Original paper

Allergic phenotypes in adult patients with atopic dermatitis, determined with the ISAC test (ImmunoCAP ISAC)

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Abstract

Introduction: Atopic dermatitis (AD) is a chronic inflammatory dermatosis, often with a concomitant allergy. The ImmunoCAP ISAC (Immuno Solid-Phase Allergen Chip) test is a novel method to determine the allergenic phenotype in a given patient.

Aim: In this study, we used the ImmunoCAP ISAC test to analyze allergic phenotypes in adult patients with AD.

Material and methods: The study included 19 adult patients with AD. The severity of AD was assessed using SCORAD index. Serum concentrations of total IgE were determined by means fluoro-enzyme immunoassay (FEIA). The levels of asIgE were measured with the ImmunoCAP ISAC kits.

Results: Positive results of the ISAC test were documented in 84.2% of the study subjects. All patients synthesized asIgE against species-specific respiratory allergens; major components of animal allergens (57.87%), tree pollen allergens (47.3%), grass pollen allergens (42.1%), dust mite allergens (26.3%) and major allergen of mugwort (26.3%). 47.3% of the subjects were sensitive to cross-reactive allergenic components, most often proteins of the lipocalin family (57.8%), followed by PR-10 (26.3%), PR-14 (21%) and PR-5 proteins (21%). asIgE against species-specific allergens were found in 10.5% of the study subjects. No statistically significant relationships were observed between the severity or duration of AD and the prevalence and levels of asIgE against the allergens included in the ISAC panel. However, duration of AD correlated significantly with the serum concentration of total IgE.

Conclusions: The ISAC test is suitable for determination of the allergenic phenotype in a given patient, and as such has an unquestioned diagnostic and therapeutic value.

Key words: atopic dermatitis, ISAC test, component-resolved diagnostics.

Introduction

Atopic dermatitis (AD) is one of the most common allergic conditions of the skin, characterized by the presence of intensive pruritus, chronic and recurrent course, and age-specific location of skin lesions. Usually, the first symptoms of AD emerge in the early childhood. The prevalence of AD in children and adults is estimated at ca. 20% and 2-5%, respectively [1, 2].

Atopic dermatitis, especially with the childhood onset, is associated with an increased risk of other allergic conditions, in particular allergic rhinitis (AR), asthma and allergic conjunctivitis (ARC); not infrequently, these diseases occur concomitantly [3, 4]. Contact allergies were detected in ca. 40% of patients with AD, and ocular allergic manifestations in 25–42% [3, 5]. The significant relation between the severity of AD and the occurrence of food hypersensitivity reactions was confirmed. Approximately 30% of children with AD present with a concomitant food allergy [5]. Some studies indicate that even 96% of patients with a severe form of AD suffer from food reactions [6]. Frequently, the respiratory, food and/or contact allergy co-existing with AD contributes to exacerbation of skin lesions due to co-exposure to other sensitizing agents [2].

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Diagnosis of AD is usually based on the Hanifin and Rajka criteria, and severity of the condition is graded using a widely accepted system, SCORing Atopic Dermatitis (SCORAD) [7, 8]. Routine evaluation for respiratory and food allergies includes skin prick tests (SPTs) with allergen extracts, and determination of total IgE and allergen-specific IgE (asIgE). Molecular diagnostics (component-resolved diagnostics – CRD) with micromatrices and biochips is a novel method providing a better insight into a complex problem of allergies. The CRD is suitable for the determination of asIgE against allergenic components synthesized by means of genetic engineering [9]. ImmunoCAP ISAC (Immuno Solid-Phase Allergen Chip) test is a complex assay for simultaneous determination of asIgE against 112 allergenic components originating from more than 50 sources. This method can be used to determine the allergic phenotype in a given patient based on the identification of sensitizing respiratory, food and cross-reactive allergens [9, 10].

Aim

In this study, we used the ImmunoCAP ISAC test to analyze allergic phenotypes in adult patients with AD.

Material and methods

Patients

The study included 19 adult (aged ≥ 18 years) patients with AD, aged between 18 and 69 years (mean age: 36.7 years). The study group was comprised of 12 women aged 18–69 years (mean age: 37.9 years) and 7 men between 19 and 68 years of age (mean age: 34.8 years). The subjects were recruited based on the analysis of ICD-10 codes recorded in a local patient database at the Department of Allergology, Clinical Immunology and Internal Diseases, Nicolaus Copernicus University in Torun, Ludwik Rydygier Medical College in Bydgoszcz (Poland). The inclusion criterion was AD as a primary diagnosis, established on the basis of the Hanifin and Rajka criteria [7]. The subjects in whom primary diagnosis of AD raised any doubts were excluded from the study. Detailed characteristics of the study participants are presented in Table 1.

Medical history of atopic dermatitis

Information obtained during history taking included duration of AD (in years) and the number of AD exacerbations during recent 12 months. Data extracted from

Table 1. Patients' characteristics

No.	Gender	Age	SCORAD index	Total IgE [kU/l]	Duration of AD [years]	Exacerbations of AD (last 12 months)	Atopic comorbidity		
							AR	ARC	Asthma
1	M	19	33.4	660	19	6	(+)	(+)	(–)
2	M	40	15	149	9	4	(–)	(–)	(–)
3	M	25	27.9	695	10	10	(+)	(+)	(–)
4	F	22	32.2	3151	22	In remission	(–)	(–)	(–)
5	F	22	38.3	1184	22	10	(–)	(–)	(–)
6	F	69	13	40.9	2	6	(+)	(+)	(–)
7	F	42	43.9	2108	5	3	(+)	(+)	(–)
8	F	31	9.4	345	12	2	(+)	(–)	(–)
9	M	33	23.4	3542	18	4	(–)	(–)	(–)
10	F	54	12.2	> 5000	54	7	(+)	(+)	(+)
11	M	68	48.9	988	68	10	(+)	(+)	(+)
12	F	18	39.5	47	18	In remission	(+)	(+)	(–)
13	F	40	41.2	53.4	7	2	(+)	(+)	(–)
14	F	21	9.8	37.5	21	10	(+)	(–)	(–)
15	F	65	25.2	236	20	2	(+)	(+)	(–)
16	F	49	27.5	1542	49	3	(+)	(+)	(–)
17	M	24	32.5	383	20	10	(–)	(+)	(–)
18	F	22	29.5	390	22	1	(+)	(+)	(–)
19	M	35	63.7	> 5000	35	10	(–)	(+)	(+)

F – female, M – male, AR – allergic rhinitis, ARC – allergic conjunctivitis.

patients medical documentation included occurrence of other allergic conditions, such as asthma, AR and ARC that have been diagnosed by a physician in charge.

Severity of AD: SCORAD index

The severity of AD of each patient was scored by a dermatologist with an aid of the SCORAD index: $A/5 + 7B/2 + C$ (where A – extent of the skin lesions determined with the rule of 9, maximum value A = 100; B – intensity of the inflammation evaluated on the representative lesions, maximum value $B = 18$; and C – severity of two subjective symptoms, pruritus and sleep disturbance, over the last 3 days, maximum value $C = 20$). Based on the SCORAD index values, currently present AD is classified as mild (< 25), moderate (25–50) or severe (> 50) [8].

IgE measurement: total IgE levels and blood testing for specific IgE

To determine total IgE and asIgE concentrations, blood samples from the basilic vein were obtained from each patient with an aid of closed-system Vacutainer sets with a clot activator. The samples were obtained outside the tree, grass and weed pollen season. Concentrations of total IgE were determined by means of fluoro-enzyme immunoassay (FEIA) with diagnostic kits from Thermo Fisher Scientific and Phadia UNICAP 100 analyzer. The results were expressed in kU/l. To make the diagnosis more accurate and to optimize the treatment of AD, the levels of asIgE in all patients were determined with ImmunoCAP ISAC kits (Thermo Fisher Scientific). The ISAC test is an assay for semi-quantitative determination of asIgE against specific allergenic components. The test has been conducted in line with the manufacturer's instruction. The solid phase of the test is the surface of a plate with 112 absorbed allergenic components (43 native and 69 recombinant ones) grouped in triplets. To detect asIgE, examined serum is applied onto the test field – chip; following incubation of the plate, asIgE bound to its surface are detected by addition of fluorescent-labeled secondary antibodies, anti-IgE. Fluorescence intensity is read with a laser scanner LuxScan – 10k/A and analyzed using microassay analysis (MIA) computer software which quantifies the fluorescence signal for each specific allergenic component. The result is expressed in standardized units, ISU-E (standardized units for specific IgE). The values < 0.3 are below the detection limit of the assay, the values of 0.3–0.9 correspond to low, 1–14.9 to moderate/high, and ≥ 15 to very high concentrations of asIgE.

Statistical analysis

Statistical characteristics of quantitative variables, i.e. age, SCORAD, total IgE and asIgE concentrations, were presented as arithmetic means (x), standard deviations (SD), minimum (min.) and maximum (max.) values. Statistical significance of intergroup differences in the values of quantitative variables was verified with Mann-Whitney U-test. Frequencies of qualitative variables were expressed as percentages. Intergroup differences in the distributions of qualitative variables were analyzed with Fisher's exact test for small sample sizes, and the power of associations between two qualitative variables was estimated on the basis of Fi coefficients. The threshold of statistical significance for all tests was set at $\alpha = 0.05$. Statistical analysis of the results was carried out with Statistica (v. 12.0, StatSoft) and MS Excel (Microsoft).

Ethics

The protocol of the study was approved by the Local Bioethics Committee at the Nicolaus Copernicus University in Torun, Ludwik Rydygier Medical College in Bydgoszcz (Poland), decision no. KB 457/2014.

Results

Medical history of atopic dermatitis

Duration of AD ranged between 2 and 68 years (mean: 22.7 years); 10 (52.6%) subjects have been diagnosed with this condition at 1 year of age. The number of AD exacerbations over the last year ranged from 1 to 10; 2 subjects did not report a remission of the skin lesions during the recent 12 months. No statistically significant relationship was found between the duration of AD and the number of its exacerbations over the last year (Mann-Whitney U-test, $p = 0.07$). The relationship between the declared number of exacerbations over the last 12 months and the severity of AD was not significant. Concomitant AR and ARC were present in 13 (68.4%) subjects each; this subset included 11 (57.8%) patients with both these conditions. Asthma was present in 3 (15.7%) patients, including 2 (10.5%) with concomitant AR and ARC, and 1 (5.2%) with co-existing ARC. Presence of concomitant chronic allergic disorders did not show a significant association with the number of AD exacerbations over the last 12 months (asthma $p = 0.2789$; AR $p = 0.1401$; ARC $p = 0.6548$).

Severity of AD: SCORAD index

SCORAD index values for individual patients are presented in Table 1. A total of 6 (31.5%) patients had SCORAD index values below 25 (mild AD), 12 (63.1%) between 25 and 50 (moderate AD), and 1 (5.2%) subject presented with severe AD (SCORAD index > 50). The severity of AD did not show a significant association with the duration of this condition ($r = 0.14$, $p = 0.56$) and the number of exacerbations over the last year ($r = 0.26$, $p = 0.26$). Patients with mild and moderate AD did not differ significantly in the prevalence of concomitant asthma ($p = 0.5686$), AR ($p = 0.5609$) and ARC ($p = 0.0573$).

Table 2. Results of the ISAC test (n = 19)

Type of allergen (n)	Allergenic molecule	Number of positive reactions (%)	ISU-E: min.–max. (mean)
Major species-specific components of food allergens:			
Egg white	nGal d 2	1 (5.2)	0.8
Milk	nBos d lact.	1 (5.2)	1.4
	nBos d 8	1 (5.2)	0.7
Kiwi fruit	nAct d 1	1 (5.2)	2.1
Cod	rGad c 1	1 (5.2)	26
Walnut	nJug r 2	1 (5.2)	2.3
Major species-specific components of airborne allergens:			
Grass pollen (8)			
Timothy-grass (8)	rPhl p 1	8 (42.1)	1.3–88 (29)
	rPhl p 2	4 (21)	2–54 (22.5)
	rPhl p 11	3 (15.7)	0.6– < 100
	nPhl p 4	2 (10.5)	22–25 (23.5)
	rPhl p 5	1 (5.2)	1.4
	rPhl p 6	1 (5.2)	1.7
Bermuda grass (6)	nCyn d 1	6 (31.5)	0.7–97 (28.9)
Grass pollen (9)			
Olive tree	rOle e 9	6 (31.5)	0.5–3.6 (2)
Birch	rBet v 1	5 (26.3)	3.7–48 (27.1)
Japanese cedar	nCry j 1	1 (5.2)	3.4
Cypress	nCup a 1	1 (5.2)	4.2
Plane-tree	nPla a 2	1 (5.2)	2.9
Weed pollen (6)			
Mugwort Parietaria	nArt v 1	5 (26.3)	1.5–17 (10.2)
	rPar j 2	3 (15.7)	0.7–1.4 (0.9)
Dust mites (6)			
D. farinae (6)	nDer f 1	5 (26.3)	0.9–75 (20.7)
	nDer f 2	5 (26.3)	1.5– < 100
D. pteronyssinus (6)	nDer p 1	5 (26.3)	0.8–71 (22.3)
	rDer p 2	5 (26.3)	1.4– < 100
Warehouse mite	rLep d 2	4 (21)	4.5–24 (11.6)
B. tropicalis (HDM)	rBlo t 5	1 (5.2)	0.8
Fungi (7)			
Alternaria	rAlt a 1	4 (21)	1.7–29 (17.1)
Aspergillus (5)	rAsp f 6	4 (21)	3–22 (12.3)
	rAsp f 3	1 (5.2)	14
Animals (11)			
Cat (9)	rFel d 1	8 (42.1)	0.7–89 (20.6)
	rFel d 4	4 (21)	0.6–8.2 (4.2)
Dog (8)	rCan f 1	7 (36.8)	1.7– < 100
	rCan f 5	3 (15.7)	4.3–95 (35)

Table 2. Cont.

Type of allergen (*n*)	Allergenic molecule	Number of positive reactions (%)	ISU-E: min.–max. (mean)
	rCan f 2	2 (10.5)	4.5– < 100
Horse (4)	rEqu c 1	4 (21)	0.7–11 (3.5)
Mouse (4)	nMus m 1	4 (21)	0.7–14 (6.1)
Cross-reactive allergenic components:			
Birch	rBet v 1	5 (26.3)	3.7–48 (27.1)
Hazelnut	rCor a 1	5 (26.3)	1.5–15 (9.3)
Apple	rMal d 1	5 (26.3)	0.6–16 (7.8)
Adler	rAln g 1	4 (21)	1.2–12 (5.7)
Hazel	rCor a 1	4 (21)	0.6–13 (6.4)
Kiwi fruit	nAct d 2	4 (21)	1.7–5.2 (3)
	rAct d 8	2 (10.5)	1.2–3.4 (2.3)
Peach	rPru p 3	4 (21)	0.6–2.1 (1.3)
	rPru p 1	3 (15.7)	3.9–5.2 (4.4)
Soy	rGly m 4	2 (10.5)	4.8–7.4 (6.1)
Peanut	rAra h 8	2 (10.5)	9.2–9.4 (9.3)
Dog	nCan f 3	2 (10.5)	9.9–81 (45.5)
Cat	nFel d 2	2 (10.5)	3.2–32 (17.6)
CCD	nMUXF3	2 (10.5)	2.7–4 (6.7)
Cow milk/beef	nBos d 6	1 (5.2)	1.2
Shrimp	nPen m10	1 (5.2)	6.9
Wheat	rTri a 14	1 (5.2)	0.6
Birch	rBet v 4	1 (5.2)	16
Timothy-grass	rPhl p 7	1 (5.2)	57
D. pteronyssinus	rDer p 10	1 (5.2)	6.4
Horse	nEqu c 3	1 (5.2)	22
Anisakis	rAni s 3	1 (5.2)	5.5
Cockroach	nBla g 7	1 (5.2)	6.7

Serum testing for total IgE

Serum concentrations of total IgE are presented in Table 1. No statistically significant associations were found between the serum level of total IgE, AD severity ($r = 0.27$, $p = 0.26$) and the number of AD exacerbations during the recent 12 months ($r = 0.13$, $p = 0.57$). However, a statistically significant correlation was observed between the duration of AD and the serum concentration of total IgE ($r = 0.51$, $p = 0.0227$). Most patients with high serum concentrations of total IgE had positive results of the ISAC test for multiple allergenic components, especially species-specific respiratory allergens.

Serum testing for specific IgE levels

The positive result of the ISAC test was documented in 16 out of 19 (84.2%) study subjects. All patients with a positive result of the ISAC test ($n = 16$) showed presence of asIgE against species-specific respiratory allergens. Two (10.5%) patients from this subset had also a positive result of the ISAC test against n-glycan MUXF3 from the carbohydrate residue group (CCD) and against native components of plant pollen, and 1 (5.2%) person additionally tested positively for IgE against a native component of plant-derived foods. asIgE against nMUXF3 may generate false positive results for antibodies to native components of plant allergens and plant-derived foods, and this fact should be considered while interpreting the results of the ISAC test. In 9 (47.3%) subjects, the test revealed antibodies against cross-reactive allergens, and in 2 (10.5%) against species-specific food allergens. The results of the ISAC test are summarized in Table 2.

In 11 (57.8%) subjects, the ISAC test yielded positive results for major allergenic components of animal origin, most often for feline component, Fel d 1 ($n = 8$, 42.1%) and canine component, Can f 1 ($n = 7$; 36.8%), as well as for equine component, Equ c 1 ($n = 4$, 21%) and murine component, Mus m 1 ($n = 4$, 21%). Presence of serum

Table 3. Positive results of the ISAC for cross-reactive components classified at a protein family level

Protein group	Number of positive reactions (%)	Cross-reactive components. including individual components of a given family
Lipocalin	11 (57.8%)	rCan f 1 7 rFel d 4 4 rEqu c 1 4 nMus m 1 4 rCan f 2 2
PR-10 protein	5 (26.3%)	rCor a 1 5 rBet v 1 5 rMal d 1 5 rAln g 1 4 rPru p 1 3 rAct d 8 2 rGly m 4 2 rAra h 8 2
nsLTP	4 (21%)	rPru p 3 4 rTri a 14 1
Thaumatin-like protein	4 (21%)	nAct d 2 4
Serum albumin	2 (10.5%)	nCan f 3 2 nFel d 2 2 nBos d 6 1 nEqu c 3 1
CCD	2 (10.5%)	nMUXF3 2
Tropomyosin	1 (5.2%)	nPen m 10 1 rDer p 10 1 rAni s 3 1 nBla g 7 1
Polcalcin	1 (5.2%)	rBet v 4 1 rPhl p 7 1
Profilin	–	–

asIgE against the allergens of tree pollen was detected in 9 (47.3%) subjects. Most often, the test revealed antibodies against Ole e 9 component of olive tree (n = 6, 31.5%) and Bet v 1 component of birch (n = 5, 26.3%); 2 patients showed antibodies against both these components. asIgE against grass pollen were found in 8 (42.1%) patients; most often these were antibodies against the major allergenic component of timothy-grass, Ph1 p 1 (n = 8, 42.1%); 6 (31.5%) patients presented with antibodies against both Ph1 p 1 and Cyn d 1 component of Bermuda-grass. The most commonly identified weed allergen was Art v 1 component of mugwort (n = 5, 26.3%). From the group of dust mite allergens, the study subjects most often (n = 5, 26.3% for each) showed antibodies against the allergenic components of *D. farinae* (Der f 1, Der f 2) and *D. pteronyssinus* (Der p 1, Der p 2); antibodies against both components were detected in 4 (21%) patients. Serum levels of asIgE turned out to be the highest in the case of respiratory allergenic components, Phl p 1 of timothy-grass (mean 29 ISU-E), followed by Cyn d 1 of Bermuda grass (mean: 28.9 ISU-E), Bet v 1 of birch (mean: 27.1 ISU-E) and canine Can f 5 (mean: 35 ISU-E). Some patients presented with extremely high (more than 100 ISU-E) levels of asIgE against canine component Can f 1, Der f 2 and Der p 2 components of dust mites, Phl p 11 of timothy-grass and another canine component, Can f 2.

asIgE for species-specific components of food allergens were detected in 2 (10.5%) subjects. One patient showed asIgE against Gad c 1 component of cod, Jug r 2 component of walnut and Bos d 8 component of milk. The other subject presented with asIgE against Gal d 2 component of egg white, Bos d component of milk lactoferrin and Act d 1 component of kiwi fruit. The highest level of asIgE was detected in the case of Gad c 1 cod's molecule (ISU-E = 26).

Detection rates of asIgE against cross-reactive components, at both protein family and individual component level, are presented in Table 3. The ISAC test most often yielded positive results for proteins of the lipocalin family (n = 11, 57.8%), primarily for canine component Can f 1 (n = 7, 36.8%). Serum asIgE against proteins of the PR-10 family were detected in 5 (26.3%) study subjects, most often against Bet v 1 component of birch, Cor a 1 component of hazelnut and Mal d 1 component of apple. A total of 4 (21%) patients showed antibodies against proteins of the nsLTP family, PR-14 family (Pru p 3 component of peach) and Act d 2 component of kiwi fruit belong to the family of thaumatin-like protein, PR-5. Patients with mild and moderate AD did not differ significantly in terms of the prevalence of allergies to specific components.

Discussion

Atopic diseases are characterized by overproduction of IgE in response to exposure to environmental allergens. Frequently, a polyvalent allergy may be triggered by a few various substances, as it was observed in this study [11]. More in-depth evaluation of patients with allergic diseases, such as AD, should include also determination of total IgE and asIgE in the serum. Santosa *et al.* [12] demonstrated that patients with atopy (asthma, ARC, AD) presented with significantly higher serum concentrations of total IgE than non-atopic controls. The Čelakovská and Bukač [13] study also found the significant relation between the severity of AD and IgE-mediated food allergy. Follow-up with periodic determination of total IgE in the serum is considered a prognostic biomarker during a long-term treatment of AD [14]. However, ca. 10–30% of AD patients may present with normal serum levels of total IgE [15]. Furthermore, it is still unclear if an increase in total IgE correlates with the severity of AD, and if this relationship is modulated by other factors, such as age, sex, exposure to allergens or family history of allergic diseases [16]. While similar to Kaminishi *et al.* [17], we did not find a significant association between

total IgE in the serum and the severity of AD; such relationship was reported by many other authors [15, 18]. However, we observed a statistically significant association between the serum concentration of total IgE and the duration of AD (time elapsed since the diagnosis); in other words, patients with a longer history of the disease presented with higher serum levels of total IgE.

The ImmunoCAP ISAC test is a novel molecular assay used in the diagnostics of allergic diseases. In our study, patients with mild and moderate AD did not differ in the prevalence of asIgE against various allergenic components and their levels. Ott *et al.* [19] demonstrated that food allergies are more common in patients with severe AD, rather than in those with mild or moderate forms of this condition; however, Röckmann *et al.* [20] did not find an association between the severity of AD and the prevalence of food allergies. In our present study, an allergy to species-specific compounds was detected in only 2 patients, and a certain proportion of the subjects synthesized antibodies against cross-reactive allergens (Table 2) of the pathogenesis-related protein (PR protein) family.

The largest proportion of the study subjects (57.8%) synthesized asIgE against major components of animal allergens. In this study, the most sensitizing animal allergen was feline component, Fel d 1. Prevalence of cat allergy in the US and EU is estimated at 17% and ca. 8%, respectively, and up to 90% of these cases may be associated with hypersensitivity to Fel d 1 [21, 22]. Fel d 1 is a protein of the uteroglobin family, synthesized in feline salivary and sebaceous glands, and transferred from the saliva to the fur during licking. Fel d 1 synthesized by the cells of sebaceous glands is transferred to basal squamous epithelial cells and stored in cat's epidermis and hair. Fel d 1 is a dimer with molecular weight of 35–39 kDa, composed of two non-covalently bound monomers. Each monomer of Fel d 1 has molecular weight of 18 kDa and is built of two peptide chains (α and β) linked with two covalent disulfide bonds; IgE binding sites are associated with a spatial structure of both chains of the Fel d 1 molecule [23]. In this study, the most commonly detected antibodies against canine allergenic components were asIgE against Can f 1. Approximately 30 canine allergenic proteins have been identified thus far, with the most commonly isolated being Can f 1 and Can f 2 of the lipocalin family, Can f 3 (albumin) and Can f 5 (kallikrein). Lipocalins form one of the largest families of mammalian allergens, including more than half of allergens originating from fur animals. Lipocalins are present in animal hair, saliva and urine [24]. According to the literature, up to 8% of human population may suffer from dog allergies [25, 26]. Can f 1 is composed of 148 amino acids and shares approximately 30% of its sequence with Can f 2. According to some authors, Can f 1 is an underlying cause of allergy more often than Can f 2 [27, 28]. Other relatively frequently sensitizing allergenic components of the lipocalin family are equine Equ c 1 and murine Mus m 1; asIgE against these proteins were detected in 21% of the study subjects. Equ c 1 is a protein with 20-kDa molecular weight, found in particularly high concentrations in equine saliva, epidermis and urine [28]. In the study conducted by Konradsen *et al.* [24], the prevalence of allergies to proteins of the lipocalin family, especially Equ c 1 turned out to be higher in children with a severe bronchial asthma than in patients with mild forms of this condition. Mus m 1 is a protein with molecular weight of 17 kDa, found primarily in mice urine, and to a lesser extent also in hair follicles. Due to their very small dimensions (0.4–10 µm) Mus m 1 molecules (as well as Mus m 2) are highly volatile and easily penetrate to human airways [29].

Regarding other major species-specific airborne allergens, our patients most often synthesized asIgE against grass pollen, including Phl p 1 and Cyn d 1 components from Grass group 1. Major allergenic components of pollen from various grass species show a considerable cross-reactivity, also with some food allergens, e.g. melon, watermelon and tomato [4]. Our subjects were also allergic to the components of tree pollen, especially Ole e 9 of the 1,3-β-glucanase family. 1,3-β-glucanase has been also found in latex, bell pepper, potatoes, tomatoes and birch, which points to likely existence of a latex-pollen-vegetable food allergy syndrome [30]. Scala *et al.* [31] suggested that Ole e 9, as well as Ole e 7 (a lipid transfer protein – LTP) can be considered as markers of sensitivity to other allergens, e.g. LTP, rather than only to specific olive tree pollens. Ole e 9 has two immunologically- and structurally-independent domains; its N-terminal domain shows the activity of 1,3-β-glucanase and therefore is classified in group 2 of pathogenesis-related proteins (PR-2) [31]. Bet v 1 is the most often described protein of the PR-10 group. This group includes small intracellular acidic proteins with molecular weights of 15-18 kDa. Homologues of Bet v 1 can be inter alia found in apple (Mal d 1), carrot (Dau c 1), celeriac (Api g 1), soy (Gly m 4), peanuts (Ara h 8) and hazelnuts (Cor a 1), as well as in the pollen of trees of the *Betulaceae* family (birch, alder, hazel) [32]. Mittermann *et al.* [33] showed that Bet v 1 triggers AD more frequently than the allergenic components of grass pollen (Phl p 1, Phl p 5, Phl p 2, Phl p 6); however, this phenomenon may be associated with geographic or climatic conditions. Sensitivity to the major allergenic component of mugwort was observed in 26.3% of patients with AD participating in our study. Art v 1 is a defensin with 60-kDa molecular weight. Sensitivity to mugwort is a vital component of a clinical pollen-food allergy syndrome (celeriac-mugwort-birch-spices syndrome, mugwort-mustard syndrome) [34]. Also sensitivity to dust mite allergens is an infrequent problem in patients with AD [9]. Nearly 30% of the study subjects synthesized asIgE against major allergenic components of dust mites. Der p 1 and Der f 1 are cysteine proteases with molecular weights of

25 kDa and nearly a 90% homology of amino acid sequence, constituting a basis for their cross-reactivity. Der p 2 and Der f 2 are NPC 2 proteins with molecular weights of 15 kDa and ca. 88% structural homology [35]. Dust mite allergens show cross-reactivity with the allergens of crustaceans, nematodes, cockroaches and shrimps; aside from allergenic properties, some of them may also produce a strong adjuvant effect [9]. Dust mite allergy constitutes a particularly important problem in patients with moderate and severe AD, especially those with the skin lesions on exposed body parts, such as the face, eyelids, neck and arms. Reduction of exposure to dust mite allergens may contribute significantly to attenuation of AD-specific skin lesions [36].

Conclusions

The ISAC test is an accurate assay suitable for determination of the allergenic phenotype in a given patient; its result may predict the risk of a severe allergic reaction and facilitate implementation of an appropriate treatment. In this study, positive results of the ISAC test were most often observed for major allergenic components of animal origin; furthermore, the test showed presence of asIgE against tree, grass and weed pollen, and dust mite allergens. Only a small proportion of the study subjects synthesized asIgE against species-specific food allergens.

Conflict of interest

The authors declare no conflict of interest.

References

1. Flohr C, Mann J. New insights into the epidemiology if childhood atopic dermatitis. Allergy 2014; 69: 3-16.
2. Wollenberg A, Oranje A, Deleuran M, et al. ETFAD/EADV Eczema task force 2015 position paper on diagnosis and treatment of atopic dermatitis in adult and pediatric patients. J Eur Acad Dermatol Venerol 2016; 30: 729-47.
3. Bruin Weller MS, Rockmann H, Knulst AC, Bruijnzeel-Koomen FM. Evaluation of the adult patient with atopic dermatitis. Clin Exp Allerg 2012; 43: 279-91.
4. Čelakovská J, Bukač J. Analysis of food allergy in atopic dermatitis patients – association with concomitant allergic diseases. Indian J Dermatol 2014; 59: 445-50.
5. Ballardini N, Kull I, Lind T, et al. Development and comorbidity of eczema, asthma and rhinitis to age 12 – data from the BAMSE birth cohort. Allergy 2012; 67: 537-44.
6. Celakovska J, Bukac J. The severity of atopic dermatitis and analysis of the food hypersensitivity reactions. Food Agricultural Immunol 2015; 26: 896-908.
7. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Dermatol Venerol (Stockh) 1980; 92: 44-7.
8. European Task Force on Atopic Dermatitis. Severity scoring of atopic dermatitis: the SCORAD index. Dermatology 1993; 186: 23-31.
9. Choi JS, Roh JY, Lee JR. Clinical availability of component-resolved diagnosis using microarray technology in atopic dermatitis. Ann Dermatol 2014; 26: 437-46.
10. Gadisseur R, Chapelle JP, Cavalier E. A new tool in the field of in-vitro diagnosis of allergy: preliminary results in the comparison of ImmunoCAP©250 with the ImmunoCAP©ISAC. Clin Chem Lab Med 2011; 49: 277-80.
11. Liu FT, Goodarzi H, Chen HY. IgE, Mast cells and eosinophils in atopic dermatitis. Clin Rev Allerg Immunol 2011; 41: 298-310.
12. Santosa A, Andiappan AK, Rotzschke O, et al. Evaluation of the applicability of the Immuno-solidphase allergen chip (ISAC) assay in atopic patients in Singapore. Clin Translational Allergy 2015; 5: 1-9.
13. Čelakovská J, Bukač J. Severity of atopic dermatitis in relation to food and inhalant allergy in adults and adolescents. Food Agric Immunol 2017; 28: 121-33.
14. Kiiski V, Karlsson O, Remitz A, Reitamo S. High serum total IgE predicts poor long-term outcome in atopic dermatitis. Acta Derm Venereol 2015; 95: 943-7.
15. Trzeciak M, Gleń J, Bandurski T, et al. Relationship between serum levels of interleukin-18, IgE and disease severity in patients with atopic dermatitis. Clin Exp Dermatol 2011; 36: 728-32.
16. Vakirlis E, Lazaridou E, Tzellos TG, et al. Investigation of cytokine levels and their association with SCORAD index in adults with acute atopic dermatitis. J Eur Acad Dermatol Venerol 2011; 25: 409-16.
17. Kaminishi K, Soma Y, Kawa Y, Mizoguchi M. Flow cytometric analysis of IL-4, IL-13 and IFN-gamma expression in peripheral blood mononuclear cells and detection of circulating IL-13 in patients with atopic dermatitis provide evidence for the involvement of type 2 cytokines in the disease. J Dermatol Sci 2002; 29: 19-25.
18. Laske N, Niggemann B. Does the severity of atopic dermatitis correlate with serum IgE levels? Pediatr Allergy Immunol 2004; 15: 86-8.
19. Ott H, Fölster-Holst R, Merk HF, Baron JM. Allergen microarrays: a novel tool for high-resolution IgE profiling in adults with atopic dermatitis. Eur J Dermatol 2010; 20: 54-61.
20. Röckmann H, van Geel MJ, Knulst AC, et al. Food allergen sensitization pattern in adults in relation to severity of atopic dermatitis. Clin Translational Allergy 2014; 4: 1-9.
21. Arbes SJ Jr, Gergen PJ, Elliott L, Zeldin DC. Prevalences of positive skin test responses to 10 common allergens in the US population: results from the third National Health and Nutrition Examination Survey. J Allergy Clin Immunol 2005; 116: 377-83.
22. Curin M, Weber M, Thalhamer T, et al. Hypoallergenic derivatives of Fel d 1 obtained by rational reassembly for allergy vaccination and tolerance induction. Clin Exp Allergy 2014; 44: 882-94.
23. Grönlund H, Saarne T, Gafvelin G, van Hage M. The major cat allergen, Fel d 1 in diagnosis and therapy. Int Arch Allergy Immunol 2010; 151: 265-74.
24. Konradsen JR, Nordlund B, Onell A, et al. Severe childhood asthma and allergy to furry animals: refined assessment using molecular-based allergy diagnostics. Pediatr Allergy Immunol 2014; 25: 187-92.
25. Custovic A, Simpson BM, Simpson A, et al. Current mite, cat and dog allergen exposure, pet ownership and sensitization to inhalant allergens in adult. J Allergy Clin Immunol 2003; 111: 402-7.
26. Kamata Y, Miyanomae A, Nakyama E, et al. Characterization of dog allergens Can f 1 and Can f 2. Comparison of Can f 1 with Can f 2 regarding their biochemical and immunological properties. Int Arch Allergy Immunol 2007; 142: 301-8.

27. Kamata Y, Miyanomae A, Nakyama E, et al. Characterization of dog allergens Can f 1 and Can f 2. Preparation of their recombinant proteins and antibodies. Int Arch Allergy Immunol 2007; 142: 291-300.
28. Arseneau AM, Hrabak TM, Waibel KH. Inhalant horse allergens and allergies: a review of the literature. Mil Med 2012; 177: 877-82.
29. Korpi A, Mäntyjärvi R, Rautiainen J, et al. Detection of mouse and rat urinary aeroallergens with an improved ELISA. J Allergy Clin Immunol 2004; 113: 677-82.
30. Palomares O, Villalba M, Quiralte J, et al. 1,3-Beta-glucanases as candidates in latex-pollen-vegetable food cross-reactivity. Clin Exp Allergy 2005; 35: 345-51.
31. Scala E, Abeni D, Pomponi D, et al. Ole e 1, Ole e 7, and Ole e 9: Identifying distinct clinical subsets of olive tree-allergic patients. J Allergy Clin Immunol 2016; 137: 629-31.
32. Kondo Y, Urisu A. Oral allergy syndrome. Allergol Internat 2009; 58: 485-91.
33. Mittermann I, Wikberg G, Johansson C, et al. IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis. Plos One 2016; 26: 1-15.
34. Figueroa J, Blanco C, Dumpiérrez AG, et al. Mustard allergy confirmed by double-blind placebo-controlled food challenges: clinical features and cross-reactivity with mugwort pollen and plant-derived foods. Allergy 2005; 60: 48-55.
35. Siwak E, Skotny A, Zbrojewicz E, et al. Allergens of mites. Adv Hyg Med Exp 2014; 68: 369-74.
36. Pónyai G, Hidvégi B, Németh I, et al. Contact and aeroallergens in adulthood atopic dermatitis. J Eur Acad Dermatol Venerol 2008; 22: 1346-55.