I.A. Deev[1], M.I. Petrovskaya[2, 3], L.S. Namazova-Baranova[2,3,4], S.G. Makarova[2,3], I.V. Zubkova[2], N.A. Mayanskiy[2, 3]
[1] Siberian State Medical University, Tomsk, Russian Federation
[2] Scientific Center of Children's Health, Moscow, Russian Federation
[3] Sechenov First Moscow State Medical University, Russian Federation
[4] Pirogov Russian National Medical Research University, Moscow Russian Federation

sIgG4 and Other Tolerance Predictors at Food Allergy in Early Age Children

Author affiliation:
Mariya Igorevna Petrovskaya, junior research fellow at the department of standardization and clinical pharmacology of the Federal State Budgetary Research Institution (FSBRI) "Scientific Center of Children's Health" (SCCH), PhD candidate at the department of allergology and clinical immunology of the faculty of pediatrics at the State Budgetary Educational Institution (SBEI) of Higher Professional Education (HPE) "Sechenov First Moscow State Medical University" (MSMU)
Address: 2/1 Lomonosovskiy Av., Moscow, 119991; **tel.:** +7 (495) 967-14-21, **e-mail:** petrovskaya_maria@mail.ru

Article received: 19.01.2014. Accepted for publication: 05.05.2015.

Despite the progress in pediatric allergology, the question of tolerance development assessment and determination of the safest time for introducing new foodstuffs to child's diet after an elimination diet remains open. This article presents results of the authors' study of practical value of specific immunoglobulins (sIg) G4 as tolerance development markers in the event of a food allergy in infants. Thus, it has been observed that high sIgG4 levels are a favorable predictor not only of mild clinical manifestations of the food allergy, but also of tolerance development. The rate of high levels of sIgG4 to food allergens in the control group infants was statistically significantly higher than in the patients with food allergy. Thus, the authors assume that sIgG4 generation is a normal physiological process preventing development of hyper-sensitivity and that high sIgG4 levels indicate a child's immune system's "contact" with one or another foodstuff. Mild clinical manifestations of food allergy (FA) (not IgE-mediated FA forms), as well as breast feeding preservation are determined as clinical predictors of tolerance development.
***Keywords**: food allergy, children, tolerance, cow milk protein allergy, milk-free diet.*
(**For citing:** I.A. Deev, M.I. Petrovskaya, L.S. Namazova-Baranova, S.G. Makarova, I.V. Zubkova, N.A. Mayanskiy. sIgG4 and other tolerance predictors at food allergy in early age children. *Pediatricheskaya farmakologiya = Pediatric pharmacology*. 2015; 12 (3): 283–289. doi: 10.15690/pf.v12i3.1352)

INTRODUCTION

Food allergy (FA) in a child requires an elimination diet, where all cause-significant foods are excluded. The today's consensus papers prescribe a minimum term of such a diet, which is 6 months [1], whereas the total duration is individual. It is believed that children allergic to cow milk proteins (CMP) during their first postnatal year have a 100% chance to develop tolerance to milk proteins by age of five, if this allergy is not IgE-mediated [2]; in case it is, such tolerance develops later [3]. According to the latest data, tolerance development in case of IgE-mediated allergy develops with a 19% chance by age of 4, with a 52% chance by age of ten [4, 5]. To

avoid unreasonably long dietary regimen, it is believed children should be checked-up periodically to find out whether any of their dietary restrictions could be lifted [1]. For food allergy and tolerance development diagnostics, the "golden standard" is the double-blind placebo-controlled provocation test, where titrated doses of a food allergen are added to the child's nutrition. However, this method often causes adverse responses. Moreover, it is not certified in Russia. This is why we have earlier proposed so-called "diagnostic introduction of foods" (L.S Namazova-Baranova, S.G. Makarova) [6] as a diagnostic trial to identify tolerance development. However, it makes sense to identify predictive laboratory and clinical-anamnestic markers of tolerance to certain foods in food allergic children, in order to avoid undesirable recrudescence of the disease and to ensure maximum safety of this technique. As of today, there are studies that suggest a probable role immunoglobulins (Ig) class G5 can play in tolerance development. This indicator is subject to analysis as a factor that can facilitate further predictions [7]. The article presents the results of our own study.

The aim of this study was to identify laboratory and clinical predictors of tolerance development for food-allergic infants and toddlers.

PATIENTS AND METHODOLOGY

Research Design and Conditions

This study was carried out from October 2013 till February 2015 at the Consultative-Diagnostic Center of the Research Institute for Preventive Pediatrics and Medical Rehabilitation of the Scientific Center of Children's Health, an institution headed by L.S. Namazova-Baranova, Doctor of Medicine and a corresponding member of the Russian Academy of Sciences.

To be included in the main group, a child should be 1-to-18-month-old and have FA manifestations (Group 1). The comparison group included healthy children of the same age without any allergic manifestations or burdened allergy history (Group 2).

This prospective monitoring study included several encounters with the food-allergic children, with clinic-laboratory and nutritional examination methods applied at different research stages. Checkups before and after the elimination diet as well as upon diagnostic introduction of foods were mandatory. See Fig.1. Decisions on whether further adherence to the dietary regimens would make sense were analyzed and overviewed every 6 to 12 months, as recommended by the EAACI [1]. Such decisions were based on the results of introducing cause-significant foods for diagnostic purposes. The level of sIgE to cause-significant foods was preliminarily identified for children with IgE-mediated allergies. As of the comparison group, sIgG4 to the allergens under research were identified on a once-only basis.

Methods

Clinical examination was carried out according to the generally accepted plan compliant with the recommendations by the European Academy of Allergy and Clinical Immunology (EAACI) for 2014. The intensity of atopic dermatitis manifestations was determined using the SCORAD index. The severity of gastrointestinal symptoms was identified by the doctors using a 0 to 3 gradation system:

- 0 stood for no symptoms;
- 1 stood for mild symptoms in the form of one-time posseting, torminas, etc., which did not really affect the children's well-being.
- 2 stood for moderate intensity of gastrointestinal symptoms, which worsened the general well-being, made children more whimsical, and affected their sleep and wakefulness patterns, but did not lead to physical retardation or insufficient body weight.
- 3 stood for severe clinical manifestations that had negative effects on children's growth and development.

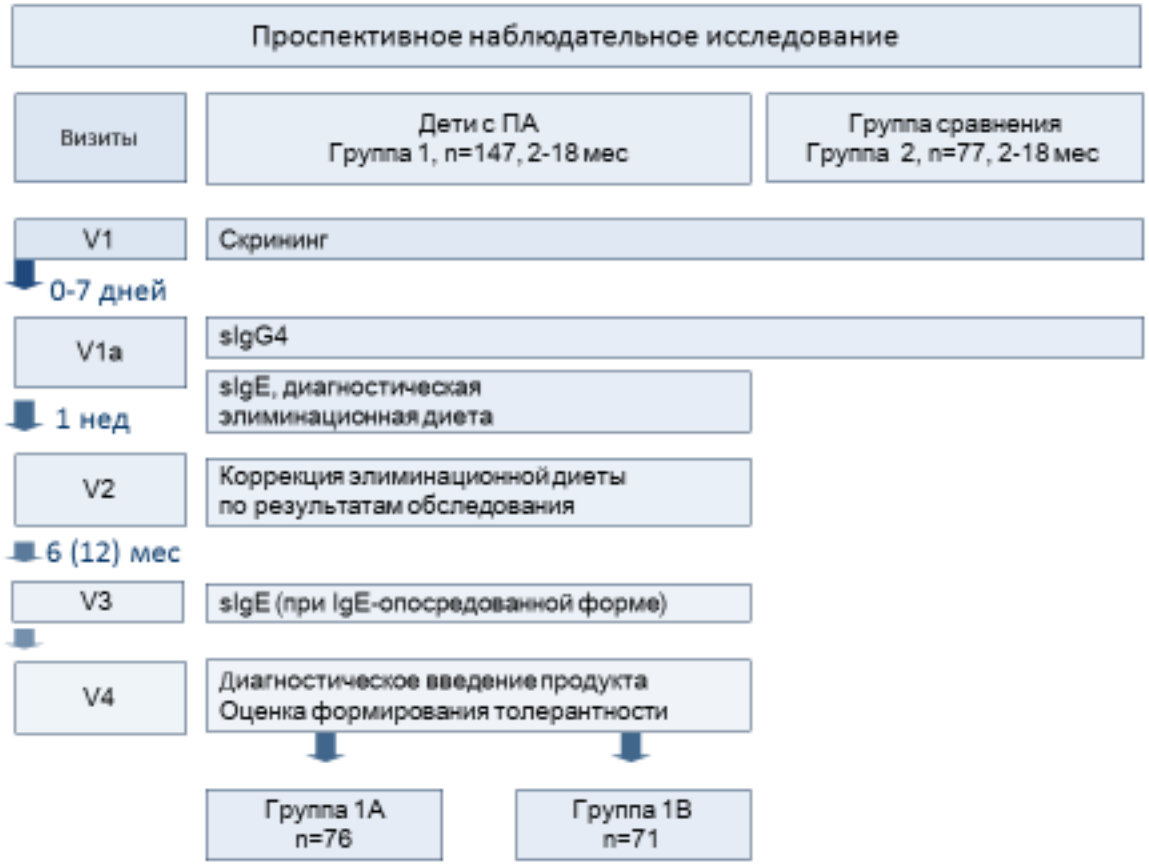


Figure 1. Research Design

Проспективное наблюдательное исследование	*Prospective monitoring study*
Визиты	*Visits*
Дети с ПА	*Food-allergic children*
Группа	*Group*
2-18 мес	*2-18 months*
Группа сравнения	*Comparison Group*
Скрининг	*Screening*
0-7 дней	*0-7 days*
диагностическая элиминационная диета	*Diagnostic elimination diet*
1 нед	*1 week*
Коррекция элиминационной диеты по результатам обследования	*Elimination diet adjustments based on monitoring results*
6 (12) мес	*6 (12) months*
при IgE-опосредованной форме	*IgE-mediated form*
Диагностическое введение продукта	*Diagnostic introduction of foods*
Оценка формирования толерантности	*Assessment of tolerance development*

Immunology and allergology examination methods included identification of blood serum content of sIgE and sIgG4 to the protein components of cow and goat milk, soy, wheat gluten, and cow milk protein fractions (bovine whey albumin, casein, β-lactoglobulin), as well as ovalbumine.

Specific IgE in blood serum were identified using ImmunoCAP laboratory diagnostics tool. The sensitization class was assessed by the sIgE concentration level (table 1).

sIgG4 to the same allergens were identified by enzyme immunoassay using the IFA-Lakttest sets (lactose trial sets for enzyme immunoassay). The concentration of specific antibodies was assessed semi-quantitatively (table 2).

Table 1. sIgE assessment by means of ImmunoCAP

Class	Level assessment	sIgE concentration, kUA/l	Concentration degree
0	Undetectable	-	-
I	Low	0.35 to 0.7	+/-
II	Average	0.7 to 3.5	+
III	Moderately high	3.5 to 17.5	++
IV	High	17.5 to 50	+++
V	Extremely high	50 to 100	++++
VI	Ultimately high	>100	+++++

Table 2. sIgG4 assessment by means of IFA-Lakttest

Class	Level assessment	sIgG4 concentration, µg/ml
0	Low	0 to 5
1	Moderate	5 to 25
2	High	25 to 100
3	Extremely high	>100

Statistical Processing

Data were processed statistically using Windows M Excel 2010 and Statistica v. 6.0, whereby descriptive statistics was applied. Whether the compared values differed significantly (i.e. the p value) was determined by means of Student's t-test. Difference was deemed statistically significant is p was less than 0.05.
Over the course of this research, we have studied the clinical-anamnestic factors and laboratory markers of tolerance development in food-allergic children. We have carried out a retrospective analysis of the clinical picture and assessed laboratory parameters as possible predictive indicators.

RESULTS

The first group comprised 147 pediatric patients, 1-to-18-month-old, affected with different FA forms. The second group comprised 77 healthy children of the same age, without any allergic manifestations or burdened allergic history.

Patients' Clinical Profile

Group 1 included children affected with three clinical forms of allergy:

- manifestations of atopic dermatitis (the AD subgroup);
- atopic dermatitis and gastrointestinal manifestations of FA (the AD & GI subgroup).
- gastrointestinal symptoms (the GI subgroup).

The nature of the main pathological condition and the age-based distribution of patients is presented in Table 3. The AD subgroup also included infants suffering from the infant-specific form of this disease.
26.5% of Group 1 children were fed artificially-only; 20.4% received mixed feeding; and 78 children (53.1%) were breast-fed at least until the 8th month of life.
An analysis of etiological factors in food-allergic children revealed the dominance of clinical responses to CMP-containing food in almost 70% of children (n=102), Fig.1; clinical responses

to eggs were dominant in 16% of children. Responses to CMP-containing foods and eggs were noted in a third of patients, including responses to foods contained in the feeding mothers' nutrition. Responses to other foods like wheat, vegetables, fruits, etc. were identified in 14% of children. No statistically significant difference was identified in the etiological factors of food allergies in children with cutaneous and cutaneous-gastrointestinal manifestations (Table 4). In patients with gastrointestinal symptoms, only CMP-triggered responses were noted, but this group was not analyzed statistically due to its small number.

53.1% of children had mild allergic responses. No significant difference of FA manifestation severity was identified in regard to the clinical picture (Fig. 2).

After sIgE identification, IgE-mediated nature of FA was confirmed for 66% of children.

Upon studying the level of wIgG4 to food proteins, we found out that sIgG4 were more frequently identified in food-allergic children as compared to their non-allergic peers (table 5). The frequency of high levels (+++) of sIgG4 to infancy allergens (cow and goat milk proteins, soy proteins, wheat gluten, ovalbumine, and CMP fractions like bovine whey albumin, casein, β-Lactoglobulin) was both in the general group and different subgroups of FA-affected children significantly lower than in the non-allergic group ($p < 0.01$). The ratio of sIgG4 values was comparable in the groups with different clinical pictures.

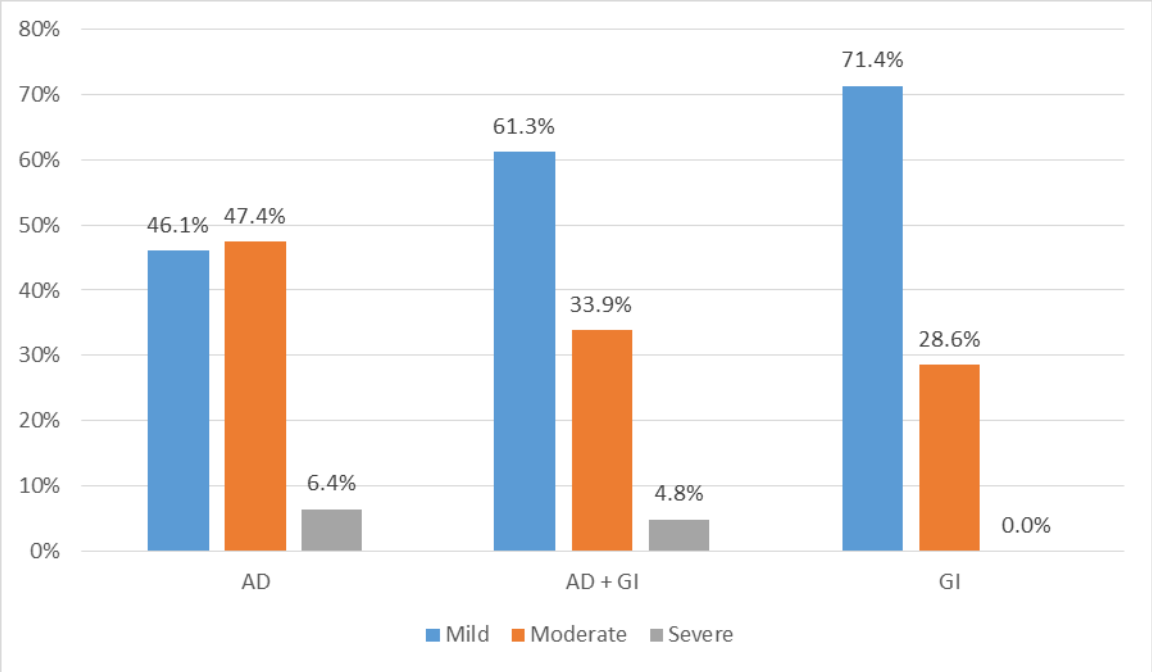


Fig. 2. Severity of FA clinical manifestations in Group 1 with different clinical manifestations of FA

Table 3. Clinical characteristics and age-based composition of Group 1

Age and number of children / **Main clinical manifestations**	**Number of children**							
	Age (months)						**Total**	
	1 to 6		**7 to 12**		**13 to 18**		**Abs.**	**%**
	n = 62		**n = 48**		**m = 37**			
	M	**F**	**M**	**F**	**M**	**F**		
Atopic dermatitis	26		28		25		79	53.7
	3	13	15	13	12	13		
Atopic dermatitis and gastrointestinal manifestations of allergy	31		17		12		60	40.8

	14	17	7	10	5	7		
Gastrointestinal manifestations of allergy	5		3		0		8	5.4
	2	3	1	2	0	0		
Percent total	62		48		37		147	100
	23	33	23	25	17	20		
	42.2		32.6		25.2			

Table 4. Frequency of clinical responses to food in children affected with food allergy of different forms

Etiological factor / Clinical forms	**Cow milk protein n = 102 (69.4%)**		**Eggs n = 24 (16.3%)**		**Responses to other foods in the absence of response to milk protein and eggs n = 21 (14.3%)**	
	n	**%**	**n**	**%**	**n**	**%**
Atopic dermatitis (n=79)	52	35.4	19	12.9	8	5.4
Atopic dermatitis & gastrointestinal manifestations (n=60)	39	26.5	9	6.1	13	8.8
Gastrointestinal manifestations (n=8)	8	5.4	0	0.0	0	0.0

Table 5. Initial sIgG4 levels in the groups under research

Groups / sIgG4 class		**0**		**1**		**2**		**3**	
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Group 1*	Atopic dermatitis (n=79)	25	17.0	31	21.1	15	10.2	8	5.4
	Atopic dermatitis & gastrointestinal manifestations (n=60)	17	11.6	23	15.6	13	8.8	7	4.8
	Gastrointestinal manifestations (n=8)	2	1.4	3	2.0	2	1.4	1	6.8
	Total, n=147	44	29.9	57	38.8	30	20.4	16	10.9
Group 2*	1-to-18-month-old, n=77	16	20.8	8	10.4	7	9.1	46	59.7

Note: * – $p < 0.01$.

Dynamic Monitoring Results and Assessment of Laboratory Predictors of Tolerance Development

Based on what diagnostic introduction of cause-significant foods resulted in, the children were divided into two groups. Group 1A consisted of those who managed to develop tolerance, Group 1B comprised those who did not.

It is nowadays recommended that food-allergic children should only be breast-fed; transition to artificial feeding is unadvisable unless well-reasoned. Breast-fed and mixed-fed children were managed by preserving breastmilk as a part of their nutrition, whereas the feeding mothers had to stick to an elimination diet. We have analyzed the correlation of feeding type with the development of tolerance to cause-significant allergens (Table 6). It has been found out that Group 1A contained significantly more breast-fed children as compared to Group 1B ($p < 0.01$). This may prove breast-feeding is protective when it comes to the development of tolerance to food allergens. Our study did not identify any correlation of the clinical picture to the type of feeding.

Table 6. Clinical tolerance development dependent on feeding type among food-allergic children

Tolerance development / **Feeding type**	**Tolerance developed n = 76 (51.7%); Group 1A**	**Tolerance not developed n = 71 (48.3%); Group 1B**
Artificial, n=39	17 (22.4)	22 (31)
Mixed, n=30	10 (13.2)	20 (28.2)
Breast-feeding, n=78	49 (64.5)	29 (40.8)

Note. In the group of children who developed tolerance, a significantly greater number of children were breast-fed, $p < 0.01$.

It is known that in case of food allergy, severe clinical manifestations are an adverse factor of tolerance development [1]. In our research, mild clinical manifestations were as well significantly more frequent in the group of children who managed to develop tolerance ($p < 0.01$, Fig. 3). Quite the contrary, severe clinical symptoms were significantly more frequent in children who did not ($p < 0.05$).

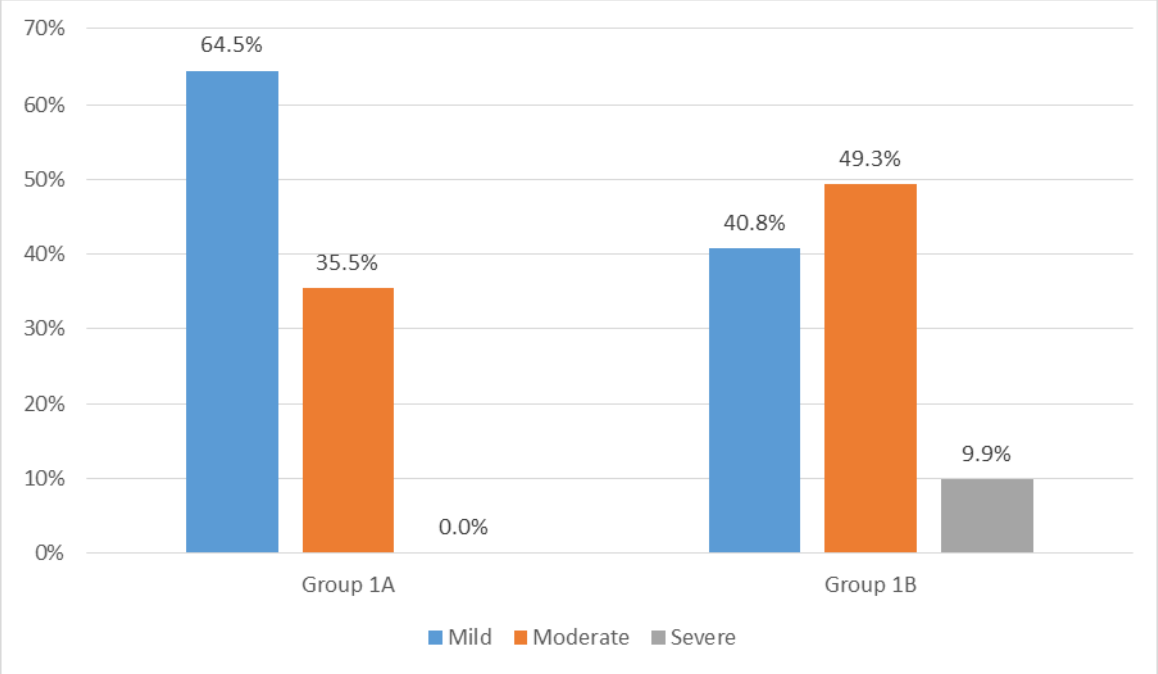


Fig. 3. Development of tolerance to cause-significant proteins dependent on the severity of clinical manifestations

Retrospective analysis did not identify any dependence of tolerance development on the FA form, whether IgE-mediated or not. Nevertheless, the frequency of non-IgE-mediated FA in the group of children who developed tolerance (Group 1A) is higher as compared to Group 1B (Table 7).

Retrospective analysis showed the frequency of initially high (+++;++) levels of sIgG4 to cause-significant allergens was significantly higher in the group of children who then developed tolerance as compared to children still sticking to elimination diet due to the persistent allergic response to cause-significant foods ($p < 0.05$). Initially high sIgG4 levels were more frequent in Group 2 as compared to the food-allergic group regardless of whether the latter developed tolerance or not (Table 8). Negative sIgG4 identification results were more frequent among the children who did not develop tolerance. This indirectly proves that high sIgG4 level is a natural physiological protective factor of food allergy development.

Our next step was to research the correlation of clinical manifestations severity and initial sIgG4 levels. We have found out that mild clinical manifestations correlated to initially high (+++; ++) sIgG4 levels ($p < 0.05$) in children who then developed tolerance (Fig. 4). On the contrary, moderate and severe clinical symptoms of FA correlated to initially low (0.1+) sIgG4 levels ($p < 0.05$) in children who did not develop tolerance.

Fig. 5 presents a general analysis of initial immunological parameters of children who developed and did not develop tolerance (monitored for 6 to 12 months).

Table 7. Frequency of IgE-mediated and non-IgE-mediated forms of FA in children who developed tolerance and who did not*

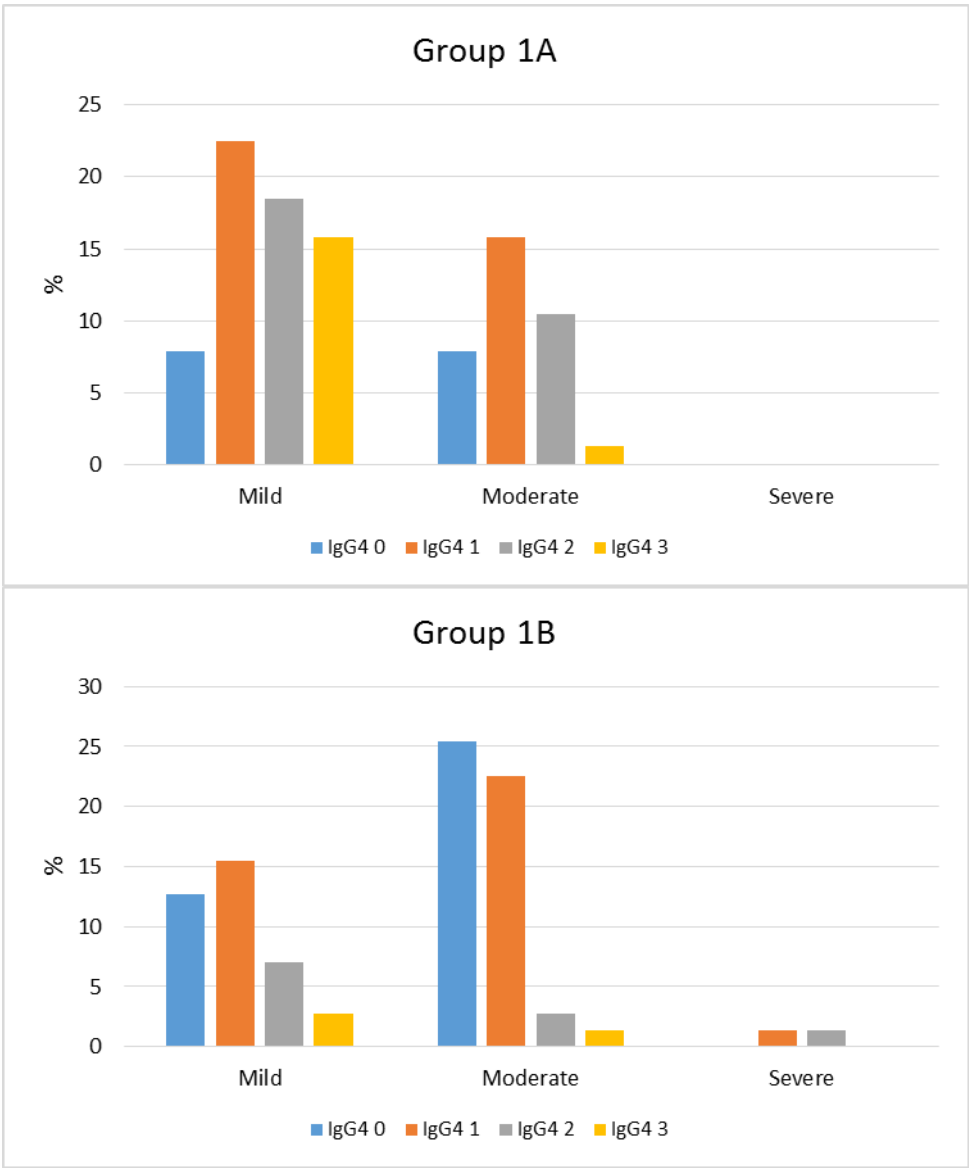
	Group 1, n = 147	**Group 1A, n = 76**	**Group 1B, n = 71**
IgE-mediated food allergy	97 (66%)	47 (61.8%)	50 (70.4%)
Non-IgE-mediated food allergy	50 (34.0 %)	29 (38.2%)	21 (29.6%)

Note. * monitored for 6 to 12 months.

Table 8. Clinical tolerance development dependent on manifestations and initial sIgG4 levels in Group 1

Groups of children / **sIgG4 level**	**Tolerance developed n = 76 (51.7%); Group 1A**				**Tolerance not developed n = 71 (48.3%); Group 1B**				**Group 2 n = 77**
	Atopic dermatitis, n = 31	**Atopic dermatitis & gastrointestinal manifestations, n=39**	**Gastrointestinal manifestations, n = 6**	**Total n (%)**	**Atopic dermatitis, n = 48**	**Atopic dermatitis & gastrointestinal manifestations, n=21**	**Gastrointestinal manifestations, n = 2**	**Total**	
0	6 (7.9)	5 (6.6)	1 (1.3)	12 (15.8)	19 (26.8)	12 (16.9)	1 (1.4)	32 (45.1)	16 (20.8)
1	12 (15.8)	15 (19.7)	2 (2.6)	29 (38.1)	19 (26.8)	8 (11.3)	1 (1.4)	28 (39.4)	8 (10.4)
2	8 (10.5)	12 (15.8)	2 (2.6)	22 (28.9)*	7 (9.9)	1 (1.4)	0 (0)	8 (11.3)*	7 (9.7)
3	5 (6.6)	7 (9.2)	1 (1.3)	13 (17.1)*	3 (4.2)	0 (0)	0 (0)	3 (4.2)*	46 (59.7)
Total, n	31 (40.8)	39 (51.3)	6 (7.9)	76 (100.0)	48 (67.6)	21 (29.6)	2 (2.8)	71 (100.0)	77 (100.0)

Note. * – $p < 0.05$.



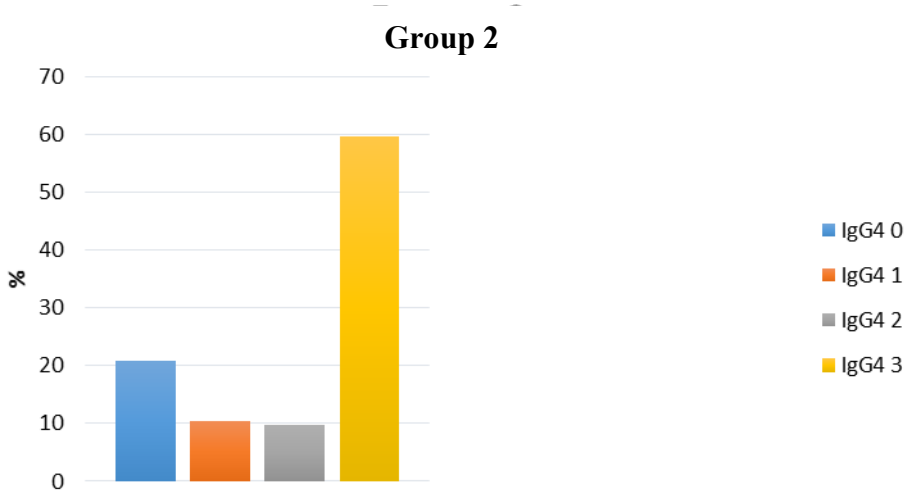


Fig. 4. Frequency of different sIgG4 levels in food-allergic children dependent on the severity of clinical manifestations and tolerance development

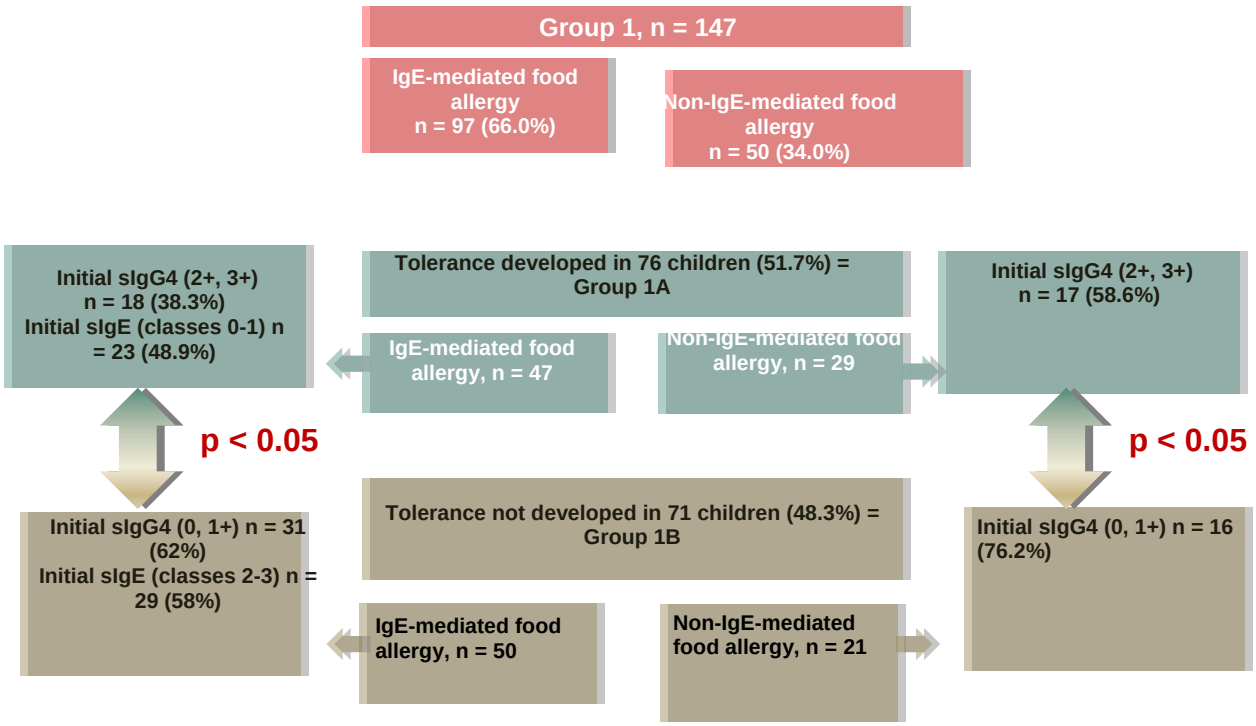


Figure 5. General analysis of initial immunological parameters of children who developed and did not develop tolerance (monitored for 6 to 12 months).

DISCUSSION

It is extremely important to develop laboratory diagnostics techniques that will allow to assess the development of tolerance to cause-significant allergens. Such techniques will simplify the management of food-allergic patients. In particular, it will help reduce the risk of recrudescence in food-allergic children upon diagnostic introduction of foods (L.S. Namazova-Baranova, S.G. Makarova), and will also allow to determine the time intervals for possible enrichment of children's nutrition, thus reducing the costs of diagnostics, treatment, and prevention.

Our study has showed that the levels of sIgG4 to food allergens were lower in food-allergic children as compared to their health peers. A comparison of specific G4 antibodies level in both children groups has showed that the frequency of high levels of sIgG4 to food allergens is significantly higher in healthy children without burdened allergic history as compared to food-allergic children of the same age. This proves the point that sIgG4 cannot be used as a food allergy diagnostic marker, as it was previously thought. Most likely, IgG4 act as "memory antibodies" and take part in tolerance development by modifying the Th2-type immune response, leading to a reduction of IgE production and elevation of IgG4 [8]. In this study, higher IgG4 levels were characteristic of children with mild FA.

In the monitored group of food-allergic children, mild clinical manifestations are a favorable factor of tolerance development.

Breast-fed children developed tolerance to food proteins more frequently compared to artificially-fed ones, which indicated the protective effects of breastmilk. However, the catamnestic monitoring of such children has to be continued.

CONCLUSION

The data we obtained by means of prospective monitoring of food-allergic infants and toddlers show that sIgG4 identification can be used in the clinical setting as an accessible technique of tolerance prediction. High sIgG4 levels seem to be a natural physiological factor of tolerance development. Mild clinical manifestaions of FA and continuous breast-feeding can be clinical predictors of food protein tolerance development in children.
Further results of this research and an analysis of catamnestic monitoring of such food-allergic children will be presented in the subsequent article.

CONFLICT OF INTEREST

The authors of this article have declared absence of reportable financial support / conflict of interest.