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## DETERMINATION OF CO-SENSITIZATION IS AN IMPORTANT STEP IN IMPROVING THE EFFECTIVENESS OF ALLERGEN-SPECIFIC IMMUNOTHERAPY IN PATIENTS WITH POLLEN DISEASE

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**Key words:** pollen disease, allergic rhinitis, sensitization, molecular allergodiagnosics, allergen-specific immunotherapy  
**Ключові слова:** поліноз, алергічний риніт, сенсibiliзація, молекулярна алергодіагностика, алерген-специфічна імунотерапія

**Ключевые слова:** поллиноз, аллергический ринит, сенсibiliзация, молекулярная алергодіагностика, алерген-специфічная імунотерапія

**Abstract. Determination of co-sensitization is an important step in improving the effectiveness of allergen-specific immunotherapy in patients with pollen disease. Dityatkovska E.M., Biletska S.V.** In order to increase the effectiveness of allergen-specific immunotherapy in patients with pollen disease, the profile of allergen sensitization was determined at the molecular level, performed in 47 patients with clinical manifestations of seasonal rhinoconjunctivitis and perennial allergic rhinitis during a long time period. Allergic examination of patients included history taking, molecular blood tests using ALEX technology to determine the level of specific antibodies class IgE of major and minor components of pollen and household allergens and diagnostic skin tests (pre-test). Assessment of the main clinical manifestations of allergic rhinitis was performed according to the recommendations of the European Association of Allergists and Immunologists. The severity of nasal symptoms was determined by the TNSS scale, and ocular symptoms - by the TOSS scale. Integral assessment of the intensity of clinical symptoms of allergic rhinitis was calculated as the sum of scores by the main symptoms. According to the results of molecular allergy diagnostics, the profile of allergic sensitization in patients with pollen disease was determined, which established the presence of specific IgE - antibodies to major allergy components of ragweed pollen (nAmb a1) – in 91.5±4.1% of patients, wormwood (nArt v3, nArt v1) – in 40.4±7.2%, meadow thyme (rPh1 p1, rPh1 p5b) – in 17.0±5.5%, house dust mites (Der p1, Der p2) – in 29.8±6.7%. Developed on the basis of molecular allergy diagnostics, the profile of allergological sensitization to allergocomponents allows to obtain complete and detailed information on patient sensitization (diagnose a real allergy), cross-reactivity to other allergens, justify the feasibility and predict the effectiveness of allergen-specific immunotherapy.

**Реферат. Определение ко-сенсibilизации – важный этап в повышении эффективности аллерген-специфической иммунотерапии у больных поллинозом. Дитятковская Е.М., Белецкая С.В.** С целью повышения эффективности аллерген-специфической иммунотерапии у пациентов с поллинозом было проведено определение у них профиля аллергенной сенсibilизации на молекулярном уровне, которое выполнено у 47 пациентов с клиническими проявлениями сезонного риноконъюнктивита и круглогодичного аллергического ринита в течение длительного времени. Аллергическое обследование больных включало сбор анамнеза, молекулярные методы исследования крови по технологии ALEX с определением уровня специфических антител класса IgE к мажорным и минорным компонентам пыльцевых и бытовых аллергенов и диагностические кожные пробы (прик-тест). Оценка основных клинических проявлений аллергического ринита проводилась по рекомендациям Европейской ассоциации аллергологов и иммунологов. Выраженность назальных симптомов определялась по шкале TNSS, глазных симптомов – по шкале TOSS. Интегральная оценка интенсивности клинической симптоматики аллергического ринита вычислялась как сумма баллов по основным симптомам. По результатам молекулярной алергодиагностики определен профиль алергологической сенсibilизации у пациентов с поллинозом, который установил наличие специфических IgE – антител к мажорным алергокомпонентам пыльцы амброзии (nAmb a1) – у 91,5±4,1% пациентов, полыни (nArt v3, nArt v1) – у 40,4±7,2%, тимopheевки луговой (rPh1 p1, rPh1 p5b) – у 17,0±5,5%, клещей домашней пыли (Der p1, Der p2) – у 29,8±6,7%. Разработанный на основании молекулярной алергодиагностики профиль алергологической сенсibilизации к алергокомпонентам позволяет получить полную и подробную информацию о сенсibilизации пациента (диагностировать настоящую алергию), перекрестную реактивность к другим алергенам, обосновать целесообразность и прогнозировать эффективность алерген-специфической иммунотерапии.

The problem of intermittent allergic rhinitis (pollen disease) remains very relevant even in the 21st century due to the significant spread of the disease (from 10% to 40% of the population suffers from it), the severity of the clinical course, ineffective therapy, which worsens the quality of life, education, and productivity of patients and is accompanied by significant economic costs [2, 4, 6].

It is well known that the basis of allergic rhinitis (AR) is the classic IgE-dependent type of hypersensitivity to pollen aeroallergens of plant origin [9-11], as well as to household allergens (house dust mites, epidermis of domestic animals, molds) [5, 9]. House dust mites of the *Pyroglyphidae* family – *Dermatophagoides pteronyssinus* (Der p) and *Dermatophagoides farinae* (Der f) are considered to be one of the powerful allergenic triggers that contribute to the development of respiratory types of allergic reactions [7]. Moreover, in the summer-

autumn period, the concentration of mites and mold fungi also increases significantly, which leads to a strengthening of the immune response to other allergens [3].

Today, allergy diagnosis and allergen-specific immunotherapy (ASIT) are mostly based on the use of allergen extracts. At the end of the 1990s, the concept of molecular or component allergy diagnostics appeared in the world, when instead of allergen extracts it was proposed to use individual allergenic proteins (allergocomponents). The composition of an allergenic substance includes not one, but several protein structures, some of which are "major" - main (allergen molecules, antibodies to which are noted in more than 50% of patients in the population), others "minor" – secondary (prevalence less than 10%). This allows you to differentiate true and cross-allergy. In contrast to skin tests (prick testing) using a mixture of allergens, when a large number of

proteins enter the human body and multiple positive reactions to allergen extracts are observed, multicomponent molecular diagnostics (Allergy Explorer – ALEX, developed by Macro Array Diagnostics) is a highly accurate, quantitative method that allows you to measure specific IgE in a wide range of concentrations, minimize the number of false-positive results, obtain an accurate profile of patient sensitization and select individual components of ASIT accordingly [6].

In connection with the above, the aim of this study was to determine at the molecular level the profile of allergen sensitization in patients with pollen disease in order to increase the effectiveness of allergen-specific immunotherapy (ASIT).

#### MATERIALS AND METHODS OF RESEARCH

The study was conducted among 47 patients, aged from 18 to 64 years (median (IQR) – 31 (26, 43) years), with clinical manifestations of seasonal rhinoconjunctival syndrome and year-round allergic rhinitis (with less pronounced clinical symptoms) for a long time (5 (3; 20) years on average). Among them there were 26 (55.3%) men and 21 (44.7%) women. All patients underwent treatment on the basis of the consultation and diagnostic center and the allergological department of the Communal Non-Commercial Enterprise "Clinical Hospital of Emergency Medical Care" of the Dnipro City Council, Dnipro, and signed a voluntary informed consent for medical examination and treatment. The study was conducted in accordance with the principles of bioethics set forth in the Declaration of Helsinki "Ethical Principles of Medical Research Involving Human Subjects" and the "Universal Declaration of Bioethics and Human Rights (UNESCO)". The protocol of this study was approved by the Biomedical Ethics Committee of the Dnipro State Medical University.

Allergological examination of patients included history taking, molecular methods of blood analysis using ALEX technology with determination of the level of specific IgE antibodies to major and minor components of pollen (wormwood, ragweed, timothy grass) and household (house dust mites, mold) allergens and diagnostic skin tests (prick test) with allergens produced by Vinnytsia LLC "Imunolog" (Ukraine).

The diagnosis of the examined persons was established on the basis of the "Native protocols for providing medical care to patients with allergic diseases", which were approved at the II Congress of Allergists of Ukraine, and then updated at the III Congress of Allergists of Ukraine.

Assessment of the main clinical manifestations of AR (nasal and conjunctival) was carried out in

accordance with the recommendations of the European Association of Allergists and Clinical Immunologists (EAACI) during the season of pollination of causative allergens [12]. The expression of the main nasal symptoms of seasonal AR (sneezing, itching in the nasal cavity, nasal congestion, rhinorrhea) was determined according to the 4-point scale TNSS (Total nasal symptom score): 0 points – the symptom is absent (no manifestations), 1 – the symptom is mildly expressed (minimal manifestations that are easily tolerated), 2 – moderately expressed (moderate manifestations that do not interfere with daily activities), 3 – strongly expressed (manifestations that are difficult to tolerate, significantly impair the quality of life and / or sleep). The expression of the TOSS (Total ocular symptom score) ocular symptoms (itching/hyperemia of the conjunctiva and sclera, lacrimation) was also assessed on a 4-point scale. Integrated assessments of the intensity of clinical symptoms of AR were calculated as a total score for the main symptoms. The total maximum score of symptom manifestation is 18.

For statistical analysis of the research results, we used the license program STATISTICA v.6.1 (Statsoft Inc., USA), serial number AGAR909E415822FA). Taking into account the deviation of the distribution of quantitative indicators from the normal law according to the Shapiro-Wilk test, the data are presented as median (Me) and interquartile range (IQR: 25%; 75% percentiles), relative indicators – as percentages with a standard error ( $f \pm m\%$ ) [1]. Indicators were compared using the Mann-Whitney (U) and Pearson ( $\chi^2$ ) tests. Correlation relationships were investigated using the Spearman rank correlation coefficient (r) [1]. The results of the statistical analysis were considered statistically significant at  $p < 0.05$ .

#### RESULTS AND DISCUSSION

According to the results of skin allergy tests conducted in 32 out of 47 patients (68.1±6.8%), sensitization of various degrees to ragweed pollen was found in 32 (100±2.1%) people, to wormwood – in 17 (53.1±8.8%), timothy grass – in 6 (18.8±6.9%), house dust mites (rDer p and rDer f) – in 15 (46.9±8.8%) patients. Combined sensitization to several pollen allergens was found in 20 (62.5±8.6%) patients.

Analysis of the clinical picture of AR in the season of pollination of causative allergens showed the presence of nasal symptoms of the disease in all 47 patients (100±1.4%), with the most pronounced manifestations (3 points on the TNSS scale) of sneezing (in 26 – 55.3±7.3%), rhinorrhea (in 28 – 59.6±7.2%), itching in the nasal cavity (in 11 – 23.4±6.2%), difficulty in nasal breathing (in 9 –

19.1±5.7% of patients). The occurrence of symptoms of conjunctivitis was also noted by almost all examined patients (44 – 93.6±3.6%), including moderate and severe manifestations (2-3 points on the TOSS scale) of itching, burning in the eye area, hyperemia of the conjunctiva and sclera was noted in 43 (91.5±4.1%), lacrimation – in 23 (48.9±7.3%).

The total symptom score ranged from 10 to 17 points, with an average of 13 (12; 14) points.

A detailed analysis of the degree of hypersensitivity to pollen and household allergens with the help of the ALEX multicomponent allergy test made it possible to determine the level of specific IgE antibodies in 47 patients who participated in the study (Table).

**Results of molecular methods of determining specific immunoglobulins E (sIgE) in blood to major allergic components of pollen and household allergens in patients with allergic rhinitis (M±m)**

Major allergic components	Not determined (<0,35 kU/L)*	Determined, sIgE level				
		low (0,35-0,69 kU/L)*	moderate (0,7-3,49 kU/L)*	high (3,5-17,4 kU/L)*	very high (>17,5 kU/L)*	on average (kU/L)**
Ragweed pollen (nAmb a1)	4/ 8,5±4,1	2/ 4,3±2,9	9/ 19,1±5,7	7/ 14,9±5,2	25/ 53,2±7,3	29,70 (3,47; 68,27)
Wormwood pollen (nArt v1)	29/ 61,7±7,1	4/ 8,5±4,1	5/ 10,6±4,5	7/ 14,9±5,2	2/ 4,3±2,9	3,27 (1,03;12,0)
Wormwood pollen (nArt v3)	43/ 91,5±4,1	2/ 4,3±2,9	–	2/ 4,3±2,9	–	7,30 (0,36;15,81)
Timothy grass pollen (rPhl p1, rPhl p5b)	39/ 83,0±5,5	1/ 2,1±2,1	–	5/ 10,6±4,5	2/ 4,3±2,9	10,50 (5,85; 20,44)
House dust mite (rDer p1)	39/ 83,0±5,5	1/ 2,1±2,1	2/ 4,3±2,9	4/ 8,5±4,1	1/ 2,1±2,1	4,16 (1,11; 14,89)
House dust mite (rDer p2)	36/ 76,6±6,2	1/ 2,1±2,1	4/ 8,5±4,1	4/ 8,5±4,1	2/ 4,3±2,9	3,86 (0,81; 13,62)
Mold fungi Alternaria alternata (rAlt A1)	44/ 93,6±3,6	1/ 2,1±2,1	–	1/ 2,1±2,1	1/ 2,1±2,1	8,06 (0,61; 20,10)

Notes: 1. \* – data are presented as abs./ f±m%; \*\* – data are presented as як Me (IQR); 2. Definition of allergen is presented in Latin letters: first three letters of gender, first letter - species name and number that defines order in which allergen was detected, n – natural, r – recombinant.

As shown in the Tble, sensitization to ragweed pollen allergen proteins (nAmb a1) was detected in the absolute majority of patients (91.5±4.1%), mainly in high and very high concentrations (32 patients – 68.1±6.8%). The median level of sIgE titer to the allergenic component of ragweed pollen was 29.70 (3.47, 68.27) kU/l. So, according to the results of various methods of determining hypersensitivity to allergens, it has been confirmed that ragweed pollen has the highest sensitization potential among residents of the Dnipro region.

19 (40.4±7.2%) patients with AR had specific IgE antibodies to major allergenic components of wormwood pollen, including to nArt v1 – 18 (38.3±7.1%), to nArt v3 – 4 (8.5±4.1%). The major components of timothy grass pollen (rPhl p1, rPhl p5b) were detected in the blood of 8 (17.0±5.5%) patients, mostly with high and very high sIgE levels – 7 (14.9±5.2%). In addition, two patients had sIgE to the minor components of timothy grass pollen (rPhl p7 rPhl p12) in the

absence of IgE to the major component of the allergen, which indicates the impracticality of using ASIT with an extract of such an allergen in them. In general, 24 (51.1±7.3%) patients had combined sensitization to the major components of several studied pollen allergens (ragweed, wormwood, timothy grass).

Specific IgE antibodies to house dust mites rDer p1 and/or rDer p2 had almost a third of the examined – 14 (29.8±6.7%), including to rDer p1 – 8 (17.0±5.5%), to rDer p2 – 11 (23.4±6.2%). In both cases, the median sIgE value (4.16 and 3.86 kU/L) corresponded to high titers (≥3.5 kU/L). Sensitization to the minor component of rDer p10 (tropomyosin) was not detected in any case. A direct correlation was established between the detection of hypersensitivity to the major allergenic components of house dust mites (rDer p1 and/or rDer p2) and the degree of severity of such key clinical symptoms of AR as sneezing attacks – r=+0.29 (p<0.05), rhinorrhea – r=+0.44 (p<0.01) and lacrimation –

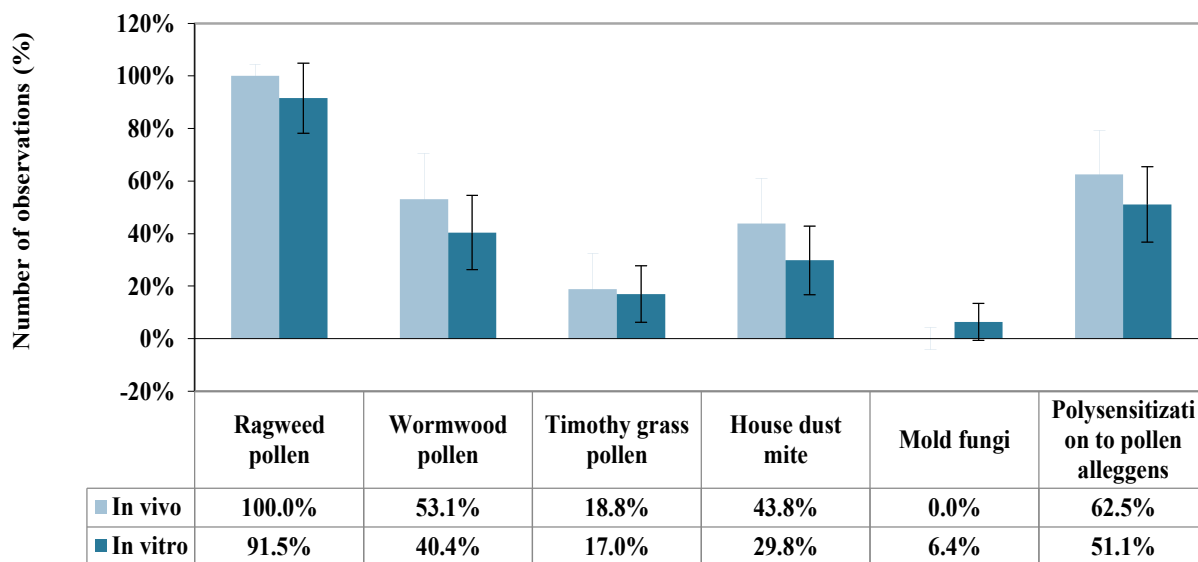
$r = +0.35$  ( $p < 0.05$ ), as well as with the total score of symptoms –  $r = +0.55$  ( $p < 0.001$ ).

Sensitization to recombinant molecules of mold allergens *Alternaria alternata* (rAlt A1) was detected in 3 ( $6.4 \pm 3.6\%$ ) patients, which in all cases was combined with allergic sensitivity to nAmb a1 and nArt v1.

The level of total IgE in the blood of patients with AR ranged from 40.9 IU/ml to 509.9 IU/ml with a median of 298.6 (117.0, 436.7) IU/ml. A weak correlation was established between the levels of total IgE and specific IgE to the major allergenic components of wormwood pollen (nArt v1) –

$r = +0.30$  ( $p < 0.05$ ), as well as with the degree of manifestations of nasal symptoms of pollinosis – sneezing and itching in the nasal cavity –  $r = +0.41$  ( $p < 0.01$ ) and  $r = +0.36$  ( $p < 0.05$ ).

Comparing the results of testing hypersensitivity to studied allergens by skin prick test (*in vivo*) and the study of the content of specific IgE in blood serum by ALEX technology (*in vitro*), some discrepancies between the results of the assessment of the same allergen *in vivo* and *in vitro* (Fig.) were established, but without statistically significant differences between them ( $p > 0.05$ ).



**Comparison of the frequency of registration of sensitization to allergens in patients with AR, determined using different diagnostic methods: frequency (f) and 95% confidence interval are indicated**

At the same time, molecular diagnostics allows to more accurately determine the causative allergen (quantitative value), cross-reactions and provide clear recommendations for the selection of ASIT components.

### CONCLUSIONS

1. According to the results of molecular allergy diagnostics, the profile of allergen sensitization in patients with pollen disease was determined, which includes the presence of specific IgE antibodies to the major allergenic components of ragweed pollen (nAmb a1) – in  $91.5 \pm 4.1\%$  of patients, wormwood pollen (nArt v1, nArt v3) – in  $40.4 \pm 7.2\%$ , timothy grass (rPhl p1, rPhl p5b) – in  $17.0 \pm 5.5\%$ , house dust mites (rDer p1, rDer p2) – in  $29.8 \pm 6.7\%$ .

2. Determination of specific antibodies to allergenic components allows you to obtain detailed information about the patient's sensitization (establish a true allergy), cross-reactivity with other allergens, justify the feasibility and predict the effectiveness of ASIT.

### Contributors:

Dityatkovska E.M. – conceptualization, methodology, management, project administration;

Biletska S.V. – research, formal analysis, resources, writing – original draft, writing – reviewing and editing, funding acquisition.

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