

PRACA ORYGINALNA
ORIGINAL ARTICLE**ALLERGEN COMPONENT TESTING – A NEW ERA IN DIAGNOSTICS OF PATIENTS WITH POLLEN ALLERGY****BADANIE ELEMENTÓW SKŁADOWYCH ALERGENÓW – NOWA ERA W DIAGNOSTYCE PACJENTÓW Z ALERGIĄ PYŁKOWĄ****Svitlana Zubchenko¹, Olena Sharikadze², Stepan Maruniak²**¹DANYLO HALYTSKY LVIV NATIONAL MEDICAL UNIVERSITY, LVIV, UKRAINE²SHUPYK NATIONAL MEDICAL ACADEMY OF POSTGRADUATED EDUCATION, KYIV, UKRAINE**ABSTRACT**

Introduction: Pollen allergy, also called hay fever or pollinosis, is referred to the most widespread allergic diseases. According to different sources, from 5 to 34% of the population in developed countries are likely to suffer from this condition. Moreover, the majority of patients are young people of working age

The aim: To demonstrate a practical decision of the choice of effective treatment tactics based on component testing in patients with pollen allergy.

Materials and methods: 126 patients with allergic rhinitis / conjunctivitis were randomly selected according to their primary visit during the first week in August of the current year. Among them, 53 (42.0%) female and 73 (58.0%) male, aged 22–47 years. General laboratory and instrumental investigations, skin prick test with allergen extracts (Diater, Spain), measurement of total serum and specific IgE by immunoenzymatic assay method using test system “Euroimmun” were performed. Immunofluorescent method ImmunoCap (Thermo Scientific, Uppsala, Swiss) was used to detect specific components of allergens. The material of investigation was blood serum.

Results: According to the results of skin prick-test, 50% of patients could receive allergen immunotherapy with two different extracts of allergens “Mixture of Weeds” and “Mixture of Grass”. On molecular investigations, it was detected that this combination was not suitable for any patient (20% of individuals had genuine sensitization to allergens of grass pollen, including a marker of cross-reactive molecules, 30% of individuals were sensitized with genuine allergens of *Artemisia* and/or *ambrosia*).

Conclusions: Based on skin prick-test and molecular diagnostics, the doctor makes a completely different decision on the choice of extracts for allergen immunotherapy conduction. Component determination of a sensitized profile and high sensitivity of this method enables to reveal a genuine protein, which is the primary cause of allergy occurrence and administer etiotropic allergen-specific immunotherapy with the allergen, to which sensitization was detected.

KEY WORDS: component diagnostics, pollen allergy, allergen immunotherapy

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INTRODUCTION

It has been over 20 years since the moment of the first concept of allergy diagnostics based on allergen components or component diagnostics (component-resolved diagnostics, CRD) [1]. Since then, the volume of knowledge in this area is constantly increasing, and the quality is improving. Allergy source is identified at a component level. Components of an allergen are proteins, which by their structure are very similar (homologous) and belong to the same protein family, though they have a completely different source. Implementation of component diagnostics into practice enables practical doctors to prognosticate possibility of occurrence of allergy symptoms, cross reactivity, determine the risks of acute conditions, to choose optimal eliminating measures, in particular diet in case of food allergy manifestations, as well as increase the efficacy of allergen immunotherapy (AIT) [2]. The Consensus on molecular diagnostics WAO-ARIA-GALEN, 2013 is the basic document to rely on during conduction of component investigations [3]. One of the evident examples, dictating necessity of conducting component investigations is allergy to plant pollen.

Pollen allergy, also called hay fever or pollinosis, is referred to the most widespread allergic diseases. According to different sources, from 5 to 34% of the population in developed countries are likely to suffer from this condition. Moreover, the majority of patients are young people of working age [4]. Thus, treatment of pollen allergy is among urgent problems of contemporary allergology. This type of allergopathy is referred to reactions of type I hypersensitivity, caused by the synthesis of specific immunoglobulins class E in the body as a response to penetration of allergen molecules, which compose pollen of wind-pollinated plants. Since pollen usually lands on the mucosa of the eyes and airways, pollen allergy, as a rule, is manifested by conjunctivitis, rhinitis, sometimes in combination with the inflammation of Eustachian tubes – eustachitis, and allergic bronchial asthma. Much rarer, pollen allergens affect the skin (urticaria, Quincke's edema, atopic dermatitis), genitourinary system (allergic vulvitis, vulvovaginitis and very rarely – cystitis and nephritis), gastrointestinal tract (gastritis, colitis with diarrhea, vomiting, other signs of digestive disorders) etc.

Worldwide, there are over 60 species of plants, pollen of which can sensitize the body. Throughout a year, three periods/seasons of exacerbations of allergic manifestations can be identified in the individuals, sensitized with pollen allergens. The first period – spring – is associated with blooming of wind-pollinated trees, among which are alder, hazel, willow, elm, aspen, poplar, birch. In the central regions, these trees blossom approximately from the second half of March to early May. Coniferous trees blossom almost at the same time. The second period – summer – is associated with blooming of meadow grasses and some weeds, for instance, dandelion, ambrosia, ryegrass, foxtail, fescue, timothy grass, saltwort, wheat-grass etc. These plants start blooming from the first week of June and to early July. The third period of pollinosis exacerbation is autumn, which is associated with weed blooming (mugwort, golden rod, orach etc.). It starts in the first weeks of September and lasts till late autumn. However, it should be remembered that pollination period of these plants can differ depending on a region, annual changes of climatic conditions, ecological problem etc. Most often, overlapping of pollination seasons is observed. Thus, diagnostics of a causative sensitizer based solely on calendars of pollination of wind-pollinated plants is relative. Another important fact is that due to a tiny size, allergenic pollen can be transmitted with wind for long distances and provoke sensitization in the regions where certain plant does not grow. Besides, according to scientific literature and our monitoring, patients with mono- or oligosensitization are quite rare, especially among adults, in whom polysensitization results in significant diagnostic difficulties [5].

Thus, component diagnostics for patients with pollen allergy is necessary, at the same time – structural approach is necessary during interpretation of the results of molecular investigation [6].

THE AIM

Thus, the aim of our research was to demonstrate a practical decision concerning the choice of effective treatment tactics based on component investigations in patients with pollen allergy.

MATERIALS AND METHODS

The investigation involved 126 the individuals, who lived in Lviv region, with allergic seasonal rhinitis / conjunctivitis, randomly selected after their first visit in the first week of August in the current year. Among them, 53 (42.0%) female and 73 (58.0%) male, aged 22–47 years. The patients sought consultations of doctors-allergists in Lviv regional center of clinical immunology and allergology. After being informed, the patients consented. The diagnosis of allergy to pollen was based on case history, which indicated a seasonal peculiarity of clinical allergic signs and data of skin prick-tests with a standard panel, which contained extracts of allergenic pollen from local sources of allergens, including a mixture of weeds (*Ambrosia artemisiifolia*, *Artemisia*,

Atriplex cana, sunflower), mixture of grasses (*Bromus*, wheat-grass, rye, timothy grass), as well as extracts of *Artemisia*, ambrosia, timothy grass (“Immunolog”, Ukraine). Physiological solution and 0.1% histamine solution were used as a negative and positive control, respectively. The results of skin prick-tests were estimated in 15 minutes according to European standards.

Total serum IgE was measured by enzyme immunoassay technique using test-system “Euroimmune” according to instructions of the manufacturer. Immuno CAP (Thermo Scientific, Uppsala, Swiss) was used for measuring the levels of specific antibodies IgE class, following instructions of the manufacturer. Five marker allergens of genuine sensitization to pollen of *Artemisia* (nArt v 1, rArt v 3), ambrosia (rAmb a 1) and grasses (rPhl p 1, rPhl p 5) were identified. Besides, two cross-reactive molecules of profilin rPhl p 12 and polcalcin rPhl p 7, which are responsible for cross reactions between pollens of different sources (including grasses and weeds), were determined [13]. Levels sIgE > 0.35 KU/l were considered positive.

The research was conducted following the 7th review of the principles of Helsinki declaration on human rights (2013) (protocol of ethical commission № 6 20.06.2016 from Danylo Halytsky Lviv National Medical University).

RESULTS AND DISCUSSION

By the data of prick-tests, positive results of prick-testing to a mixture of weeds, *Artemisia*, ambrosia and a mixture of grass were found in 50% of patients indicating co-sensitization to various sources of allergens. Monosensitization to allergens of weed pollen was revealed in 30% of patients, and monosensitization to pollen components of grasses was detected in 20% of individuals. It should be mentioned that prick-test to an extract of weed mixture was negative in 40% of patients with positive skin reaction to pollen extracts of *Artemisia* and ambrosia. Negative results of skin reaction to an extract of grass mixture were revealed in 10% of patients with high response (+++++) to pollen components of timothy grass.

Based on serological measuring of IgE to genuine components of *Artemisia* (Art v 1, Art v 3), ambrosia (Amb a 1) and timothy grass (Phl p 1, Phl p 5), the results obtained in these patients differed from the data of skin tests. Thus, simultaneous sensitization to pollen allergens of *Artemisia*, ambrosia and timothy grass, which was detected in 50% of patients in skin tests, was not confirmed at molecular level. Instead, sensitization to *Artemisia* and ambrosia was found in 20% of individuals, monosensitization to *Artemisia* – in 30%, and monosensitization to allergens of ambrosia pollen – in 20%. In the majority (70%) of patients with monosensitization to allergens of weed pollen, specific IgE to Art v 1 and/or Art v 3, and/or Amb a 1 were identified.

The results of our component investigation have regional peculiarities, which, first of all, depend on climatic and geographic conditions of a region. By our long-term monitoring, sensitization to *Artemisia*, which is common on the territory of Ukraine, is most often observed in

patients sensitive to weed pollen. Due to geographic location of Western Ukraine, prevalence of sensitization to this weed is similar to a number of European countries. In particular, ambrosia pollen is a significant cause of allergy among residents of Germany, where sensitization to it constituted 23% (76, 77). In reports from Italy, prevalence of sensitization to ambrosia is increasing annually and constitutes approximately 17.08% (78, 79). Prevalence of sensitization to this pollen also increased in France (82). Particularly, in the north of France, ambrosia pollen is considered the third most common cause of pollinosis in children after grasses and platan, and the percentage of sensitized individuals reaches 21% (83). Concerning Poland, which borders on Western Ukraine, the research showed that sensitivity to ambrosia constituted 82.9% (91). In other investigation, conducted in Poland, sensitization to ambrosia was observed in 42% of individuals; in 71% of them, clinical symptoms appeared only after 20 years of age.

Thus, it has been established that the doctor makes completely different decisions on the choice of extracts for conduction of allergen-specific immunotherapy based on skin prick-test and molecular diagnostics. By the results of skin prick-test, 50% of patients could receive AIT with two different extracts of allergens “Mixture of weeds” and “Mixture of grasses”. However, based on molecular investigations, it was found out that such combination was suitable for none of the patients (20% of individuals suffered from genuine sensitization to allergens of grass pollen, including a marker of cross-reactive molecules, 30% were sensitive to genuine allergens of *Artemisia* and/or ambrosia). Since diagnostics based on skin prick-tests was less accurate than conducted molecular investigations for identification of exact sources of allergens, optimal choice of AIT tactics for such patients was elaborated based on molecular investigations. The tactics was as follows:

1. Patients with detected components of grass – Phl p 1/ Phl p 5 (+), Phl p 7/ Phl p 12 (±) were recommended AIT with an extract of grass pollen;
2. Patients with detected components of ambrosia – Amb a 1 (+), Art v 1/Art v 3 (-) were recommended AIT with an extract of ambrosia pollen;
3. Patients with detected major components of ambrosia pollen – Amb a 1 (+) and *Artemisia* pollen – Art v 1/ Art v 3 (+) were recommended AIT with extracts of ambrosia and *Artemisia* pollen;
4. Patients with detected components of *Artemisia* – Amb a 1 (-), Art v 1/Art v 3 (+) were suggested AIT with an extract of *Artemisia* pollen;
5. Patients with detected only minor components of grasses – Phl p 1/ Phl p 12 (+) were not recommended an extract of grass pollen.

Exact identification of causative sensitizers in patients with mono- or oligosensitization to pollen, without overlapping of pollination seasons, is achieved by means of traditional diagnostics using whole extracts of allergens. In most cases, patients are sensitized to major components of pollen (for example, Ole e 1, Bet v 1, Phl p 1, Phl p 5). However, this rule does not work in the areas with a high allergenic load, for

example, in case of olive pollen in Mediterranean countries, where Ole e 1 and Ole e 9 are usually considered minor allergens and can be the main sensitizers [7]. Thus, administering AIT in the areas with a high degree of sensitization to “minor allergens”, molecular diagnostics can be very important, since commercial extracts for immunotherapy are standardized only for major allergens. Thus, patients, sensitized only to minor allergens, basically, will not be able to receive a sufficient amount of allergen for successful result of immunotherapy with the allergen. Even worse consequences are possible – side effects can occur in patients in case of high concentration of these minor allergens in the extract [8].

In recent clinical investigations, it has been shown that AIT with grass pollen extract with one allergen is safe and effective both in poly- and monosensitized patients [9]. It means that the allergen extract, used for AIT, is suitable for highly sensitized patients. CRD helps to obtain information about a patient's sensitization to choose an extract for AIT, which supports the concept of “therapy based on allergen components”. Administration of AIT will be expedient if sensitization to species-specific allergens is confirmed, while in selective recognition of cross-reactive allergens, in particular, profilins or CCD – administration of CCD is doubtful [10]. Cross-reactive allergens are considered to have a limited clinical value and their qualitative amount in extracts is usually not estimated. In case of sensitization to “raw” extract (skin prick-tests and/or positive test for specific IgE), AIT administration is controversial if response to all components is negative, since extracts, most likely, will not contain sensitizing molecules.

Proving the importance of immunotherapy administration based on component diagnostics in three recent prospective investigations of adults and children it was shown that the results with the use of component investigations modified previously administered AIT in approximately half of patients [11, 12].

The data obtained by us confirm the results of our scientific literature on the correct choice of the extract for AIT on the basis of molecular research. Thus, according to the results of SPT – 50% of patients in the study group could receive AIT with two different extracts of allergens “Mixture of weeds” and “Mixture of grasses”. However, according to molecular studies, none of these patients had this combination (20% had true sensitization to grass pollen allergens, including cross-reactive molecule markers, 30% were sensitive to genuine allergens of *artemisia* and/or ambrosia). Thus, in half of patients the correct choice of drug for AIT with the prediction of its high effectiveness was based on molecular studies.

CONCLUSIONS

1. Patients with pollen allergy, especially those polysensitized, require mandatory conduction of component allergy diagnostics for proper choice of treatment tactics, correction of therapy at the stage of patient monitoring and estimation of its efficacy.
2. Component identification of a sensitizing profile according to Consensus on molecular allergy diagnostics and high sensitivity of this method enables to detect a

genuine protein, which is a primary cause of allergy occurrence and administer etiotropic allergen-specific immunotherapy with the allergen, to which sensitization was found out.

3. In the treatment of patients, it is important to use allergens standardized by activity and controlled for the presence of major components, which enables to achieve a maximally high effect of treatment.

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According to the order of the Authorship.

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The Authors declare no conflict of interest

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