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MOLECULAR ALLERGEN AND CROSS-ALLERGEN COMPONENT STUDIES AMONG PEOPLE LIVING IN THE NORTHEASTERN HUNGARIAN REGION

ÉVA KISS-TÓTH DOJCSÁK[1] – DÁVID NÉMETH[1] – ADRIENN J. SZALAI[1] – BERTALAN FODOR[1, 2]

[1]*Institute of Clinical Methodology, Faculty of Health Sciences, University of Miskolc, Miskolc*

[2]*Borsod-Abaúj-Zemplén County Hospital and University Teaching Hospital, Department of Laboratory Medicine.*

Summary: Background: The availability of multiplex microarray-based diagnostic systems in the field of allergology contributes to the determination of personalized, component- based diagnosis. It enables allergen source and accurate allergen component identification even in patients with ambiguous symptoms and Prick skin test suggesting simultaneous positivity of two or more related allergens. **Aim:** In our study, we determined the presence and type of sensitization in the adult population of northeastern Hungary using a new type of multiple allergy diagnostic system and identified the molecular components most frequently involved in the background of sensitization and cross sensitization. **Methods:** The study was a cross-sectional study involving 229 adult volunteers. An ELISA- based multiplex molecular diagnostic system was used to determine sensitization. **Results:** Sensitization to an allergen was confirmed in nearly 70% of the subjects. 22.70% were sensitized to a single allergen, while 46.72% were sensitized to multiple allergens simultaneously. The degree of sensitization and total IgE were moderately correlated. In both groups, significant seasonal inhaled allergens were the molecular components of ragweed, grasses and birch. As perennial allergens, dust and food mite proteins caused frequent sensitization. The most common components responsible for cross-sensitization were proteins from ragweed, Timothy grass, birch and dust mite. **Discussion:** Multiplex technology and component-based diagnosis can assist the clinician in determining targeted, patient-centred and cost-effective allergy treatment.

Keywords: *allergy, sensitization, allergenic component, molecular diagnostics, cross-allergen*

INTRODUCTION

Foreign structures, antigens, which enter our body from outside, trigger a regulated immune response to restore or maintain immune homeostasis. In the meantime, certain cells of the immune system produce allergen-specific immunoglobulin E (IgE) antibodies to ensure a humoral immune response to the allergen. This process is called allergen sensitization. In some cases, however, the immune system does not respond adequately to the antigen and a hypersensitivity reaction develops. A hypersensitivity

(allergic) reaction is usually the result of a second or repeated exposure to the same allergen. [1]

Several epidemiological studies have found that atopic sensitization is a strong risk factor for asthma, hay fever or allergic conjunctivitis. In a person sensitized to an allergen, the specific IgE produced against the allergen is the initial triggering component for the activation of a complex inflammatory cascade that can lead to the development of specific symptoms. However, it should be noted that not all sensitized individuals develop allergy. [2]

The presence of IgE produced in response to a particular allergen is considered a qualitative response, but it can also be interpreted as a quantitative marker. Some people show a positive immune response to only one allergen (monosensitized), while others are sensitized to a wide range of allergens (polysensitized). It is also necessary to distinguish between the two phenotypes because they are characterised by important clinical and immunological differences. [2–3] Monosensitized children often become polysensitized in adulthood, but the trend persists in monosensitized adults. However, polysensitized patients do not necessarily develop an allergic symptom complex, but several studies have demonstrated the presence or development of polysensitization in allergic individuals over time. [4]

In the case of a polysensitized individual, it is useful to distinguish cross-reactivity from cosensitization. [4] The term cosensitization was introduced to describe multiple, independent sensitization to multiple, structurally unrelated groups of allergens. This is particularly important in the case of panallergenic groups, where the proteins representing each group are evolutionarily conserved molecules that show a high degree of molecular identity and are found in members of several different plant genus. Although the representatives of the panallergens are not numerous, the high degree of homology can cause problems in making the correct diagnosis.

Cross-reactivity to a particular allergen may develop if its three- dimensional structure is similar to that of another allergen previously encountered by the immune system. In this case, the same IgE may bind to several different allergens. In general, at least 70% amino acid sequence identity is required for this phenomenon to occur.[4] A practical example of this is the development of cross-allergies associated with pollen-food (Oral Allergy Syndrome – OAS). In such cases, it is difficult to identify the initial sensitizing allergen responsible for hypersensitization by conventional diagnostic methods. [5]

Further complicating the identification of the main allergenic component are cross- reactive carbohydrate components (CCDs), which are non-protein molecules but can induce IgE- driven cross-reactivity. These glycoprotein-based asparagine-related oligosaccharides are found in varying amounts in insect venoms, plant pollen, house dust mites, crustaceans and vegetables. [4, 6]

The Prick Skin Test (SPT) and extract-based allergen-specific IgE blood tests have been at the front line of allergy diagnostics for decades. The latter procedures are quantitative and cost-effective, but the number of allergens that can be tested is limited and in many cases detection of sensitization is either missed or underestimated. [7–8]

Over the past two decades, important innovations in allergy diagnostics have taken place. One direction of progress has been the identification, characterization and production of an increasing spectrum of molecules representing unique allergens of clinical relevance. These recombinant components have enabled the availability of molecular-resolution diagnostics and component- resolved diagnosis (CRD) in the field of allergy. [9] In addition, multiplex microarray- based diagnostic systems have been developed, which can detect specific IgE antibodies produced against 100–300 different allergens from small samples. The whole allergen extract helps to identify the allergen source to which the patient is sensitized, while the allergen component allows the separation of specific and cross-reactive sensitization in polysensitized individuals. [7] The microarray technique can represent an added value for diagnosis when symptoms are ambiguous and SPT indicates the simultaneous positivity of two or more related allergens.

The objective of our cross-sectional study was to determine the extent and type of sensitization among the population living in the Northeastern Hungarian region and to identify the molecular components most frequently involved in the background of sensitization and cross-sensitization.

MATERIALS AND METHODS

Study design and study population

The study obtained approval from the Regional/Institutional Scientific and Research Ethics Committee of the Borsod-Abaúj-Zemplén County Central Hospital and University Teaching Hospital, with approval number IG-117-24/2020. The subjects for the study were selected from the citizens of the University of Miskolc by recruitment through voluntary application. The 229 subjects recruited received verbal and written information and completed a patient consent form. They were assured of their anonymity, the voluntary nature of their participation, and their right to withdraw from the survey at any point. Patient data were collected by completing a questionnaire. To perform molecular allergy testing, 2 tubes of blood per person were collected and the tests were performed at the Clinical Diagnostic Laboratory of the Faculty of Health Sciences between February 2020 and June 2021.

Questionnaire recording

Our questionnaire recorded demographic and lifestyle data. Patients provided information about their self-assessed health status, family history of diseases and allergies.

Multiplex microarray analysis

A serum sample was obtained from whole blood drawn in a BD Vacutainer Native sterile blood collection tube by centrifugation (15 min, 2700 rpm). The samples were stored at –80 °C until use. For sample preparation, an ALEX2 Allergy Explorer kit (Macro Array Diagnostics, Vienna, Austria) was used, following the manufacturer's

protocol. The solid phase of the ALEX2 Allergy Explorer consists of allergens (295 allergens, including 117 allergen extracts and 178 molecular components) bound to nanoparticles on a nitrocellulose membrane on the surface of a chip. For sample preparation, 100 µl of serum was transferred to the solid phase with the addition of 400 µl of serum diluent. The serum diluent contains an inhibitor of CCDs. After two hours of incubation, the chip was washed thoroughly and alkaline phosphatase-labelled anti-human IgE solution was added and incubated for 30 minutes. After further washing, the enzyme substrate was added, and the reaction was stopped after eight minutes. The membrane was dried at room temperature and the intensity of the colour reaction for each allergen was measured using the Image Xplorer instrument (Macro Array Diagnostics, Vienna, Austria) and evaluated using Raptor v1.5.4.16 software. Total IgE and specific IgE were determined. The measurement range of ALEX2 Allergy Explorer is 0.35–50 kUA/L for specific IgE (quantitative determination) and 1–2500 kU/L for total IgE (semi-quantitative determination).

Data evaluation and statistical analysis

Results were considered negative if the specific IgE produced against any allergen did not exceed 0.35 kUA/L. For positive results, a person with specific IgE antibodies against an allergenic protein was considered monosensitized. A person was considered polysensitized if two or more specific IgE antibodies to different allergenic proteins were detected simultaneously. Among the polysensitized, a result where specific IgE antibodies were detected against several different proteins with no structural homology or relatedness to each other was considered to be cosensitized. Cross-sensitization was considered to be results where specific IgE antibodies directed against structurally homologous allergens from taxonomically related allergen sources gave positive results.

In advance of analyzing the data, we observed a partial non-response for the questionnaire item concerning family allergies. A logical imputation technique was used for the treatment of the missing data.

Before starting the analysis of the datasets, the conditions for the parametric tests were always checked. Normality testing (e.g. Shapiro–Wilk test) and variance homogeneity testing (e.g. Levene's test) were performed.

Clinical parameters and demographic data are presented as means and standard deviations of continuous variables. Relative frequencies of categorical variables were compared using an χ^2 test for dichotomous variables. Unpaired Student's t-test was utilized for continuous variable analysis, alongside a one-way ANOVA for the assessment of three group comparisons, both employing a 95% confidence interval. Spearman correlation analysis was used to determine the correlation between two variables. The effect size metrics (e.g., Cohen's d for t-tests, eta-squared for ANOVA, and Cramer's V for χ^2 tests) are presented exclusively for differences that reach statistical significance. In instances where the conditions of normality and homogeneity were not satisfied, non-parametric alternatives, including the Mann–Whitney U-test and Kruskal–Wallis's test, were employed.

GraphPad Prism 8.0.1 statistical software (GraphPad Software, San Diego, USA) was used for statistical analysis of the results. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Characterisation of the study population

The study population consisted of 229 people, divided into two groups based on the results obtained. The non-sensitized group included 30.56% of the subjects, while the sensitized group included 69.43%. No significant differences were found in the mean age, gender distribution and number of family members with allergies *(Table 1)*. The sensitized group had significantly higher total immunoglobulin E (t-IgE) values (23.94 ±15.04 kU/L vs. 80.86 ±146.52 kU/L, p = 0.0001, Cohens' *d* = 3.8333).

Table 1

Characterisation of patient sample by sensitization

	Non sensitized	**Sensitized**	**p- value**
Number of patients n, (%)	70 (30.56)	159 (69.43)	
Age (years), mean (SD)	44.32 (14.11)	43.39 (11.31)	0.3873
Gender M/F n, (%)	20/50 (28.57/71.43)	65/94 (40.88/59.12)	0.0757
Serum t-IgE, kU/L mean (SD)	23.94 (15.04)	80.86 (146.52)	**<0.0001**
Allergic in the family, n (%)	39 (55.71)	93(58.49)	0.8053

Serum total Immunoglobulin E (t-IgE); A person who showed a positive reaction to at least 1 allergenic protein was considered sensitized. $p < 0.05$, n = 229

Characterisation of the study population by degree of sensitization

Table 2 illustrates the characteristics of the groups sensitized to one allergen, sensitized to several allergens and not sensitized. Of the 229 subjects, 70 (30.56%) showed no sensitization, 52 (22.70%) showed sensitization to one allergen, while 107 (46.72%) showed sensitization to 2 or more allergens. The age distribution of the groups was homogeneous. In general, more females enrolled in the study, which was typical in the non-sensitized and monosensitized groups, whereas the proportion of males in the polysensitized group was almost equal to the proportion of females.

Table 2

Characterisation of the study population by degree of sensitization

		Sensitized (n = 159)			
	Non sensitized	**Mono-sensitized**	**Poly-sensitized**	***p-value****	***p-value*** **
Number of patients n, (%)[1]	70 (30.56)	52 (22.70)	107 (46.72)		
Age (year), mean (SD)	44,33 (14.11)	45.32 (10.63)	42.47 (11.52)	0.1355	0.3361
Gender, n (%)					
M, n (%)	20 (28.57)	12 (23.1)	55 (51.40)	**0.001**	0.3713
F, n (%)	50 (71.43)	40 (76.9)	52 (48.60)	**0.001**	0.3713
Total IgE ≥100 kU/L, n (%)	0 (0)	1 (1.92)	27 (25.23)	**0.0001**	0.2338
Serum t-IgE, kU/L (SD)	23.94 (15.04)	21,80 (11.93)	109.57 (171.43)	**<0.0001**	**<0.0001**
Allergic in the family, n (%)	39 (55.71)	26 (50.98)	67 (62.6)	0.1298	0.2924

Serum total immunoglobulin E (t- IgE). Those who showed a positive reaction to only 1 allergen were considered monosensitized, those who showed positive reaction to 2 or more unrelated allergens were considered polysensitized. $p < 0.05$, [1] n = 229, p-value* comparison between mono- and polysensitized groups. p-value** comparison between non sensitized, mono- and polysensitized groups.

We observed significantly higher t- IgE levels in the sensitized groups compared to the non sensitized group ($p < 0.0001$, $R^2 = 0.1198$), and in the polysensitized group compared to the monosensitized group ($p < 0.0001$, Cohens' $d = 0.73$) (*Table 2* and *Figure 1*).

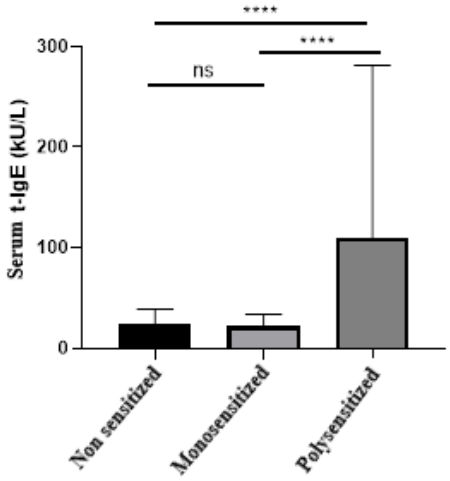

Figure 1. *Serum t-IgE values in the different sensitization groups*

In the polysensitized group, there were significantly (p = 0.0001, Cramer *V* = 0,0581 (CI 0,0055–0,3331) more people with t-IgE levels above 100 kU/L. A correlation test between the degree of sensitization and t- IgE levels demonstrated a moderate degree of association (r = 0.5138, p = 0.0001, 95% CI 0.4084–0.6057) between the two variables.

The most frequently occurring molecular components in the two sensitized groups

The prominent seasonal inhalation allergen in the monosensitized group was the protein of ragweed pectate lyase (Amb a 1), to which 4 individuals was sensitized. The birch pollen- derived protein PR-10 (Bet v 1) induced sensitization in only one person in this group, and the same rate of sensitization was observed for grass allergens *(Table 3)*. The dust mite and yeast proteins, which are perennial inhaled allergens, were equally detected in this population. Sensitization to common wasp antigen 5 (Ves v 5) was detected in 10 individuals. Sensitization to the 2S protein of sesame seed albumin (Ses i 1) was found in 5 individuals. Additional sensitizations were detected to allergenic extracts and are not included in the table.

In the polysensitized group, the most common seasonal inhaled molecular allergen components were mainly pollen from weeds, grasses and trees (Amb a 1, Phl p 1, Lol p, 1Cyn d 1), while in the group of perennial inhaled allergens, they were NPC2 proteins from dust mites (Der p 2, Der f 2, Lep d 2). The cat uteroglobin protein (Fel d 1) was highly abundant among the perennial inhalant allergens. The frequency of sensitization to common wasp Antigen 5 was equally high in this group.

Table 3

Molecular allergen frequency in the mono- and polysensitized group

Monosensitized (n = 52)			
Allergen source	**Molecular allergen**	**Protein family**	**Number of cases (n =)**
Seasonal inhalation allergens			
Ragweed	Amb a 1	Pectate lyase	4
Birch tree	Bet v 1	PR-10	1
Bermuda grass	Cyn d 1	A Beta-expansin	1
Saltwort	Sal k 1	Pectin methyl esterase	1
European ash	Fra e 1	Ole e-1-family	1

Perennial inhalation allergens			
Malassezia sympodialis	Mala s 11	Mn superoxide dismutase	1
American house dust mite	Der f 2	NPC2	1
Nutritive allergens			
Sesame seeds	Ses i 1	Albumin 2S	5
Kiwi	Act d 1	Cysteine protease	1
Insect venom			
Common wasp	Ves v 5	Antigen 5	10
Common wasp	Ves v 1	Phospholipase A1	1
European paper wasp	Pol d 5	Antigen 5	1

Polysensitized (n = 107)			
Allergen source	**Molecular allergen**	**Protein family**	**Number of cases (n =)**
Seasonal inhalation allergens			
Ragweed	Amb a 1	Pectate lyase	36
Timothy grass	Phl p 1	A Beta-expansin	35
Perennial ryegrass	Lol p 1	A Beta-expansin	31
Bermuda grass	Cyn d 1	A Beta-expansin	26
Timothy grass	Phl p 5.0101	Grass group 5/6	21
Japanese cedar	Cry j 1	Pectate lyase	21
Birch tree	Bet v 1	PR-10	19
Timothy grass	Phl p 6	Grass group 5/6	17
European beech	Fag s 1	PR-10	16
European hazelnut	Cor a 1.0103	PR-10	15
Timothy grass	Phl p 2	Expansin	15
Ragweed	Amb a 4	Plant defensin	14

Perennial inhalation allergens			
European domestic dust mite	Der p 2	NPC2 family	32
American house dust mite	Der f 2	NPC2 family	29
Lepidoglyphus destructor	Lep d 2	NPC2 family	23
Cat	Fel d 1	Uteroglobin	21
European domestic dust mite	Der p 23	Peritrophin-like protein domain	21
European domestic dust mite	Der p 1	Cysteine protease	18
American house dust mite	Der f 1	Cysteine protease	16
Nutritive allergens			
European hazelnut	Cor a 1.0401	PR-10	16
Cantaloupe	Cuc m 2	Profilin	15
Insect venom			
Common wasp	Ves v 5	Antigen 5	20

Molecular components most involved in cross-sensitization

In the monosensitized group, cross sensitization was detected in a total of 9 individuals (17.30%). Cross-sensitization was observed in 3 cases with the major component of ragweed Amb a 1 and the antigen of common wasp Ves v 5. The major component Bet v 1 of birch pollen induced cross-sensitization in only one case, similar to American house dust mite and Malassezia yeast *(Table 4)*.

Table 4

Most frequent molecular components underlying cross-sensitization in the two sensitized groups

Monosensitized (n = 52)			
Allergen source	**Molecular allergen**	**Protein family**	**Number of cases (n =)**
Seasonal inhalation allergens			
Ragweed	Amb a 1	Pectate lyase	3
Birch tree	Bet v 1	PR-10	1
Perennial inhalation allergens			
American house dust mite	Der f 2	NPC2 family	1
Malassezia sympodialis	Mala s 11	Mn superoxide dismutase	1

Insect venom			
Common wasp	Ves v 5	Antigen 5	3

Polysensitized (n = 107)			
Allergen source	**Molecular allergen**	**Protein family**	**Number of cases (n=)**
Seasonal inhalation allergens			
Timothy grass	Phl p 1	A Beta-expansin	28
Ragweed	Amb a 1	Pectate lyase	22
Birch tree	Bet v 1	PR-10	16
Timothy grass	Phl p 12	Profilin	12
Common mugwort	Art v 1	Plant defensin	5
Timothy grass	Phl p 5.0101	Grass group 5/6	4
Olive	Ole e 1	Ole e 1- family	3
Perennial inhalation allergens			
American/European domestic dust mite	Der p 2/Der f 2	NPC2 family	28
American/European domestic dust mite	Der p 1/ Der f 1	Cysteine protease	9
Cat	Fel d 4	Lipocalin	3
European domestic dust mite	Der p 10	Tropomyosin	3
Cat	Fel d 1	Uteroglobin	3
German cockroach	Bla g 9	Arginine kinase	3
Insect venom			
Common wasp	Ves v 5	Antigen 5	6

In the polysensitized group, only 31 (28.97%) were found to be cosensitized, while 76 [71.03%, $p < 0.0001$, Cramer *V*: 0,1224 (CI 0,0558–0,2642)] were also found to be cross- sensitized. Only the most common cross- allergenic components are shown in *Table 4*. The most frequent major components causing cross-sensitization were found to be β- expansin (Phl p1) of Timothy grass pollen and NPC2 proteins (Der p2/ Der f 2) of dust mites.

The main allergenic component of ragweed (Amb a 1) was responsible for cross-sensitization in 22 cases, the main component of birch pollen Bet v 1 in 16 cases and the profilin component of Timothy grass Phl p 12 in 12 cases. Among the perennial allergens, lipocalin and uteroglobin proteins of feline outer coat origin were also cross-sensitising components in the population.

DISCUSSION

The MeDALL international study on the mechanisms underlying the development of allergy found that the presence of allergen specific IgE is important for the development of allergic disease, with many cases of IgE sensitization occurring without symptoms. [2] In 2018, a new multiplex-based molecular diagnostic system, the MADX ALEX2 Allergy Explorer, was introduced as a laboratory diagnostic tool for allergy. It has made available at the University of Miskolc the possibility to perform component-based allergy testing among the inhabitants of the region.

Specific IgE antibodies to a molecular component were detected in almost 70% of the subjects. The serum t-IgE level was significantly higher in sensitized individuals ($p < 0.0001$). 22.7% of the subjects showed positivity to only one molecular component, they were monosensitized. Our results agree with the study of Kadocsa and Juhász published in 2000. They investigated the changes in the allergen spectrum of hay fever sufferers in the Southern Great Plain among young adults between 1990 and 1998 using the Prick Skin Test. In their study, 17.3% of the subjects were monosensitized, a value that did not change significantly over the 9-year study period. [10] The higher value we found can be partly explained by the more sensitive diagnostic method and the increasing number of allergic patients.

Clinical studies have demonstrated that a small proportion of symptomatic individuals are monosensitized, while more than 70% are polysensitized, even when cross-reactivity between allergens and panallergens is considered. [11] The proportion of polysensitized individuals increases with age. [12] In our case, the study population consisted of adults of working age and polysensitization was confirmed in nearly 46.72% of the subjects by multiplex testing. Despite the female predominance in the study population, the sex ratio was balanced in this group. Serum t-IgE levels showed an increase depending on the degree of sensitization, being significantly higher in the polysensitized group. However, this group also had the highest number of cases with t-IgE levels above 100 kU/L. Based on the MeDALL study, mono- and polysensitized individuals differ in their IgE immune response, suggesting a dichotomy of low and high IgE responders. Monosensitized individuals have lower serum t-IgE and in many cases allergen specific IgE levels. [2–3] In our study, a moderate positive correlation between polysensitization and serum t-IgE levels was found by correlation analysis, which is in agreement with the literature. [11, 13] The trend of association between IgE sensitization levels and the risk of allergic symptoms is general, however, IgE thresholds are far from absolute. [14]

In the case of polysensitization, we should expect simultaneous immune responses to several allergenic components and a high rate of positivity. It is important to identify the marker components, the main allergen, cross-sensitization and cosensitization for accurate diagnosis and appropriate treatment.

In Hungary, studies have been carried out since the 1970s, mostly to identify the allergenic components underlying hay fever. Most of the studies were conducted among children and in a defined geographical area (Southern Great Plain, Somogy County, Budapest). These studies were most often performed by questionnaire and Prick Skin Test, rarely by specific IgE determination. [15–16] Without exception, the studies concluded that the most common seasonal inhaled allergens in our country belong to the group of weeds, grasses and tree pollens.

Mezei and colleagues found weeds (64.8%), including ragweed (59.0%), to be the most common seasonal inhalant allergen among children with rhinitis in Budapest. Grass pollen allergy was confirmed in 67.6% of patients, and wood pollen allergy was also detected in 7.6%. They found that weed and grass pollen allergy were of equal importance in the population, whereas the allergenicity of wood pollen had clinically minor importance. [15] In 1997, Balogh and colleagues identified the allergens underlying the symptoms of rhinitis in 105 adults in Budapest. Again, the most common allergen was found to be ragweed (70%), followed by grass (50%) and mugwort (45%) pollen. [17] Based on allergological studies in Debrecen in 2005, Sipka and colleagues also ranked ragweed, lawn grass and mugwort in the top three of the most common seasonal inhalant allergens in Hungary. [16] Kadocsa and colleagues in their study covering a 9-year period found that ragweed, grasses and early tree pollen were the most common causes of hay fever symptoms in young adult hay fever sufferers. [10]

The most common seasonal inhalant allergen component in both the mono- and poly- sensitized groups we studied was ragweed Amb a 1. In the monosensitized group, molecular allergens of trees and grasses were equally prevalent, whereas in the polysensitized group, grass pollens (Phl p 1, Lol p1, Cyn d 1) were more predominant. Among the polysensitized, the pollen of Japanese cypress (pectate lyase protein), which is not a native flora constituent in our country, stands out in the tree group. In this case, the cross-sensitizing effect of the main component of ragweed is suggested. In addition, the sensitising effect of birch, beech and hazelnut can be highlighted.

There is no consensus among national studies on the order of prevalence of perennial inhalant allergens. According to Gállfy, the prevalence of house dust mite allergy has varied over the years of the study, while mould positivity has shown an upward trend. [18] Balogh et al. reported a 40% prevalence of dog and cat hair hypersensitivity among Budapest residents, while house dust mite showed a 30% prevalence in the population. [17] In a study published in 2005 concluded that, the prevalence of mould allergies showed an increase compared to the previous decade, while the prevalence of house dust mite allergy did not change significantly, although its prevalence is significant everywhere in Hungary. [19] In our study, we found equal levels of yeast and house dust mite sensitization in monosensitized

individuals. However, in the polysensitized individuals, sensitization to house dust mite and food mite, and sensitization to cat hair were prominent.

Cross-reactivity is a common phenomenon, especially among those sensitized to pollen, which can lead to misdiagnosis and inappropriate immunotherapy. [20] The polysensitized group had a markedly higher incidence of cross- sensitization. The major allergenic component of Timothy grass (Phl p 1) and proteins of dust mites (Der f 2, Der p 2) were confirmed as the most common components. Cross-sensitization of ragweed is significant in both groups. In total, the Amb a 1 (pectate lyase protein) component of ragweed caused cross- sensitization to fruit (e.g. banana, melon) in 25 individuals. This invasive weed species appeared in Hungary in the 1920s. Due to its rapid spread, the Carpathian Basin is now the most contaminated region in Europe. In a representative questionnaire survey carried out in autumn 2013, Márk and colleagues found that 22% of adult respondents suffer from hay fever symptoms during the period of ragweed flowering. The proportion of people with ragweed allergy in Borsod-Abaúj-Zemplén County was 13% at the time of the survey. Their study also showed that 29% of people with ragweed allergies also had other allergies.[21] In our study, the most common cross- sensitizing allergen of the tree group was the major birch pollen component (Bet v 1 – PR10 protein family), which caused cross-sensitization mostly to hazelnut, apple and strawberry. Nearly 70% of people with birch pollen allergy experience the "birch-fruit-vegetable syndrome" mainly when eating Rosacea fruits (apples, cherries, peaches, pears), nuts (hazelnuts) and vegetables of the Apiacea family (carrots, celery). [5]

Some potential limitations of the study should be considered when interpreting the results. The study is not a large population study. The recruitment of individuals was based on voluntary enrolment, resulting in a predominance of female sex. Allergen testing was not preceded by a specialist examination and Prick Skin Test. Blood samples were not taken at the same period of year. In addition to the hundreds of allergenic components, the system does not provide the detection of allergens related to drugs, bird feathers, metals, contrast agents. Due to their absence, marker identification is incomplete.

CONCLUSION

The present cross-sectional study is the first allergen study based on multiplex microarray technology in Hungary, which found a correlation between the degree of sensitization and the amount of total IgE in serum. It revealed the prevalence of different allergens among people living in the northeastern Hungarian region and identified the allergen components most frequently responsible for cross-sensitization. The described technology will greatly facilitate and support specialist decision- making and the determination of targeted immunotherapy. In the future, it is proposed to introduce component-based diagnostics into precision medicine approaches in allergy, as it provides individual molecular data for better phenotyping and selection of personalized treatments.

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