3.1 kU/L against Bos d 5. This confirms the most important role of casein (Bos d 8) in anaphylaxis. Despite the lack of statistical significance in this respect, the results presented in Tab. 5 indicate that both in the group of children with asthma, gastrointestinal symptoms and AD, the highest levels of asIgE against the Bos d 8 component were found, followed by antibodies against Bos d 5 and Bos d 4, while only in the group of children with a history of anaphylaxis there was a slightly higher concentration of asIgE against Bos d 4 compared to Bos d 5.
The natural history of food allergy shows that cow's milk allergy is often outgrown – in up to 80–90% of allergic children, the symptoms disappear by the end of 3 years of age[18,28]. This is confirmed by our own study, in which children aged 1–10 years were recruited, but the majority were children aged 1–6 years (63%).
The authors of the study also showed a statistically significant relationship between the concentration of Bos d 4 and the duration of clinical symptoms of allergy to cow's milk protein. In the case of the other components, this relationship was not significant (Tab. 3). This does not confirm the importance of monitoring the concentration of asIgE against Bos d 8 to monitor the course of sensitisation and the acquisition of immunotolerance to cow's milk proteins, as previously described in the literature[8]. These observations require further research, and our study is still ongoing.

CONCLUSIONS

The results of the presented study confirm that molecular diagnostics plays a very important role in confirming food allergy in children. The demonstration of casein (Bos d 8) as the most common sensitising component and the correlation between the concentration of antibodies against Bos d 4 and the duration of symptoms suggest a key role of these two components in the pathogenesis of CMPA. More studies must be carried out to confirm our data with larger samples of patients so that the results exhibit more consistency.
The limitations of the study were: small group size, fairly large age range, and incomplete information about the patients' diet (whether any form of milk had already been introduced).

Conflict of interest

The authors report no financial or personal relationships with other individuals or organisations that could adversely affect the content of the publication and claim ownership of this publication.

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Author contributions

Original concept of study: AR, AT, AB, BK. Collection, recording and/or compilation of data: AR, ALO, AES, MP, AB. Analysis and interpretation of data: AR, ALO, AT, AES, AB. Writing of manuscript: AR, ALO. Critical review of manuscript: ALO, AT, BK. Final approval of manuscript: AR, AT, BK.

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