

Atopy patch test in the diagnosis of food allergy in children with gastrointestinal symptoms

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ABSTRACT

Purpose: To evaluate the diagnostic accuracy of the atopy patch test (APT) in detection of delayed-type sensitization to different food allergens, in children with digestive symptoms and with/ or without cow's milk allergy.

Material and methods: 28 children suspected of food allergy-related gastrointestinal symptoms were underwent diagnostic procedures as skin prick test (SPT), specific immunoglobulin E (sIgE), APT, oral food challenge and then divided into two groups according to the results of oral food challenge: A – 17 children with cow's milk allergy, B – 11 children without cow's milk allergy. Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of APT were calculated.

Results: A positive challenge response to milk was found in 60,7% of examined children. No statistical difference in the prevalence of positive SPT and sIgE to milk has been found between the groups. The prevalence of positive APT to other food allergens was significantly higher in children with allergy to milk ($p < 0,005$). No statistical difference of positive APT to other food allergens has been found in children with/ or without cow's milk allergy. APT sensitivity and specificity in children with cow's milk allergy obtained on 77%/73%; where PPV obtained on 71%.

Conclusions: High sensitivity and specificity of APT confirm its accuracy in diagnostics of delayed-type reactions in children with gastrointestinal symptoms. Combined atopy patch test and oral food challenge is essential for avoidance of unnecessary elimination diet. Delayed-type sensitization to other food allergens is common in children with cow's milk allergy.

Key words: cow's milk allergy, gastrointestinal symptoms, atopy patch test, oral food challenge

INTRODUCTION

The atopy patch test (APT) allow to identify the sensitization to harmful food and inhalant allergens [1,2]. They are introduced in the diagnosis of delayed-onset reactions among infant with atopic eczema, where despite routine treatment the improvement is not observed. It is difficult to find the correlation between ingestion of food and clinical symptoms [3,4]. Additional evidence from recent researches show that APT-reactions are associated with T lymphocyte-mediated allergen-specific immune responses. The published data indicate that patients with atopic eczema and late-phase reaction, have elevated serum concentration of interleukin 2, interferon gamma, tumor necrosis factor alfa. This fact confirms the role of lymphocytes T in pathogenesis of atopic eczema. The study performed by Majamaa et al. demonstrated

that a lot of infants with skin lesions and negative results of skin prick tests and specific immunoglobulin E (sIgE) to milk, have positive APT [5].

The same immunological mechanism is observed in gut lamina propria of gastrointestinal tract. Antigen which induces local immune-complex reactions or T-cell mediated immunity probably reaches the bloodstream via the mucosus membrane of digestive tract. The mucosus membrane is the main penetration route of harmful allergens [6]. Lymphocytes T produce the cytokines and inflammatory mediators activating others cells (macrophages, basophiles) and developing a variety of allergic (IgE-mediated and T-cell-mediated) reactions. Patients with food allergy syndrome and protein-induced intestinal disorders, demonstrate different clinical symptoms as: diarrhea, vomiting, loss of appetite, abdominal pain, gut dysmotility, gastroenterocolitis [6,7,8]. Among infant

Table 1. Population characteristics of children with (A) and without (B) cow's milk allergy.

	Group A (n=17)	Group B (n=11)	P
Age			ns
Min-max (months):	6-144	15-114	
Mean:	39,12±39,28	57,36±33,36	
(CI 95%)	(18,92-59,31)	(34,95-79,77)	
Positive family allergic history (n,%)	8 (47,1)	4 (36,4)	ns
Breast feeding time			ns
Min-max (months):	1-15	1-15	
Mean:	6,65±4,14	5,45±4,20	
(CI 95%)	(4,52-8,77)	(2,63-8,28)	
Onset of clinical symptoms			ns
Min-max (months):	1-60	1-110	
Mean:	10,82±14,86	19,27±34,48	
(CI 95%)	(3,18-18,46)	(-3,89-42,43)	
Serum IgE concentration (IU/ml)			ns
Min-max (IU/ml):	2-507	3-302	
Mean (IU/ml):	99,41±130,91	74,73±103,61	
(CI 95%)	(32,10-166,72)	(5,12-144,33)	
Inhalant allergy (n,%)	4 (23,5)	5 (45,5)	ns
Milk-free diet (n,%)	13 (76,5)	5 (45,5)	ns

CI – confidence interval

and young children the most important role play following allergens: milk, hen's egg; where wheat, soy and cereals are more significant among older children [2,4,9].

Skin prick test and specific IgE in patients with delayed-type reactions do not imply a role for potential harmful allergens. They are reserved for immediate reactions and remain negative in non-IgE-mediated allergy. Currently, a diagnostic food challenge (double blind placebo food controlled challenge) is the best method to confirm the diagnosis of food allergy [10-12]. Sometimes, the results of test are not easy to interpret (especially in polysensitized children) and it is difficult to establish the correlation between harmful allergens and clinical symptoms. Different study demonstrated that many patients with negative prick test results and delayed-onset clinical reactions could be diagnosed by atopy patch test. The T-cell mediated immunity in skin and gut lamina propria suggests the usefulness of APT in patients with digestive symptoms [10,12]. There is only few data indicating the accuracy of APT in these disorders and they concerned infants with cow's milk allergy [13], eosinophilic esophagitis [10]. Last data published by Canani et al. suggest the usefulness of APT in children with different food allergy-related gastrointestinal symptoms and sensitization to other allergens than milk [7].

The aim of our study was to evaluate the accuracy of the APT in diagnosing of delayed-type food allergy to milk and other vegetable and animal allergens, among children suffering from digestive symptoms. The diagnostic value (sensitivity and specificity) of APT in correlation to skin prick test (SPT) and sIgE in children with positive results of milk oral food challenge was estimated. We compared the incidence of immediate and delayed-onset food sensitization to other food allergens, in children with or without cow's milk allergy.

MATERIAL AND METHODS

Patients

The prospective, nonrandomized study were carried out on the group of 28 children (21 boys, 7 girls) aged from 6 to 144 months, referred to the Department of Pediatrics, Gastroenterology and Allergology in Białystok, for evaluation of digestive symptoms suspected of food hypersensitivity. Children with suspicion of food-related symptoms were enrolled to the study according to the inclusion and exclusion criteria. The other diagnostic procedures were performed for excluding other gastrointestinal disorders.

Inclusion criteria were: digestive symptoms, personal history indicated the time correlation between food ingestion and clinical symptoms. Exclusion criteria were: systemic diseases, active atopic eczema (diagnosis stated on the basis of criteria Hanifin and Rajka), infection of gastrointestinal tract (positive laboratory indicators of infection, stool bacteriology, anus smear test), anatomical and autoimmune disorders (immunological examinations, endoscopy), chronic inflammatory bowel diseases, lactose intolerance, actual and earlier treatment with antihistaminic and antiallergic drugs (in dependence of wash-out period for different types of antihistaminics).

Children were underwent standardized diagnostic procedures provided for food allergy as: SPT, sIgE, APT, oral food challenge. Diagnosis of gastroesophageal reflux was performed with usage of pH-metry monitoring; the exacerbation was assessed by relapse of symptoms being observed before treatment. Sigmoidoscopy combined with

additionally tests was performed to diagnose food protein enterocolitis and exclude organic disorders.

To make clinical and statistical analysis, examined children were divided into 2 groups depending on the results of oral food challenge to cow's milk: group A– children with cow's milk allergy, group B- without allergy to cow's milk.

Population characteristics is presented in *Tab. 1*.

Skin-prick test

The skin-prick tests with cow's milk and other food allergens were performed in all examined children, by the same person on the volar side of the forearm in accordance with the instructions of the European Academy of Allergy and Clinical Immunology [14]. Food allergens were tested in fresh form by means of a modified skin prick technique (prick by prick tests). To perform tests with aeroallergens (mites, tree, grass and weed pollen, dog and cat epithelia, wool, cotton) commercial extracts from Allergopharma Company were used - a 1 mm, single-peak lancet with shoulder and negative (NaCl 0.9%) and positive (9% Codeine) as control. Reactions were read after 15 minutes by the author. The mean weal diameter was calculated and a reaction of at least 3 mm was considered as positive without reaction of negative control.

Atopy patch test

Atopy patch tests were applied on uninvolved skin of the child's back, according to the method described by Isolauri and Turjanmaa [15,16]. We used 8-mm diameter aluminium cups for children younger than 3 years and 12-mm aluminium cups for children older than 3 years (Finn Chamber, Epitest Ltd., Finland). Microcrystalline cellulose was used as negative control. Application sites were checked after 20 minutes for immediate reactions. The occlusion time of patch test was 48 hours. The results were read for the first time 15 min after removal of the cups. If irritative redness was found in the test area, the results were read after 30 min. The second evaluation was done 72 hours after attaching the patch tests. Reactions were classified according to standards and considered as: 0 - negative – no reaction, either visible or palpable; + - redness – negative or doubtful reaction; ++ - redness and palpable infiltration with papules – positive reaction; +++ - redness, palpable infiltration with many papules and eczema – strong positive reaction. If the reaction was very intensive as: redness, edema, palpable infiltration and vesicles, the result was marked as +++++. Reactions 0 and + (redness without infiltration) were regarded as negative. Occurrence of alone redness can be the results of local irritation.

Determination of total IgE and specific IgE antibodies

Serum samples (2 ml) were analyzed for concentration of total IgE and specific IgE antibodies to food allergens as: cow's milk, egg white, soy, wheat, maize, rice (Pharmacia Upjohn) with a fluoroenzymatic assay (UniCAP) according to the manufacture's instruction. The detection limit of the CAP

Table 2. Positive results to milk (SPT, sIgE, APT, oral food challenge) in the examined group of children (n=28).

Diagnostic test	Positive results n (%)
SPT	5 (17,9)
sIgE	6 (21,4)
APT	14 (50,0)
Oral food challenge	17 (60,7)
Immediate-onset reaction	4/17 (23,5)
Delayed-onset reaction	13/17 (76,5)

system is 0.35 kU/L IgE; measurable specific IgE was defined as a positive test result if >0.7 kU/L.

Oral food challenge

Standardized oral challenge was performed according to guidelines [17,18], with the increasing amounts of milk at 30 min intervals until intake appropriate for their age was reached. A challenge was taken on all children after 4 week on an elimination milk-free diet. Cow milk challenge was performed as the open challenge being a reliable method for infants and young children (<1 year) and as a blinded test for older children (>1 year). The foodstuff was blinded in apple pulp or rice if there was no sensitization (SPT, negative history of consumption) to these allergens. The same doses of milk formula were given in open and blinded challenges.

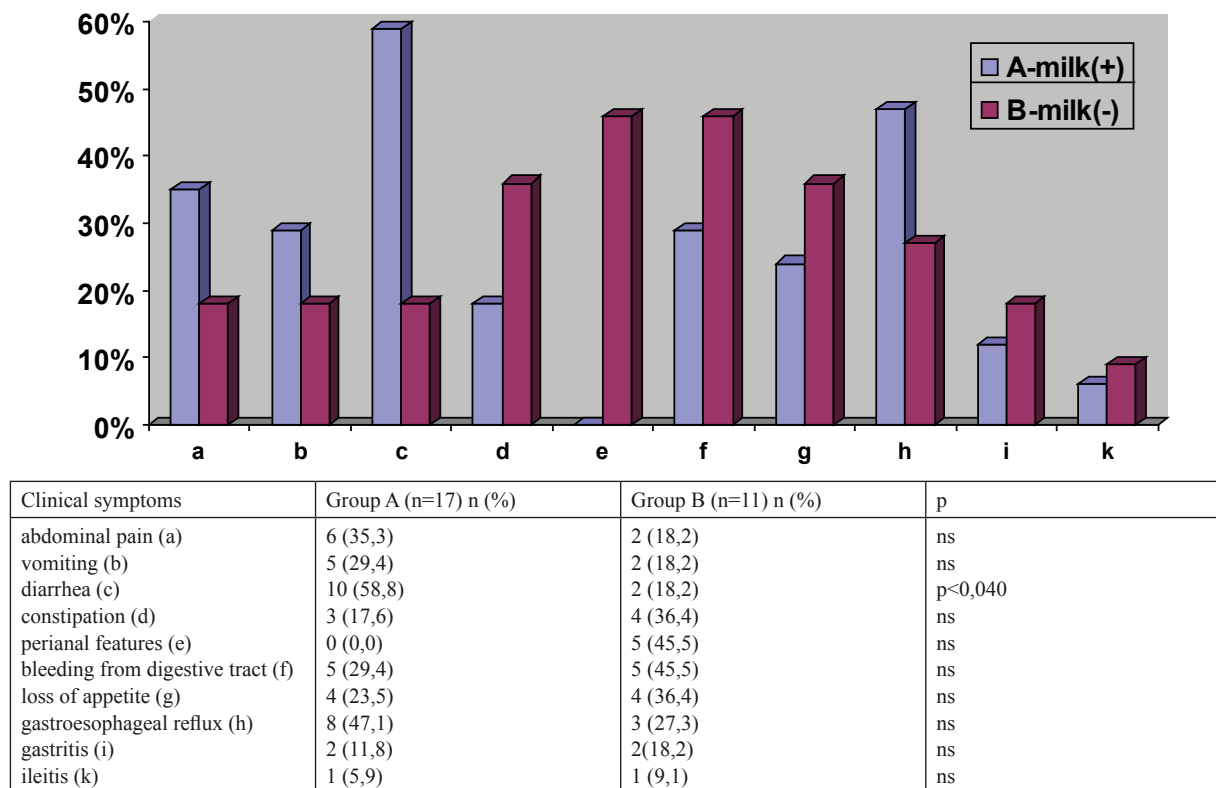
Immediate reactions were defined as reactions within 2 hours after the last dose of milk was administered. The challenge was started in the hospital when rising doses of the infant formula were given and continued after 24 hours of observation in the patient's home, where the parents recorded the symptoms of the child being with telephone contact with the author. The challenge was discontinued when a clinical reaction was noticed. If one of the following symptoms such as skin eruptions, pruritus, edema, urticaria, vomiting, diarrhea, irritability, rhinitis, wheezing, anaphylactic shock, was noticed, the challenge was regarded as positive. The evaluation of symptoms and decision to stop a challenge was made by the investigator. All children were examined once a week to verify reported symptoms and seen 1 month after the beginning of challenge to confirm the diagnosis.

Statistical analysis

Statistical analysis was performed by using SPSS (version 8.0; PL) for Windows. Two-by-two tables were used to calculate sensitivity, specificity, positive (PPV) and negative predictive value (NPV), likelihood ratio (LR) individually for SPT, sIgE for immediate-onset reactions and for APT in patients with delayed-onset reactions and combined for immediate and delayed reactions. The Mann-Whitney nonparametric test, χ^2 -test, Fisher test were used to compare the results in children with or without cow's milk allergy. Data is presented as mean with ranges for 95% confidence interval (CI). Statistical significance was defined by a level of 0,05.

Table 3. SPT, sIgE and APT in children with and without cow's milk allergy.

Diagnostic test	Group A (n=17)n (%)	Group B (n=11)n (%)	p
SPT	4/17 (23,5)	1/11 (9,1)	ns
sIgE	5/17 (29,4)	1/11 (9,1)	ns
APT	13/17 (76,5)	1/11 (9,1)	p<0,0005

Figure 1. Clinical symptoms from digestive tract in children with (A) and without (B) cow's milk allergy.

RESULTS

APT in the diagnosis of sensitization to milk and other food allergens

A positive challenge response to milk was found in 17/28 (60,7 %) of examined children. According to these results children were divided into two groups: A – with cow's milk allergy, B- without cow's milk allergy (*Tab. 2*). In the group of children with positive reactions, 23,5% demonstrated immediate-type reactions. 3 children had dyspeptic symptoms (abdominal pain, diarrhea, vomiting) and one of them allergic eczema. Among patients with delayed-onset reactions (76,5 %) symptoms were confined to the gastrointestinal tract as: abdominal pain, diarrhea, vomiting, loss of appetite. 23,1 % of children demonstrated symptoms from respiratory tract (rhinitis, cough). The exacerbation of gastroesophageal reflux was observed in 30,8 % of patients.

Positive APT to milk was noticed in 14/28 (50,0%) of investigated children; significantly more often in the group A (76,5%/9,1%; p<0,0005 (*Tab. 3*). In children from the group

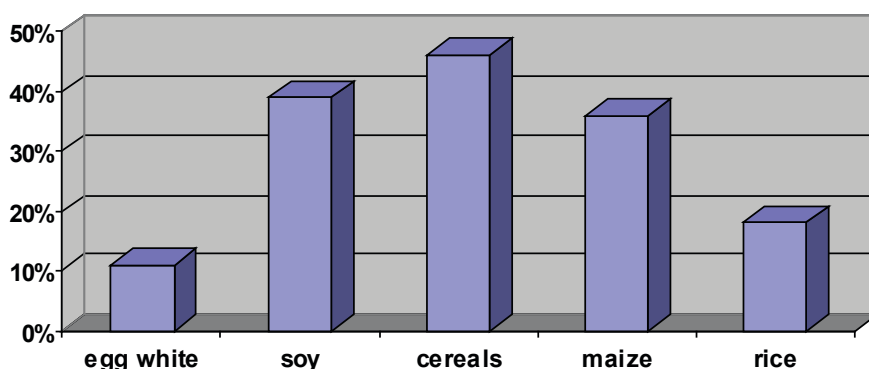
B, the results of APT were verified by oral food challenge. The lack of correlation between positive results to milk in APT and positive delayed-onset reaction in oral food challenge was registered in 7 on 28 (25,0%) of children. False positive reactions were noticed in 4 cases.

Positive results of SPT, sIgE were higher in the group of children with cow's milk allergy, but the difference was not statistically significant (p<0,3293 and <0,2135). We did not notice correlation between positive result of SPT/sIgE to milk and immediate-onset reaction in oral food challenge was noticed in 3/28 (10,7%) of examined children. The prevalence of diarrhea was also statistically higher in the group of children with cow's milk allergy (p<0,040) (*Fig. 1*).

Polysensitization to other than milk foods confirmed by APT was detected in 17/28 (60,7%) of examined children (*Tab. 4*). Patients were mostly sensitized to soy, cereals, maize (*Fig. 2*).

Table 4. Coincidence of sensitization to other food allergens in children with (A) and without (B) cow's milk allergy.

	Group A (n=17) n (%)	Group B (n=11) n (%)	p
Food allergy (n,%)	14 (82,4)	8 (72,7)	ns
Immediate-type reaction (SPT, sIgE)	5 (29,4)	7 (63,6)	ns
Delayed-type reaction (APT)	12 (70,6)	5 (45,5)	ns

Figure 2. Positive APT to other food allergens in the investigated group of children (n=28).

Food polysensitization among children with/without cow's milk allergy

IgE-sensitization to other food allergens was observed more often in the group B of children (63,6%), but the difference was not statistically significant ($p < 0,3205$) (Tab. 4). Results of APT to other food allergens among children with and without cow's milk allergy are presented in Tab. 5. In the group A the results of prick tests to milk correlated with the results of APT. To other food allergens the correlation was not observed.

Using all tests, food allergy was not confirmed among three of children. Two of them were diagnosed with constipation according to diagnostic guidelines, one of them with gastritis and gastroesophageal reflux.

Diagnostic value of APT in correlation between SPT/sIgE and oral food challenge

Sensitivity of APT in children with cow's milk allergy (delayed-onset reactions) was 77%, specificity 73%. Sensitivity and specificity of SPT/sIgE in correlation between immediate-onset reactions was 75%, 83% (Tab. 6).

Oral food challenge

Immediate-type reactions to milk were stated among 4 children in the form of diarrhea and vomiting. The symptoms were noticed within 2 hours of starting oral food challenge. 13 children have been diagnosed with delayed-type reactions to milk (Tab. 2).

DISCUSSION

The role of food allergy in pathogenesis of symptoms from digestive tract is very common and described. A lot of published data suggest the influence of harmful food allergens on the mucous membrane of gut, motoric and digestive function.

The presence of T-cells in the intestinal mucosa allows to the initiation of immune response [6,8,9]. However it is still difficult to explain how T-cells reactions in the gastrointestinal tract could be detected in the skin. The rational interpretation of this fact is the migration of T cells throughout the circulatory system or the interaction of T cells in regional lymph nodes [6,8]. Reaginic antibodies cause only a part of digestive symptoms and most of allergic symptoms have rather delayed than immediate type. Therefore, allergy testing such as prick skin testing or specific IgE are not useful in the diagnosis of non-IgE-mediated reactions [10,11].

Current data indicate that the atopy patch test can be useful in suspected delayed-onset clinical reactions; especially among children with atopic eczema. The role of APT in the diagnosis of cow's milk allergy in patients with digestive symptoms is still underestimated. Majamaa et al. were the first who investigated children with APT and isolated gastrointestinal symptoms suspected of cow's milk allergy [5]. Previous studies with APT concerned patients with atopic eczema [2,5,7,10,15]. In our investigations positive APT was demonstrated in 70,6% of children with cow's milk allergy in comparison to 9,1% of children with negative results of oral food challenge. Our observations are in accordance with studies by de Boissieu et al. indicating high sensitivity and specificity of APT (79%/91%) [13]. In the examined group of

Table 5. Positive APT to other food allergens in children with and without cow's milk allergy.

Food allergens	Group A (n=17) n (%)	Group B (n=11) n (%)	p
Egg white	3 (17,6)	0 (0,0)	ns
Soy	7 (41,2)	4 (36,4)	ns
Cereals	9 (52,9)	4 (36,4)	ns
Maize	7 (41,2)	3 (27,3)	ns
Rice	4 (23,5)	1 (9,1)	ns

Table 6. Sensitivity, specificity, PPV, NPV, LR for SPT/sIgE ag patients with positive oral food challenge to milk (IR/DR-immediate/delayed reaction, DR-delayed reaction).

	SPT/sIgE	APT	
	IR	IR/DR	DR
Sensitivity (%)	75	77	77
Specificity (%)	83	91	73
PPV (%)	43	93	71
NPV (%)	5	29	21
LR+	4,50	8,41	2,88
LR -	0,30	0,26	0,31

PPV - predictive value, NPV – negative predictive value, LR-like-hood ratio

children sensitivity and specificity of APT was 77% and 73% in correlation to delayed-type reactions. In the comparison to positive oral food challenge (immediate and delayed-type reactions), specificity was higher (91%). The comparable results were achieved earlier in our patients with atopic eczema (80%/70%). Diagnostic value of APT in children with atopic eczema and isolated symptoms from gastrointestinal tract confirm its accuracy in diagnosing clinical symptoms with allergic background, independent on their characters. Some of authors concluded that APT together with the measurement of levels of specific IgE makes DBPCFC superfluous for suspected food allergy, especially in patients with allergy to milk and egg white [19].

APT are not standardized and most clinicians use food allergens in native form. The diameter of aluminium cups is still controversial. Niggemann et al. advice to use the large cup (12 mm) for APT with food even in infants and small children [2]. However according to the EAACI position paper, some of investigator have found good correlation between APT results using the 8 mm cups and challenge test [12,15,16]. We performed the test by method described by Isolauri and Turjanmaa and used 8-mm diameter aluminium cups for children younger than 3 years (small area of the back) and 12-mm aluminium cups for children older than 3 years. The lack of standardization and other opinions about limited usefulness of APT [20-22], remain food challenge as the gold standard for diagnosing clinically relevant food allergy [17,18,23].

Sensitivity and specificity of APT in late-phase reaction were comparable to SPT/sIgE in immediate-type reactions.

This fact confirm the role of APT in diagnostics of non-IgE-dependent allergy. From another side, sensitivity and specificity of SPT/sIgE in children with digestive symptoms in comparison to atopic eczema patients was lower. Only some of them presented immediate-onset reactions in food challenge. These two facts confirm the implication of delayed-onset immunological reactions in the pathogenesis of digestive symptoms and the accuracy of APT as a diagnostic procedure.

Cow's milk allergy is the first manifestation in a children life and concerns about 2-7% of infants [4,5,9]. 50% of the youngest patients presented symptoms from gastrointestinal tract; usually vomiting, diarrhea [9]. These facts were confirmed in the investigated group of patients. Diarrhea was noticed statistically more often in children with cow's milk allergy. Excluding patients with lactose intolerance allowed to define the cause of symptoms. The difference in positive SPT/sIgE between both groups was not statistically significant. The percentage of positive APT to milk ($p<0,005$) confirm higher prevalence of delayed-type clinical reactions in the pathogenesis of digestive symptoms. Combined atopy patch test and oral food challenge is essential for avoidance of unnecessary elimination diet. After diagnostic procedures, it was possible to change the diet restriction in patients from group B. The study performed by Sinagra et al. confirmed that milk elimination diet is often overprescribed for children suspected of food allergy [24].

No statistical difference in sensitization to other food allergens between investigated groups suggests that milk allergy is not the trigger factor to food polysensitization. The prevalence of positive APT to other food allergens was higher in cow's milk allergy children, however positive sIgE in children without cow's milk allergy. It confirms allergic background of digestive symptoms in both groups.

The APT seems to be valuable additional tool in the diagnostic work up of food allergy in infants and children with non-IgE-dependent food allergy. In our study, positive APT to other, than milk, foods were stated in 45,5 % of children. APT was helpful in diagnosis of food allergy in patients with growth disturbance, bleeding from the gastrointestinal tract and gastroesophageal reflux. No statistical relationship between the prevalence of GER and cow's milk allergy has been stated, what indicate the role of other allergens in the pathogenesis of disease. Positive results of SPT or APT indicate only sensitization to food allergens and it should be verified by oral food challenge.

CONCLUSIONS

Our investigations confirmed the accuracy of the APT in the diagnosis of food allergy in children with digestive symptoms. Its sensitivity and specificity had shown high predictive value of APT for patients with delayed-type reactions. Our results suggest also the role of APT in allergy to wheat and other cereals [25]. Coincidence of IgE-mediated and non-IgE-mediated hypersensitivity to foods indicated the allergic background of symptoms from gastrointestinal tract [26,27].

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