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Sensitisation to molecular components in patients with atopic dermatitis, relation to asthma bronchiale and allergic rhinitis

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ABSTRACT

Background and aim: To evaluate the sensitisation to molecular components in atopic dermatitis patients with the use of Multiplex ISAC testing. Method: The complete dermatological and allergological examination including the examination of the sensitisation to molecular components with Multiplex ISAC testing was performed. Results and conclusion: Eighty one patients were examined – 40 men, 41 women, average age 41.7 years. The positivity was recorded most frequently to molecular components of timothy, birch, house dust mite, animals dander, bermuda grass, peach, apple, hazel pollen, kiwi, peanut and hazelnut, *Alternaria* and *Aspergillus*. Molecular components such as Equ c 1 (Horse), Alt a 6 (*Alternaria*), Fel d 1, Fel d 4 (Cat) and Can f 1, Can f 5 (Dog) are recorded with the significant higher occurrence simultaneously in patients suffering from severe form of AD, asthma bronchiale and allergic rhinitis; these molecular components play the most important role in the atopic march.

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Atopic dermatitis; molecular components; multiplex ISAC testing; SCORAD; severity of atopic dermatitis; underlying probability

Introduction

Atopic dermatitis (AD) constitutes together with allergic rhinitis (AR) and asthma, the triad of atopic diseases. The pathogenesis of AD involves interactions among multiple factors, including susceptibility genes, environmental factors (food and inhalant allergens), skin barrier defects, and immunologic factors (Czarnowicki et al., 2017; Paller et al., 2019). The progression of atopic disorders from AD in infants to AR and asthma in children is used to describe as an atopic march and this concept has been supported by cross-sectional and longitudinal studies as well as the inter-relationships between subsequent allergic manifestations (Czarnowicki et al., 2017; Paller et al., 2019). Mechanisms for developing atopic comorbidities after AD onset are poorly understood but can involve the impaired cutaneous barrier, which facilitates cutaneous sensitisation. The association can also be driven or amplified in susceptible subjects by a systemic T_H2-dominant immune response to cutaneous inflammation (Paller et al., 2019).

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The initial laboratory approach in the diagnosis of allergies (such as AD, rhinitis, and wheezing disorders) is to detect the type of allergic reaction, i.e. whether the patient's allergy is mediated by immunoglobulin E (IgE) or not. Various allergens play a role in the elicitation or exacerbation of eczematous skin lesions in AD, and much research effort has been focused on improving diagnostic tests to identify causative allergens. The main allergenic sources are foods, fungi, trees, weeds, grasses, mites, and finally animals; with the largest number of allergenic proteins being found in foods and the smallest in animals (He et al., 2014). Currently, allergens could be defined as proteins, glycoproteins, lipoproteins, or protein-conjugated haptens, which have unique molecular and structural properties (He et al., 2014).

Progress in laboratory diagnostics of IgE-mediated allergy is the use of component-resolved diagnosis (CRD) or molecular diagnosis of allergies. The CRD approach has been developed when highly purified or recombinant allergen molecules have become available. These molecules are the allergenic proteins towards which the specific and clinically relevant IgE immune response is directed. 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. The major advantage of ISAC is the comprehensive IgE pattern obtained with a minute amount of serum (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). Determination of sIgE against allergenic components may significantly improve current diagnostics of allergy (Dodig & Čepelak, 2018). In our previous studies, we evaluated also the occurrence of sensitisation to food and inhalant allergens in relation to the severity of AD, but the sensitisation was determined according to the extract-specific IgE, atopy patch test, and skin prick tests without using CRD (Čelakovská & Bukač, 2017). There is a lack of reports focusing on the course of AD with respect to its evolution and association with sensitisation to foods and to aeroallergens in adolescent and adult patients suffering from AD. Only few reports demonstrate the comorbidity of allergy to food and inhalant allergens and the severity of AD evaluated with SCORAD index (Ott et al., 2010; Röckmann et al., 2014). The SCORAD index has been developed on consensus by the European Task Force on Atopic Dermatitis (ETFAD) in 1993. The acronym SCORAD was proposed by Arnold Oranje and stands for SCORing Atopic Dermatitis (European Task Force on Atopic Dermatitis, 1993). The use of this instrument makes different studies more comparable in routine practice, as well as in observational or double-blind randomised clinical trials.

Aim of the study

The aim of this study is to evaluate the sensitisation to molecular components in AD patients with the use of Multiplex ISAC testing and to evaluate the relation to the severity of AD, occurrence of asthma bronchiale (AB), and AR in this group of patients. Also, we evaluate if there is some relation between the level of specific IgE to molecular components and the severity of AD, the occurrence of AB and AR.

Patients and methods

In the period 2018–2019, 81 patients suffering from AD 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 with the Hanifin–Rajka criteria (Hanifin & Rajka, 1980). Exclusion criteria were long-term therapy with cyclosporin or systemic corticoids, pregnancy, and breast-feeding. Patients with AD having other systemic diseases were excluded from the study as well. The complete dermatological and allergological examination was performed in patients included in the study. This study was approved by Ethics committee of Faculty Hospital Hradec Králové, Charles University of Prague, Czech Republic.

Examination of specific IgE to molecular components

The serum level of the sIgE was measured by the CRD 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 (Choi et al., 2014; Jakob et al., 2015). 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 >0.3 ISU-E was assessed as positive (Jakob et al., 2015). The level of molecular components in ISU-E was evaluated: <0.3 – negative, 0.3–0.9 ISU-E low positivity, 0.9–15 ISU-E moderate positivity, and >15 ISU-E very high positivity (Choi et al., 2014; Jakob et al., 2015).

Severity of atopic dermatitis

The severity of AD was scored in agreement with SCORAD (Scoring of atopic dermatitis) with the assessment of topography items (affected skin area), intensity criteria, and subjective parameters. The severity of AD was evaluated with SCORAD as a mild form to 25 points, as moderate over 25–50 points, as a severe form over 50 points (European Task Force on Atopic Dermatitis, 1993). This examination was performed during one year every two months and the average SCORAD index was calculated.

The diagnosis of AB was made according to the results in spirometry at allergological outpatients department.

The evaluation of allergic rhinitis (AR) (seasonal or perennial) was made according to the allergological examination.

Statistical analysis

We analysed the data to determine whether there is some difference of sensitisation to examine molecular components in the mild, moderate and severe form of AD, in patients suffering from AB and AR. Also, we evaluated if there is some difference between the severity of AD and the level of specific IgE to molecular components. Allergens were ordered in the decreasing order to find out the one with the highest probability. We continued by testing the difference between the highest underlying probability and the following one and we found out that the null hypothesis of equality of those underlying probabilities was confirmed. It follows that we have to consider a set of allergens with the highest probability. The question of calculating which allergens fall in the set of those with the highest probability is found in the study of the change-point problem. A typical setting of this

branch of statistics appears as a time series. The independent variable plotted on the time-axis on which it is represented by equidistant points. This is definitely not our case because we are looking for a change in underlying probabilities of events that are sorted in a decreasing order with respect to the magnitude of their relative frequencies. In our application of finding the change-point, it really does not matter that we do not use the time series model.

We prefer to use an intuitive approach. We consider events with the highest probabilities, denote their probabilities as $p(1), p(2), \dots, p(k)$, and ask if we may include one more event with probability $p(k+1)$. The null hypothesis may be written as $H_0: p(i) = p$, for $i = 1, \dots, k+1$ for some probability p common to all the $k+1$ events, whereas the alternative hypothesis is $H_a: p(i) = p$ for $i = 1, \dots, k$ but $p(k+1)$ is not equal to p .

This says that the event with this distinct probability $p(k+1)$ is not in the set of events with the highest probability. Since the probabilities $p(1), \dots, p(k)$ are the same, we can add all the numbers of successes (positive results of tests) and all the numbers of trials in binomial distributions with the same parameter p and, provided the trials are independent, the cumulative sum of the numbers of successes out of the cumulative sum of trials have a binomial distribution with the same parameter p . We compare this probability p with the probability $p(k+1)$ of the next $k+1$ th event.

As Worsley would put it, essentially, we are dealing with the association in a 2×2 contingency table. Our conclusion is that such an association may be tested by implementing the well-known chi-squared test (Worsley, 1983). Essentially, the method of maximum likelihood estimates K by choosing the value of k that gives the strongest association in a 2×2 contingency table formed by combining all periods up to and including k and all periods after k , against success and failure (Worsley, 1983).

For the evaluation of the relation between the severity of AD and the level of specific IgE, we formed a contingency table with the severity of AD as one classification and level of specific IgE of the molecular component as the other classification (0.3–0.9 ISU-E low positivity, 0.9–15 ISU-E moderate positivity, >15 ISU-E very high positivity). The p -value is on the bottom line.

For the evaluation of the relation between the occurrence of AB (AR) and the level of specific IgE, we formed a contingency table with the occurrence of AB (AR) as one classification and level of specific IgE of the molecular component as the other classification.

We tested if there are significant differences in relative frequencies of molecular components in mild, moderate, and severe forms of AD, in patients suffering from AB and AR. The results are recorded in tables. The heading of each table contains the column sums (enclosed in parentheses), numbers of patients with positive test results are recorded on each separate line next to their molecular component abbreviations. The negative test results would be obtained by subtracting the positive results from the column sum in the headings. This is the way we form a 2 by 3 (in the case of severity of AD) or 2 by 2 (in the case of AB and AR). Such a table could be evaluated by the usual Chi-squared test. The p -value is displayed in the right-hand column. Unfortunately, zeroes appeared in many of the tables and one has to be careful in making conclusions.

Results

Patients

In 81 patients included in the study (40 men and 41 women with the average age 41.7 years and with the average SCORAD 39, s.d.13.1 points), 112 different components from 51

allergen sources were examined. The mild form of AD was recorded in 11 patients (13.6%), moderate form in 45 patients (55.6%), and severe form in 25 patients (30.9%). In the included patients, 46 patients (56.8%) suffer from AB and 65 patients (80.2%) suffer from rhinitis. The positive results in sensitisation to molecular components were recorded in 75 patients (92.6%). The characteristics of patients is shown in [Table 1](#).

The number of patients with positive reactions to different types of allergens, number of positive reactions to molecular components, and the level of molecular components in ISU-E (min., max., mean) are recorded in [Table 2](#). The order of allergens according to the positivity at least in one of the molecular component in 81 patients is shown in [Table 3](#). The positivity to some of the molecular components was recorded to timothy in 60 patients (74.1%), in 48 patients (59.3%) to birch, in 45 patients (64.2%) to house dust mite, in 40 patients (49.4%) to the dog, in 40 patients (49.4%) to the cat, and in 39 patients (48.1%) to peach followed by others allergens.

The list of molecular components with the level of specific IgE >15 ISU-E (high positivity) is shown in [Table 4](#). The highest level of specific IgE was recorded to these molecular components: Der p 10 (House dust mite), Pla l 1 (Plantain), Phl p 1 (Timothy), Can f 1 (Dog), and Fel d 1 (Cat), from food allergens to Pen m 1 (Shrimp), to Mal d 1 (Apple), and Ani s 3 (Anisakis).

The number of negative and positive reactions (low, moderate, and high positivity of the level of specific IgE) in a mild, moderate, and severe form of AD is shown in [Table 5](#). The significant relationship between the severity of AD and the level of specific IgE to molecular components was confirmed (p -value = .000).

The list of positive results to molecular components in all included patients and the list in the mild, moderate, and severe form of AD is shown in [Table 6](#). The leading molecular components are Bet v 1 (Birch) in mild and moderate form of AD, and Phl p 1 (Timothy) in severe form of AD followed by other molecular components as shown in [Table 6](#). According to the statistical evaluation, the order of molecular components in mild, moderate, and severe form (the underlying probability of the occurrence) is not statistically significant. We compared also the conditional relative frequency of positivity of molecular components in patients suffering from a mild, moderate and severe form of AD. The increasing relative frequency of positive reactions to molecular components from mild to moderate and to severe form was recorded ([Table 7](#)). The significant relation between the severity of AD and the positivity to these molecular components was confirmed: Bet v 2 (Birch), Alt v 6 (Alternaria), Asp f 6 (Aspergillus), Der f 2 and Der p 2 (House dust mite), Fel d 4 (Cat), Can f 1 (Dog), Equ c 1 (Horse), Ves v 5 (Common wasp venom), Tri a A (Wheat), Gal d 2 (Egg white), Pen m 2 (Shrimp), and Ara h 1 (Peanut).

Table 1. The characteristic of patients with atopic dermatitis.

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- 81 patients examined – 40 men, 41 women, the average age 41 years
 - The average SCORAD 39 s.d. 13.1 points
 - The positive results in sensitisation to molecular components – 75 patients (92.6%)
 - Mild form of AD patients 11 (13.6%)
 - Moderate form of AD patients 45 (55.6%)
 - Severe form of AD patients 25 (30.9%)
 - Number of patients with asthma bronchiale – 46 (56.8%) number of patients with rhinitis – 65 (80.2%)
-

Table 2. Results of examination in 81 patients. Number of patients with positive reactions to different types of allergens, number of positive reactions to molecular components and the level of molecular components in ISU-E (min., max., mean). The mean of sIgE above 15 ISU-E is marked extra bold.

Type of allergen (n)	Allergen component	No. of positive reactions (%)	ISU-E: min.–max. (mean)	No. of patients with positive reactions (%)
Egg white	nGal d 1	6 (7.4)	0.3–3.3 (1.4)	11 (13.6)
	nGal d 2	5 (6.2)	0.6–3.6 (1.3)	
	nGal d 3	6 (7.4)	0.4–21 (5.2)	
Egg yolk/chicken meat	nGal d 5	3 (3.7)	0.3–6.4 (2.4)	3 (3.7)
Cow's milk	nBos d 4	0	0	1 (1.2)
	nBos d 5	1 (1.2)	0.6	
Cow's milk/meat	nBos d 6 ^a	2 (2.5)	0.3–2.4 (1.4)	2 (2.5)
Cow's milk	nBos d 8	2 (2.5)	1.0–1.4 (1.2)	4 (4.9)
	nBos d lactoferrin	2 (2.5)	0.4–0.4 (0.4)	
Cod	rGad c 1	4 (4.9)	0.7–24 (15.9)	4 (4.9)
Shrimp	nPen m 1 ^a	7 (8.6)	0.4–100 (26.1)	22 (27.2)
	nPen m 2	21 (25.9)	0.4–63 (17.6)	
	nPen m 4	2 (2.5)	1.7–4.8 (3.3)	
Cashew nut	rAna o 2	4 (4.9)	0.3–2.4 (1.2)	4 (4.9)
Brazil nut	rBer e 1	0	0	0
Hazelnut	rCor a 1.0401 ^a	34 (42.0)	0.3–40 (10.2)	35 (43.2)
	rCor a 8 ^a	4 (4.9)	0.4–3.2 (1.5)	
	nCor a 9	1 (1.2)	0.6	
Walnut	rJug r 1	2 (2.5)	5.4–6.6 (6.0)	13 (16.0)
	nJug r 2	7 (8.6)	0.5–3.7 (1.6)	
	nJug r 3 ^a	6 (7.4)	1.3–4.5 (2.5)	
Sesame seed	nSes i 1	4 (4.9)	0.4–14 (5.7)	4 (4.9)
Peanut	rAra h 1	3 (3.7)	0.4–22 (9.8)	35 (43.2)
	rAra h 2	2 (2.5)	17–17 (17.0)	
	rAra h 3	3 (3.7)	0.6–1.1 (0.9)	
	nAra h 6	1 (1.2)	11	
	rAra h 8 ^a	33 (40.7)	0.4–40 (8.7)	
	rAra h 9 ^a	7 (8.6)	0.8–6.2 (2.6)	
	rGly m4 ^a	28 (34.6)	0.3–26 (5.4)	
	nGly m 5	2 (2.5)	0.4–0.5 (0.5)	
Soybean	nGly m 6	0	0	29 (35.8)
	nFag e 2	0	0	
Buckwheat	rTri a 14 ^a	1 (1.2)	1.4	4 (4.9)
	rTri a 19.0101	0	0	
	nTri a aA_T1	3 (3.7)	0.4–1.0 (0.6)	
Kiwi	nAct d 1	9 (11)	0.4–9.7 (3.2)	36 (44.4)
	nAct d 2 ^a	16 (19.8)	0.5–17 (3.3)	
	nAct d 5	0	0	
Celery	rAct d 8	18 (22.2)	0.3–13 (2.7)	18 (22.2)
	rApi g 1 ^a	18 (22.2)	0.7–29 (7.1)	
Apple	rMal d 1 ^a	38 (46.9)	0.4–100 (18.3)	38 (46.9)
Peach	rPru p 1 ^a	38 (46.9)	0.3–92 (11.8)	39 (48.1)
	rPru p 3 ^a	7 (8.6)	0.4–15 (4.5)	
Bermuda grass	nCyn d 1	39 (48.1)	0.4–39 (11)	39 (48.1)
Timothy grass	rPhl p 1	47 (58.0)	0.4–100 (34.8)	60 (74.1)
	rPhl p 2	32 (39.5)	0.3–72 (17.3)	
	nPhl p 4	41 (50.6)	0.3–41 (11.3)	
	rPhl p 5	31 (38.2)	1.2–100 (39.6)	
	rPhl p 6	33 (40.7)	0.3–100 (17.5)	
	rPhl p 7 ^a	5 (6.2)	0.5–100 (25.7)	
	rPhl p 11	15 (18.5)	0.4–87 (23.2)	
	rPhl p 12 ^a	3 (3.7)	0.7–7.3 (4.2)	
	rAln g 1 ^a	35 (43.2)	0.8–43 (11.5)	
	rBet v 1 ^a	47 (58.0)	0.3–100 (34.1)	
	rBet v 2 ^a	9 (11.1)	0.5–17 (4.6)	
	rBet v 4 ^a	3 (3.7)	0.9–56 (19.6)	

(Continued)

Table 2. Continued.

Type of allergen (<i>n</i>)	Allergen component	No. of positive reactions (%)	ISU-E: min.–max. (mean)	No. of patients with positive reactions (%)
Hazel pollen	rCor a 1.0101 ^a	37 (45.7)	0.5–39 (10.6)	37 (45.7)
Japanese cedar	nCry j 1	13 (16.0)	0.4–21 (2.7)	13 (16.0)
Cypress	nCup a 1	12 (14.8)	0.6–5.4 (1.8)	12 (14.8)
Olive pollen	rOle e 1	13 (16.0)	0.4–59 (17.0)	29 (35.8)
	nOle e 7 ^a	1 (1.2)	0.3	
	rOle e 9	17 (21.0)	0.4–7.8 (1.8)	
Plane tree	rPla a 1	0	0	21 (25.9)
	nPla a 2	19 (23.5)	0.4–14 (2.5)	
	rPla a 3 ^a	5 (6.2)	1–5 (2.3)	
Ragweed	nAmb a 1	4 (4.9)	3.5–64 (26.4)	4 (4.9)
Mugwort	nArt v 1	24 (29.6)	0.4–100 (11.6)	25 (30.9)
	nArt v 3 ^a	5 (6.2)	0.5–59 (12.6)	
Goosefoot	rChe a 1	7 (8.6)	0.4–3.9 (2.5)	7 (8.6)
Annual mercury	rMer a 1 ^a	11 (13.6)	0.5–40 (7.7)	11 (13.6)
Wall pellitory	rPar j 2	9 (11.1)	0.3–2.8 (1.2)	9 (11.1)
Ribwort plantain	rPla l 1	9 (11.1)	0.4–100 (53.2)	9 (11.1)
Saltwort	nSal k 1	2 (2.5)	1.1–5.2 (3.2)	2 (2.5)
Dog	rCan f 1	32 (39.5)	0.5–100 (31.2)	40 (49.4)
	rCan f 2	13 (16.0)	0.4–56 (22.8)	
	nCan f 3 ^a	6 (7.4)	0.5–11 (3.3)	
	rCan f 5	21 (25.9)	0.4–100 (20.2)	
Horse	rEqu c 1	24 (29.6)	0.4–58 (16.8)	26 (32.1)
	nEqu c 3 ^a	3 (3.7)	0.8–11 (4.5)	
Cat	rFel d 1	35 (43.2)	0.7–100 (29.4)	40 (49.4)
	nFel d 2 ^a	6 (7.4)	0.4–2.5 (1.3)	
	rFel d 4	25 (30.9)	0.6–64 (14.6)	
Mouse	nMus m 1	17 (21.0)	0.4–95 (27.7)	17 (21.0)
Alternaria	rAlt a 1	24 (29.6)	1.1–100 (25.0)	30 (37.0)
	rAlt a 6	14 (17.3)	0.4–28 (8.4)	
Aspergillus	rAsp f 1	5 (6.2)	0.4–4.8 (2.2)	26 (32.1)
	rAsp f 3	6 (7.4)	0.5–15 (4.5)	
	rAsp f 6	19 (23.5)	0.4–27 (8.3)	
Cladosporium	rCla h 8	3 (3.7)	1.2–6.4 (3.0)	3 (3.7)
Storage mite	rLep d 2	11 (13.6)	0.7–100 (30.4)	12 (14.8)
House dust mite	rBlo t 5	7 (8.6)	0.4–23 (8.8)	52 (64.2)
	nDer f 1	30 (37.0)	0.4–96 (30.2)	
	rDer f 2	41 (50.6)	0.6–100 (37.4)	
	nDer p 1	31 (38.2)	0.4–100 (37.1)	
	rDer p 2	41 (50.6)	0.5–100 (43.5)	
	rDer p 10 ^a	4 (4.9)	6.7–100 (59.2)	
Cockroach	rBla g 1	2 (2.5)	0.5–0.8 (0.7)	12 (14.8)
	rBla g 2	3 (3.7)	0.5–1.6 (0.9)	
	rBla g 5	3 (3.7)	0.3–0.8 (0.5)	
	nBla g 7 ^a	4 (4.9)	6.2–94 (44.8)	
Honey bee venom	rApi m 1	2 (2.5)	0.7–2.9 (1.8)	2 (2.5)
	nApi m 4	0	0	
Paper wasp venom	rPol d 5	5 (6.2)	0.4–1.4 (0.8)	5 (6.2)
Common wasp venom	rVes v 5	7 (8.6)	0.3–2.8 (1.1)	7 (8.6)
Anisakis	rAni s 1	1 (1.2)	47	4 (4.9)
	rAni s 3 ^a	3 (3.7)	5.7–35 (17.6)	
Latex	rHev b 1	3 (3.7)	0.4–11 (4.7)	18 (22.2)
	rHev b 3	2 (2.5)	0.3–4.1 (2.2)	
	rHev b 5	2 (2.5)	2.3–2.6 (2.5)	
	rHev b 6.01	6 (7.4)	0.5–7.2 (2.5)	
	rHev b 8 ^a	12 (14.8)	0.3–41 (7.8)	
Sugar epitope from Bromelain (CCD)	nMUXF3 ^a	18 (22.2)	0.4–24 (3.5)	18 (22.2)

^aMainly cross-reactive components.

Table 3. The list of allergens according to positivity to one or more of molecular components in the allergen (81 patients = 100%).

Type of allergen	Number of patients with positive results to one or more of molecular components of this allergen
Timothy	60 (74.1%)
Birch	48 (59.3%)
House dust mite	45 (64.2%)
Dog	40 (49.4%)
Cat	40 (49.4%)
Peach	39 (48.1%)
Bermud	39 (48.1%)
Apple	38 (46.9%)
Hazel pollen	37 (45.7%)
Kiwi	36 (44.4%)
Peanut	35 (43.2%)
Hazelnut	35 (43.2%)
Alternaria	30 (37.0%)
Soybean	29 (35.8%)
Oliva pollen	29 (35.8%)
Horse	26 (32.1%)
Aspergillus	26 (32.1%)
Shrimp	22 (27.2%)
Plane tree	21 (25.9%)
Latex	18 (22.2%)
CCD	18 (22.2%)
Mouse	17 (20.9%)
Wallnut	13 (16.0%)
Japanec	13 (16.0%)
Cedar	13 (16.0%)
Cypres	12 (14.8%)
Cocroach	12 (14.8%)
Storage mite	12 (14.8%)
Annual mercury	11 (13.6%)
Egg white	11 (13.6%)
Wall pelitory	9 (11.1%)
Platan	9 (11.1%)
Ves	7 (8.7%)

Regarding the occurrence of AB in our group of patients, we evaluated the level of specific IgE to molecular components (low, moderate, and high positivity of specific IgE), the underlying probability of the positive results to molecular components and the relative frequency of sensitisation to molecular components in patients with AB and without AB. The number of positive reactions to molecular components (low, moderate and high positivity of specific IgE) in patients with and without AB is shown in [Table 8](#). The significant difference in the level of specific IgE to molecular components between the patients with and without AB was confirmed (p -value = .000).

We evaluated the order of molecular components in patients suffering from AB and in patients without AB. The first 25 molecular components with the highest underlying probability in patients suffering from AB were found to be different from those not suffering from AB. Molecular components such as Der f 2 (House dust mite), Fel d 1, Fel d 4 (Cat), Mal d 1 (Apple), Phl p 2, Phl p 5, (Timothy), Cora 1.0101, Cora 10401 (Hazelnut), Der f 1, Der p 1 (House dust mite), Ara h 8 (Peanut), Aln g 1 (Alder), Gly m 4 (Soy), Pen m 2 (Shrimp), and Alt a 1 (Alternaria) are not recorded among the first 25 molecular components in patients without AB ([Table 9](#)). The relative frequency of the positive results in molecular components in patients with AB and without AB is shown in [Table 10](#). These

Table 4. The list of allergens and molecular components with the level of specific IgE above 15 ISU-E (high positivity).

Allergen	Molecular component	ISU-E (mean)
House dust mite	Der p 10	59.2
Plantain	Pla l 1	53.2
Anisakis	Ani s 1	47.0
Cockroach	Bla g 7	44.8
House dust mite	Der p 2	43.5
Timothy	Phl p 5	39.6
House dust mite	Der f 2	37.4
House dust mite	Der p 1	37.1
Timothy	Phl p 1	34.8
Birch	Bet v 1	34.1
Dog	Can f 1	31.2
Storage mite	Lep d 2	30.4
House dust mite	Der f 1	30.2
Cat	Fel d 1	29.4
Shrimp	Pen m 1	26.1
Mouse	Mus m 1	27.7
Ragweed	Amb a 1	26.4
Timothy	Phl p 7	25.7
Alternaria	Alt a 1	25
Timothy	Phl p 11	23.2
Dog	Can f 2	22.8
Dog	Can f 5	20.2
Birch	Bet v 4	19.6
Apple	Mal d 1	18.3
Anisakis	Ani s 3	17.6
Shrimp	Pen m 2	17.6
Timothy	Phl p 6	17.5
Timothy	Phl p 2	17.3
Olive pollen	Ole e 1	17.0

Table 5. The number of negative and positive reactions (low, moderate, and high positivity of the level of specific IgE to molecular component) in mild, moderate and severe form of atopic dermatitis. The significant relation between the severity of AD and the level of specific IgE to molecular components was confirmed (p -value = .000).

Severity of atopic dermatitis	Number of reactions			
	Negative < 0.3	0.3–0.9 ISU-E – low positivity	0.9–15 ISU-E – moderate positivity	Above 15 ISU-E – high positivity
Mild	1186	17	60	13
Moderate	4513	129	365	213
Severe	2331	76	255	238
Number of reactions in %				
Mild	92.9	1.3	4.7	1.0
Moderate	86.5	2.5	7.0	4.1
Severe	80.4	2.6	8.8	8.2
Number of reactions in %				
Mild	14.8	7.7	8.8	2.8
Moderate	56.2	58.1	53.7	45.9
Severe	29.0	34.2	37.5	51.3
	100.0%	100.0%	100.0%	100.0%
p -value	.000			

molecular components were recorded with the significant higher occurrence in AD patients suffering from AB: Ara h 8, Ara h 9 (Peanut), Equ c 1 (Horse), Mus m 1 (Mouse), MUXF 3 (Sugar epitope from Bromelain (CCD)), Pen m 2 (Shrimp), Alt a 1 (Alternaria), Ole e 9 (Oliva pollen), Art v 1 (Mugwort), Can f 3 (Dog), Cora 1.0101,

Table 6. The list of molecular components in all included patients and the list in mild, moderate and severe form of AD (81 patients = 100%). According to the statistical evaluation, the order of molecular components in mild, moderate and severe form (the underlying probability of the occurrence) is not statistically significant.

Positive frequency of molecular components in patients suffering from AD (81 patients = 100%)				Positive frequency of molecular components in all form of AD%			
Severity of AD							
Mild form 11 patients (13%)		Moderate form 45 patients (46%)		Severe form 25 patients (31%)			
Molecular component	No. of patients (%)	Molecular component	No. of patients (%)	Molecular component	No. of patients (%)		
rBetr 1	7 (8.6%)	rBetr 1	26 (32.1%)	rPhlp1	17 (20.9%)	rPhlp1	47 (58.0%)
nCynd1	5 (6. %)	rPhlp1		nCynd1	16 (19.7%)	rBetr 1	47 (58.0%)
rAlng1		rPhlp4	23(28.3%)	Derf2		rPhlp4	41 (50.6%)
rCora10401		Derf2		rDerp2		Derf2	
rPhlp1	4 (4.9%)	rDerp2		rPhlp4	15 (18.5%)	rDerp2	
rPhlp2		rMald1	21 (25.9)	rAlng1	14 (17.3%)	nCynd1	39 (48.1%)
rCora1.0101		rPrup1	20 (24.7)	rCora1.0101		rMald1	38 (46.9%)
rAlta1		rCora10401	19 (23.5%)	rPrup1		rPrup1	
rPrup1		rCora1.0101		rMald1		rCora1.0101	37 (45.7%)
rArah8		rFeld1		rFeld1		rAlng1	35 (43.2%)
rPhlp4	3 (3.7%)	rCanF1		rFeld4		rFeld1	
rDerp1		nCynd1	18 (22.2%)	rBetr 1	13 (16.1%)	rCora10401	34 (42.0%)
rMald1		rPhlp6		rPhlp2		rPhlp6	33 (40.7%)
rPhlp5	2 (2.5%)	rDerp1	17 (20.9%)	rArah8		rArah8	
rPhlp6		rPhlp5		rPhlp6		rPhlp2	32 (39.5%)
nOLee1		rDerf1		nPenm2		rPhlp5	31 (38.2%)
rDerf1		rAlng1	16 (19.7%)	rEguc1		rDerp1	
Derf2		rArah8		rPhlp5	12 (14.8%)	rCanF1	
rDerp2		rPhlp2	15 (18.5%)	rGlym4		rDerf1	30 (37.0%)
rFeld1		rGlym4	14 (17.3%)	rCanF1		rGlym4	28 (34.6%)
rGlym4		rArtv 1		rDerp1	11 (13.5%)	rFeld4	25 (30.9%)
rPhlp11	1 (1.2%)	rAlta1	12 (14.8%)	rDerf1		rArtv 1	24 (29.6%)
rPhlp7		rPhlp11	11 (13.6%)	rCora10401	10 (12.4%)	rAlta1	
nCupa1		rCanf5		rArtv 1		rEguc1	
nCryj1		nMUXF3		rAspf6		rCanf5	21 (25.9%)
rPlaa2		rFeld4		rCanf5	9 (11.1%)	nPenm2	
rBetr 4		rEguc1		nMusm1		rPlaa2	19 (23.5%)
rArtv 1		rPlaa2	10 (12.3%)	rAlta1	8 (9.8%)	rAspf6	
rBlot5		nActd8		rPlaa2		nActd8	18 (22.2%)
rDerp10		rApig1		nOlee9		rApig1	
rlepd2		nActd2	9 (11.1%)	rAlta6		nMUXF3	
rCanf5		nOlee9		rApig1		nOlee9	17 (21.0%)
nActd2		rAspf6		nActd8	7 (8.6%)	nMusm1	
nActd8		nCupa1	8 (9.8%)	rCanf2		nActd2	16 (19.8%)
rAnis 3		nMusm1		nOLee1		rPhlp11	15 (18.5%)
nPenm1		nCryj1	7 (8.6%)	nActd2	6 (7.4%)	rAlta6	14 (17.3%)
nPenm2		nPenm2		nMUXF3		nCryj1	13 (16.0%)
nPenm4		rAlta6	6 (7.4%)	rBetr2		nOLee1	
nMUXF3		rCanf2		rMera1		rCanf2	
rBlag7		nHevb8		nHevb8		nCupa1	12 (14.8%)
						Hev b 8	
		rlepd2	5 (6.2%)	rlepd2	5 (6.1%)	rMera1	11 (13.6%)
		rPlal1		rVesv 5		rlepd2	
		rMera1		ngald2		rPlal1	9 (11.1%)
		rparj2		rBlot5	4 (4.5%)	rparj2	
		rFeld2		rPlal1		nActd1	
		nActd1		rparj2		rBetr2	
		n/rPrup3		rAspf3		rhea1	7 (8.6%)
		nJugr3		nGald3		rBlot5	
		rPlaa3	4 (4.9%)	nActd1		rVesv 5	
		rhea1		nJugr2		n/rPrup3	
		rArtv 3		rPhlp11	3 (3.7%)	rArah9	

(Continued)

Table 6. Continued.

nCanf3		nCupa1		nJugr2	
rArah9		nPenm1		nPenm1	
hevb6.01		rchea1		rAspf3	6 (7.4%)
nPenm1	3 (3.7%)	rAspf1		rFeld2	
rAmba1		rPold5		nCanf3	
nGald1		nSesi1		nGald1	
rAnao2		nTriaaA		nGald3	
rCora8		nGald1		nJugr3	
nJugr2		nArah1		hevb6.01	
rPhlp7	2 (2.5%)	rArah9		rPhlp7	5 (6.2%)
rBlot5		rPhlp7	2 (2.5%)	rPlaa3	
rBetv2		rDerp10		rArtv 3	
rAspf1		rBlag7		rAspf1	
rAspf3		rPhlp12		rPold5	
rBlag1		rClah8		ngald2	
rBlag2		rBlag5		rAmba1	4 (4.9%)
nEquc3		nCanf3		rDerp10	
rVesv 5		nBosd8		rBlag7	
rPold5		n/rPrup3		nSesi1	
nGald3		nArah2		rAnao2	
nGald5		rGlym5		rCora8	
nArah3		nJugr1		rGadc1	
rGadc1		rGadc1		rPhlp12	3 (3.7%)
rHevb1		hevb6.01		rBetv 4	
rHevb3		rBetv 4	1 (1.2%)	rClah8	
rHevb5		rAnis 3		rBlag2	
rBetv 4	1 (1.2%)	rPlaa3		rBlag5	
rDerp10		rArtv 3		nEquc3	
rAnis 3		rAmba1		nTriaaA	
nPenm4		nSalk 1		nGald5	
rBlag7		rBlag2		nArah1	
rPhlp12		rFeld2		nArah3	
nOlee7		nEquc3		rAnis 3	
nSalk 1		r/nApim1		rHevb1	
rClah8		nGald5		nSalk 1	2 (2.5%)
rBlag5		nBosd5		rBlag1	
r/nApim1		nBosd		r/nApim1	
nSesi1		rAnao2		nBosd8	
rTria14		rCora8		nBosd	
nBosd		nCora9		nArah2	
nBosd6		nArah3		rGlym5	
		nJugr3		nPenm4	
		rAnis 1		nBosd6	
		nBosd6		rHevb3	
		rHevb1		rHevb5	
				nOlee7	1 (1.2%)
				rTria14	
				nBosd5	
				nCora9	
				nArah6	
				rAnis 1	
				nLolp1	0
				rAlng4	
				rPlaa1	
				rPla1	
				nApim4	
				nFage2	
				nTriaGliadin	
				nBosd4	
				nActd5	
				nBere1	
				nGlym6	
				rhevb602	

Table 7. The conditional relative frequency of positivity of molecular components in patients suffering from mild, moderate and severe form of atopic dermatitis.

Allergen	Molecular component	Severity of atopic dermatitis – number of patients (relative frequency, given severity)			p-value
		Mild form (11 patients = 100%)	Moderate form (45 patients = 100%)	Severe form (25 patients = 100%)	
Bermuda grass	nCyd1	5 (45.5%)	18 (40.0%)	16 (64.0%)	.153
Perennial regrass	nLolp1	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Timothy	rPhlp1	4 (36.4%)	26 (57.8%)	17 (68.0%)	.207
	rPhlp2	4 (36.4%)	15 (33.3%)	13 (52.0%)	.301
	rPhlp4	3 (27.3%)	23 (51.1%)	15 (60.0%)	.193
	rPhlp5	2 (18.2%)	17 (37.8%)	12 (48.0%)	.236
	rPhlp6	2 (18.2%)	18 (40.0%)	13 (52.0%)	.161
	rPhlp11	1 (9.1%)	11 (24.4%)	3 (12.0%)	.301
	rPhlp7	1 (9.1%)	2 (4.4%)	2 (8.0%)	.764
	rPhlp12	0 (0.0%)	1 (2.2%)	2 (8.0%)	.369
Alder	rAlng1	5 (45.5%)	16 (35.6%)	14 (56.0%)	.251
	rAlng4	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Cypress	nCupa1	1 (9.1%)	8 (17.8%)	3 (12.0%)	.685
Japanese cedar	nCryj1	1 (9.1%)	7 (15.6%)	5 (20.0%)	.707
Hazelnut	rCora1.0101	4 (36.4%)	19 (42.2%)	14 (56.0%)	.432
	rCora10401	5 (45.5%)	19 (42.2%)	10 (40.0%)	.953
	rCora8	0 (0.0%)	3 (6.7%)	1 (4.0%)	.636
	nCora9	0 (0.0%)	0 (0.0%)	1 (4.0%)	.321
Plane tree	rPlaa1	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
	rPlaa2	1 (9.1%)	10 (22.2%)	8 (32.0%)	.313
	rPlaa3	0 (0.0%)	4 (8.9%)	1 (4.0%)	.472
Olive pollen	nOlee1	2 (18.2%)	5 (11.1%)	6 (24.0%)	.363
	nOlee7	0 (0.0%)	1 (2.2%)	0 (0.0%)	.666
	nOlee9	0 (0.0%)	9 (20.0%)	8 (32.0%)	.091
Birch	rBetv 1	7 (63.6%)	26 (57.8%)	13 (52.0%)	.793
	rBetv2	0 (0.0%)	2 (4.4%)	6 (24.0%)	.015*
	rBetv 4	1 (9.1%)	1 (2.2%)	1 (4.0%)	.554
Goosefoot	rchea1	0 (0.0%)	4 (8.9%)	3 (12.0%)	.496
Mugwort	rArtv 1	1 (9.1%)	13 (28.9%)	10 (40.0%)	.171
	rArtv 3	0 (0.0%)	4 (8.9%)	1 (4.0%)	.472
Ragweed	rAmba1	0 (0.0%)	3 (6.7%)	1 (4.0%)	.636
	rPla1	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Plantain	rPlal1	0 (0.0%)	5 (11.1%)	4 (16.0%)	.371
Annual mercury	rMera1	0 (0.0%)	5 (11.1%)	6 (24.0%)	.117
Saltwort	nSalk 1	0 (0.0%)	1 (2.2%)	1 (4.0%)	.765
Wall pelitory	rparj2	0 (0.0%)	5 (11.1%)	4 (16.0%)	.371
Alternaria	rAlta1	4 (36.4%)	12 (26.7%)	8 (32.0%)	.780
	rAlta6	0 (0.0%)	6 (13.3%)	8 (32.0%)	.037*
Aspergillus	rAspf1	0 (0.0%)	2 (4.4%)	3 (12.0%)	.297
	rAspf3	0 (0.0%)	2 (4.4%)	4 (16.0%)	.125
	rAspf6	0 (0.0%)	9 (20.0%)	10 (40.0%)	.023*
Cladosporium	rClah8	0 (0.0%)	1 (2.2%)	2 (8.0%)	.369
House dust mite	rDerf1	2 (18.2%)	17 (37.8%)	11 (44.0%)	.331
	Derf2	2 (18.2%)	23 (51.1%)	16 (64.0%)	.040*
	rBlot5	1 (9.1%)	2 (4.4%)	4 (16.0%)	.256
	rDerp1	3 (27.3%)	17 (37.8%)	11 (44.0%)	.632
	rDerp2	2 (18.2%)	23 (51.1%)	16 (64.0%)	.040*
	rDerp10	1 (9.1%)	1 (2.2%)	2 (8.0%)	.446
Cockroach	rBlag1	0 (0.0%)	2 (4.4%)	0 (0.0%)	.440
	rBlag2	0 (0.0%)	2 (4.4%)	1 (4.0%)	.779
	rBlag5	0 (0.0%)	1 (2.2%)	2 (8.0%)	.369
	rBlag7	1 (9.1%)	1 (2.2%)	2 (8.0%)	.446
Sorage mite	rlepd2	1 (9.1%)	5 (11.1%)	5 (20.0%)	.521
Cat	rFeld1	2 (18.2%)	19 (42.2%)	14 (56.0%)	.105
	rFeld2	0 (0.0%)	5 (11.1%)	1 (4.0%)	.332

(Continued)

Table 7. Continued.

Allergen	Molecular component	Severity of atopic dermatitis – number of patients (relative frequency, given severity)			<i>p</i> -value
		Mild form (11 patients = 100%)	Moderate form (45 patients = 100%)	Severe form (25 patients = 100%)	
Dog	rFeld4	0 (0.0%)	11 (24.4%)	14 (56.0%)	.001*
	rCanf1	0 (0.0%)	19 (42.2%)	12 (48.0%)	.017*
	rCanf2	0 (0.0%)	6 (13.3%)	7 (28.0%)	.082
	nCanf3	0 (0.0%)	4 (8.9%)	2 (8.0%)	.595
	rCanf5	1 (9.1%)	11 (24.4%)	9 (36.0%)	.223
Horse	rEquc1	0 (0.0%)	11 (24.4%)	13 (52.0%)	.003*
	nEquc3	0 (0.0%)	2 (4.4%)	1 (4.0%)	.7794
Mouse	nMusm1	0 (0.0%)	8 (17.8%)	9 (36.0%)	.036*
Common wasp venom	rVesv 5	0 (0.0%)	2 (4.4%)	5 (20.0%)	.046*
Honey bee venom	r/nApim1	0 (0.0%)	1 (2.2%)	1 (4.0%)	.765
	nApim4	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Paper wasp venom	rPold5	0 (0.0%)	2 (4.4%)	3 (12.0%)	.297
Buckwheat	nFage2	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Sesame seed	nSesi1	0 (0.0%)	1 (2.2%)	3 (12.0%)	.139
Wheat	rTria14	0 (0.0%)	1 (2.2%)	0 (0.0%)	.666
	nTriaaA	0 (0.0%)	0 (0.0%)	3 (12.0%)	.030*
	nTriaGliadin	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Egg white	nGald1	0 (0.0%)	3 (6.7%)	3 (12.0%)	.430
	nGald2	0 (0.0%)	0 (0.0%)	5 (20.0%)	.002*
	nGald3	0 (0.0%)	2 (4.4%)	4 (16.0%)	.125
Egg yolk	nGald5	0 (0.0%)	2 (4.4%)	1 (4.0%)	.779
Cow 's milk	nBosd4	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
	nBosd5	0 (0.0%)	0 (0.0%)	1 (4.0%)	.321
	nBosd8	0 (0.0%)	0 (0.0%)	2 (8.0%)	.100
	nBosd	0 (0.0%)	1 (2.2%)	1 (4.0%)	.765
Cow 's milk/meat	nBosd6	0 (0.0%)	1 (2.2%)	1 (4.0%)	.765
Apple	rMald1	3 (27.3%)	21 (46.7%)	14 (56.0%)	.281
Kiwi	nActd1	0 (0.0%)	5 (11.1%)	4 (16.0%)	.371
	nActd2	1 (9.1%)	9 (20.0%)	6 (24.0%)	.584
	nActd5	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
	nActd8	1 (9.1%)	10 (22.2%)	7 (28.0%)	.453
Peach	rPrup1	4 (36.4%)	20 (44.4%)	14 (56.0%)	.489
	n/rPrup3	0 (0.0%)	5 (11.1%)	2 (8.0%)	.496
Brazil nut	nBere1	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Cashew nut	rAnao2	0 (0.0%)	3 (6.7%)	1 (4.0%)	.636
Peanut	nArah1	0 (0.0%)	0 (0.0%)	3 (12.0%)	.030*
	nArah2	0 (0.0%)	0 (0.0%)	2 (8.0%)	.100
	nArah3	0 (0.0%)	2 (4.4%)	1 (4.0%)	.779
	nArah6	0 (0.0%)	0 (0.0%)	1 (4.0%)	.321
	rArah8	4 (36.4%)	16 (35.6%)	13 (52.0%)	.386
	rArah9	0 (0.0%)	4 (8.9%)	3 (12.0%)	.496
Soy	rGlym4	2 (18.2%)	14 (31.1%)	12 (48.0%)	.170
	nGlym5	0 (0.0%)	0 (0.0%)	2 (8.0%)	.100
	nGlym6	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
Walnut	nJugr1	0 (0.0%)	0 (0.0%)	2 (8.0%)	.100
	nJugr2	0 (0.0%)	3 (6.7%)	4 (16.0%)	.225
	nJugr3	0 (0.0%)	5 (11.1%)	1 (4.0%)	.332
Anisakis	rAnis 1	0 (0.0%)	0 (0.0%)	1 (4.0%)	.321
	rAnis 3	1 (9.1%)	1 (2.2%)	1 (4.0%)	.554
Cod	rGadc1	0 (0.0%)	2 (4.4%)	2 (8.0%)	.578
Shrimp	nPenm1	1 (9.1%)	3 (6.7%)	3 (12.0%)	.747
	nPenm2	1 (9.1%)	7 (15.6%)	13 (52.0%)	.001*
	nPenm4	1 (9.1%)	1 (2.2%)	0 (0.0%)	.266
Celery	rApig1	0 (0.0%)	10 (22.2%)	8 (32.0%)	.104
Latex	rHevb1	0 (0.0%)	2 (4.4%)	1 (4.0%)	.779
	rHevb3	0 (0.0%)	2 (4.4%)	0 (0.0%)	.440

(Continued)

Table 7. Continued.

Allergen	Molecular component	Severity of atopic dermatitis – number of patients (relative frequency, given severity)			<i>p</i> -value
		Mild form (11 patients = 100%)	Moderate form (45 patients = 100%)	Severe form (25 patients = 100%)	
	rHevb5	0 (0.0%)	2 (4.4%)	0 (0.0%)	.440
	rhevb602	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA
	hevb6.01	0 (0.0%)	4 (8.9%)	2 (8.0%)	.595
	nHevb8	0 (0.0%)	6 (13.3%)	6 (24.0%)	.160
Sugar epitope from Bromelain (CCD)	nMUXF3	1 (9.1%)	11 (24.4%)	6 (24.0%)	.529

*Significant difference (NA, not available).

Table 8. The number of positive reactions to molecular components (low, moderate and high positivity of specific IgE) in patients with and without AB. The significant difference in the level of specific IgE to molecular components between the patients with and without AB was confirmed (*p*-value = .000).

	Number of reactions			
	0.3–0.9 ISU-E low positivity	0.9–15 ISU-E moderate positivity	above 15 ISU-E high positivity	
Asthma bronchiale (AB)				
AB yes	143	397	315	
AB no	79	283	149	
	Number of reactions in %			
AB yes	16.8	46.4	36.8	100%
AB no	15.5	55.4	29.2	100%
<i>p</i> -value	.000			

Cora 1.0401 (Hazelnut), Der f 1, Der f 2 (House dust mite), Fel d 1, Fel d 4 (Cat), Gly m 4 (Soy), Hev b 5, Hev b 8 (Latex), Mal d 1 (Apple), Phl p 2, Phl p 4, Phl p 5, Phl p 11 (Timothy), Pla l 1 (Plantain), and Act d 2 (Kiwi). The positivity to molecular components such as Der f 2 (House dust mite), Phl p 2 (Timothy), Cora1.0101 (Hazelnut), and Fel d 4 (Cat) is recorded only in patients suffering from AB (Table 10). On the other hand, other molecular components were recorded with the significant higher occurrence in AD patients without AB: Ara h 6 (Peanut), Api m 4 (Honey bee venom), Bos d 6 (Cow's milk), Gal d 5 (Egg yolk), Ole e 1 (Oliva pollen), Tri gliadin (Wheat), Ani s 1 (Anisakis), Bla g 7 (Cockroach), Can f 5 (Dog), Blot 5 (House dust mite), Par j 2 (Wall pelitory), Che a 1 (Gossefoot), and Hev b 6 and Hev b 6 (Latex) (Table 10).

Regarding the occurrence of AR in our group of patients, we evaluated the level of specific IgE to molecular components (low, moderate, and high positivity of specific IgE), the underlying probability of the positive results to molecular components, and the relative frequency of sensitisation to molecular components in patients with and without AR. The number of positive reactions to molecular components (low, moderate, and high positivity of specific IgE) in patients with and without AR is shown in Table 11. The significant difference in the level of specific IgE to molecular components between the patients with and without AR and was not confirmed (*p*-value = .099). The list of positive results to molecular components in patients suffering from AR and in patients without AR was recorded. According to the statistical evaluation, the order of molecular components in these groups of patients is not statistically significant, it means that there is not the statistically important difference in the underlying probability in the order of molecular components in patients suffering from AR and in patients without AR. On the other hand, in

Table 9. The order of molecular components with positive results in patients suffering from AB and in patients without AB. The first 25 molecular components with the highest underlying probability in patients suffering from AB were found to be different from those not suffering from AB.

Positive results of molecular components in 81 patients according to the occurrence of asthma bronchiale (81 patients = 100%)

Order of molecular component	Asthma bronchiale yes 46 patients (56.8%)			Asthma bronchiale no 35 patients (43.2%)		
	Molecular components	Number of patients	Frequency %	Molecular components	Number of patients	Frequency %
1.	rPhlp1	28	34.6	rEquc1	23	28.4
2.	rDerf2	26	32.1	rDerp2	19	23.5
3.	rDerp2	26	32.1	rPrup1	18	22.2
4.	rPhlp4	25	30.9	rCanF1	17	21.0
5.	nCynd1	24	29.7	nBosd	17	21.0
6.	rBetv 1	23	28.4	nCynd1	16	19.7
7.	rPhlp6	22	27.2	rBlag7	16	19.7
8.	rFeld1	22	27.2	rPhlp1	15	18.6
9.	rMald1	22	27.2	nOLee1	15	18.6
10.	rPhlp2	21	25.9	rVesv 5	15	18.6
11.	rPrup1	21	25.9	nBosd6	15	18.6
12.	rCora1.0101	20	24.7	nArah6	14	17.3
13.	rDerf1	20	24.7	rBetv 1	13	16.0
14.	rDerp1	19	23.5	nCanf3	13	16.0
15.	rCanF1	19	23.5	rparj2	12	14.9
16.	rCora10401	19	23.5	rchea1	12	14.9
17.	rArah8	19	23.5	rPhlp4	11	13.6
18.	rPhlp5	18	22.2	rPhlp6	11	13.6
19.	rAlng1	17	21.0	rPlaa2	11	13.6
20.	rFeld4	17	21.0	rBlot5	11	13.6
21.	rGlym4	17	21.0	rAnis 1	11	13.6
22.	rEquc1	16	19.7	nCryj1	10	12.4
23.	nPenm2	15	18.6	nCupa1	10	12.4
24.	rAlta1	14	17.3	nGald5	9	11.1
25.	nMUXF3	14	17.3	rPlaa3	8	9.9

patients suffering from AR, we can observe the increasing relative frequency of positive results in all molecular components (Table 12). These molecular components were recorded with the significant higher occurrence in AD patients suffering from AR: Pru p 1 (Peach), Cyn d 1 (Bermuda grass), Equ c 1 (Horse), Mus m1 (Mouse), Ole e 9 (Oliva pollen), Alt a 6 (Alternaria), Asp f 6 (Aspergillus), Can f 1 (Dog), Cor a 1.0101 (Hazelnut), Fel d 1 and Fel d 4 (Cat), Mal d 1 (Apple), and Phl p 4, 5, 6 (Timothy).

The summary list of allergens and molecular components with significantly higher occurrence in patients suffering from AB, AR, and severe form of AD is shown in Table 13. Molecular components such as Equ c 1 (Horse), Alt a 6 (Alternaria), Fel d 1, Fel d 4 (Cat), and Can f 1, Can f 5 (Dog) are recorded with the significant higher occurrence simultaneously in patients suffering from a severe form of AD, AB, and AR.

Discussion

This study evaluates the sensitisation and the level of specific IgE to molecular components with the use of Multiplex ISAC testing in the group of 81 AD patients. We evaluated this sensitisation with the underlying probability of the occurrence according to the severity of AD, according to the occurrence of AB, and AR. There are few studies dealing with this question in adolescents and adults suffering from AD according to the database in Medline, Pubmed, and Web of Science. Although we found some studies dealing with

Table 10. The relative frequency of positivity to molecular components in patients with AB (46 patients = 100%) in comparison to patients without AB (35 patients = 100%).

Allergen	Molecular component	Asthma bronchiale negative (35 patients = 100%)	Asthma bronchiale positive (46 patients = 100%)	p-value
Peach	n/rPrup3	0 (0.0%)	4 (8.7%)	.073
	rPrup1	18 (51.4%)	21 (45.7%)	.606
Kiwi	nActd1	2 (5.7%)	7 (15.2%)	.177
	nActd2	2 (5.7%)	11 (23.9%)	.027*
	nActd5	2 (5.7%)	0 (0.0%)	.100
	nActd8	6 (17.1%)	9 (19.6%)	.780
Honey bee venom	nApim4	6 (17.1%)	0 (0.0%)	.003*
	r/nApim1	5 (14.3%)	2 (4.3%)	.114
Peanut	nArah1	3 (8.6%)	2 (4.3%)	.433
	nArah2	0 (0.0%)	2 (4.3%)	.211
	nArah3	0 (0.0%)	1 (2.2%)	.380
	nArah6	14 (40.0%)	1 (2.2%)	1.41E-05*
	rArah8	6 (17.1%)	19 (41.3%)	.019*
	rArah9	0 (0.0%)	6 (13.0%)	.026*
Brazil nut	nBere1	0 (0.0%)	0 (0.0%)	NA
Cow 's milk	nBosd	17 (48.6%)	2 (4.3%)	3.27E-06*
	nBosd4	1 (2.9%)	0 (0.0%)	.2486
	nBosd5	1 (2.9%)	0 (0.0%)	.2486
	nBosd6	15 (42.9%)	2 (4.3%)	2.48607E-05*
	nBosd8	0 (0.0%)	1 (2.2%)	.380
Japanese cedar	nCryj1	10 (28.6%)	10 (21.7%)	.479
Cypress	nCupa1	10 (28.6%)	8 (17.4%)	.230
Bermuda grass	nCynd1	16 (45.7%)	24 (52.2%)	.564
Horse	nEquc3	2 (5.7%)	2 (4.3%)	.778
	rEquc1	23 (65.7%)	16 (34.8%)	.005*
Buckwheat	nFage2	1 (2.9%)	0 (0.0%)	.2486
Egg	nGald1	6 (17.1%)	4 (8.7%)	.252
	ngald2	1 (2.9%)	3 (6.5%)	.450
	nGald3	0 (0.0%)	3 (6.5%)	.123
	nGald5	9 (25.7%)	2 (4.3%)	.005*
Walnut	nJugr1	1 (2.9%)	2 (4.3%)	.724
	nJugr2	2 (5.7%)	6 (13.0%)	.273
	nJugr3	0 (0.0%)	4 (8.7%)	.073
Perennial regrass	nLoip1	1 (2.9%)	0 (0.0%)	.248
Mouse	nMusm1	0 (0.0%)	11 (23.9%)	.001*
Sugar epitope from Bromelain (CCD)	nMUXF3	3 (8.6%)	14 (30.4%)	.016*
Olive pollen	nOlee1	15 (42.9%)	7 (15.2%)	.005*
	nOlee7	0 (0.0%)	1 (2.2%)	.380
	nOlee9	1 (2.9%)	13 (28.3%)	.002*
Shrimp	nPenm1	2 (5.7%)	5 (10.9%)	.413
	nPenm2	3 (8.6%)	15 (32.6%)	.009*
	nPenm4	0 (0.0%)	1 (2.2%)	.380
Saltwort	nSalk 1	0 (0.0%)	2 (4.3%)	.211
Sesame seed	nSesi1	0 (0.0%)	0 (0.0%)	NA
Wheat	nTriaaA	0 (0.0%)	2 (4.3%)	.211
	nTriaGliadin	6 (17.1%)	0 (0.0%)	.003*
	rTria14	1 (2.9%)	1 (2.2%)	.844
Honey bee venom	r/nApim1	5 (14.3%)	2 (4.3%)	.114
Alder	rAlng1	6 (17.1%)	17 (37.0%)	.051
	rAlng4	0 (0.0%)	0 (0.0%)	NA
Alternaria	rAlta1	3 (8.6%)	14 (30.4%)	.016*
	rAlta6	0 (0.0%)	13 (28.3%)	.0005*
Ragweed	rAmba1	1 (2.9%)	2 (4.3%)	.7249
Cashew nut	rAnao2	2 (5.7%)	3 (6.5%)	.881
Anisakis	rAnis 1	11 (31.4%)	1 (2.2%)	.0002*
Anisakis	rAnis 3	1 (2.9%)	1 (2.2%)	.844

(Continued)

Table 10. Continued.

Allergen	Molecular component	Asthma bronchiale negative (35 patients = 100%)	Asthma bronchiale positive (46 patients = 100%)	<i>p</i> -value
Celery	rApig1	4 (11.4%)	12 (26.1%)	.100
Mugwort	rArtv 1	2 (5.7%)	13 (28.3%)	.009*
	rArtv 3	4 (11.4%)	4 (8.7%)	.682
Aspergillus	rAspf1	1 (2.9%)	4 (8.7%)	.279
	rAspf3	0 (0.0%)	3 (6.5%)	.123
	rAspf6	3 (8.6%)	13 (28.3%)	.0274*
Birch	rBetv 1	13 (37.1%)	23 (50.0%)	.2486
	rBetv 4	1 (2.9%)	0 (0.0%)	.2486
	rBetv2	5 (14.3%)	5 (10.9%)	.6433
Cockroach	rBlag1	1 (2.9%)	2 (4.3%)	.7249
	rBlag2	0 (0.0%)	2 (4.3%)	.211
	rBlag5	0 (0.0%)	3 (6.5%)	.123
	rBlag7	16 (45.7%)	2 (4.3%)	9.16E–06*
Dog	rCanf1	17 (48.6%)	19 (41.3%)	.514
	rCanf2	3 (8.6%)	9 (19.6%)	.167
	rCanf5	1 (2.9%)	10 (21.7%)	.013*
	nCanf3	13 (37.1%)	5 (10.9%)	.004*
Cladosporium	rClah8	4 (11.4%)	3 (6.5%)	.436
Hazelnut	rCora1.0101	0 (0.0%)	20 (43.5%)	6.950E–06*
	rCora10401	0 (0.0%)	19 (41.3%)	1.387E–05*
	rCora8	2 (5.7%)	3 (6.5%)	.881
	nCora9	2 (5.7%)	1 (2.2%)	.403
House dust mite	rDerf1	4 (11.4%)	20 (43.5%)	.001*
	Derf2	0 (0.0%)	26 (56.5%)	6.75E–08*
	rDerp1	3 (8.6%)	19 (41.3%)	.001*
	rDerp10	1 (2.9%)	2 (4.3%)	.724
	rDerp2	19 (54.3%)	26 (56.5%)	.840
	rBlot5	11 (31.4%)	4 (8.7%)	.009*
Cat	rFeld1	6 (17.1%)	22 (47.8%)	.004*
	rFeld2	8 (22.9%)	4 (8.7%)	.075
	rFeld4	0 (0.0%)	17 (37.0%)	5.24E–05*
Cod	rGadc1	3 (8.6%)	3 (6.5%)	.727
Soy	rGlym4	4 (11.4%)	17 (37.0%)	.009*
	rGlym5	1 (2.9%)	1 (2.2%)	.844
	nGlym6	2 (5.7%)	0 (0.0%)	.100
Latex	rHevb1	1 (2.9%)	2 (4.3%)	.724
	rHevb3	0 (0.0%)	1 (2.2%)	.380
	rHevb5	3 (8.6%)	0 (0.0%)	.043*
	rhevb602	4 (11.4%)	0 (0.0%)	.018*
	hevb6.01	0 (0.0%)	4 (8.7%)	.073
	nHevb8	0 (0.0%)	9 (19.6%)	.005*
Goosefoot	rchea1	12 (34.3%)	4 (8.7%)	.004*
Storage mite	rlepd2	1 (2.9%)	6 (13.0%)	.106
Apple	rMald1	5 (14.3%)	22 (47.8%)	.001*
Annual mercury	rMera1	3 (8.6%)	7 (15.2%)	.367
Wall pellitory	rparj2	12 (34.3%)	7 (15.2%)	.044*
Timothy	rPhlp1	15 (42.9%)	28 (60.9%)	.107
	rPhlp11	1 (2.9%)	9 (19.6%)	.023
	rPhlp12	1 (2.9%)	3 (6.5%)	.450
	rPhlp2	0 (0.0%)	21 (45.7%)	3.4E–06*
	rPhlp4	11 (31.4%)	25 (54.3%)	.039*
	rPhlp5	1 (2.9%)	18 (39.1%)	.0001*
	rPhlp6	11 (31.4%)	22 (47.8%)	.136
	rPhlp7	2 (5.7%)	0 (0.0%)	.100
	rPla1	2 (5.7%)	0 (0.0%)	.100
Plane tree	rPlaa1	2 (5.7%)	0 (0.0%)	.100
	rPlaa2	11 (31.4%)	13 (28.3%)	.757
	rPlaa3	8 (22.9%)	4 (8.7%)	.075

(Continued)

Table 10. Continued.

Allergen	Molecular component	Asthma bronchiale negative (35 patients = 100%)	Asthma bronchiale positive (46 patients = 100%)	<i>p</i> -value
Plantain	rPlal1	0 (0.0%)	6 (13.0%)	.0263*
Paper wasp venom	rPold5	1 (2.9%)	4 (8.7%)	.279
Common wasp venom	rVesv 5	15 (42.9%)	7 (15.2%)	.005*

*The significant difference in the relative frequency of positivity to molecular components (NA, not available).

Table 11. The number of positive reactions to molecular components (low, moderate, and high positivity of specific IgE) in patients with and without AR. The difference between the patients with and without AR and the level of molecular components was not confirmed (*p*-value = .099).

	Number of reactions			
	0.3–0.9 ISU-E low positivity	0.9–15 ISU-E moderate positivity	Above 15 ISU-E high positivity	
Allergic rhinitis (AR)				
AR yes	205	608	431	
AR no	17	72	33	
	Number of reactions in %			
AR yes	16.5	48.9	34.7	100%
AR no	13.9	59.0	27.0	100%
<i>p</i> -value	.099			

the examination of molecular components in AD patients, we did not find any similar one going to the detailed description of the results with CRD examination with the use of the statistical method as used in our study. The most common positivity of molecular components were observed to allergens such as timothy, birch, house dust mite, dog, cat, peach, Bermuda grass, apple, hazel pollen, kiwi, peanut, hazelnut, alternaria, soybean. As Bermuda grass pollen is not present in our region, possible cross-reactivity with β -expansins from other grasses could be the explanation for the results with high sensitisation to Cyn d 1 in our study. In a more detailed examination of the results, we found some significant differences in the positivity to molecular components in patients suffering from a mild, moderate, and severe form of AD and in subgroup of patients suffering from AB and AR. Patients suffering from the severe form of AD differ from patients with moderate and mild form in several characteristics. First, severe AD patients were characterised by the fact that they reacted to a larger panel of environmental allergens than patients with moderate AD and mild AD. Although we observe the difference in the order of molecular components in the different form of AD, there is no statistically important difference in the underlying probability in the mild, moderate, and severe form of AD, and in patients suffering from AR. So far, we cannot decide which allergen has the highest probability in different forms of AD and AR. In patients suffering from a different form of AD (mild, moderate, severe), the set of allergens is large and we believe it makes no sense to discuss the differences due to the severity of AD. An approach like this would require a much larger number of patients. The main reason for this approach was to determine which allergens (molecular components) should be tested first. On the other hand, we show that in some allergens (molecular components), the difference in frequencies depending on the severity of AD was found. The same would hold true in the case of AR. Only in patients suffering from AB, we can see that the first 25 molecular components

Table 12. The increasing relative frequency of positive results in molecular components in patients with AR (65 patients = 100%) in comparison to patients without AR (16 patients = 100%).

Allergen	Molecular component	Allergic rhinitis negative (16 patients = 100%)	Allergic rhinitis positive (65 patients = 100%)	p-value
Peach	n/rPrup3	1 (6.2%)	6 (9.2%)	.703
	rPrup1	4 (25.0%)	34 (52.3%)	.049*
Kiwi	nActd1	0 (0.0%)	9 (13.8%)	.114
	nActd2	3 (18.8%)	13 (20.0%)	.910
	nActd5	0 (0.0%)	0 (0.0%)	NA
	nActd8	3 (18.8%)	15 (23.1%)	.709
Honey bee venom	nApim4	0 (0.0%)	0 (0.0%)	NA
Peanut	nArah1	0 (0.0%)	3 (4.6%)	.381
	nArah2	0 (0.0%)	2 (3.1%)	.477
	nArah3	0 (0.0%)	3 (4.6%)	.381
	nArah6	0 (0.0%)	1 (1.5%)	.617
	rArah8	4 (25.0%)	29 (44.6%)	.152
	rArah9	1 (6.2%)	6 (9.2%)	.703
Brazil nut	nBere1	0 (0.0%)	0 (0.0%)	NA
Cow 's milk	nBosd	0 (0.0%)	2 (3.1%)	.477
	nBosd4	0 (0.0%)	0 (0.0%)	NA
	nBosd5	0 (0.0%)	1 (1.5%)	.617
	nBosd6	0 (0.0%)	2 (3.1%)	.477
	nBosd8	0 (0.0%)	2 (3.1%)	.477
Japanese cedar	nCryj1	1 (6.2%)	12 (18.5%)	.233
Cypress	nCupa1	1 (6.2%)	11 (16.9%)	.281
Bermuda grass	nCynd1	4 (25.0%)	35 (53.8%)	.038*
Horse	nEquc3	0 (0.0%)	3 (4.6%)	.381
Horse	rEquc1	1 (6.2%)	23 (35.4%)	.022*
Buckwheat	nFage2	0 (0.0%)	0 (0.0%)	NA
Egg	nGald1	0 (0.0%)	6 (9.2%)	.206
	ngald2	0 (0.0%)	5 (7.7%)	.252
	nGald3	0 (0.0%)	6 (9.2%)	.206
	nGald5	1 (6.2%)	2 (3.1%)	.547
Walnut	nJugr1	0 (0.0%)	2 (3.1%)	.477
	nJugr2	0 (0.0%)	7 (10.8%)	.169
	nJugr3	1 (6.2%)	5 (7.7%)	.843
Perennial reygrass	nLolp1	0 (0.0%)	0 (0.0%)	NA
Mouse	nMusm1	0 (0.0%)	17 (26.2%)	.0213*
Sugar epitope from Bromelain (CCD)	nMUXF3	1 (6.2%)	17 (26.2%)	.086
Olive pollen	nOlee1	1 (6.2%)	12 (18.5%)	.233
	nOlee7	0 (0.0%)	1 (1.5%)	.617
	nOlee9	0 (0.0%)	17 (26.2%)	.021*
Shrimp	nPenm1	1 (6.2%)	6 (9.2%)	.703
	nPenm2	2 (12.5%)	19 (29.2%)	.171
	nPenm4	1 (6.2%)	1 (1.5%)	.276
Saltwort	nSalk 1	0 (0.0%)	2 (3.1%)	.477
Sesame seed	nSesi1	0 (0.0%)	4 (6.2%)	.308
Wheat	nTriaaA	0 (0.0%)	3 (4.6%)	.388
	nTriGliadin	0 (0.0%)	0 (0.0%)	NA
	rTria14	0 (0.0%)	1 (1.5%)	.617
Honey bee venom	r/nApim1	0 (0.0%)	2 (3.1%)	.477
Alder	rAlng1	4 (25.0%)	31 (47.7%)	.100
	rAlng4	0 (0.0%)	0 (0.0%)	NA
Alternaria	rAlta1	3 (18.8%)	21 (32.3%)	.287
	rAlta6	0 (0.0%)	14 (21.5%)	.041*
Ragweed	rAmba1	0 (0.0%)	4 (6.2%)	.308
Cashew nut	rAnao2	0 (0.0%)	4 (6.2%)	.308
Anisakis	rAnis 1	0 (0.0%)	1 (1.5%)	.617
	rAnis 3	1 (6.2%)	2 (3.1%)	.547
Celery	rApig1	1 (6.2%)	17 (26.2%)	.086
Mugwort	rArtv 1	3 (18.8%)	21 (32.3%)	.287
	rArtv 3	0 (0.0%)	5 (7.7%)	.252

(Continued)

Table 12. Continued.

Allergen	Molecular component	Allergic rhinitis negative (16 patients = 100%)	Allergic rhinitis positive (65 patients = 100%)	p-value
Aspergillus	rAspf1	0 (0.0%)	5 (7.7%)	.252
	rAspf3	0 (0.0%)	6 (9.2%)	.206
	rAspf6	0 (0.0%)	19 (29.2%)	.013*
Birch	rBetv 1	6 (37.5%)	40 (61.5%)	.082
	rBetv 4	1 (6.2%)	2 (3.1%)	.547
	rBetv2	1 (6.2%)	7 (10.8%)	.587
Cockroach	rBlag1	0 (0.0%)	2 (3.1%)	.477
	rBlag2	0 (0.0%)	3 (4.6%)	.381
	rBlag5	0 (0.0%)	3 (4.6%)	.381
	rBlag7	1 (6.2%)	3 (4.6%)	.786
Dog	rCanf1	1 (6.2%)	30 (46.2%)	.003*
	rCanf2	0 (0.0%)	13 (20.0%)	.050
	rCanf5	2 (12.5%)	19 (29.2%)	.171
	nCanf3	0 (0.0%)	6 (9.2%)	.206
Cladosporium	rClah8	0 (0.0%)	3 (4.6%)	.381
Hazelnut	rCora1.0101	3 (18.8%)	34 (52.3%)	.015*
	rCora10401	5 (31.2%)	29 (44.6%)	.331
	rCora8	1 (6.2%)	3 (4.6%)	.786
	nCora9	0 (0.0%)	1 (1.5%)	.617
House dust mite	rDerf1	4 (25.0%)	26 (40.0%)	.265
	Derf2	5 (31.2%)	36 (55.4%)	.083
	rDerp1	4 (25.0%)	27 (41.5%)	.222
	rDerp10	1 (6.2%)	3 (4.6%)	.786
	rDerp2	5 (31.2%)	36 (55.4%)	.083
	rBlot5	2 (12.5%)	5 (7.7%)	.539
Cat	rFeld1	3 (18.8%)	32 (49.2%)	.027*
	rFeld2	0 (0.0%)	6 (9.2%)	.206
	rFeld4	1 (6.2%)	24 (36.9%)	.017*
Cod	rGadc1	0 (0.0%)	4 (6.2%)	.308
Soy	rGlym4	3 (18.8%)	25 (38.5%)	.137
	rGlym5	0 (0.0%)	2 (3.1%)	.477
	nGlym6	0 (0.0%)	0 (0.0%)	NA
Latex	hevb6.01	0 (0.0%)	6 (9.2%)	.206
	rHevb1	0 (0.0%)	3 (4.6%)	.381
	rHevb3	0 (0.0%)	2 (3.1%)	.477
	rHevb5	0 (0.0%)	2 (3.1%)	.477
	rhevb602	0 (0.0%)	0 (0.0%)	NA
	nHevb8	1 (6.2%)	11 (16.9%)	.281
Goosefoot	rchea1	0 (0.0%)	7 (10.8%)	.169
Storage mite	rlep2	1 (6.2%)	10 (15.4%)	.339
Apple	rMald1	4 (25.0%)	34 (52.3%)	.049*
Annual mercury	rMera1	1 (6.2%)	10 (15.4%)	.339
Wall pellitory	rparj2	0 (0.0%)	9 (13.8%)	.114
Timothy	rPhlp1	6 (37.5%)	41 (63.1%)	.063
	rPhlp11	1 (6.2%)	14 (21.5%)	.158
	rPhlp12	0 (0.0%)	3 (4.6%)	.381
	rPhlp2	3 (18.8%)	29 (44.6%)	.057
	rPhlp4	4 (25.0%)	37 (56.9%)	.023*
	rPhlp5	2 (12.5%)	29 (44.6%)	.017*
	rPhlp6	2 (12.5%)	31 (47.7%)	.01*
	rPhlp7	1 (6.2%)	4 (6.2%)	.988
	rPla1	0 (0.0%)	0 (0.0%)	NA
Plane tree	rPlaa1	0 (0.0%)	0 (0.0%)	NA
	rPlaa2	2 (12.5%)	17 (26.2%)	.248
	rPlaa3	1 (6.2%)	4 (6.2%)	.988
Plantain	rPlal1	0 (0.0%)	9 (13.8%)	.114
Paper wasp venom	rPold5	0 (0.0%)	5 (7.7%)	.252
Common wasp venom	rVesv 5	1 (6.2%)	6 (9.2%)	.703

Table 13. The summary list of allergens and molecular components with significant higher occurrence in patients suffering from AB, AR and severe form of AD.

The allergens and molecular components with significant difference in the occurrence in patients suffering from asthma bronchiale, rhinitis and severe form of AD

Asthma bronchiale		Rhinitis		Severe form of AD	
Allergen	Molecular component	Allergen	Molecular component	Allergen	Molecular component
Peanut	Ara h 6 Ara h 8	Peach Bermuda grass	Pru p 1 Cyn d 1	Birch Alternaria ^a	Bet v 2 Alt v 6
Horse ^a	Ara h 9 Equ c 1	Horse ^a Mouse Oliva pollen	Equ c 1 Mus m 1 Ole e 9	Aspergillus House dust mite Cat ^a	Asp f 6 Der f 2 Der p 2
Mouse	Mus m 1	Alternaria ^a	Alt a 6	Shrimp Peanut Dog ^a	Fel d 4 Pen m 2 Ara h 1 Can f 1
Sugar epitope from Bromelain	MUXF 3	Aspergillus	Asp f 6		
Shrimp	Pen m2	Apple	Mal d 1	Horse ^a	Equ c 1
Alternaria ^a	Alt a 1 Alt a 6	Timothy	Phl p 4 Phl p 5 Phlp 6 Can f 1	Common wasp venom	Ves v 5
Mugwort	Art v 1	Dog ^a	Can f 1	Wheat	Tri a 1
Hazelnut	Cora 10401	Hazelnut	Cor a 1.0101	Egg yolk	Gal d 2
House dust mite	Der f 1 Der f 2	Cat ^a	Fel d 1 Fel d 4		
Cat ^a	Fel d 1 Fel d 4				
Dog ^a	Can f 5 Can f 3 ^a				
Soy	Gly m 4				
Latex	Hev b 8				
Apple	Mal d 1				
Timothy	Phl p 2 Phl p 4 Phl p 5 Phl p 11				
Plantain	Pla l 1				
Kiwi	Act d 2				
Olive pollen	Ole e 9				

^aMolecular components such as Equ c 1 (Horse), Alt a 6 (Alternaria), Fel d 1, Fel d 4 (Cat), Can f 1, Can f 5 (Dog) are recorded with the significant higher occurrence simultaneously in patients suffering from severe form of AD, AB and AR.

with the highest underlying probability were found to be different from those not suffering from AB.

After calculation of the relative frequency, we can observe, in the majority of molecular components, the increase in relative frequency from mild to moderate and to severe form of AD and in patients with AR. But the statistically significant difference was confirmed only in some of the molecular components. Regarding the severity of AD, the significant difference was confirmed in molecular components such as Bet v 2 (Birch), Alt v 6 (Alternaria), Asp f 6 (Aspergillus), Der f 2 and Der p 2 (House dust mite), Fel d 4 (Cat), Can f 1 (Dog), Equ c 1 (Horse), Ves v 5 (Common wasp venom), Tri a A (Wheat), Gal d 2 (Egg white), Pen m 2 (Shrimp), and Ara h 1 (Peanut). Regarding the occurrence of AR, we can also observe the increasing relative frequency of positive results in all molecular components in patients with AR. The significant difference was confirmed in molecular

components such as Pru p 1 (Peach), Cyn d 1 (Bermuda grass), Equ c 1 (Horse), Mus m 1 (Mouse), Ole e 9 (Oliva pollen), Alt a 6 (Alternaria), Asp f 6 (Aspergillus), Can f 1 (Dog), Cor a 1.0101 (Hazelnut), and Fel d 1 and Fel d 4 (Cat). On the other hand, in patients suffering from AB, we calculated the underlying probability and we show the first 25 molecular components with higher underlying probability in these patients. Regarding the subgroup of patients suffering from AB, these molecular components were recorded with significantly higher occurrence: Ara h 6, Ara h 8, Ara h 9 (Peanut), Equ c 1 (Horse), Mus m 1 (Mouse), Pen m 2 (Shrimp), Alt a 1 (Alternaria), Ole e 9 (Oliva pollen), Art v 1 (Mugwort), Can f 3 (Dog), Cora 1.0101, Cora 1.0401 (Hazelnut), Der f 1, Der f 2 (House dust mite), Fel d 1, Fel d 4 (Cat), Gly m 4 (Soy), Hev b 8 (Latex), Mal d 1 (Apple), Phl p 2, Phl p 4, Phl p 5, Phl p 11 (Timothy), Pla l 1 (Plantain), and Act d 2 (Kiwi). On the other hand, the other molecular components were recorded with significant higher occurrence in AD patients without AB: Api m 4 (Honey bee venom), Bos d (Cow 's milk), Gal d 5 (Egg yolk), Ole e 1 (Oliva pollen), Tri gliadin (Wheat), Ani s 1 (Anisakis), Bla g 7 (Cockroach), Can f 5 (Dog), Blot 5 (House dust mite), Par j 2 (Wall pelitory), Che a 1 (Gossefoot), Hev b 6, and Hev b 5 (Latex). Interestingly, molecular components such as Der f 2 (House dust mite), Phl p 2 (Timothy), Cora 1.0101 (Hazelnut), and Fel d 4 (Cat) are positive only in patients suffering from AB. Thus, these components might be useful serological marker allergens for the identification of a subgroup of AD patients suffering from AB.

In the similar study, Wojciechowska et al. used the ImmunoCAP ISAC test to analyse allergic phenotypes in adult patients with AD. They included 19 adult patients with AD and the severity of AD was assessed using the SCORAD index. Positive results of the ISAC test were documented in 84.2% of the study subjects. All patients synthesised sIgE against species-specific respiratory allergens; major components of animal allergens (57.8%), tree pollen allergens (47.3%), grass pollen allergens (42.1%), dust mite allergens (26.3%), and major allergen of mugwort (26.3%). In their study, no statistically significant relationships were observed between the severity or duration of AD and the prevalence and levels of sIgE against the allergens included in the ISAC panel (Wojciechowska et al., 2018 Aug). Choi et al. evaluated the serum samples of 25 AD patients by using ISAC and a multiple allergen simultaneous test-enzyme immunoassay (MAST-EIA). ISAC results in AD correlated well with the SPT results, and compared favourably to the MAST-EIA results (Choi et al., 2014). According to Mothes, the novel concept "from molecules to clinic" offers a reliable diagnostic workup in shorter time. It is especially applicable for young children and seniors, in atopic patients, and whenever skin tests get difficult or unreliable (Mothes-Luksch et al., 2018). Patients reporting hazelnut allergy ($n = 423$) from 12 European cities were tested for IgE against individual hazelnut allergens; a model combining CRD with a clinical background and extract-based serology was superior to CRD alone in assessing the risk of severe reactions to hazelnut, particularly in ruling out severe reactions (Datema et al., 2018). In the research project MeDALL, IgE reactivities towards a large number of micro-arrayed allergen molecules have been determined in several European birth cohorts using the MeDALL allergen chip (Lupinek et al., 2014). Data obtained in the MeDALL project seem to confirm that patients with AD are often polysensitised towards a large number of different allergen molecules and thus exhibit extremely complex IgE sensitisation profiles (Anto et al., 2017; Asarnoj et al., 2016; Asarnoj et al., 2017; Bousquet et al., 2015; Lupinek et al.,

2014; Posa et al., 2017; Westman et al., 2015). Multiallergen tests, mainly chip tests based on micro-arrayed allergen molecules utilizing the ImmunoCAP-ISAC technology, have been used for the analysis of IgE reactivity profiles in cohorts of children with AD and adult patients with AD (Banerjee et al., 2015; Fedenko et al., 2016; Foong et al., 2016; Gray et al., 2016; Mittermann et al., 2016). Banerjee et al. studied the importance of the high-molecular-weight group 11 allergen from *Dermatophagoides pteronyssinus* (Der p 11) in house dust mite (HDM) allergy. Interestingly, they found that Der p 11 is a major allergen for patients suffering from AD, whereas it is only a minor allergen for patients suffering from respiratory forms of HDM allergy. Thus, rDer p 11 might be a useful serological marker allergen for the identification of a subgroup of HDM-allergic patients suffering from HDM-associated AD (Banerjee et al., 2015). In ISAC testing, the molecular component Der p 11 is not present, so we cannot compare it with our results. According to our results, the main molecular component from HDM is Der f 2 in patients suffering from moderate and severe form of AD and in patients suffering from AB.

Mitterman et al. characterised in their study the specificities of IgE reactivity in patients with AD to a broad panel of exogenous allergens including microbial and human antigens. In their study, adult patients with AD were grouped according to the SCORAD index, into severe ($n = 53$) and moderate AD ($n = 126$). IgE reactivity was detected in 92% of patients with severe and 83% of patients with moderate AD. Sensitisation to cat allergens occurred most frequently, followed by sensitisation to birch pollen, grass pollen, and to the skin commensal yeast *M. sympodialis*. Patients with severe AD showed a significantly higher frequency of IgE reactivity to allergens like cat (Fel d 1) and house dust mite (Der p 4 and 10), there were no significant differences in the frequencies of IgE reactivity to the grass pollen allergens Phl p 1, 2, 5 and 6 between the two AD groups and the IgE reactivity profile of patients with severe AD was more spread towards several different allergen molecules when compared with patients with moderate AD. The conclusion of their study is that they revealed a hitherto unknown difference regarding the molecular sensitisation profile in patients with severe and moderate AD. Molecular profiling towards allergen components may provide a basis for future investigations aiming to explore the environmental, genetic and epigenetic factors which could be responsible for the different appearance and severity of disease phenotypes in AD (Mittermann et al., 2016). According to the Mitterman's study, it would suggest that grass pollen allergens are less important as trigger factors for AD compared to birch pollen and indoor allergens in their studied population. On the other hand, in our study, we confirmed that the molecular component Phl p1 is the leading allergen in patients suffering from moderate and severe form of AD. The difference between birch pollen and grass pollen is unexpected because grass pollen contains several very potent allergens whereas birch contains only one major allergen (Bet v 1). Furthermore, the grass pollen season lasts longer than the birch pollen season and allergen loads are typically higher for grass pollen (Asarnoj et al., 2017; Schappi et al., 1997). Since the route of exposure should be the same for both pollens, it is quite conceivable that other factors play a role such as climatic effects where low humidity and cold temperatures negatively affect skin barrier functions and increase the risk of dermatitis (Engelbrechtsen et al., 2016; Schappi et al., 1997, 1999). According to other studies, the analysis of complex IgE-reactivity profiles by a molecular diagnosis can improve disease management following the principle of precision and personalised medicine approaches in allergy

(Ferrando et al., 2017; McGhee, 2011; Riccio et al., 2016). Flohr performed the first systematic review analysing the evidence on the diagnostic accuracy of CRD for a range of food allergies. This systematic review included 11 studies that assessed the accuracy of CRD in diagnosing cow's milk, hen's egg, peanut, hazelnut, and shrimp allergies. According to Flor, the last findings suggest that some CRD components have the potential to diagnose cow's milk, hen's egg, peanut, hazelnut, and shrimp allergies with high specificity, but low sensitivity. Nevertheless, at present, there is not enough methodologically robust evidence to draw definite conclusions. Further studies employing double-blind, placebo-controlled food challenge test as the reference standard are urgently needed to effectively evaluate the effectiveness of CRD, as well as standardisation of the components assessed and CRD assays used, and consensus on study reporting (Flores et al., 2018 Aug). Röckmann et al. (2014) investigated the pattern of food sensitisation in 211 adults with AD in relation to AD severity using multiplexed allergen microarray. Specific IgE levels were routinely measured using a microarray immunoassay (Immuno Solid-phase allergen Chip (ImmunoCAP ISAC®, VBC Genomics and Phadia)) with 103 allergens. Sensitisation to PR-10 related food allergens occurred most frequently (63.5%) and was independent from AD severity. Of all plant food allergens, only sensitisation to *nAra h 1* was significantly more frequent in patients with severe AD. In the total group, 75 (35.5%) patients with AD showed sensitisation to any animal food allergen. The percentage was significantly higher in patients with severe AD (51.4%) compared to patients with mild/moderate AD (27.7%) (Röckmann et al., 2014). According to our results, the sensitisation to Bet v 2 (Birch), Alt v 6 (Alternaria), Asp f 6 (Aspergillus), Der f 2 and Der p 2 (House dust mite), Fel d 4 (Cat), Can f 1 (Dog), Equ c 1 (Horse), Ves v 5 (Common wasp venom), Tri a A (Wheat), Gal d 2 (Egg white), Pen m 2 (Shrimp), and Ara h 1 (Peanut) was significantly higher in patients with moderate and severe AD. In our study, the positivity to PR10 protein allergens (Gly m 4 – soy, Cora 10101 – hazelnut, Pru p 1 – peach, Ara h 8 – peanut, Mal d 1 – apple) is higher in severe form in comparison to moderate and mild form AD, but the difference is not significant. On the other hand, the relative frequency Bet v 1 is higher in the mild form in comparison to the moderate and severe form, but the difference is not significant as well. Unfortunately, zeroes appeared in many of the tables and one has to be careful in making definite conclusions. Regarding the level of specific IgE (low, moderate, high positivity) to molecular components in mild, moderate, and severe form of AD, the difference is statistically significant. The moderate positivity and high positivity of specific IgE to molecular components were recorded significantly more often in patients suffering from moderate and severe form of AD and in subgroup of patients suffering from AB.

In our previous studies, we evaluated the occurrence of sensitisation to food and inhalant allergens according to the severity of AD. Two hundred and eighty-three patients were examined, the significant relation was recorded between the severity of AD and sensitisation to tested inhalant allergens. The significant relation was also found between the severity of AD and IgE-mediated food allergy. It turns out that the higher time to reaction, the higher the severity of AD (Čelakovská & Bukač, 2011, 2016, 2017; Čelakovská et al. 2015a). Also, we evaluated the relation between the severity of AD and the occurrence of AB and rhinitis. A significant relationship was confirmed between the occurrence of AB, the occurrence of AR, the duration of the skin lesions, and the severity of AD evaluated with the SCORAD index. The occurrence of bronchial asthma, AR, and persistent

eczematous lesions was significantly higher in moderate and severe forms of AD evaluated by using the SCORAD index (Čelakovská & Bukač, 2017; Čelakovská et al., 2017). According to our results in another study, AD patients with sensitisation to mites and animal dander suffer significantly more often from AB, rhinitis, persistent eczematic lesions and have positive data about atopy in their family history. Patients with the sensitisation to bird feather have the onset of AD more often >5 years of age. Sensitisation to dust is in significant relationship with the occurrence of rhinitis, but not with the occurrence of AB (Čelakovská et al. 2015b).

Negaoui et al. tried to investigate the allergenic properties of bovine lactoferrin (bLf) at different doses. The lactoferrin (LF) allergenicity was explored using the murine model of allergy through measuring anti-LF IgG, IgG1 and IgE antibodies (Abs) responses and by *in vivo* anaphylactic reactions in LF-sensitised mice. Concerning the *in vivo* reactions, all groups developed clinical symptoms of anaphylactic reactions at different stages. Their findings show that LF-sensitised mice at 5% and 10% developed important clinical symptoms after intraperitoneal challenge with LF (Negaoui et al., 2016).

Little is known about pollen-food allergy syndrome (PFS) in China. Ma Shikun et al. investigated the clinical characteristics, as well as sensitisation patterns, of PFS in China. Clinical parameters and serum IgE responses to prevalent pollens, plant foods, and corresponding allergen components were evaluated. The top three most common pollen-associated allergenic foods were peach, apple, and pear. Peach was the most common allergenic food in PFS patients. Patients with PFS in China showed an LTP-dominant sensitisation profile and usually presented systemic reactions upon consumption of the allergenic foods (Ma et al., 2018).

Wheat is one of the major cereals consumed throughout the world; there has been a radical increase in the population suffering from many wheat-related disorders. The Kumar's study was conducted to screen low immunogenic hexaploid and tetraploid wheat varieties. A total of 34 different wheat varieties were tested for its total protein content, gliadin content, and immunoreactivity with immunoglobulins of celiac (IgA) and wheat allergy patients (IgE). The immunoblot assays for IgE reactivity have revealed that gliadin subunits present in all varieties are found to be allergic proteins. The principal component analysis biplot showed that tetraploid wheat varieties are less immunoreactive than hexaploids. The low immunogenic wheat varieties are suitable for plant breeding and preparation of low gluten or hypo-immunogenic products (Kumar et al., 2017). Food allergies, including kiwi fruit allergy, have been the subject of extensive research in the last few years. The aim of Gavrovic-Jankulovic's study was to examine a possible relationship between the developmental stage of kiwi fruit and its allergenic potency. The protein and allergen patterns of kiwi fruit extracts in September, October, November, and December fruit in the period from 2000 to 2002 were analysed. One of the factors that may contribute to the difficulties in proposing well-defined and standardised fruit extracts should also be the time of fruit harvesting. In this particular case, when the kiwi fruit was edible throughout November and December, they showed discrepancies in allergen content and potencies both in qualitative and quantitative terms. Two major allergens of kiwi fruit, Act c 1 and Act c 2, mainly accounted for the highest allergenic potential of November kiwi extract *in vivo* and *in vitro*. Not only the content of major allergens but also the ratio of different proteins and even isoforms of the same allergen (Act c 2) changes with fruit

ripening. These findings should be taken into account during the preparation of extracts for allergy diagnosis (Gavrovic-Jankulovic et al., 2005).

The prevalence of fish allergies has become a serious health problem and has increased alarmingly over the past few years. Liu's study attempted to identify and purify the major allergen implicated in the allergic response to largemouth bass (*Micropterus salmoides*), a freshwater fish widely consumed in China. According to their results, nucleoside diphosphate kinase B was identified as a novel fish allergen in largemouth bass. This finding is important for allergy diagnoses and the treatment of freshwater fish-allergic disorders (Liu et al., 2014). More recently, other proteins such as arginine kinases, myosin light chains, troponins, and sarcoplasmic calcium-binding proteins have been regarded as relevant allergens in fish, crustaceans, and molluscs. Fernandes' review focuses on seafood allergens, reporting an updated and compiled list of allergens from fish, crustaceans, and mollusc species, with an overview on the most representative analytical methods for their detection (Fernandes et al., 2015). Wan's study determined whether food allergen sensitivity is a marker for increased asthma incidence in primary school children in Taiwan. Food allergen sensitivity was evaluated in 6- to 8-year-old primary school students ($n=1010$) using the Phadia ImmunoCAP, Phadiatop Infant, and radioallergosorbent tests. The most prevalent food allergies were scallop, abalone, lobster, pork, casein, alpha-lactalbumin, and garlic. According to their results, food allergen hypersensitivity may be an important marker of inhaled allergen-induced respiratory allergy in later childhood (Wan & Chiu, 2012).

The quality of an applied protein extract is important in both serological and *in vivo* diagnosis of allergy, and for allergen detection methods. In Dooper's study, the effects of the extraction procedure and hazelnut source on antibody binding to hazelnut (*Corylus avellana*) proteins were investigated. This study indicates that for the production of hazelnut protein extracts, the use of fresh hazelnuts is important. A quick, vigorous extraction in a tris buffer might contribute positively, at least for extraction of Cor a 9 (Dooper et al., 2008).

Mechanisms for developing atopic comorbidities after AD onset are poorly understood but can involve the impaired cutaneous barrier, which facilitates cutaneous sensitisation. The association can also be driven or amplified in susceptible subjects by a systemic T_H2 -dominant immune response to cutaneous inflammation (Paller et al., 2019). However, these associations might merely involve shared genetic loci and environmental triggers, including microbiome dysregulation, with the temporal sequence reflecting tissue-specific peak time of occurrence of each disease, suggesting more of a clustering of disorders than a march. Prospective longitudinal cohort studies provide an opportunity to explore the relationships between postdermatitis development of atopic disorders and potential predictive phenotypic, genotypic, and environmental factors. Early intervention studies to repair the epidermal barrier or alter exposure to the microbiome or allergens might elucidate the relative roles of barrier defects, genetic locus alterations, and environmental exposures in the risk and sequence of occurrence of T_H2 activation disorders (Paller et al., 2019).

The purpose of our study was to evaluate the sensitisation to molecular components in AD patients and to find some molecular components, which may play the important role in a severe form of AD and in a subgroup of patients suffering from AB and AR. Molecular components such as Equ c 1 (Horse), Alt a 6 (Alternaria), Fel d 1, Fel d 4 (Cat), and Can f

1, Can f 5 (Dog) are recorded with the significant higher occurrence simultaneously in patients suffering from a severe form of AD, AB and AR. It seems that these molecular components play the most important role in the atopic march.

Conclusion

In patients suffering from AD, the positivity was recorded most frequently to molecular components of timothy, birch, house dust mite, animals dander, peach, apple, hazel pollen, kiwi, peanut and hazelnut, *Alternaria*, and *Aspergillus*. The highest level of specific IgE was recorded to molecular components of House dust mite, Timothy, Dog, Cat, Shrimp, Apple, and *Anisakis*. The moderate and high positivity of specific IgE to molecular components were recorded significantly more often in patients suffering from a moderate and severe form of AD and in the subgroup of patients suffering from AB, not in patients suffering from AR. Molecular components such as Equ c 1 (Horse), Alt a 6 (*Alternaria*), Fel d 1, Fel d 4 (Cat), and Can f 1, Can f 5 (Dog) are recorded with the significant higher occurrence simultaneously in patients suffering from a severe form of AD, AB, and AR. It seems that these molecular components play the most important role in the atopic march.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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