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Molecular Approach to Optimal Choice of Specific Immunotherapy for Patients with Sensitization to Weed Pollen Allergens

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Abstract
Introduction: *The problem of pollen allergy, particularly pollen of weeds is urgent for the population of Western Ukraine, including Lviv region.*
Aim: *To compare possibility of SPT and component diagnostics for the selection of appropriate specific immunotherapy.*
Materials and Methods: *Forty eight patients of both sexes, aged 23.0±2.7 years, residents of Lviv region with seasonal allergic rhinitis / conjunctivitis, were selected according to primary stay in the first week of August of the current year. SPT to extracts of pollen allergens from local sources was performed, including a mixture of weeds, grasses and extracts of mugwort, ragweed, timothy ("Immunologist", Ukraine). Immuno CAP (Thermo Scientific, Uppsala, Sweden) was used for molecular investigations of sIgE.*
Results: *Positive SPT to weed mixture, extracts of ambrosia, ragweed and grass mixtures was found in 50% of patients. This indicated co-sensitization to various sources of allergens; 29.2% of patients had monosensitization to weed pollen, and 20.8% – monosensitization to grass pollen. However, simultaneous sensitization to pollen of mugwort, ragweed and timothy was not proven by molecular investigations. Instead, it was found that 20.8% of patients had sensitization to ragweed and mugwort, 29.2% of individuals – monosensitization to ragweed, and 20.8% – monosensitization to mugwort. Most (70.8%) patients with monosensitization to weed pollen had specific IgE to Art v1 and/or Art v3, and / or Amb a1. False positive*

results of SPT indicated that co-sensitization to grasses and weeds can be explained by the presence of sIgE for cross-reactive markers of profilin Phl p 12 and polcalcin – Phl p 7.
Conclusion: *Based on SPT and molecular investigations the doctor makes a fundamentally different decision on the selection of extracts for specific allergen immunotherapy. Optimal allergic immunotherapy is based on identification of primary sensitizer and cross-reactivity markers.*
Keywords: *weeds, component diagnostics, primary sensitizer, cross-reactivity, allergen immunotherapy.*

Introduction

The problem of pollen allergy, particularly pollen of weeds is urgent for the population of Western Ukraine, including Lviv region.

Weeds are undesirable plants without any economic or aesthetic value. A large number of them spread uncontrollably, depriving cultivated plants of space, light and nutrients. Thus, the term "weed" does not belong to any taxonomic group of plants[(1)]. Moreover, due to growth where they are not needed, weeds can pose a threat to human health, both as a secondary consequence - through the use of herbicides to fight them, and directly - as a source of allergens[(2)]. Therefore, the weeds are the main "victims" of human control of the environment, as they are traditionally eradicated (if possible), and in some cases used as ornamental plantings and medicinal plants [(3)].

Weed pollen, which is responsible for allergic reactions, belongs to several botanical families. Clinically important pollen allergens are found in *Ambrosia, Artemisia, Parietaria, Chenopodium album, Kali turgida and Plantago*. These can produce up to a million pollen grains per day, which easily spread depending on weather conditions[(4)]. The "allergic season" for weed pollen usually occurs later than for trees and grasses: as a rule, from midsummer to late autumn [(5)].

Currently, 35 weed allergens have been identified which may play an important role in the development of allergic rhinitis, pollen allergy, asthma and food allergy (www.allergen.org)[(6)]. It is interesting to note, that main pollen allergens are confined to four main families of proteins: pectate lyases, defensin-like proteins, Ole e 1-like proteins, and a group of nonspecific lipid transfer proteins (nsLTP). In addition to major allergens, profilin and polcalcin panalergens, responsible for the development of cross-reactivity in pollen-sensitized patients, are also found in the pollen of weeds [(7)].

Determination of the major component of weed pollen is the key for choosing a more accurate personified allergen-specific immunotherapy (ACIT), as well as for the monitoring of vaccination efficacy and improvement of the manufacture of new vaccines.

Aim

To compare the possibilities of skin prick tests and diagnosis based on the definition of IgE to individual allergenic components and evaluate the effectiveness of the selected allergy-immunotherapy (AIT).

Materials and Methods

The research was conducted in 2014-2015 years in Lviv Regional Medical Center of Clinical Immunology and Allergy. The study involved 48 patients of both sexes, aged 23.0 ± 2.7 years, residents of Lviv region with seasonal allergic rhinitis/conjunctivitis, randomly selected according to their initial visit during the first week of August of the current year, among them 56.25% – women and 43.75% – men. During the period of clinical remission, the skin prick tests (SPT) were performed to allergenic pollen extracts from local sources of allergens, including mixtures of weeds, herbs, and pollen of mugwort, ragweed, timothy ("Immunologist", Ukraine). Negative and positive controls (1% histamine solution) were also produced by "Immunologist", Ukraine. SPT results were evaluated in 15 minutes in accordance with European requirements[(8)]. For the detection of species-specific allergen components, immunofluorescence method Immuno CAP ("Phadia AB", Sweden) was used. The material of the study was blood serum.

The effectiveness of AIT was evaluated using a 5-point visual analogue scale (VAS), Huskisson (up): before treatment; after 1 year of AIT; after 2 years of therapy[9].

The study was conducted according to the 7th revision of the principles of the Helsinki Declaration of Human Rights (2013). Patients gave an informed consent.

Results

The diagnosis of allergy to pollen was based on the disease history, which indicated the seasonal nature of clinical allergic manifestations. All patients complained of difficulty breathing through the nose, rhinorrhea, itching and swelling in the nasal cavity, and sneezing. Among them, the nasal symptoms were combined with conjunctivitis in 66.7% of individuals, and 6.25% of patients complained of breathlessness. Most patients clearly noticed worsening of clinical symptoms in late June and relief at the end of September to the beginning of October. However, year-round symptoms were observed in 6.25%, with characteristic peak in summer. One patient complained of discomfort in the oral cavity (swelling or numbness of the tongue, lips, itching of the palate, etc.) after consuming raw peaches; 33.3% of patients had an aggravating family history and in most cases (68.75%) it was on the mother's side. Rhinocytogram data confirmed the allergic nature of rhinorrhea: the presence of eosinophils> 10% – in 81.25% of individuals.

The next stage of the research was the conduction of SPT to allergenic pollen extracts from local sources of allergens. There were 50% of patients with a positive (> 3mm) result to the extracts of a mixture of weeds, mugwort, ragweed and a mixture of grasses, which indicated co-sensitization to various sources of allergens. In the remaining 29.2% of patients, monosensitization to weed pollen was detected, and in 20.8% – monosensitization to grass pollen.

It should be mentioned that in 39.6% of patients with a positive skin reaction to pollen extract of mugwort and ragweed, prick test to weed mixture extract was negative. In 10.4% of patients with high reaction (++++) to timothy pollen, negative results of skin reaction to grasses mixture extract were detected.

Based on the serological identification of IgE to true components of *Artemisia* (Artv 1, Artv 3), *Ambrosia* (Amb a 1) and *Phlenum* (Phl p 1, Phl p 5), the results in the same patients were different from their skin tests. Thus, the simultaneous sensitization to pollen of mugwort, ragweed and timothy, which was detected in 50% of skin tests, was not confirmed at the molecular level. Instead, sensitization to mugwort, ragweed was detected in 20.8% of individuals, in 29.2% of persons – monosensitization to mugwort, and in 20.8% – monosensitization to ragweed. In the majority (70.8%) of patients with monosensitization to weed pollen, specific IgE to Art v 1 and / or Art v 3 and / or Amb a 1 were identified. False positive results of skin prick tests that indicated the co-sensitization to grass and weeds can be explained by the presence of sIgE to cross-reactive markers of profilin Phl p 12 and polcalcin – Phl 7.

Thus, based on skin prick tests and molecular diagnostics, the doctor makes a fundamentally different decision on the selection of extracts for the specific allergy-immunotherapy. According to SPT, 50% of patients could receive AIT with two different extracts of allergens "Weed mixture" and "Mixture of grasses". However, based on molecular studies, it was found that such combination was not appropriate for any patients (20.8% of individuals had genuine sensitization to grass pollen, including the marker of cross-reactive molecules, 29.2% were sensitized to true allergens of mugwort/ragweed).

Since the SPT-based diagnosis was not precise enough as compared with the molecular studies to identify the true source of allergens, the choice of AIT tactics was based only on component research. Therefore, patients were recommended the following AIT with sublingual administration:

- 1 group - 29.2% of individuals - extract of a mixture of meadow grasses (Diater, Spain);

- 2 group - 20.8% of individuals - ragweed pollen extract (Diater, Spain);
- 3 group - 20.8% of individuals - Phase-treatment – pollen mixture extract of mugwort, and during the second year of treatment - addition of a mixture of ragweed pollen (Diater, Spain);
- 4 group - 29.2% of individuals - mugwort pollen extract (Diater, Spain).

According to international recommendations, identification of specific therapy efficacy was performed using a 5-point Visual Analogue Scale (VAS): comparing the results prior AIT, after the first and second years of therapy (Table 1).

As the results of the research showed: patients noticed clinical symptoms typical for this pathology before the beginning of treatment. On the background of conducted AIT, a significant decrease in the severity of symptoms in all study groups was revealed ($p < 0.05$). In particular, after one year of treatment, a positive effect was observed in 69.8% of individuals: in the first group – 71.4%, in the second – 70.0%, in the fourth – 78.5% of persons. It is logical that in patients of the 3rd group with a true sensitization to mugwort and ragweed only a tendency (59.6%) to improvement of clinical symptoms was observed. From the listed symptoms, nasal congestion lasted the longest. Also in patients with year-round symptoms (polysensitized), clinical manifestations persisted due to co-sensitization to allergens of pets and home dust mites. These patients were offered elimination measures and hygiene of everyday life, as well as AIT, particularly with the extract "Mixture of home dust mites" during the second year of treatment.

After 2 years of treatment a positive clinical effect was observed in 88.5% of patients, without significant difference in the 1st, 2nd and 4th groups: 85.7% – 1st group, 90.0% – 2nd group, 92.7% – 4th group. Concerning the patients of the 3rd group, a significant (85.5%) improvement of the condition ($p < 0.05$) was observed when AIT with ragweed pollen extract was added. All patients noticed a significant improvement in life quality. Therefore, all individuals were encouraged to continue therapy to consolidate a positive effect of AIT.

Table 1 Estimation of allergen immunotherapy efficacy by VAS scale

Sign		Difficult nasal breathing	Rhinorrhea	Sneezing	Swelling	Itching in the nasal cavity (palate)	Life quality
1 group (n=14)	Before treatment	4.21±0.36	4.76±0.27	3.24±0.16	2.45±0.17	1.65±0.09	3.35±0.08
	After 1 year of treatment	2.34±0.17*	2.12±0.11*	0.65±0.03*	1.87±0.06*	1.45±0.17	1.15±0.08
	After 2 years of treatment	0.94±0.01*^	0.85±0.02*^	0.3±0.01*^	0.45±0.05*^	0.95±0.04*^	0.4±0.01*^
2 group (n=10)	Before treatment	4.28±0.91	4.80±1.01	3.22±0.07	2.52±0.06	1.65±0.20	3.45±0.09
	After 1 year of treatment	1.45±0.09*	1.06±0.08*	0.25±0.02*	0.32±0.03*	1.30±0.11	1.35±0.08*
	After 2 years of treatment	0.55±0.02*^	0.68±0.03*^	0.10±0.01*^	0.30±0.04*	0.87±0.08*^	0.20±0.01*^
3 group (n=10)	Before treatment	4.45±0.32	4.56±0.23	3.04±0.18	2.17±0.11	1.67±0.13	3.47±0.06
	After 1 year of treatment	2.83±0.15	2.93±0.13	1.68±0.04	1.82±0.08	1.42±0.27	1.35±0.07
	After 2 years of treatment	0.89±0.02*^	0.78±0.03*^	0.42±0.04*^	0.48±0.04*^	0.87±0.06*^	0.47±0.02*^
4 group (n=14)	Before treatment	4.68±0.96	4.76±1.21	3.28±0.07	2.72±0.16	1.84±0.40	3.75±0.19
	After 1 year of treatment	2.05±0.19*	1.36±0.05*	0.45±0.03*	1.35±0.03*	1.38±0.15	1.69±0.12*
	After 2 years of treatment	0.75±0.02*^	0.48±0.04*^	0.16±0.01*^	0.38±0.03*	0.82±0.06*^	0.28±0.02*^

*Note** -$p<0.05$ – comparison with the group before treatment
^ - $p<0.05$ – comparison between groups of the 1st and 2nd years of treatment

Conclusions

1) Based on skin prick tests and molecular diagnosis, the doctor makes a fundamenta-lly different decision on the choice of extracts for specific allergy-immuno-therapy.
2) The choice of optimal allergen immuno-therapy is based on identification of a primary sensitizer and cross-reactivity markers.
3) AIT with sublingual vaccines - mixture of allergens of meadow grass, ragweed, magwort (Diater, Spain) in patients with pollen allergy demonstrates safety and high efficacy.

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