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Assessment of the Efficacy of Molecular Diagnostics in Children with Cow's Milk Protein Allergy: Results of Observation

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Abstract

Introduction. Cow's milk protein allergy is an urgent problem in pediatrics and affects from 0.5% to 3% of infants under one year. IgE-dependent form is present in 60% of children with cow's milk protein allergy and occurrence of symptoms is usually observed from several minutes to several hours from the time of allergen consumption. Molecular diagnostics opens many new opportunities for diagnosing different forms of allergy and prognosticating efficacy of treatment.
Purpose. To assess the effectiveness of molecular diagnostics in children with cow's milk allergy in different therapeutic tactics.
Materials and methods. Inclusion criteria of the investigation: children from six month to three years old, positive skin test to milk (papule ≥3 mm with prick method) and specific IgE to milk ≥0.35 kUa/L in the serum, positive oral provocation test (OPT). The children had clinical changes associated milk consumption. The research did not include patients with anaphylaxis in anamnesis, present severe concomitant or autoimmune diseases, contraindications to OPT. All 60 patients and their parents or custodians gave written consent to participation in the investigation. Assessment of a molecular profile was performed for detecting major components of milk (Bos d 8 – casein, Bos d 5 – β-Lactoglobulin, Bos d 4 – α-lactalbumin), minor (Bos d 6 – bovine serum albumin) and cross-reactive ones with serum albumin (Fel d 2 – felis domesticus allergen 2, Can f 3 – canis familiaris allergen 3).
Results. The study cohort consisted of 60 cow's milk allergic patients, randomized into two groups, compared based on age (p=0.88) and gender (p=0.3).
Analysis of a molecular profile of milk components permitted to detect the highest indices in the group of major molecules: Bos d 4 (2.10 kUa/L in specific oral tolerance group and 2.00 kUa/L in elimination diet group), Bos d 8 (1.90 kUa/L and 1.55 kUa/L respectively) and Bos d 5 (0.85 kUa/L and 1.60 kUa/L respectively).
A skin prick test papule diameter had a statistically significant correlation with sIgE to milk (r=0.51) and Bos d 8 (r=0.44). Bos d 8 had a statistically significant correlation with

papule diameter (r=0.44), as well as with Can f 3 (r=0.39) and Bos d 4 (r=0.28). Bos d 6 had a statistically significant correlation with Fel d 2 (r=0.64) and Can f 3 (r=0.44). The obtained data confirm that bovine serum albumin indicates cross-reactivity with animals, particularly a cat and a dog. The children were observed for three years.

Conclusions. 1. Assessment of a molecular profile is important for diagnosis and treatment of cow's milk protein allergy irrespective of a choice of therapeutic tactics – specific oral tolerance induction or elimination diet. Identification of individually significant food allergens makes it possible to determine individual indications and contraindications for oral provocation test and select the most appropriate treatment, in particular, specific oral tolerance induction as a disease-modifying immunotherapy. 2. Cow's milk protein allergy is basically accompanied by the presence of major molecules, in particular α-lactalbumin (Bos d 4), casein (Bos d 8) and β-lactoglobulin (Bos d 5). 3. Casein (Bos d 8) is a major thermostable component of cow's milk protein and has a statistically significant positive correlation with a papule diameter (r=0.44), Can f 3 (r=0.39) and Bos d 4 (r=0.28). 4. Bovine serum albumin (Bos d 6) is a minor component of milk and has a statistically significant positive correlation with Fel d 2 (r=0.64) and Can f 3 (r=0.44), which indicates cross-reactivity with animals, particularly a cat and a dog.

Keywords: cow's milk protein allergy, molecular diagnostics, children

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Оценка эффективности молекулярной диагностики у детей с аллергией к белкам коровьего молока: результаты собственного наблюдения

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Резюме

Введение. Аллергия к белкам коровьего молока является актуальной проблемой в педиатрии и охватывает от 0,5% до 3% детей в возрасте до 1 года. У 60% детей с аллергией к белкам коровьего молока наблюдаются IgE-зависимая форма и появление

симптомов преимущественно от нескольких минут до нескольких часов с момента потребления аллергена. Молекулярная диагностика открывает много новых возможностей для диагностики различных форм аллергии и прогнозирования эффективности лечения.

Цель. Оценить эффективность молекулярной диагностики у детей с аллергией к белкам коровьего молока при различных терапевтических тактиках.

Материалы и методы. Критерии включения в исследование: дети в возрасте 6 месяцев – 3 лет, положительный кожный прик-тест к молоку (папула ≥3 мм методом прик) и уровень специфических IgE к молоку ≥0,35 kUa/L в сыворотке крови, положительная оральная провокационная проба (ОПП). У детей наблюдались клинические изменения при употреблении молока. В исследование не привлекали пациентов, у которых в анамнезе была анафилаксия, имелись тяжелые сопутствующие или аутоиммунные заболевания, противопоказания к проведению ОПП. Все 60 пациентов, а также их родители или опекуны дали письменное согласие на участие в исследовании. Оценка молекулярного профиля проводилась по выявлению мажорных компонентов молока (Bos d 8 – казеин, Bos d 5 – β-лактоглобулин, Bos d 4 – α-лактальбумин), минорного (Bos d 6 – бычий сывороточный альбумин) и перекрестно-реактивных с альбумином (Fel d 2 – аллерген кошки домашней 2, Can f 3 – аллерген собаки домашней 3).

Результаты. Когорта исследования состояла из 60 пациентов с аллергией на коровье молоко, рандомизированных на две группы, сопоставимые по возрасту (p=0,88) и полу (p=0,3). Анализ молекулярного профиля компонентов молока позволил выявить самые высокие уровни показателей в группе мажорных молекул: Bos d 4 (2,10 kUa/L в группе детей, получающих специфическую оральную индукцию толерантности, и 2,00 kUa/L в группе детей, находящихся на элиминационной диете), Bos d 8 (1,90 kUa/L и 1,55 kUa/L соответственно) и Bos d 5 (0,85 kUa/L и 1,60 kUa/L соответственно).

Диаметр папулы кожного прик-теста имел статистически значимую корреляционную связь с sIgE к молоку (r=0,51) и Bos d 8 (r=0,44). Bos d 8 имел статистически значимую корреляционную связь с диаметром папулы (r=0,44), а также с Can f 3 (r=0,39) и Bos d 4 (r=0,28). Bos d 6 имел статистически значимую корреляционную связь с Fel d 2 (r=0,64) и Can f 3 (r=0,44). Полученные данные подтверждают, что бычий сывороточный альбумин свидетельствует о перекрестной реактивности с аллергенами животных, в частности кота и собаки. Детей наблюдали в течение трех лет.

Выводы. 1. Оценка молекулярного профиля важна для диагностики и лечения аллергии к белкам коровьего молока, независимо от выбора терапевтической тактики – специфической оральной индукции толерантности или элиминационной диеты. Выявление индивидуально значимых пищевых аллергенов дает возможность определения индивидуальных показаний и противопоказаний для проведения оральных провокационных проб и выбора наиболее оптимального лечения, в частности специфической оральной индукции толерантности как болезнь-модифицирующей иммунотерапии. 2. Аллергия к белкам коровьего молока сопровождается наличием мажорных молекул, в частности α-лактальбумина (Bos d 4), казеина (Bos d 8) и β-лактоглобулина (Bos d 5). 3. Казеин (Bos d 8) является мажорным термостабильным компонентом белка коровьего молока и имеет статистически значимую положительную корреляционную связь с диаметром папулы (r=0,44), Can f 3 (r=0,39) и Bos d 4 (r=0,28). 4. Бычий сывороточный альбумин (Bos d 6) является минорным

компонентом молока и имеет статистически значимую положительную корреляционную связь с Fel d 2 (r=0,64) и Can f 3 (r=0,44), что свидетельствует о перекрестной реактивности с животными, в частности котом и собакой.
Ключевые слова: аллергия к белкам коровьего молока, молекулярная диагностика, дети

■ INTRODUCTION

Currently, molecular diagnostics opens many new opportunities for diagnosing different forms of allergy and prognosticating efficacy of treatment.

Cow's milk protein allergy is a topical issue in pediatrics and affects from 0.5% to 3% of children under one year of age [1, 2]. IgE-dependent form is found in 60% of children with cow's milk protein allergy and development of symptoms is usually observed from a few minutes to several hours from the moment of allergen consumption [3, 4].

Over 40 proteins are identified in milk, which can be classified into two groups of various chemical and physical compositions – casein (80%) and whey proteins (20%). Whey includes α-lactalbumin and β-lactoglobulin, which are produced by mammary gland and proteins, which enter from bloodstream – bovine serum albumin, lactoferrin, immunoglobulins [5, 6]. Polysensitization to different proteins is detected in 75% of children with cow's milk protein allergy [7]. Casein, β-lactoglobulin and α-lactalbumin are common in development of cow's milk protein allergy.

Molecular characteristics of the major milk proteins are given in Table 1 [8].

Clinical manifestations, their intensity, response to treatment largely depend on the type of cow's milk protein, which provoked sensitization.

Symptoms of cow's milk protein allergy can vary depending on affected target organ (skin, digestive tract, respiratory tract, systemic manifestations) [9]. While recording anamnesis, it is necessary to conduct complex assessment of complaints and a child's general condition.

There are different approaches to treatment of cow's milk protein allergy: elimination and specific oral tolerance induction (SOTI). In fact, most doctors choose elimination, but

Table 1
Molecular characteristics of the major milk proteins (Valenta R. et al.) [6]

Milk protein		Concentration in milk (g/L)	Molecular mass (kDa)
20% of whey (approximately 5 g/L)			
β-lactoglobulin (Bos d 5)	10%	3–4	18.3
α-lactalbumin (Bos d 4)	5%	1–1.5	14.2
Immunoglobulins Bos d 7	3%	0.6–1.0	150
Bovine serum albumin (Bos d 6)	1%	0.1–0.4	66.3
Lactoferrin	Traces	0.09	80
80% total casein (approximately 30 g/L)			
αS1-casein	32%	12–15	23.6
αS2-casein	10%	3–4	25.2
β-casein	28%	9–11	24.0
κ-casein	10%	3–4	19.0

SOTI is a new and perspective opportunity [8]. This therapy method is a relatively new, perspective method of treating IgE-dependent form of food allergy, which implies regular introduction of a product from lower to higher doses for a prolonged period (specific immunotherapy) to achieve complete tolerance of the product and administration of liberal diet.

Elimination of milk from a diet deprives a child of essential nutritive components. Dairy products are necessary and valuable elements of a child's daily nutrition, especially at early age. They contain nutrition elements important for the body, which, in balanced amounts, are easily absorbed in the body (proteins, fats, carbohydrates, vitamins, minerals, enzymes etc.) [10]. Dairy products contain large amount of calcium, which plays an indispensable role in many physiological processes: participation in building the skeleton and tooth mineralization, maintenance of proper heart activity (heart rate and heart muscle strength), appropriate blood clotting, activity of muscles and nervous system [11]. This significance explains the importance of SOTI and a need in studying and implementing this treatment method into clinical practice by doctors-allergists.

■ PURPOSE OF THE STUDY

To assess the effectiveness of molecular diagnostics in children with cow's milk allergy in different therapeutic tactics.

■ MATERIALS AND METHODS

Criteria of inclusion in the research: children aged from six months to three years, positive skin test to milk (papule ≥3 with prick method) and specific IgE to milk ≥0.35 kUa/L, positive oral provocation test (OPT). The research did not include patients with anaphylaxis in anamnesis, present severe concomitant or autoimmune diseases, contraindications to OPT. All patients / their parents or custodians gave written consent to participation in the investigation.

The study cohort consisted of 60 cow's milk allergic patients, randomized into two groups: group A (n=30) with SOTI, and group B (n=30) treated by milk elimination diet (ED).

For diagnosis, all patients underwent skin-prick testing for the most common allergens. Blood samples were collected to test total IgE and specific IgE levels to individual cow's milk allergens α-lactalbumin – Bos d 4, β-lactoglobulin – Bos d 5, bovine serum albumin – Bos d 6 and casein – Bos d 8. In addition, potential cross-reactive inhalant allergens were tested: Fel d 2 – cat and Can f 3 – dog (ALEX 1).

In general, three visits to a medical establishment were planned: at the beginning of the investigation, in 12 months and 36 months after therapy onset.

Methods of statistics

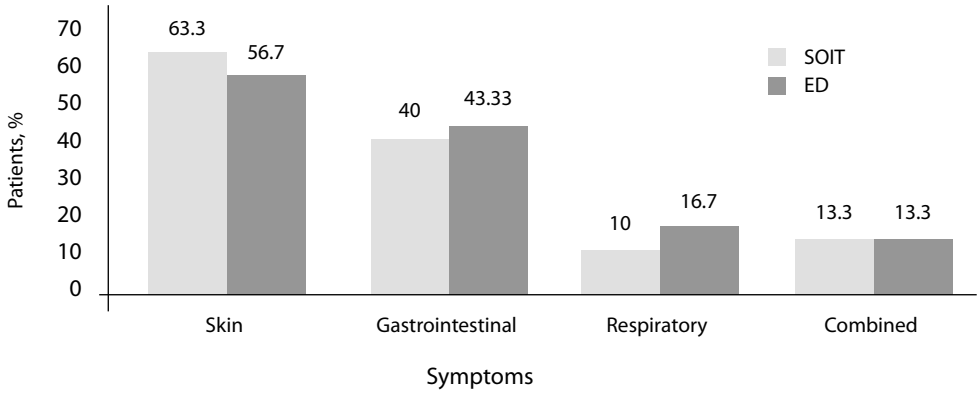
Description of the results was performed by means of mediana, 25 and 75 percentiles: Me [25%; 75%], as well as a fraction (%) and 95% confidence interval. Comparison of indices of two discrete totals was conducted by Mann – Whitney U-test, comparison of fractions – by means of χ^2 test. Difference between groups was significant at values $p<0.05$. Determination of correlations between quantitative values of variants was conducted by Spearman's correlation. Software RStudio v.1.1.442 was used for statistical analysis of the obtained data.

RESULTS

Experimental groups were compared by age (p=0.88) and gender (p=0.3).

In patients of both groups, skin symptoms (rash, swelling, itching) dominated (63.3% in SOTI group and 56.7% in ED group; p=0.60), the second place – gastrointestinal (nausea, stomachache, vomiting and diarrhea) (40% and 43.33% respectively; p=0.79), the third – respiratory symptoms (sneezing, itching of the nose, wheezing, rhinorrhea, dry cough, breathlessness) (10% and 16.7% respectively; p=0.45). In 13.3% (p=1) symptoms were combined (Figure).

Signs of skin symptoms included rash, swelling, itching, redness of the outer skin. Gastrointestinal manifestations were characterized by nausea, stomachache, vomiting and diarrhea, deficit body's mass. Respiratory symptoms were manifested by sneezing, itching of the nose, wheezing, rhinorrhea, dry cough, breathlessness. Combination of predominantly skin and other symptoms was observed in combined symptoms.



Distribution of symptoms in patients

Table 2
Characteristics of indices at the beginning of the investigation

Index	Groups		P
	SOTI	ED	
Amount of consumed milk during oral provocation test, g	1.11 [0.73; 1.49]	1.14 [0.78; 1.37]	0.67
Skin test to milk, papule (mm)	5.75 [4.78; 6.68]	5.50 [4.70; 6.50]	0.5538
Specific IgE to milk, IU/ml	3.22 [2.36; 5.68]	3.45 [2.20; 5.14]	0.8650
Total IgE, IU/ml	80.00 [41.00; 203.75]	44.50 [18.93; 138.00]	0.1008
Bos d 8, kUa/L	1.90 [0.78; 2.95]	1.55 [0.52; 2.10]	0.2034
Bos d 5, kUa/L	0.85 [0.13; 2.13]	1.60 [0.09; 2.78]	0.4768
Bos d 4, kUa/L	2.10 [1.23; 2.30]	2.00 [0.28; 2.10]	0.1015
Bos d 6, kUa/L	0.75 [0.09; 2.08]	0.40 [0.00; 1.18]	0.1629
Fel d 2, kUa/L	0.39 [0.00; 1.95]	0.00 [0.00; 0.86]	0.0767
Can f 3, kUa/L	0.46 [0.00; 1.88]	0.31 [0.00; 1.68]	0.6140

Note: p – value p by the results of calculation of Mann – Whitney test.

At the beginning of the investigation, all patients were subjected to complex laboratory investigation, and oral provocation tests were performed in dynamics in 12 and 36 months.

Characteristics of indices at the beginning of the investigation in experimental groups are presented in Table 2.

Data of table 2 demonstrate that, on average, patients in both groups tolerated 1 g of cow's milk protein at the beginning of the research. Assessment of a molecular profile was conducted for revealing major components of milk (Bos d 8, Bos d 5, Bos d 4), minor (Bos d 6) and cross-reactive ones with whey albumin (Fel d 2, Can f 3). It should be emphasized that the highest indices were recorded in the group of three major molecules: Bos d 4 (2.10 kUa/L in group SOTI and 2.00 kUa/L in group ED), Bos d 8 (1.90 kUa/L and 1.55 kUa/L respectively) and Bos d 5 (0.85 kUa/L and 1.60 kUa/L respectively).

Assessment of a child's tolerance to cow's milk proteins (g) was also performed. A profile of patient's tolerance was divided into three groups: does not tolerate (0 – 0.99 g), partially tolerates (1 – 3.99 g), and completely tolerates (over 4 g). Assessment of the results of tolerance formation in experimental groups is presented in Table 3.

At the beginning of the research, patients of both groups could not tolerate dairy products, among them 43.3% hardly tolerated, and 56.7% partially tolerated.

In SOTI patients, complete tolerance could be achieved in 12 months in 66.7% (20) of children, and in 96.7% (29) – in 36 months. Partial tolerance of cow's milk proteins was observed only in one patient at the end of the investigation. It should be mentioned that this patient tolerated 3.705 g of protein, which allowed consuming 140 ml of cow's milk daily without symptoms.

In ED patients, complete tolerance could not be achieved in any patient in 12 months, partial tolerance developed in 96.7% (29) of children and 3.33% (1) did not have milk tolerance. In 36 months, partial tolerance was observed in 83.3% (25), and complete tolerance – in 16.7% (5).

Thus, the process of forming milk tolerance occurred much more rapidly and in larger amount in SOTI group and a significant difference was observed in dynamics in 12 months and 36 months from the onset of therapy.

Amount of protein, which patients tolerated, depending on a group and visit is given in Table 4.

Table 3
Assessment of the results of tolerance formation in children of experimental groups

Group	Specific oral tolerance induction			Elimination diet		
	At the beginning of the investigation	In dynamics in 12 months	In dynamics in 36 months	At the beginning of the investigation (p=1)*	In dynamics in 12 months (p<0.001)*	In dynamics in 36 months (p<0.001)*
Does not tolerate	13 (43.3%)	0	0	13 (43.3%)	1 (3.3%)	0
Partially tolerates	17 (56.7%)	10 (33.3%)	1 (3.3%)	17 (56.7%)	29 (96.7%)	25 (83.3%)
Completely tolerates	0	20 (66.7%)	29 (96.7%)	0	0	5 (16.7%)

Note: * value p in comparison with a corresponding level of tolerance in SOTI group.

Table 4
Amount of protein, which patients tolerated depending on a group and visit

Group	Specific oral tolerance induction			Elimination diet		
	At the beginning of the investigation	In dynamics in 12 months	In dynamics in 36 months	At the beginning of the investigation	In dynamics in 12 months	In dynamics in 36 months
Does not tolerate	0.51 [0.38; 0.75]	0	0	0.68 [0.60; 0.86]	0.91 [0.91; 0.91]	0
Partially tolerates	1.43 [1.25; 1.53]	**2.92 [2.26; 3.54]**	3.70 [3.70; 3.70]	1.26 [1.16; 2.25]	**1.69 [1.25; 2.08]***	2.54 [2.08; 2.80]
Completely tolerates	0	4.44 [4.20; 5.26]	6.01 [6.01; 6.01]	0	0	6.01 [6.01; 6.01]

Note: * p<0.05 in comparing values of a corresponding tolerance degree in SOTI group by the results of Mann – Whitney criterion calculations.

Data in table 4 demonstrate that in the patients who received SOTI, there was a significant difference in the group of partial protein tolerance, which constituted 2.92 g, in 12 months from the onset of therapy compared with elimination diet group, where this index was 1.69. It should be emphasized that average amount of protein in SOTI group increased by 1.82 g compared with an initial index at the beginning of the research and by 0.33 in ED group.

Table 5 demonstrates coefficients of Spearman's rank correlation, which show the degree of correlation between investigated signs.

Coefficients of Spearman's correlation, which indicate statistically significant correlation between two corresponding signs, are highlighted in the table. In particular, the larger the size of a papule, the higher its correlation was with an investigated positive index. Thus, a papule diameter had a statistically significant correlation with sIgE to milk (r=0.51) and Bos d 8 (r=0.44).

Bos d 8 had a statistically significant positive correlation with a papule diameter (r=0.44), as well as with Can f 3 (r=0.39) and Bos d 4 (r=0.28).

Bos d 6 had a statistically significant positive correlation with Fel d 2 (r=0.64) and Can f 3 (r=0.44). The obtained data confirm that bovine serum albumin proves cross-reactivity with animals, in particular, a cat and a dog.

Table 5
Coefficients of Spearman's rank correlation in children between investigated signs

	Skin	sIgE to milk	IgE Total	Bos.d8	Bos.d5	Bos.d4	Bos.d6	Cat	Dog
Skin	1.00	0.51	0.19	0.44	–0.05	0.08	–0.06	0.02	0.05
IgE Milk	0.51	1.00	0.08	0.25	–0.01	–0.15	0.01	–0.09	–0.16
IgE Total	0.19	0.08	1.00	–0.03	0.08	–0.02	0.09	–0.06	–0.09
Bos d 8	0.44	0.25	–0.03	1.00	–0.02	0.28	–0.09	–0.06	0.39
Bos d 5	–0.05	–0.01	0.08	–0.02	1.00	–0.02	0.09	0.16	–0.15
Bos d 4	0.08	–0.15	–0.02	0.28	–0.02	1.00	–0.05	0.07	0.40
Bos d 6	–0.06	0.01	0.09	–0.09	0.09	–0.05	1.00	0.64	0.44
Fel d 2	0.02	–0.09	–0.06	–0.06	0.16	0.07	0.64	1.00	0.34
Can f 3	0.05	–0.16	–0.09	0.39	–0.15	0.40	0.44	0.34	1.00

Table 6
Characteristics of patients, who achieved complete tolerance of cow's milk protein (fraction and 95% confidence interval)

Group of complete tolerance	SOTI	
Index	**In dynamics in 12 months (n=20)**	**In dynamics in 36 months (n=29)**
Papule diameter ≥5 mm	55.0% (33.2–76.8)	65.5% (48.2–82.8)
Total IgE ≥60 IU/ml	60.0% (38.5–81.5)	62.1% (44.4–79.7)
Bos d 8 ≥0.35 kUa/L	80.0% (62.5–97.5)	86.2% (73.7–98.8)
Bos d 5 ≥0.35 kUa/L	70.0% (49.9–90.1)	65.5% (48.2–82.8)
Bos d 4 ≥0.35 kUa/L	90.0% (76.8–100)	86.2% (73.7–98.8)
Bos d 6 ≥0.35 kUa/L	65.0% (44.1–85.9)	55.2% (37.1–73.3)
Fel d 2 ≥0.35 kUa/L	60.0% (38.5–81.5)	51.7% (33.5–69.9)
Can f 3 ≥0.35 kUa/L	55.0% (33.2–76.8)	55.2% (37.1–73.3)

The group of children, who developed complete tolerance, is of utmost importance, since it is the main criterion of successful treatment of cow's milk protein allergy. We have analyzed changes in clinically important indices in dynamics: a papule diameter over 5 mm, total IgE IU/ml and molecular profile of components to milk (Bos d 8, Bos d 5, Bos d 4, Bos d 6, Fel d 2, Can f 3) in concentration ≥0.35 kUa/L (Table 6).

Data in table 6 permit to analyze that in children of SOTI group, who managed to achieve complete tolerance in 12 months from the onset of treatment, clinically significant indices were Bos d 4 (90%), Bos d 8 (80%), Bos d 5 (70%), Bos d 6 (65%), Fel d 2 (60%), Can f 3 (55%), papule diameter (55%). In 36 months from the onset of treatment, clinically significant indices in this group were Bos d 4 (90%), Bos d 8 (86.2%), Bos d 5 (65.5%), Fel d 2 (51.7%), Can f 3 (55.2%), papule diameter (65.5%).

Any patient from ED group did not achieve complete tolerance in 12 months of monitoring, thus, discrete indices could not be determined in this group. In 36 months from the onset of treatment, clinically significant indices were Bos d 4 (40%), Bos d 5 (20%), Fel d 2 (20%), Can f 3 (40%) in ED group, individuals of which managed to achieve complete tolerance.

■ DISCUSSION

Study of a molecular profile permitted to assess peculiarities of the disease course in children with cow's milk protein allergy depending on a choice of therapeutic tactics – specific oral tolerance induction or elimination diet. Knowing composition of molecules and their characteristics, it is possible to explain mechanisms of symptom formation exactly, choose schemes of treatment and form correct predictions [12].

We have analyzed basic characteristics of each molecular component of milk, since clinical manifestations of symptoms, their intensity, and response to treatment largely depend on a type of cow's milk protein, which provoked sensitization.

Casein (Bos d 8) is a stable protein, which does not change its configuration under the influence of high temperature and hydrochloric acid. It is the main milk protein (constitutes 75–80%), which is found in milk of all mammals, and, in case of allergy, cross-reaction can develop to milk of all other species. Casein family has different primary structure and functions. Three of them (αS1-casein, αS2-casein, β-casein) are calcium-sensitive whereas κ-casein does not possess such property. Depending on a type of casein sensitization,

there is specificity and intensity of a clinical response. Sensitization to αS1-casein and αS2-casein is present in all patients with allergy, κ-casein – 91.7%; β-casein – 66.7% [8].

Proteins of casein group are highly resistant to heating, thus, pasteurization and boiling processes have little effect on them. It is associated with the fact that basic epitopes have linear and non-conformational features. Casein has a lack of well-defined structure and a significant number of secondary and tertiary structures. Protein molecules form the so-called casein micelle, in which hydrophobic central and hydrophilic outer parts are differentiated. It has six basic and three secondary IgE-binding regions and five basic and one secondary IgG-binding region. It was assumed that the majority of linear IgE-epitopes in casein can promote stable allergy [4].

Children having stable allergy to milk have considerably higher level of specific antibodies IgE to linear epitopes of αS1-casein and β-casein than children with acquired tolerance to milk proteins do. Casein is a protein, which allows, in most cases, differentiating persistent and transitory forms of allergy. The chances "to outgrow" allergy are low [6].

β-lactoglobulin (Bos d 5) – is one of the main cow's milk allergens belonging to lipocalin family. It is the most common whey protein (constitutes 50% of its fraction) and approximately 10% of cow's milk.

Protein is a mixture of dimers and monomers, besides, the number of monomers increases on heating to 70 °C. β-lactoglobulin is a lipid-binding protein and a transport vehicle for vitamin A. Two forms of β-lactoglobulin have been studied, which differ by two point mutations, thus, there is different intensity and duration of response. A molecule has many binding epitopes, some of which have short linear sequence, while others – large fragments with three-dimensional structure. Three main groups of epitopes have been distinguished, which have high binding property. The majority of epitopes are a marker of persistent allergy to cow's milk protein [13]. Although most formulas are attempted to adjust to breast milk by composition, β-lactoglobulin is detected in breast milk, but it is a dominant whey protein in formulas. Many companies are attempting to change ratio of whey proteins, in particular to replace β-lactoglobulin with α-lactalbumin. Study of children's formula composition showed that there is a significant difference between amount of β-lactoglobulin in cow's milk and formulas with partial and complete hydrolysis [5].

β-lactoglobulin is relatively stable to action of acid hydrolysis and intestinal proteases, thus, a part of the protein can remain intact and penetrate through the intestinal wall. Thermal processing decreases binding ability proportionally to heating degree. However, new antigenic components that are unavailable for binding in a native molecule can form in denaturated proteins in the process of a chemical reaction. After hydrolysis, binding property of protein can largely remain, thus, reaction may occur even after consumption of fermented dairy products [6].

β-lactoglobulin is predominantly sensitive to heat denaturation. Patients have a high risk of reaction while consuming fresh milk and lower risk in thermally processed milk. Average chances "to outgrow" allergy.

α-Lactalbumin (Bos d 4) is a calcium-binding protein constituting 25% of whey and 5% of the total cow's milk protein. It is referred to hydrolase family. It consists of amino acids, located in two structural regions (α and β domains), connected by four disulfide bridges. Three-dimensional (conformational) epitopes for binding immunoglobulin exist in a native molecule. However, under the influence of denaturating agents, linear

epitopes with high binding property are determined, which, in their turn, increase protein stability [14].

α-Lactalbumin is a species-specific protein, therefore, in case of allergy to this protein, cross reaction to other mammal's milk is impossible. However, there exists a high probability of meat allergy [2].

Patients have a high risk of reaction while consuming fresh milk and low risk in case of thermally processed milk. High chances "to outgrow" allergy.

Bovine serum albumin (Bos d 6) is a protein of whey albumin family present in milk and meat. Quite often, it can be a cause of allergy to veal and beef. It can prove cross-reactivity with animals – a dog (Can f 3) and a cat (Fel d 2) [8].

■ CONCLUSIONS

1. Assessment of a molecular profile is important for diagnosis and treatment of cow's milk protein allergy irrespective of a choice of therapeutic tactics – specific oral tolerance induction or elimination diet. Identification of individually significant food allergens makes it possible to determine individual indications and contraindications for oral provocation test and select the most appropriate treatment, in particular, specific oral tolerance induction as a disease-modifying immunotherapy.
2. Cow's milk protein allergy is basically accompanied by the presence of major molecules, in particular α-lactalbumin (Bos d 4), casein (Bos d 8) and β-lactoglobulin (Bos d 5).
3. Casein (Bos d 8) is a major thermostable component of cow's milk protein and has a statistically significant positive correlation with a papule diameter (r=0.44), Can f 3 (r=0.39) and Bos d 4 (r=0.28).
4. Bovine serum albumin (Bos d 6) is a minor component of milk and has a statistically significant positive correlation with Fel d 2 (r=0.64) and Can f 3 (r=0.44), which indicates cross-reactivity with animals, particularly a cat and a dog.

■ REFERENCES

1. Kaczmarski M., Korotkiewicz-Kaczmarska E. *Allergy and food intolerance. Milk and other food.* Help-Med s.c. 2015. 210 p. (in Polish)
2. De Greef E, Hauser B, Devreker T. Diagnosis and management of cow's milk protein allergy in infants. *World J Pediatr.* 2012;8(1):19–24.
3. Koletzko S, Niggemann B, Arato A. Diagnostic approach and management of cow's-milk protein allergy in infants and children: ESPGHAN GI committee practical guidelines. *JPGN.* 2012;55:221–229.
4. Lozinsky AC, Meyer R, Anagnostou K. Cow's milk protein allergy from diagnosis to management: a very different journey for general practitioners and parents. *Children.* 2015;2:317–329.
5. Katz A, Hundal NV, Yuan Q. Cow's milk allergy: a new approach needed? *J Pediatrics.* 2013;163(3):620–622.
6. Valenta R, Karaulov A, Niederberger V, Gattinger P, van Hage M, Flicker S, Linhart B, Campana R, Focke-Tejkl M, Curin M, Eckl-Dorna J, Lupinek C, Resch-Marat Y, Vrtala S, Mittermann I, Garib V, Khaitov M, Valent P, Pickl WF. Molecular Aspects of Allergens and Allergy. *Adv Immunol.* 2018;138:195–256. doi: 10.1016/bs.ai.2018.03.002.
7. MacGinnitie A, Kamalia R, Alvernaz L, Timmons K, Hamel V, Lafreniere A. A quality improvement initiative to increase access to food challenges. *Pediatr Allergy Immunol.* 2018 Jun;29(4):447–9. doi: 10.1111/pai.12882.
8. Muraro A. EAACI food allergy and anaphylaxis guidelines. Primary prevention of food allergy. *Allergy.* 2014;69(5):590–601.
9. Sampson HA. Food allergy: past, present and future. *Allergol Int.* 2016;65(4):363–369.
10. Besh L, Muraro A, Matsyura O, Bonaguro R. Experience in diagnostics and treatment of milk allergy in children in Padua (Italy). *Asthma and Allergy.* 2017;3:64–71.
11. Aguilar A, Serra J. When a patient with suspected food intolerance has to be studied. *Aten Primaria.* 2020 Mar;52(3):140–1. doi: 10.1016/j.aprim.2020.02.002.
12. Campana R, Dzoro S, Mittermann I, Fedenko E, Elisyutina O, Khaitov M, Karaulov A, Valenta R. Molecular aspects of allergens in atopic dermatitis. *Curr Opin Allergy Clin Immunol.* 2017 Aug;17(4):269–277. doi: 10.1097/ACI.0000000000000378.
13. Nowak-Wegrzyn A, Fiocchi A. Is oral immunotherapy the cure for food allergies? *Curr Opin Allergy Clin Immunol.* 2010 Jun;10(3):214–9. doi: 10.1097/ACI.0b013e3283399404.
14. Vandenplas Y, Dupont Ch, Eigenmann Ph, Host A, Kuitunen M, Carmen Ribes-Koninckx C. A workshop report on the development of the Cow's Milk-related Symptom Score awareness tool for young children. *Acta Paediatr.* 2015 Apr;104(4):334–9. doi: 10.1111/apa.12902.