Діагностичні предиктори формування толерантності при елімінаційній дієті у дітей з алергією до білків коров'ячого молока

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Вступ. Алергія до білків коров'ячого молока є актуальною проблемою педіатрії. Це захворювання охоплює 2–3 % дітей раннього віку.

Метою роботи є покращити прогнозування ймовірності формування толерантності до білків коров'ячого молока у дітей раннього віку, які отримують елімінаційну молочну дієту, на основі визначення діагностичних предикторів.

Матеріали і методи. У дослідження включено 30 дітей з підтвердженою алергією до білків коров'ячого молока та призначеною елімінаційною молочною дієтою. Спостереження за дітьми проводилося впродовж 36 місяців. Оцінка молекулярного профілю проводилася із виявлення мажорних компонентів молока (Bos d 8, Bos d 5, Bos d 4), мінорного (Bos d 6) та перехресно реактивних з сироватковим альбуміном (Fel d 2, Can f 3).

Результати. У досліджуваній групі 46,7 % були хлопці. Середній вік досліджуваних становив $14,5 \pm 3,18$ місяців. У ході виконання роботи за методом логістичної регресії з прогресивною селекцією було виокремлено із 25 аналізованих чинників дев'ять, які при поєднаній дії мають вплив на формування толерантності до білків коров'ячого молока у дітей з алергією. Перевірка результатів прогнозування згідно отриманих коефіцієнтів у наявній базі даних дозволяє констатувати, що точність опрацьованої моделі становить 100 %. Представлено три клінічні приклади підрахунку прогнозування розвитку повної толерантності при елімінаційній дієті у дітей з алергією до білків коров'ячого молока.

Висновки. Незалежними предикторами, які зменшують ймовірність формування толерантності при елімінаційній дієті, виявлені: шкірний тест до молока, Bos d 8, Bos d 5, Bos d 4, Bos d 6. Незалежними предикторами розвитку толерантності при елімінаційній дієті є: специфічні IgE до молока, загальний IgE, Fel d 2, Can f 3. Низька ймовірність формування толерантності методом елімінаційної терапії є рекомендацією для призначення пацієнту лікування методом специфічної оральної індукції толерантності

Ключові слова: алергія до білків коров'ячого молока, елімінаційна дієта, діагностичні предиктори, діти раннього віку.

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Diagnostic predictors of tolerance development in the elimination diet in children with cow's milk protein allergy

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Background. Cow's milk protein allergy is a topical issue in pediatrics. This disease affects 2–3% of young children.

Methods. The study included 30 children with a confirmed cow's milk protein allergy and a prescribed milk elimination diet. The children were observed for 36 months. The molecular profile was evaluated by detecting major (Bos d 8, Bos d 5, Bos d 4), minor (Bos d 6), and cross-reactive with serum albumin (Fel d 2, Can f 3) components of milk.

Results. The study group included 46.7% of boys. The mean age of subjects was 14.5±3.18 months. Nine of the 25 analyzed factors were identified in the course of our work by the method of logistic regression with progressive selection, which in combined action affect the formation of tolerance to cow's milk proteins in allergic children. There are three clinical examples of calculating the prediction of complete tolerance development in the elimination diet in children with cow's milk protein allergy.

Conclusions. Independent predictors that reduce the likelihood of tolerance development in the elimination diet, have been identified: skin test to milk, Bos d 8, Bos d 5, Bos d 4, Bos d 6. Independent predictors of tolerance development in the elimination diet are: milk-specific IgE, total IgE, Fel d 2, Can f 3. Low probability of tolerance development by elimination therapy is a recommendation for prescribing treatment to a patient by specific oral tolerance induction.

Keywords: cow's milk protein allergy, elimination diet, diagnostic predictors, young children.

Introduction. Cow's milk protein allergy (CMPA) is a topical issue in pediatrics. This disease affects 2–3% of young children [7]. Clinical symptoms can be manifested in different ways, involving different target organs — skin, gastrointestinal, respiratory tracts [2]. In this case, the doctor should conduct a broad differential diagnosis. Patients are often unduly prescribed grueling diets at the initial stage.

It should be noted that diet is a big challenge for a family with a young child, as the lack of a balanced diet can negatively affect physical development, emotional background, and quality of life in general [13].

To date, there are two treatment options for CMPA: elimination and specific oral tolerance induction. In practice, unfortunately,

elimination is prescribed much more often. Such a therapeutic approach creates many problems because young children cannot understand why they are forbidden to eat what others eat around them [12].

According to the literature, 87% of children develop a cow's milk protein allergy over the age of 3, but the percentage of children with persistent allergies is increasing [14]. The development of atopic march can begin with food allergy, so all efforts of physicians should be aimed at preventing the transformation of skin forms into respiratory and providing the patient and his/her parents with a full quality of life [8].

The aim of our study was to improve the prediction of the probability of the development of tolerance to cow's milk proteins in young children keeping to a milk elimination diet, based on determining diagnostic predictors.

Materials and methods.

Criteria for inclusion in the study: children aged 6 months — 3 years, positive skin test to milk (papule ≥ 3 mm by skin prick) and milk-specific IgE ≥ 0.35 kUa/L, positive oral food challenge test (OFC). The study did not include patients with a history of anaphylaxis, severe comorbidities or autoimmune diseases, contraindications to OFC. All patients/their parents or foster parents gave written consent to participate in the study. The molecular profile was evaluated by detecting the major milk components (Bos d 8, Bos d 5, Bos d 4), minor (Bos d 6) and cross-reactive with serum albumin (Fel d 2, Can f 3). Among the list of other tests, the specific immunoglobulin E to food, household, fungal, and epidermal allergens were determined.

The study included 30 children with a confirmed cow's milk protein allergy and a prescribed milk elimination diet. The children were observed for 36 months. Assessment of clinical symptoms and oral milk challenge test were performed at the start of the study and over time after 12 and 36 months. The endpoint for calculating predictors of tolerance development was the assessment of symptoms and the results of the oral challenge test as of 36 months of follow-up.

Separately, the child's tolerance to cow's milk protein (g) was assessed. The tolerance profile

of patients was divided into three groups: no tolerance (0 — 0.99 g), partial tolerance (1 — 3.99 g), full tolerance (more than 4 g).

According to the literature and our own observations, we have selected factors that could influence the tolerance development to cow's milk proteins in children with allergies. A step-by-step approach of logistic regression with progressive selection (Forward) was used to isolate the factors that, when combined, have a significant effect on the development of tolerance to cow's milk proteins in allergic children. The obtained model coefficients were analyzed using the chi-square criterion. To determine the proportion of variance that can be explained by the factors included in the regression model, we analyzed the Nagelkerke R square.

The calculations were performed with the SPSS Statistics 23 software.

The probability of full tolerance to cow's milk proteins (m) depending on the factors we selected, was calculated using the following formula 1:

$$m = \frac{1}{1 + e^{-R}} \times 100\% \quad (1)$$

where $e = 2.72 \dots$ is the basis of natural logarithms,

R is the value calculated by formula 2:

$$R = K + \beta_1 v_1 + \beta_2 v_2 + \dots \beta_n v_n,$$

where K is a constant,

β_i is coefficients for each factor,
 v_i is the value of factors.

Theoretically, m can take values from 0% (impossible event) to 100% (event always occurs). The range that divides the probability into high and low is 50%.

Results of the study.

The study group included 46.7% of boys. The mean age of the subjects was 14.5 ± 3.18 months.

Skin symptoms (56.7%) mostly dominated in patients, gastrointestinal (43.44%) ranked second, respiratory (16.7%) ranked third. The symptoms combined in 13.3%. It should be noted that the manifestations of skin symptoms were rash, oedema, itching,

redness of the outer skin. Gastrointestinal — nausea, abdominal pain, vomiting, diarrhea. Respiratory symptoms — sneezing, runny nose, wheezing, rhinorrhea, dry cough, shortness of breath. A combination of skin symptoms with others was mainly observed in the case of combined symptoms. The average duration of symptoms was 9 months [7 — 11].

Nine of 25 analyzed factors were selected in the course of our study by logistic regression with progressive selection, which in combination affect the development of tolerance to cow's milk proteins in children with allergies, as shown in Table 1.

The probability of calculated model coefficients (p) is 0.0014. The Nagelkerke R square is 0.98, i.e., 98.0% of the variance in the development of tolerance to cow's milk proteins in children by specific oral desensitization can be explained by the factors included in the regression model. At the same time, checking the prediction results according to the obtained coefficients in the existing database allows us to state that the accuracy of our model is 100.0%, i.e., full tolerance was predicted for all children who achieved it by specific oral desensitization.

By substituting the results in the formula that have been obtained by logistic regression, we can obtain the R -value to determine the probability of the development of tolerance

to cow's milk proteins in children receiving therapy by specific oral desensitization:

$$R = -46.33 \cdot V1 + 11.78 \cdot V2 + 0.32 \cdot V3 - 35.37 \cdot V4 - 27.78 \cdot V5 - 49.87 \cdot V6 - 41.94 \cdot V7 + 19.46 \cdot V8 + 31.30 \cdot V9 + 281.10 \quad (1)$$

Among the above mentioned nine factors, five were identified that reduce the likelihood of developing oral tolerance in the elimination diet: skin test to milk, Bos d 8, Bos d 5, Bos d 4, Bos d 6. That is, the probability of tolerance development in children by specific oral desensitization reduces with increasing rates of Bos d 8, Bos d 5, Bos d 4, Bos d 6. At the same time, other factors (milk-specific IgE, total IgE, Fel d 2, Can f 3) are predictors of tolerance development — it is more likely to develop oral tolerance to cow's milk proteins in the elimination diet at their higher levels.

We can give some clinical examples to understand the functioning of the proposed model better.

Clinical example No.1

A 14-month-old boy suffers from gastrointestinal symptoms of food allergy from 11 months of age.

During the oral challenge test, he consumed 46 ml of cow's milk (equivalent to 0.598 g of protein) at the beginning of the study. The following indicators were analyzed: skin

Table 1

Predictors of the tolerance development to cow's milk proteins in children keeping to a milk elimination diet

No	Factors	Conventional signs	Regression coefficient (β_i)
1	Skin test to milk, papule in mm	V1	-46.33
2	Milk-specific IgE, IU/ml	V2	11.78
3	Total IgE, IU/ml	V3	0.32
4	Bos d 8, kUa/L	V4	-35.37
5	Bos d 5, kUa/L	V5	-27.78
6	Bos d 4, kUa/L	V6	-49.87
7	Bos d 6, kUa/L	V7	-41.94
8	Fel d 2, kUa/L	V8	19.46
9	Can f 3, kUa/L	V9	31.30
10	b		281.10

Note: p of the model is 0.0014; Nagelkerke — 0.98; threshold — 50%; sensitivity — 100%; specificity — 100%; accuracy — 100%.

test to milk — 7.20 mm papule; milk-specific IgE — 7.25 IU/ml; total IgE — 25 IU/ml; Bos d 8 — 7.3 kUa/L; Bos d 5 — 3.2 kUa/L; Bos d 4 — 0 kUa/L; Bos d 6 — 2.11 kUa/L; Fel d 2 — 0.8 kUa/L; Can f 3 — 0.22 kUa/L.

By substituting the data in equation 1, the following results are obtained:

$$R = -46.33 \cdot 7.20 + 11.78 \cdot 7.25 + 0.32 \cdot 25 - 35.37 \cdot 7.3 - 27.78 \cdot 3.2 - 49.87 \cdot 0 - 41.94 \cdot 2.11 + 19.46 \cdot 0.8 + 31.30 \cdot 0.22 + 281.10 = -333.576 + 85.405 + 8 - 258.201 - 88.896 - 0 - 88.4934 + 15.568 + 6.886 + 281.10 = -372.2 \quad (1)$$

$$m = \frac{1}{1 + 2.72^{372.2}} \times 100\% = 0.01\% \quad (1)$$

Therefore, according to these calculations, such a child has an extremely low probability of developing tolerance to milk by elimination therapy.

In comparison with the database, the boy tolerated only 0.91 g of protein at 12 months of follow-up and 1.768 g of protein at 36 months. That is, our prediction is valid.

Clinical example No.2

A 17-month-old girl has been suffering from skin allergy in the form of urticaria since the age of 3 months.

During the oral challenge test, she consumed 75.5 ml of cow's milk (equivalent to 0.9815 g of protein) at the beginning of the study.

The following indicators were analyzed: skin test to milk — 6.50 mm papule; milk-specific IgE — 6.45 IU/ml; total IgE — 19 IU/ml; Bos d 8 — 0 kUa/L; Bos d 5 — 0.8 kUa/L; Bos d 4 — 1.4 kUa/L; Bos d 6 — 1.2 kUa/L; Fel d 2 — 0.6 kUa/L; Can f 3 — 0 kUa/L.

By substituting the data in equation 1, the following results are obtained:

$$R = -46.33 \cdot 6.50 + 11.78 \cdot 6.45 + 0.32 \cdot 19 - 35.37 \cdot 0 - 27.78 \cdot 0.8 - 49.87 \cdot 1.4 - 41.94 \cdot 1.2 + 19.46 \cdot 0.6 + 31.30 \cdot 0 + 281.10 = -301.145 + 75.981 + 6.08 - 0 - 22.224 -$$

$$69.818 - 50.328 + 11.676 + 0 + 281.10 = -68.68$$

$$m = \frac{1}{1 + 2.72^{68.68}} \times 100\% = 0.01\%$$

Therefore, according to these calculations, such a child has an extremely low probability of developing tolerance to milk by elimination therapy.

Compared to the database, the girl tolerated only 1.248 g of cow's milk protein at 12 months of follow-up and 2.08 g of protein at 36 months. That is, our prediction is valid.

Clinical example No.3

A 13-month-old boy has been suffering from respiratory allergies for the past 5 months in the form of a dry paroxysmal cough.

At the beginning of the study, he consumed 233 ml of cow's milk (equivalent to 3.029 g of protein) during an oral challenge test.

The following indicators were analyzed: skin test to milk — 4.50 mm papule; milk-specific IgE — 4.11 IU/ml; total IgE — 38 IU/ml; Bos d 8 — 0.3 kUa/L; Bos d 5 — 2.4 kUa/L; Bos d 4 — 0 kUa/L; Bos d 6 — 0.8 kUa/L; Fel d 2 — 0 kUa/L; Can f 3 — 0 kUa/L.

By substituting the data in equation 1, the following results are obtained:

$$R = -46.33 \cdot 4.50 + 11.78 \cdot 4.11 + 0.32 \cdot 38 - 35.37 \cdot 0.3 - 27.78 \cdot 2.4 - 49.87 \cdot 0 - 41.94 \cdot 0.8 + 19.46 \cdot 0 + 31.30 \cdot 0 + 281.10 = -208.485 + 48.416 + 12.16 - 10.611 - 66.672 - 0 - 33.552 + 0 + 0 + 281.10 = 22.36$$

$$m = \frac{1}{1 + 2.72^{22.36}} \times 100\% = 0.01\% \quad (1)$$

Therefore, according to these calculations, such a child has an extremely high probability of developing tolerance to milk by elimination therapy.

In comparison with the database, the boy reached full milk tolerance during the

observation after 12 months and tolerated 3.77 g of protein, and after 36 months 6.006 g of protein. Therefore, our prediction is valid.

Discussion. Despite the growing knowledge about oral tolerance, a strict elimination diet remains the current standard of care in the treatment of food allergies following international guidelines [11]. However, the dietary approach has several limitations. First, the risk of severe systemic reactions due to the presence of latent allergens in food, despite all efforts to strictly avoid food allergens [4, 16]. Second, elimination diets have the risk of nutrient deficiencies and, as a result, impaired growth [9]. Cow's milk is an important source for the growth and development of young organism [3, 5]. Third, food allergies significantly reduce the quality of life of the child and his/her parents [17]. Fourth, there is a possibility of disease progression and transformation of skin forms in respiratory, so-called «atopic march». All facts, mentioned above, include potentially negative influences of elimination and serve as convincing arguments for the prescription of alternative therapy for allergy to cow's milk protein — specific oral induction of tolerance [4, 13].

Today, there are ongoing discussions about the possibility of developing oral tolerance in children with allergies to cow's milk proteins, as there are fundamental differences in the mechanisms of immune response in this disease associated with changes in basophils, IgG4, allergen-specific Th2 cells and allergen-specific cells with regulatory markers [14].

Food tolerance is a state of active immunological reactivity to the antigen, given enterally. In children, who are fully tolerant to cow's milk protein, clinical symptoms will not develop despite the frequency and amount of milk consumption. In the presence of allergy to cow's milk protein, a minimum amount of milk, even traces, is enough to provoke the appearance of pathological symptoms. Taking into consideration the growing prevalence of food allergies, there are active attempts to modify the immune response, especially in severe food allergies [10].

The priority treatment of food allergy is the formation of clinical tolerance, i.e., loss of reactivity to the antigen or allergen, in which

repeated exposure to the antigen does not lead to the development of a pathological reaction. Clinical tolerance is natural and induced. From the point of view of the medical worker, natural tolerance is a passive process, and induced involves the active intervention and cooperation of the child — parents — doctor. It should be noted that the formation of oral tolerance can be performed only in patients with high adherence to treatment [13].

The results of meta-analyses showed, that in the case of food allergy, the scheme of specific oral induction of tolerance in children with allergies to milk, eggs and peanuts works especially well and the course of the disease changes [8, 12]. At least 80% of children with allergy to cow's milk protein reach spontaneous clinical tolerability at school age, while others develop persistent forms of skin and respiratory allergies [6]. In fact, 20% of children who fail to «outgrow» allergies are the biggest focus for scientists and doctors, as this group requires the development of preventive measures and optimization of diagnostic predictors of the disease [5].

The literary data confirms the hypothesis, that special oral induction of tolerance can accelerate the resolution of food allergy due to suppressing the reactivity of mast cells and basophils, reducing allergen-specific IgE and simultaneous increase in allergen-specific IgG4 antibodies. In parallel, changes in allergen-specific cells with regulatory markers are observed, in particular Foxp3 + and latency of the associated peptide + Tregs, which play a central role in stimulating long-term tolerance [1, 14]. Oral tolerance is an active regulatory immune response aimed to return the product into the diet in a relatively short time. The prescription of an elimination diet distances the child from the complete diet and increases the risk of accidental contamination of the product and the development of adverse reactions [11].

The study of natural tolerance deserves particular attention. An interesting study was conducted by the Consortium for Food Allergy Research, which involved 512 children aged 3-15 months with moderate or severe atopic dermatitis and sensitization to milk or eggs. At an average age of 53 months, 53% developed tolerance [8].

An eighteen-year study of a cohort of people on the Isle of Wight ($n = 1456$) led by Venkataraman D. et al. showed, that the manifestation of IgE-dependent reaction to milk (manifested 4 hours after consumption of the product) decreases from 3.5% at the age of 1 year to 0.3% at 18 years [18].

Sensitization decreases with age. Skripak et al. have analyzed data from patients, observed at private and university institutions ($n = 4117$). Allergy to cow's milk protein was confirmed in 1073 patients. For further analyses, 807 patient cards were selected, which contained complete medical information. Retrospective evaluation of data was performed at 4, 8, 12, and 16 years of age. Complete recovery was observed in 19%, 42%, 64% and 79%, respectively. The highest incidence of allergy to cow's milk protein is observed during the first year of life and with a tendency to decreased sensitization and clinical manifestations with age [15].

The state of a young child's health and clean environment are the foundations for further life, developing proper eating habits [1, 6]. Physicians should be motivators and support patients and their parents, help shape the right diet, identify and eliminate risk factors, monitor harmonious child's development, and most importantly — work to prevent diseases, rather than passively monitor now to fight less successfully with consequences later [12].

However, our study had some limitations. First, the examination was performed in a group of early age children, which caused difficulties in receiving blood samples and performing skin allergological test. Secondly, conducting the oral provocative test to confirm the diagnosis of allergy to cow's milk proteins, is a very responsible method of examination, which requires an experienced team of medically trained professionals for emergency care in case of the development of pathological reactions. The most difficult is to interpret the results of a test when the symptoms are mild, subjective or atypical. In particular, mild symptoms can be provoked by emotional factors or be the first signs of severe reactions. In case of mild symptoms, it is necessary to switch the patient's attention and at the same time stay attentive to the child's condition, behavior, symptoms. Third, in Ukraine, there

is no structured study of food allergies in children, and separate data from different regions are difficult to compare. There is a need for a unified study of food allergies in our country determining causal factors of disease, which would be a basis for the formation of the correct therapeutic approach, preventive measures and development of prognostic criteria. It should be noted that the study confirms the importance of determining certain external factors, studying the provoking and preventive factors of the disease, with the subsequent development of recommendations. In the future, we may recommend using the diagnostic predictors of the development of tolerance to cow's milk proteins in children before choosing a treatment approach.

Taking into account the obtained results, we can summarize that:

1. Independent predictors, which reduce the likelihood of the tolerance development in the elimination diet, have been identified by a skin test to milk, Bos d 8, Bos d 5, Bos d 4, Bos d 6.
2. Independent predictors of the tolerance development in the elimination diet are: milk-specific IgE, total IgE, Fel d 2, Can f 3.
3. Low probability of tolerance development by elimination therapy (less than 50%) is a recommendation for prescribing a patient treatment in the form of specific oral tolerance induction.

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Conflict of interest

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