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Egg allergy in atopic dermatitis patients-analysis of results of specific extract IgE and specific IgE to molecular components. Bird-egg syndrome

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ABSTRACT

Aim: To evaluate the occurrence of egg allergy in atopic dermatitis patients, to analyse the results of specific IgE. We show the occurrence of bird-egg syndrome and the relation between the sensitisation to some molecular components and egg allergy. **Methods:** Egg allergy was confirmed according to the results of the challenge test (history) together with positive results of specific IgE to molecular components of egg and/or extract specific IgE to egg. **Results and conclusion:** Altogether 100 atopic dermatitis patients were examined – 48 men, 52 women, the average age 40.9 years. Reaction to egg was confirmed in 23 patients (23%); egg allergy was confirmed in 18 patients (18%). In these patients we recorded the significantly higher sensitisation to molecular components Der f 2, Der p 2, Equ c 1 and Phl p 7. The bird – egg syndrome was confirmed in 3 patients (3%).

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Introduction

Egg allergy is one of the most common food allergies in infants and young children, affecting up to 9% of children worldwide (Wei-Liang Tan et al., 2017). Spontaneous resolution and tolerance to egg allergy is common and occurs in 60–75% of children prior to their teenage years (Dang et al., 2019; Wei-Liang Tan et al., 2017). Egg allergy in adults is likely to be severe and long-lasting and is due either to persistent childhood egg allergy or to true adult-onset egg allergy, such as occupational, for example, in workers from the baking industry who develop sensitization by inhalation, part of the bird-egg syndrome with an allergy to egg yolk (Dang et al., 2019; Wei-Liang Tan et al., 2017). Egg sensitization is closely associated with atopic dermatitis (AD), particularly in infants who develop AD in the first year of life (Hill et al., 2008; Hill & Hosking, 2004; Wolkerstorfer et al., 2002). Egg-sensitized children have been shown to have more severe and persistent

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dermatitis, and the level of sensitization correlated with the severity of disease (Hill et al., 2008; Hill & Hosking, 2004; Wolkerstorfer et al., 2002). A few reports demonstrate the occurrence of egg allergy in adolescent and adult patients suffering from atopic dermatitis. Other numerous trigger factors for AD have been identified, including inhalable respiratory allergens, irritative substances and infectious microorganisms, such as *Staphylococcus aureus*. Psychogenic and climatic factors may also cause exacerbation of AD (Breuer et al., 2004; Dang et al., 2019; Hill et al., 2008; Hill & Hosking, 2004; Wei-Liang Tan et al., 2017; Wolkerstorfer et al., 2002). An allergic reaction or response encompasses two distinct stages based on the contact of the allergen with the immune system, referred to as the humoral or primary response and the cell-mediated or secondary response (Calzada et al., 2018; Jabs et al., 2018; Kandikattu et al., 2019; Niggemann et al., 1999; Steinman, 1991). Delayed exacerbations of atopic dermatitis (>24 h after egg consumption) may occur in patients without evidence of sensitization to egg, most likely because of T-cell mediated allergic reactions (Breuer et al., 2004; Niggemann et al., 1999). The risk of sensitization to aeroallergens and asthma is also increased in egg-allergic children (Breuer et al., 2004; Niggemann et al., 1999).

The most common symptoms of egg allergy are IgE-mediated erythema, urticaria and eczematous rash (Matricardi et al., 2016); gastrointestinal symptoms, abdominal pain and vomiting (mostly in conjunction with other immediate-type symptoms) occur in 40–50% of cases following egg ingestion (Matricardi et al., 2016). In addition, egg is one of the most common food allergens in allergic eosinophilic esophagitis and allergic eosinophilic gastroenteritis (Matricardi et al., 2016).

Correct diagnosis of food allergy is of great importance so as to avoid falsely labeling the patient as food - allergic or not allergic; there is the need to improve and to evaluate the approach on the diagnosis of food allergy (Flammarion et al., 2011; King et al., 2009; Luengo & Cardona, 2014; Sastre, 2010; Soares-Weiser et al., 2014). Diagnosis of food allergy is based on personal history, measurement of specific IgE (serum-specific IgE level – sIgE, skin prick tests – SPTs), atopy patch tests (APT), challenge tests (open exposure test – OET, double-blind, placebo-controlled food challenge test – DBPCFC). Progress in laboratory diagnostics of IgE-mediated allergy is the use of component-resolved diagnosis (CRD) or molecular diagnosis (MD) of allergies (Luengo & Cardona, 2014; Matricardi et al., 2016; Sastre, 2010; Wolthers, 2012). There is growing evidence that the use of MD in combination with conventional sensitization testing improves analytical and diagnostic performance and can lead to a reduction in the need for diagnostic oral food challenges in selected cases (Čelakovská & Josef, 2014; Čelakovská et al., 2011, 2015a, 2015b, 2015c, Dang et al., 2012; Sampson, 2001; Sporik et al., 2000). The introduction of allergen molecules has had a major effect on analytic specificity and allergy diagnosis. They are used in both singleplex ImmunoCAP and multiplex ImmunoCAP ISAC assays (van Hage et al., 2017). ImmunoCAP ISAC (Thermo Fisher), based on 112 different molecular components (both extracted and recombinant), is the most studied and most frequently used molecular diagnostic tool based on a microarray (Melioli et al., 2011).

Five proteins most commonly involved in allergic reactions to hen's egg have been identified and characterized (Gal d 1–5), Table 1 (Boyano Martínez et al., 2001). Egg white, which is generally considered more allergenic than egg yolk, has four major allergenic proteins namely, ovomucoid, ovalbumin, ovotransferrin and lysozyme which are defined as immunodominant egg white proteins. Although ovomucoid comprises only

Table 1. Allergenic molecules of hen's egg and clinical relevance of specific proteins Adapted from (Boyano Mart_inez et al., 2001).

Protein name	Mr* (kDa)	Protein family	Biologic function(s)	Resistance to heating and chemical denaturation	Clinical relevance
Egg White Proteins					
ovomucoid (Gal d 1)	28	Kazal-type serine protease inhibitor	Serine protease inhibition activity	High	Heat-stable and highly allergenic; Risk for reaction to all forms of egg; High levels of specific IgE may indicate sustained egg allergy
Ovalbumin (Gal d 2)	45	Serine protease inhibitor	Storage protein?	Low	Heat-labile; Most abundant egg white protein; Risk for clinical reaction to raw or slightly heated egg and certain vaccines
ovotransferrin or conalbumin (Gal d 3)	76–77	Transferrin	Iron-binding capacity with antimicrobial activity	Low	Heat-labile; Risk for clinical reaction to raw or slightly heated egg
egg lysozyme (Gal d 4)	14.3	Glycoside hydrolase family 22	Antibacterial activity	moderate	Risk for clinical reaction to raw or slightly heated egg
Ovomucin	165	Contains trypsin inhibitor-like domains	Heavily glycosylated protein with potent antiviral activities	n.a.	n.a.
Egg Yolk Proteins					
phosvitin	35	transferase?	Metal-chelating agent	n.a.	n.a.
a-livetin (Gal d 5)	65–70	Serum albumin	Bind ions, fatty acids, hormones in physiological condition	n.a.	n.a.
apovitellenins I	9.5	Very low density lipoprotein	Potent lipoprotein lipase inhibitor	n.a.	n.a.
apovitellenins VI (or apoprotein B)	170	Unknown	Lipid-binding activity	n.a.	n.a.

10% of the total egg white protein, it has been shown to be the dominant allergen (Boyano Martínez et al., 2001; Cooke & Sampson, 1997). The most reported allergen from egg yolk seems to be serum albumin alpha-livetin Gal d 5. Serum albumins are highly cross-reactive proteins conserved in amino acid sequence and protein structure with a molecular weight around 70 kDa. They can be found in all tissues, including muscle tissue, as well as in high amounts in egg yolk (Eigenmann, 2000; Quirce et al., 2001). The relationship between asthma and rhinoconjunctivitis due to bird feather sensitization and egg allergy has been described as a bird-egg syndrome related to the cross-reactive IgE antibody able to bind the egg yolk livetin and bird feather allergens (Eigenmann, 2000; Quirce et al., 2001).

The aim of our study

- (1) to evaluate the egg allergy in atopic dermatitis patients
- (2) to evaluate, if there is the significant relation between the occurrence of egg allergy and the sensitisation to some molecular components in ISAC Multiplex testing
- (3) to evaluate the occurrence of bird – egg syndrome.

Method

Patients and methods

In the period of 2018–2019, 100 patients suffering from atopic dermatitis at the age of 14 years and older were examined. All these patients were examined in the Department of Dermatology, Faculty Hospital Hradec Králové, Charles University, Czech Republic. The diagnosis of AD was made using the Hanifin-Rajka criteria (Hanifin & Rajka, 1980). Exclusion criteria were systemic therapy (cyclosporin, systemic corticoids, biological therapy), pregnancy, breastfeeding. Patients with atopic dermatitis having other systemic diseases were excluded from the study as well. Complete dermatological and allergological examination was performed in all patients included in the study. This study was approved by Ethics committee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic.

Confirmation of egg allergy

- (1) The diagnosis of egg allergy was confirmed according to the challenge test (or convincing history with the clinical reaction after egg ingestion) together with the positive result of specific IgE (molecular components Gal d 1 and/or, Gal d 2 and/or Gal d 3 and/or Gal d 5 in ISAC Multiplex testing and/or extract specific IgE to egg yolk and white).
- (2) Sensitisation to egg white and/or egg yolk was confirmed in patients with no clinical reaction after ingestion of egg, but with positive results of specific IgE to molecular components of egg in ISAC Multiplex testing and/or extract specific IgE to egg yolk and white. It means, that although the positive result of specific IgE was recorded, these patients are without clinical symptoms. All these patients are informed about the positive results of specific IgE and they are always monitored.

Confirmation of bird egg syndrome

Patients with reaction to egg with positive results of specific IgE to Gal d 5 and positive results of extract specific IgE to mix of bird-feather.

Examination of specific IgE (sIgE) to molecular components

The serum level of the sIgE was measured by the component-resolved diagnosis microarray-based sIgE detection assay ImmunoCAP ISAC (Phadia, Thermo Fisher Scientific, Uppsala, Sweden). ImmunoCAP ISAC is a solid-phase multiple immunoassay which enables to determine 112 different components from 51 allergen sources (Melioli et al., 2011; van Hage et al., 2017). The allergens are applied in triplicates to ensure the test reproducibility. The specific IgE values are presented in arbitrary units called ISAC Standardized Units (measuring range of 0.3-100 ISU-E). The level of specific IgE higher than 0.3 ISU-E was assessed as positive. The level of molecular components in ISU – E was evaluated: < 0.3- negative, 0.3-0.9 low positivity, 0.9-15 moderate positivity, above 15 ISU – E very high positivity (Melioli et al., 2011; van Hage et al., 2017).

Specific extract IgE

The serum level of the specific IgE to yolk and white and to mix of bird feather has been measured using the CAP method (system FEIA; Pharmacia Diagnostics, Uppsala, Sweden). A level of specific IgE higher than 0.35 U/ml was assessed as positive.

Personal history

The detailed personal history of possible food allergy was taken in all included patients. The patients answered if they suffered from immediate or late food adverse reactions affecting the skin, gastrointestinal tract, or respiratory tract not only after the ingestion of egg but also after ingestion of other foods also.

Recommendation of performing the open exposure test (OET) with egg

The OET with egg after the diagnostic hypoallergenic diet was recommended to be performed in patients suffering from moderate and severe form of AD. In patients with mild form of AD, we recommended the elimination of egg in the period of four weeks (according to the positive results in extract specific IgE and according to the positive results to molecular components to egg in ISAC Multiplex testing). In patients with early reactions after egg ingestion in their history, the open exposure test was not performed because of anaphylactic reaction danger.

Diagnostic hypoallergenic diet:

In the period of four weeks we recommended these foods: gluten free foods, potatoes, rice, meat-beef, pork, vegetable, and fruits only after thermal modification, but parsley, celery, and seasoning were not allowed. The patients are allowed to drink only drinking water, mineral water, or black tea (Čelakovská et al., 2015a; Čelakovská et al., 2015b).

Open exposure test (OET)

Consecutive OET with egg was performed. This test was performed in intervals without symptoms or during a consistent period with regard to AD. When it was impossible to avoid steroids, the patient was allowed to treat himself with a low-potency topical corticosteroid. No other antiinflammatory substances, antihistamines or UV therapy were applied. OETs took two days with three doses of examined food in the interval of 12 h, but one dose was divided into incremental dosages given over 80 min. The well-cooked egg was applied at 20-minutes intervals with incremental dosages – 50, 500 mg, 5, 50, and 100 g. On the first day, the patient consumed a dose of egg at 08:00 h, the reaction was observed, and in the case of no response, a second dose of food was consumed at 18:00 h. If no response resulted, the last dose challenge was administered the next day at 08:00 h. The patient also recorded the reaction during the 48 h after the last dose. The skin was scored by the SCORAD system before the diet, during the diet, before OET, and then 24 and 48 h after OET. The food challenge results were scored as positive if one or more of the following objective and subjective clinical reactions were noted: urticaria, angioedema, vomiting, wheezing, abdominal pain, diarrhea, oral allergic syndrome, pruritus, erythema, rashes, or worsening of AD (evaluated with SCORAD). Early reactions were defined as clinical symptoms within two hours after ingestion of the dose in OET and late symptoms if occurring after more than two hours. In the case where the physician or the patient recorded worsening AD or some other reactions during the test with egg, the patient continued in the elimination of egg, and the severity of AD was evaluated every three months for one year. If the test with egg was negative, the patient introduced egg in the diet regimen. The severity of AD was evaluated during the average daily intake of food over a period of three months for one year.

Scoring of AD

The severity of AD was scored in agreement with the SCORAD score (European Task Force on Atopic Dermatitis, 1993 Čelakovská et al., 2015a; Čelakovská et al., 2015b): as a mild form – 25 points; moderate – over 25–50 points; severe – over 50 points. The severity of AD was evaluated by this system at the beginning of the study and then at the end of the specific hypoallergenic diet before the open exposure test (OET) and during the OET until 48 h after the challenge. The severity of AD was evaluated in patients with a confirmed food allergy three, six, nine, and 12 months after elimination of egg from the diet. In patients with negative reactions in the OET, egg was introduced in a common meal, and the severity of AD was evaluated over a period of three months in one year also.

Statistical analysis

The comparison of the results in the examination of ISAC Multiplex testing and extract specific IgE to egg white and egg yolk and really confirmed food reactions in the open exposure (patient's history) test was performed; according to these results, we calculated the sensitivity of the ISAC Multiplex testing and the sensitivity of extract specific IgE.

Sensitivity was calculated as proportion of positive IgE antibody tests in patients with clinical reaction to egg. We also tested if there is the significant relation between the clinical reactions to egg and the sensitisation to some molecular components. Pairs of these categories were enrolled in the contingency tables and the Chi-square independence test was performed. The significance level was set to 5%. The *p* – value is displayed in the right hand column.

Results

Patients

One hundred patients suffering from atopic dermatitis were included in the study – 48 men and 52 women with the average age 40.9 years and with the average SCORAD 39 (s.d.13.1 points). The mild form of AD was recorded in 14 patients (14%), moderate form in 58 patients (58%), severe form in 28 patients (28%); 55 patients (55%) suffer from asthma bronchiale and 78 patients (78%) suffer from rhinitis. The characteristics of patients is shown in Table 2. The reaction to egg with clinical symptoms was confirmed in 23 patients (23%): open exposure test confirmed the reaction in 9 patients (9%), convincing history with clinical symptoms confirmed the reaction in 14 patients (14%). We recorded in 16 patients (16%) combined reactions (early reaction such as pruritus, maculopapulous rash and late reaction such as worsening of atopic dermatitis). We recorded in 4 patients (4%) only late reaction with the worsening of atopic dermatitis; only early reaction such as erythema of the face, oedema of the face, pruritus of the skin with maculopapulous rash and anaphylactic reaction was recorded in 3 patients (3%). Table 3. The diagnosis of food allergy was confirmed in 18 patients (18%); in these

Table 2. The characteristic of patients with atopic dermatitis.

-
- 100 patients examined – 48 men, 52 women, the average age 40.9 years
 - the average SCORAD 39 s.d. 13.1 points
 - the positive results in sensitisation to molecular components – 93 patients (93%)
 - mild form of AD patients 14 (14%)
 - moderate form of AD patients 58 (58%)
 - severe form of AD patients 28 (28%)
 - number of patients with asthma bronchiale – 55 (55%)
 - number of patients with allergic rhinitis – 78 (78%)
-

Table 3. The results of open exposure test (or history) with the symptoms of food reaction to egg in 23 patients (23%). GIT = gastrointestinal symptoms; Combined reactions (early + late reactions) – 16 patients (16%); Only early reactions – 3 patients (3%); Only late reactions – 4 patients (4%).

Early reactions 19 patients		Late reactions 20 patients	
- 3 patients only early reaction		- 4 patients only late reaction	
-16 patients late + early reaction		-16 patients late + early reactions	
Maculo-papulous rash	7 (7%)	Late pruritus	15 (15%)
Pruritus	13 (13%)	New eczematic lesions	20 (20%)
GIT symptoms	2 (2%)		
Urticaria	0 (0%)		
Oedema of the face	2 (2%)		
Anaphylactic reaction	1 (1%)		

Table 4. The analysis of results of specific IgE to molecular components of egg. We show the number of patients with positive results of specific IgE in the whole group of patients, in patients with reactions to egg and in patients with no reactions to egg. Sensitivity of molecular components Gal d 1, Gal d 2, Gal d 3, Gal d 5 was calculated as proportion of positive results of IgE in patients with clinical reaction to egg.

Molecular component	Protein family	Number of patients with positive results to molecular components, 100 patients (= 100%)			Sensitivity of molecular components
		in the whole group of 100 patients	in patients with reactions to egg (23 patients)	in patients with no reactions to egg (77 patients) - sensitisation	
Gal d 1	Ovomucoid	7 (7%)	7 (7%)	0 (0%)	29.2%
Gal d 2	Ovoalbumin	5 (5%)	4 (4%)	1 (1%)	16.7%
Gal d 3	Conalbumin/Ovotransferrin	6 (6%)	6 (6%)	0 (0%)	25.0%
Gal d 5	Livetin/Serum albumin	3 (3%)	3 (3%)	0 (0%)	12.5%
Gal d 1, 2, 3, 5*		12 (12%)	12 (12%)	1 (1%)	52.2%

Explanation:* positivity of Gal d 1 and/or Gal d 2 and/or Gal d 3 and/or Gal d 5.

patients we confirmed the reaction to egg together with positive results of sIgE (to molecular components Gal d 1 and/or, Gal d 2 and/or Gal d 3 and/or Gal d 5 in ISAC Multiplex testing and/or to extract specific IgE to egg yolk and white).

In the whole group of 100 patients we analysed the results of specific IgE to molecular components of egg, Table 4. We compare the results of specific IgE to molecular components of egg from the whole group of 100 patients with the occurrence of clinical reactions to egg. The sensitivity of ISAC Multiplex testing is 52.2%; it was calculated as the positivity at least to one of molecular components (positivity to Gal d 1 and/or Gal d 2 and/or Gal d 3 and/or Gal d 5) in patients with reaction to egg, (Table 4). We compare the results of extract specific IgE to egg white and to egg yolk from the whole group of 100 patients with the occurrence of clinical reactions to egg, (Table 5). The use of the ISAC Multiplex testing in combination with conventional sensitization testing such as extract specific IgE improves the sensitivity to 73.9%. In 5 patients (5%) suffering from food reaction to egg, we recorded no positive results in specific IgE (extract specific IgE, Multiplex ISAC examination); these patients suffer from moderate and severe form of AD and eliminate egg from their diet for a long time.

Table 5. The analysis of results of extract specific IgE to egg yolk and egg white. We show the number of patients with positive results of specific IgE to egg yolk and egg white in the whole group of patients, in patients with reactions to egg and in patients with no reactions to egg. Sensitivity of extract specific IgE to egg white and egg yolk was calculated as proportion of positive results of IgE in patients with clinical reaction to egg.

Extract specific IgE (sIgE)	Number of patients with positive results to extract specific IgE, 100 patients (= 100%)			Sensitivity of specific IgE
	in the whole group of 100 patients	in patients with reactions to egg (23 patients)	in patients with no reactions to egg (77 patients) - sensitisation	
sIgE white	14 (14%)	10 (10%)	4 (4%)	43.5%
sIgE yolk	12 (12%)	7 (7%)	5 (5%)	30.4%

Sensitisation to egg (no clinical reaction after ingestion of egg, but positive results of specific IgE to molecular components of egg and/or extract specific IgE to egg yolk and white) was confirmed to Gal d 2 in 1 patient (Table 4), to extract specific IgE to egg yolk in 4 patients and to egg white in 5 patients (Table 5).

From 23 patients (=100%) suffering from reactions to egg, the positive result of specific IgE to mix of bird-feather was confirmed in 12 patients (=52.2%). Regarding the confirmation of bird-egg syndrome, positive results of specific IgE to Gal d 5 together with positive results of extract specific IgE to mix of bird-feather were confirmed in 3 patients (3%). The overview of 23 patients (23%) suffering from reactions to egg with is recorded in Table 6. We show 18 patients (18%) with confirmed egg allergy, 3 patients (3%) with bird – egg syndrome. We record reaction to egg,

Table 6. The overview of 23 patients (23%) with reactions to egg (according to the results in the open exposure test or according to the history). We show reaction to egg (early, combined, late), severity of AD (mild, moderate, severe), the results of the ISAC Multiplex testing (the level of specific IgE to Gal d 1, 2, 3, 5 in ISU -E), the results of extract specific IgE to egg white, egg yolk and to bird feather (the level of specific IgE in U/ml), n. = negative reaction

FA+: Food allergy is confirmed in 18 patients (18%) with positive results of specific IgE to molecular components and/or extract specific IgE to egg (yolk, egg white)

FA-: Reaction to egg in 5 patients (5%) with no positive results of specific IgE to molecular components and/or extract specific IgE.

Bird – egg syndrome: 3 patients (3%) with positive results of specific IgE to Gal d 5 and positive results of extract specific IgE to mix of bird – feather (**shown extra bold***).

No. of patient	Severity of AD	Reaction	FA	Molecular components ISU -E				Extract specific IgE U/ml		
				Gal d 1	Gal d 2	Gal d 3	Gal d 5	Egg white	Egg yolk	Bird feather
1	Mild	Early	FA +	n.	n.	n.	n.	0.69	0.5	n.
2	Severe	Combined	FA +	n.	0.6	n.	n.	n.	n.	0.44
3	Severe	Combined	FA +	n.	n.	2.4	n.	0.44	n.	0.94
4	Moderate	Combined	FA +	0.3	n.	1.7	n.	n.	n.	1.35
5	Mild	Combined	FA +	n.	n.	n.	n.	0.93	1.19	n.
6	Moderate	Late	FA +	0.4	n.	n.	0.5*	n.	n.	0.807*
7	Severe	Combined	FA +	3.3	0.7	21	6.4*	n.	n.	21.3*
8	Severe	Combined	FA +	n.	0.9	n.	n.	n.	n.	1.89
9	Moderate	Early	FA +	1.0	n.	0.4	n.	0.77	n.	0.813
10	Moderate	Combined	FA -	n.	n.	n.	n.	n.	n.	n.
11	Severe	Combined	FA+	n.	n.	n.	n.	0.9	0.43	n.
12	Severe	Combined	FA+	1.0	n.	4.5	n.	5.79	n.	2.97
13	Severe	Late	FA+	2.6	n.	1.1	n.	n.	n.	1.1
14	Moderate	Combined	FA -	n.	n.	n.	n.	n.	n.	n.
15	Mild	Early, anaphylactic reaction	FA +	n.	n.	n.	n.	6.28	8.03	n.
16	Severe	Combined	FA +	n.	3.6	n.	n.	35.6	15.9	n.
17	Severe	Late	FA +	n.	n.	n.	n.	0.35 – border level	n.	n.
18	Moderate	Combined	FA +	n.	n.	n.	n.	4.19	4.36	n.
19	Moderate	Late	FA +	n.	n.	n.	0.3*	n.	n.	0.688*
20	Severe	Combined	FA -	n.	n.	n.	n.	n.	n.	0.772
21	Moderate	Combined	FA -	n.	n.	n.	n.	n.	n.	n.
22	Moderate	Combined	FA -	n.	n.	n.	n.	n.	n.	n.
23	Severe	Combined	FA +	1.8	n.	n.	n.	6.68	3.38	1.58

severity of AD, the results of the ISAC Multiplex testing with the level of specific IgE to Gal d 1, 2, 3, 5, the results of extract specific IgE to egg white and egg yolk and to bird feather.

We evaluate, if the positive results of specific IgE to some molecular components are recorded with the significantly higher occurrence in patients with reactions to egg (Table 7).

In patients with reactions to egg we confirmed the significantly higher occurrence of positive results of specific IgE to molecular components Der f 2 (NPC2 family, house dust mite), Der p 2 (NPC2 family, house dust mite), Phl p 7 (Polcalcin, Timothy grass) and Equ c 1 (Lipocalin, horse).

Discussion

The purpose of our study was to show the occurrence of egg allergy in atopic dermatitis patients, to analyze the data of ISAC Multiplex examination and extract specific IgE and to evaluate the occurrence of bird egg syndrome in AD patients. Our results show the high occurrence of egg allergy in atopic dermatitis patients (in 18%); in other 5 patients (5%) we confirmed the clinical reaction to egg without positive results to specific IgE. The explanation of negative specific IgE in these patients may be in fact, that all these patients eliminate egg from childhood. Patients with reactions to egg don't tolerate the heated and raw egg; they eliminate egg from their meal and during the mild breaking of the diet regimen they observe the reactions on the skin, such as the pruritus, erythema and new eczematous lesions. The severity of AD in patients with positive results in OET to egg was evaluated after egg elimination every 3 month during 1 year. The skin finding improved in majority of them. These patients also suffered from exacerbations of AD, but it was the consequence of other triggering factors (stress, inhalant allergens, pollen-associated foods). Late eczematous reaction on the skin in patients with a persistent moderate or severe form of AD can be observed with difficulty. The worsening of AD occurs several hours after ingestion of food allergen and other factors can contribute to the deterioration of AD at the same time also.

Bird – egg syndrome was confirmed in 3 patients, (3%); these patients (women) suffer from egg allergy, they have positive results of specific IgE to Gal d 5 and positive results of specific IgE to mix of bird-feather. These patients have been suffering from allergic asthma bronchiale from childhood and from acute allergic rhinitis and shortness of breath in contact with birds, such as canary or parrot, and animals such as a horse, a cat or a dog. We confirmed in these patients in ISAC Multiplex testing also the positive results of specific IgE to molecular components of dog, cat, horse and house dust mites. Bird-egg syndrome is characterized by respiratory and gastrointestinal symptoms (Szépfalusi et al., 1994; Williams, 1962 May, De Maat-Bleeker et al., 1982; Mandallaz et al., 1988). The patients diagnosed as having bird-egg syndrome were usually adult females and they also had sensitization to animal fur (Szépfalusi et al., 1994; Williams, 1962 May, De Maat-Bleeker et al., 1982; Mandallaz et al., 1988), as it was confirmed also in our study. Asthma is the main symptom as a consequence of contact with birds and rhinoconjunctivitis may develop. The initial symptoms are usually asthma with or without rhinoconjunctivitis due to contact with birds. Individuals first become sensitized to bird proteins (feathers, excrements, serum, meat) and subsequently develop egg allergy, although sometimes the order of appearance is reversed (De Maat-Bleeker et al., 1982;

Table 7. The relation between the occurrence of reactions to egg and the sensitisation to some molecular components in ISAC Multiplex examination. We show the number of patients with positive results of specific IgE to molecular components in the whole group of 100 patients, in patients with reaction to egg (23 patients = 100%), and in patients without reaction to egg (77 patients = 100%). Pairs of these categories were enrolled in the contingency tables and the Chi-square independence test was performed. The significance level was set to 5%. The *p* – value is displayed in the right hand column. The significant relation is shown extra bold*.

Allergen Source	Molecular component	Protein group	Number of patients with positive results of specific IgE to molecular component			<i>p</i> – value
			In the whole group of 100 patients	In patients with no egg reaction - 77 patients (= 100%)	In patients with egg reaction – 23 patients (= 100%)	
Alternaria	Alt a 1	Acidic glycoprotein	29	22(28.6%)	7(30.4%)	0.863
Aspergillus	Alt a 6	Enolase	16	10(13.0%)	6(26.1%)	0.133
	Asp f 1	Mitogillin family	6	4(5.2%)	2(8.7%)	0.535
	Asp f 3	Peroxisomal protein	6	4(5.2%)	2(8.7%)	0.535
	Asp f 6	Mn superoxide dismutase	21	14(18.2%)	7(30.4%)	0.206
Cladosporium	Cla h 8	Mannitol dehydrogenase	4	2(2.6%)	2(8.7%)	0.190
House dust mite	Der f 1	Cysteine protease	34	23(29.9%)	11(47.8%)	0.111
	Der f 2	NPC2 protease	45	29(37.7%)	16(69.6%)	0.007*
	Der p 1	Cysteine protease	36	25(32.5%)	11(47.8%)	0.178
	Der p 2	NPC2 family	46	30(39.0%)	16(69.6%)	0.010*
	Der p 10	Tropomyosin	5	2(2.6%)	3(13.0%)	0.044
Cat	Fel d 1	Uteroglobin	42	33(42.9%)	9(39.1%)	0.751
	Fel d 2	Serum albumin	7	4(5.2%)	3(13.0%)	0.195
	Fel d 4	Lipocalin	29	20(26.0%)	9(39.1%)	0.222
Dog	Can f 1	Lipocalin	39	29(37.7%)	10(43.5%)	0.616
	Can f 2	Lipocalin	17	11(14.3%)	6(26.1%)	0.186
	Can f 3	Serum albumin	8	6(7.8%)	2(8.7%)	0.889
	Can f 5	Arginine esterase	26	18(23.4%)	8(34.8%)	0.274
Horse	Equ c 1	Lipocalin	27	17(22.1%)	10(43.5%)	0.043*
Timothy grass	Phl p 1	Beta expansin, Timothy	61	44(57.1%)	17(73.9%)	0.148
	Phl p 2	Expansin, Timothy	39	31(40.3%)	8(34.8%)	0.637
	Phl p 4	Berberine bridge enzyme	52	40(51.9%)	12(52.2%)	0.985
	Phl p 5	Grass group 5	43	32(41.6%)	11(47.8%)	0.594
	Phl p 6	Grass group 6	42	30(39.0%)	12(52.2%)	0.260
	Phl p 11	Ole e 1-related protein	20	16(20.8%)	4(17.4%)	0.722
Olive pollen	Ole e 7	Lipid transfer protein	1	1(1.3%)	0(0.0%)	0.583
Plane tree	Pla a 3	Lipid transfer protein	5	5(6.5%)	0(0.0%)	0.210
Mugwort	nArt v 3	Lipid transfer protein	5	4(5.2%)	1(4.3%)	0.870
Birch	rBet v 2	Profilin	9	5(6.5%)	4(17.4%)	0.109
Timothy	Phl p 12	Profilin	3	2(2.6%)	1(4.3%)	0.666
Annual mercury	Mer a 1	Profilin	13	9(11.7%)	4(17.4%)	0.475
Timothy	rPhl p 7	Polcalcin	7	3(3.9%)	4(17.4%)	0.026*
Birch	Bet v 4	Polcalcin	4	2(2.6%)	2(8.7%)	0.190

Mandallaz et al., 1988; Szépfalusi et al., 1994; Williams, 1962). Typically, patients develop upper and lower respiratory symptoms on exposure to birds and gastrointestinal symptoms with chicken meat or lightly cooked eggs. The likely allergen is chicken serum albumin Gal d 5 and the egg allergy is due to IgE cross-reactivity with livetin found in egg yolk (Wolkerstorfer et al., 2002). Egg yolk contains significant quantities of serum proteins, since livetins are derived from the blood of the hen. Williams identified livetin as chicken serum albumin (CSA), a protein of 65–70 kDa (Williams, 1962). Szépfalusi et al. (1994) pointed out that CSA is a cross-reactive allergen in the bird-egg syndrome. De Maat-Bleeker et al. first reported the association of hypersensitivity to ingested egg yolk with rhinitis and asthma caused by exposure to a parrot in an older woman (1982). By RAST inhibition, Mandallaz et al. (1988) demonstrated that livetin, the water-soluble fraction of egg-yolk proteins, was the major cross-reacting antigen found in bird feathers and egg yolk, and they coined the term “bird-egg syndrome” to designate this IgE-mediated association of inhalant and food allergy.

Although the unequivocal diagnosis of egg allergy requires a double blinded placebo controlled food challenge test (DBPCFC), open food challenges, which are less resource-intensive, are generally considered sufficient in clinical practice (Bock et al., 1988). Once a diagnosis confirms an allergy, in particular an egg allergy, the current treatment involves strict dietary avoidance or minimised contact with the allergen (Centers for Disease Control and Prevention (CDC)). Immunotherapy is considered to be the most efficient way to treat and relieve the symptoms of allergies, with clinical studies indicating immunotherapy approaches have the potential to not only improve allergic symptoms but to eventually prevent allergies (Centers for Disease Control and Prevention (CDC); Wood, 2016).

The diagnostic accuracy of CRD-specific tests in comparison to tradition diagnosis needs to be further investigated as current studies show high specificity but low sensitivity during analysis. According to our results, the examination of specific IgE to molecular components and extract specific IgE to egg yolk and egg white improves the sensitivity to 73.9%.

The systematic review conducted by Kim and associates in 2018 focus on CRD diagnosis of the “big eight” food allergies. The review summarizes the diagnostic accuracy measures emphasizing the need for a methodologically dynamic but uniform approach (Canonica et al., 2013; Flores Kim et al., 2018). The current lack of relevant allergen variants needed for diagnostics assays leads to lower levels of specificity and sensitivity than that of open food challenge. Consequently, open food challenge remains the gold standard in food allergy diagnosis, limiting the progress of CRD (Calamelli et al., 2019).

According to our results, AD patients suffering from egg reactions are significantly higher sensitized to molecular components Der f 2 (NPC2 family, house dust mite), Der p 2 (NPC2 family, house dust mite), Phl p 7 (Polcalcin, Timothy grass) and Equ c 1 (Lipocalin, horse).

Der p 2, Der f 2 are from NPC2 (Niemann-Pick Type C2) protein family (epidermal secretory proteins). NPC2 proteins are secreted early in response to an IgE-mediated stimulus and co-localizes with the lysosomal membrane marker, CD63. NPC2 belongs to the ML (MD-2-related lipid-recognition) protein family (155 members), which includes the Toll-like receptor co-factors, MD-1 and MD-2, and seven major house dust mite allergens of unknown function (including Der p 2 and Der f 2), (Pathak &

Helm, 2010 Mar 8). Group 2 allergens from the *Dermatophagoides* house dust mites causes IgE-mediated responses in over 80% of the dust mite allergic individuals and are therefore classified as major allergens. For the last 20 years, researchers have been interested to uncover the biological role of group 2 dust mite allergens. Der p 2 is a major dust mite allergen and >80% of mite allergic individuals have specific IgE to this allergen (Platts-Mills et al., 1997; Thomas & Smith, 1998). Although it is well characterized in terms of allergenicity, there is still some ambiguity in terms of its biological function. Three-dimensional structural analysis of Der p 2 and its close homologues indicate the presence of a hydrophobic cavity which can potentially bind to lipid molecules. Initial reports on the binding of Der p 2 to the surface of *E. coli* (Ichikawa et al., 1998) and the presence of Gram-negative bacterial DNA in washed *D. pteronyssinus* mites (Valerio et al., 2005) led to the hypothesis that Der p 2 may be involved in the mite's innate anti-bacterial defence system. Later, studies on the identification of the ligand of group 2 allergens were focused on lipopolysaccharide (LPS), which is a major component of bacterial cell wall. Among the various proteins that belong to the ML domain family, Der p 2 shows the highest sequence similarity to NPC2 (Inohara & Nunez, 2002). The structures of group 2 allergens and NPC2 are made up of a single domain β -sandwich protein, with 6 anti-parallel β -strands stabilized by 3 disulfide bonds (Derewenda et al., 2002; Ichikawa et al., 2005).

Significantly higher sensitisation to Equ c 1 (Horse, lipocalin) was recorded in patients with reaction to egg. The majority of the mammalian allergens are lipocalins (Dang et al., 2012). Lipocalins are proteins that are ubiquitous; they are present also in arthropods, plants and bacteria, and have very diverse functions. They are characterized by a common tertiary structure composed of a central β -barrel formed of eight antiparallel β -strands. Lipocalin allergens are present in dander, saliva, and urine, (Hilger et al., 2012; Nilsson et al., 2012; Saarelainen et al., 2008). Lipocalins are small, secreted molecules of 150–250 amino acids. Representatives of this cross-reactive group are Equ c 1, Fel d 4 and Can f 6. However, even between molecules of low general sequence identity such as Fel d 4 and Can f 2 (25% identity), single epitopes may have short stretches of sequence identity and lead to patient-dependent IgE cross-reactivity (Hilger et al., 2012; Nilsson et al., 2012; Saarelainen et al., 2008).

Significantly higher sensitisation to Phl p 7 (Timothy, polcalcin) was recorded in patients with reaction to egg. Polcalcin is a calcium-binding protein present in many different pollens. An IgE response to Phl p 7 is observed only infrequently among grass pollen-allergic patients and usually many years after the disease onset. IgE to Phl p 7 marks a relatively distinct category of grass pollen-allergic patients, with more severe symptoms, a higher prevalence of asthma, and a higher frequency of additional allergic comorbidities. Moreover, many other pollens and allergenic sources contain polcalcin so that the origin sensitization to polcalcin in a grass pollen-allergic patient must be carefully searched. These allergenic sources could be indeed responsible of a more severe disease (Bousquet et al., 2007; Davies, 2014; Devanaboyina et al., 2014; Popescu, 2014).

Transcriptome profile of spleen from BALB/c mice was analysed in response to egg ovomucoid (OVM) sensitisation and challenge (Husain et al., 2012). Micro-array analysis identified several differentially expressed genes that were not previously characterised in food allergic conditions; results from this study may contribute as

reference for future studies that may want to identify biomarkers of egg allergy (Husain et al., 2012).

In order to detect ovalbumin in foods, a highly sensitive sandwich enzyme-linked immunosorbent assay (ELISA) based on two monoclonal antibodies was established (Peng et al., 2014). The developed ELISA may provide a valuable tool for the sensitive determination of ovalbumin in processed foods and for future studies on the mechanism of how ovalbumin functions in anaphylaxis (Peng et al., 2014).

Conclusion

In atopic dermatitis patients, the food allergy to egg was confirmed in 18% of patient; in another 5% of patients we confirmed food reactions to egg without positive results of specific IgE to molecular components of egg and/or to extract specific IgE to egg. The use of the ISAC Multiplex testing in combination with conventional sensitization testing such as extract specific IgE improves the sensitivity to 73.9%. The occurrence of bird-egg syndrome was confirmed in 3 patients (3%). Significantly higher occurrence of positive results of specific IgE to molecular components Der f 2 (NPC2 family, house dust mite), Der p 2 (NPC2 family, house dust mite), Phl p 7 (Polcalcin, Timothy grass) and Equ c 1 (Lipocalin, horse) was recorded in patients with reactions to egg.

We recommend to evaluate the egg allergy (the use of the elimination diet or diagnostic hypoallergenic diet, the challenge test) in all patients suffering from moderate and severe form of atopic dermatitis.

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