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DIAGNOSTIC VALUE OF METHODS FOR SENSITIZATION DETERMINATION IN PEOPLE WITH ALLERGIC RHINITIS

Key words: skin prick testing, allergy, western-blotting, IgE.

Nowadays, attention to the epidemic of allergic diseases is shown not only by professional medical communities, but also by governments around the world. In 2025, it is predicted that 400 million of individuals will suffer from allergic asthma and 500 million from allergic rhinitis [1, 2]. It may occur not only in developed countries, but also in developing countries [3].

Traditionally, the diagnosis of allergic diseases is based on the sequential (stage) application of a number of research methods, which include: 1) collection of complaints and anamnesis; 2) physical examination; 3) skin tests with allergens; 4) provocative (bronchial, nasal, conjunctival, sublingual, gastrointestinal, etc.) tests; 5) laboratory and instrumental (general laboratory methods and specific laboratory tests with allergens) tests; 6) functional and instrumental (rhinopneumometry, spirometry, peak flow measurement, endoscopy of the nasal cavity and bronchial tree, X-Ray and tomography of sinuses and chest organs); 7) counseling patients with other specialists. In this article we will try to summarize data and compare diagnostic values of traditional skin prick testing, serological IgE determination and, partially, component resolved diagnostic methods.

The basis of modern laboratory allergy diagnostics should be based on modern standardized and well-tested methods that have an evidence base. This is especially important in such situations:

- When it is impossible to cancel antiallergic drugs in the testing period;
- There is a pronounced hypersensitivity to allergens and anaphylactic reactions in anamnesis;
- The patient is examined in a period of acute disease;
- It is necessary to establish a diagnosis of allergy in early childhood;
- There is a multiple sensitivity to allergens, and the timing of the patient's examination is limited;
- Skin reactivity is changed, which leads to false negative and false positive results of skin testing;
- Simultaneous examination of the patient with the use of a large number of drugs and other chemicals;
- There is a difficult differential diagnostic case;
- Examination is necessary during the period of allergen-specific immunotherapy (ASIT);
- Remote examination of the patient.

The main tasks of the laboratory diagnosis of allergy are:

- determination of the types of allergic reaction (AR), the establishment of MS to Al (specific allergic diagnosis);
- identification of the nature and extent of immune disorders (immunodiagnostics);
- characterization of pathogenetic links typical for a specific allergies (clinical laboratory diagnostics).

World Allergy Organization (WAO) mentions that skin prick test (SPT) is the gold standard in detecting IgE [4]. It provides evidence for sensitization and can help to confirm the diagnosis of a suspected type I allergy. It is minimally invasive, inexpensive, results are immediately available and when carried out by trained health professionals, reproducible. Since the first publication about SPT by Helmtraud Ebruster in 1959 [5], who extensively researched this diagnostic test, it has been used as a primary diagnostic tool to detect type I hypersensitivity reactions. Although the principle of SPT still largely resembles the original methods described, a wide array of interpretations and modifications has led to diminished comparability when SPT results are reported [6].

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SPT is indicated if a type I (immediate type) allergy is suspected, based on the medical history and clinical symptoms; they can identify sensitivity to inhalant, food, drug or occupational allergens. SPTs thus provide objective confirmation of sensitivity, whereas the relevance of such sensitivity to allergens should always be carefully interpreted in the light of the clinical history so that appropriate advice concerning avoidance measures can be given and, as necessary, the correct allergen(s) prescribed for specific immunotherapy (SIT). The chief advantage of SPT as compared to an in vitro measurement of specific IgE antibodies is that the test can be interpreted within 15 to 20 minutes after the reagent is applied to the skin. Moreover, the test gives a visual indication of the sensitivity which can be used in order to impact the patient's behavior. SPT can also be utilized to test less common allergens, such as certain medications, and fresh fruits and vegetables where no specific IgE antibody measurements are available.

Specific serum IgE measurement aim is at detecting IgE in serum. The price of specific serum IgE measurement is higher than skin prick test. However, it is not influenced by antihistamine drugs, skin disease, or skin adverse reactions [7]. The advantages of methods for determining specific IgE (sIgE) for diagnosis of allergies are: safety, lack of influence on the skin condition of pharmacopoeia, independence from cooperation with the patient (especially with children), good reproducibility, elimination of false positive and false negative results of skin tests, single invasiveness during blood sampling , the possibility of remote examination of the patient.

However, these methods have drawbacks: expensiveness and lower sensitivity compared to skin tests, lack of laboratories, a sufficiently long time for obtaining results, the possibility of only circulating IgE fixing, the presence of cross-reactions between inhalant and food allergens, inability to recognize non-protein allergens. In addition, the determination of the level of specific IgE is usually less sensitive than skin prick tests with allergens, the sIgE titer is not always associated with the severity of symptoms, the assessment of the significance of increasing the concentration of serum IgE depends on the research method, the type of allergen, the patient's age and the nature of the disease. In some cases, patients have false positives (due to an increased level of total IgE, the formation of IgG-IgE immune complexes and the generation of false IgE binding) or false negatives (due to the production of specific anti-IgE antibodies of the IgG class, the possibility of binding part of the total IgE level by cross-linking allergens, binding of mast cells with sIgE until they are detected in serum). In connection with all this, this type of laboratory testing is not recommended to be carried out in isolation without taking anamnesis and skin tests with allergens.

Within the framework of the SAPALDIA survey (Swiss study on Air Pollution and Lung Diseases in Adults), a total of 8,344 subjects aged 18-60 years studied and the sensitivity of SPT was significantly higher (68.4 % vs 43.9 % $P < 0.001$) than that of IgE to diagnose seasonal allergic rhinitis. In conclusion, SPT have the best positive predictive value and the best efficiency to diagnose respiratory atopic diseases [8]. Another study shows poor to moderate agreement between SPT and sIgE level when diagnosing inhalant and food allergen sensitization in young children, which was particularly poor for food sensitization and deteriorated with age [8]. Kumar R. et al. showed that SPTs did not have good agreement with serum-specific IgE in early childhood. Both tests (SPT and specific IgE) should be used. Skin allergenic reactivity increased with age and was transient at 1 year but associated with the occurrence of atopic dermatitis [9].

The area under the curve (AUC) obtained with SPT was not significantly different from that obtained with sIgE in recent research made by Chauveau A. et al. [10, 11]. For asthma and hay fever, SPT (cutoff value at 3 mm) had a significantly higher specificity ($P < 0.0001$) than sIgE (cutoff value at 0.35 IU/mL) and the specificity was not different between both tests (P=.1088). Regarding the method chosen for SSIgE, Wood et al. [12] showed some differences in SSIgE results according to the method of assay. However, Herzum et al. [13] found that the Allergy Screen panel yields reliable results in the detection of allergic sensitization to common allergens.

However, the higher sensitivity of SSIgE detection, previously reported in a prospective 18-month study [14], does not alone explain why the agreement between both tests was so poor in our study. The strength of agreement was only substantial [15] for aeroallergens at 6 years of age and for cutoffs of 0.7 and 3.5 IU/ml. The recent meta-analysis by Soares-Weiser et al. [16] reported that very few studies have compared the tests head-to-head in the same population over time. In their populations of food-allergic subjects without age limits, SPT and SSIgE were both sensitive but not specific. In the DARC birth cohort, Kjaer et al. [17] showed that SPTs were better correlated with a diagnosis of allergic disease at 6 years than SSIgE. In a recent article on the COPSAC2000 at-risk birth cohort, Schoos et al. [18] also found a substantial disagreement between SPT and SSIgE in early childhood with all κ-coefficient under 0.60 without increasing with age.

The analysis shows that the results of the diagnostic parameters of various tests in studies vary widely. Below we present the data of our own research conducted by us in patients with allergic rhinitis in 2015-2018.

Materials and methods. During this research, 88 patients with allergic rhinitis were examined by three different methods of specific allergic diagnosis (in vivo and in vitro). The inclusion criteria were allergic rhinitis diagnosis (both intermittent and persistent) with proven sensitivity to domestic allergens. SPT was carried out according to the classical testing procedure in accordance with regulatory documents with commercial extracts of allergens (Immunolog, Vinnitsa, Ukraine). For the test, a positive (histamine dihydrochloride solution 0.01 % – Solutio histamini dihydrochloridi 0.01 % pro diagnostica cutanea morborum allergicorum) and

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negative (sodium chloride, disodium phosphate dodecahydrate (sodium phosphate dibasic), potassium dihydrogen phosphate (potassium phosphate monosubent phenol, tween 80, water for injection) controls (Immunolog, Vinnitsa, Ukraine) were used. The following allergen extracts were used – Dermatophagoides farinae and Dermatophagoides pteronyssinus. SPT results were assessed in 15 min visually using a ruler in mm and were classified according to the existing scale as negative, doubtful, weak (+), strong (++) and very strong (++).

A standard medical interview and the qualification of patient were performed during an earlier visit, and then, 15 mL of blood for the sIgE test was collected. Western blot testing for specific IgE levels was performed using RIDA qLine test systems (R-Biopharm AG, Darmstadt, Germany) and Euroline (Euroimmun) system. The sIgE concentration was converted to a nominal scale (grades) according to the following rules: < 0.35 IU mL-1-level 0 (negative), (0.36-0.69) IU mL-1-level 1 (boundary levels), (0.7- 3.49) IU mL-1-level 2 (slightly elevated), (3.50-17.4) IU mL-1-level 3 (moderately elevated), (17.5-49,9) IU mL-1-level 4 (high levels), (50-100) IU mL-1-level 5 (very high levels) and > 100 IU mL-1-level 6 (extremely high levels).

Results. In the examined patients, the sensitization to the allergen D. Pteronissinus was 36.4 % (32 subjects) by the presence of specific IgE by Rida AllergyScreen, 34.1 % (30 cases) by the presence of specific IgE by Euroline and 38.6 % (34 persons) according to the data of skin test by a blind test method with an appropriate allergen.

In Table 1 the results of the comparison of the determination of the specificity of the specific IgE method Rida AllergyScreen to the D. Pteronissinus mite with the data of skin testing by the prick test method are presented. When comparing two different types of specific allergic diagnosis by the method of establishing the correlation relations of the population with D. pteronissinus, the dominance of the elements of the main diagonal is noted, which indicates a rather close coincidence of the results of two different methods (the validity of the coincidence of results was 86.4% – 76 cases).

The results of two different methods of specific allergic diagnosis to determine the sensitization to the D. pteronissinus allergy are closely identical, but there is a certain asymmetry of the differences in the results of skin testing by the blind test method and the determination of specific IgE blood when one test gives negative results and the other one is positive or doubtful.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The results of the analysis of the consistency of the results of two different methods of allergic diagnosis to determine the sensitization to the D. pteronissinus mite through the construction of the confidence interval (Table 2) showed that the coefficient indicates a good agreement (r = 0.740) of the findings of the two different tests. The limits of the 95 % confidence interval (0.618-0.863) exclude 0, which indicates the accuracy of the match. The lower limit is in the range of good coherence, and the upper one is in the area of excellent coherence.

Table 1. Sensitization to D. Pteronissinus mites based on skin testing and the detection of specific IgE method Rida AllergyScreen

Prick test	Specific IgE (ku/l)			Total
	< 0,35 (negative)	0,35–0,7 (doubtful)	> 0,7 (positive)	
PAPULA 0 мм (NEGATIVE)	48	2	4	54
PAPULA 1–2 мм (DOUBTFUL RESULT)	0	0	0	0
PAPULA ≥ 3мм (POSITIVE RESULT)	0	6	28	34
TOTAL	48	8	32	88

The results of the statistical estimation of the null hypothesis of the lack of agreement between the results of two different methods of specific allergic diagnosis for the determination of sensitization to the mite D. Pteronissinus are given in Table 3.

The hypothesis is rejected both in one-sided and bilateral tests, which testifies to the true consistency of both allergic tests. Thus, there is a good degree of agreement between the results of the skin testing of

D. Pteronissinus allergens and the detection of specific IgE by the Rida AllergyScreen method.

In Table 4 the results of the comparison of the determination of the presence of specific IgE to the

D. pteronissinus mite according to the Euroline method with the data of skin testing by the blind test method are presented. When comparing two different types of specific allergic diagnosis by the method of establishing correlation relations of the suffering to D. pteronissinus, a moderate dominance of the elements of the main diagonal is noted, indicating an average coincidence of the results of two different methods (the validity of the results was 61.4 % – 54 cases).

Table 2. Results of statistical estimation of the consistency of the results on the results of skin testing and the detection of specific IgE by the Rida AllergyScreen method to determine the sensitization to the allergen D. Pteronissinus

Kappa coefficient	0,740
Asymptotic error kappa	0,063
Lower border 95% confidence interval (√*var*)	0,615
Upper border 95% confidence interval	0,851

The results of two different methods of specific allergic diagnosis for the determination of sensitization to the D. pteronissinus allergen are in part identical, but a certain asymmetry of the differences in the results of skin testing by the blind

Table 3. Results of 'null hypothesis' checking between the results of skin testing and Rida AllergyScreen to D. Pteronissinus

Asymptotic error kappa H_0, $\sqrt{var_0(\kappa)}$	0,0721
Z	7,7944
One-way test *Pr* > *Z*	< 0,0001
Two-way test *Pr* > |*Z*|	< 0,0001

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Table 4. Sensitization to D. Pteronissinus mites based on skin tests and detection of specific IgE method Euroline

Specific IgE (ku/l) < 0,35 (negative)	Specific IgE (ku/l)			Total 0,35–0,7 (doubtful)
	< 0,35 (negative)	0,35–0,7 (doubtful)	< 0,35 (negative)	
Specific IgE (ku/l)	36	8	10	54
< 0,35 (negative)	0	0	0	0
Specific IgE (ku/l)	8	8	18	34
< 0,35 (negative)	44	16	28	88

test method and the determination of specific IgE blood is noted when one test gives negative results and the other one is positive or questionable.

To obtain conclusions about the reliability of this asymmetry, we conducted an in-depth statistical analysis of the correlation of laboratory allergic and skin tests. The results of the analysis of the consistency of the results of two different methods of allergic diagnosis for the determination of sensitization to the D. pteronissinus mite through the construction of the confidence interval (Table 5) showed that the coefficient indicates a satisfactory agreement (r = 0,322) of the findings of the two different tests. The limits of the 95 % confidence interval (0.168-0.461) exclude 0, which indicates the accuracy of the correspondence. The lower limit lies in the range of poor consistency, and the upper one - in the area of moderate coherence.

Results of the statistical estimation of the null hypothesis of the lack of agreement between the results of two different methods of specific allergic diagnosis for the determination of sensitization to the tick D. Pteronissinus are shown in Table 6.

Table 5. Results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the Euroline method for the determination of sensitization to the allergen D. Pteronissinus

Kappa coefficient	0,322
Asymptotic error kappa	0,077
Lower border 95 % confidence interval	0,168
Upper border 95 % confidence interval	0,461

Table 6. Results of 'null hypothesis' checking between the results of skin testing and Euroline to D. Pteronissinus

Asymptotic error kappa H_0, $\sqrt{var_0(\kappa)}$	0,152
Z	7,7944
One-way test $Pr > Z$	< 0,001
Two-way test $Pr > \|Z\|$	< 0,001

The hypothesis is rejected both by one-sided, and by two-way testing, which testifies the consistency of tests with each other.

That is, between the data of skin testing with allergens D. Pteronissinusta detection of specific IgE by Euroline, there is a satisfactory degree of consistency between the results of studies.

To evaluate such a difference between the results of two systems for the determination of specific IgE to D. Pteronissinus by Rida AllergyScreen and Euroline, we conducted a comparative analysis according to the Blend-Altman charts. The comparison results are shown in Fig. 1

First, the systematic error of measurement results is -1.27ku / l, which indicates the presence of a systematic difference. In this case, the distribution graph corresponds to the type of graphs of the absolute systematic error. Secondly, the standard deviation of the differences was 5.2, which is significantly compared with the values themselves. Thirdly, there is a certain dependence of the difference in measurements on the

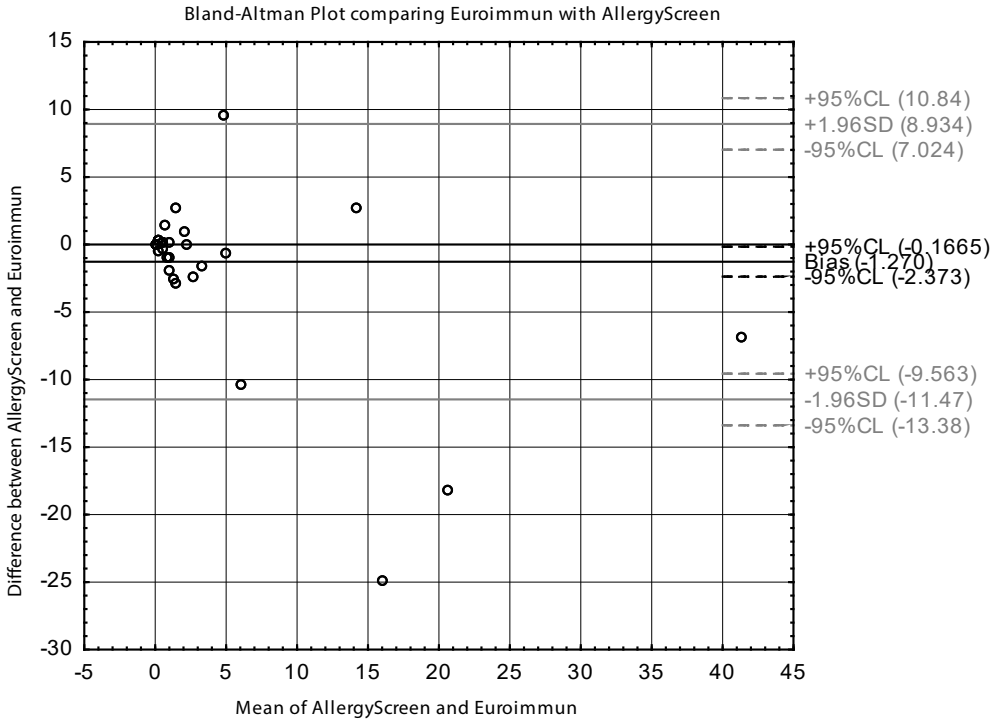

Figure 1. Bland – Altman plot to determine the specific IgE to D. Pteronissinus methods Rida AllergyScreen and Euroline

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number of specific IgE in the blood, as with the increase in the numerical values of the signs the number of discrepancies increases. In addition, some of the values do not fit into the confidence interval of ± 95 %.

Thus, the results of two systems for determining the specific IgE to D. Pteronissinus by Rida AllergyScreen and Euroline have a systematic difference in rates (-1.27 kU / l).

Conclusions. Between the data of skin testing with D. Pteronissinus allergens and detection of specific IgE by the Rida AllergyScreen method, there is good agreement between the results, there is satisfactory agreement between the results of the research between the data of skin testing with allergens D. Pteronissinus and the detection of specific IgE by the Euroline method.

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DIAGNOSTIC VALUE OF METHODS FOR SENSITIZATION DETERMINATION IN PEOPLE WITH ALLERGIC RHINITIS
(Review of literature and own data)

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Abstract

Objective was to review the literature on the diagnostic value of various methods for determining sensitization in patients with allergies, to study the parameters of the specificity and sensitivity of skin testing and laboratory determination of specific IgE.

Materials and methods. 88 patients with allergic rhinitis were examined by three different methods of specific allergic diagnosis (in vivo and in vitro) in accordance with the guidelines of the ethics committee of the National Pirogov memorial medical university, all were beyond the acute period. The inclusion criteria were allergic rhinitis diagnosis (both intermittent and persistent) with proven sensitivity to domestic allergens. Skin prick test was carried out according to the classical testing procedure in accordance with regulatory documents with commercial extracts of allergens.

Western blot testing for specific IgE levels was performed using RIDA qLine test systems (R-Biopharm AG, Darmstadt, Germany) and Euroline (Euroimmun). The sIgE concentration was converted to a nominal scale (grades) according to the following rules: < 0.35 IU mL-1-level 0 (negative), (0.36-0.69) IU mL-1-level 1 (boundary levels), (0.7-3.49) IU mL-1-level 2 (slightly elevated), (3.50-17.4) IU mL-1-level 3 (moderately elevated), (17.5-49,9) IU mL-1-level 4 (high levels), (50-100) IU mL-1-level 5 (very high levels) and > 100 IU mL-1-level 6 (extremely high levels).

Results and discussion. The results of two systems for determining the specific IgE to D. Pteronissinus by Rida AllergyScreen and Euroline have a systematic difference in rates (-1.27 kU / l).

Between the data of skin testing with D. Pteronissinus allergens and detection of specific IgE by the Rida AllergyScreen method, there is good agreement between the results, there is satisfactory agreement between the results of the research between the data of skin testing with allergens D. Pteronissinus and the detection of specific IgE by the Euroline method.

Key words: skin prick testing, allergy, western-blotting, IgE.

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ДИАГНОСТИЧЕСКАЯ ЦЕННОСТЬ МЕТОДОВ ОПРЕДЕЛЕНИЯ СЕНСИБИЛИЗАЦИИ У ЛИЦ С АЛЛЕРГИЧЕСКИМ РИНИТОМ
(Обзор литературы и собственные данные)

А. Е. Богомолов

Резюме

Целью исследования было провести обзор литературы по тематике диагностической ценности различных методов определения сенсибилизации у пациентов с аллергией, изучить параметры специфичности и чувствительности кожного тестирования и лабораторного определения специфического IgE.

Материалы и методы. В ходе исследования 88 пациентов с аллергическим ринитом были обследованы тремя различными методами специфической аллергической диагностики (in vivo и in vitro) в соответствии с рекомендациями комитета по этике Винницкого национального медицинского университета имени Пирогова, причем все они были вне острого периода. Критериями включения были диагноз аллергического ринита (как интермиттирующего, так и персистирующего) с доказанной чувствительностью к домашним аллергенам. Прик-тест проводился по классической методике тестирования в соответствии с нормативными документами с коммерческими экстрактами аллергенов.

Вестерн-блоттинг для определения уровней IgE проводили с использованием тест-систем RIDA qLine (R-Biopharm AG, Дармштадт, Германия) и Euroline (Euroimmun). Концентрацию sIgE переводили в номинальную шкалу (оценки) в соответствии со следующими правилами: < 0,35 МЕ мл-1-уровень 0 (отрицательный), (0,36–0,69 МЕ) мл-1-уровень 1 (граничные уровни), (0,7–3,49) IU mL-1-level 2 (слегка повышенный), (3,50–17,4) IU mL-1-level 3 (умеренно повышенный), (17,5–49, 9) IU mL-1-level 4 (высокие уровни), (50–100) МЕ мл-1-уровня 5 (очень высокие уровни) и > 100 МЕ мл-1-уровня 6 (очень высокие уровни).

Результаты и обсуждение. Результаты двух систем определения специфического IgE к *D. Pteronissinus* по данным Rida AllergyScreen и Euroline имеют систематическую разницу в результатах (–1,27 кЕ/л).

Между данными кожного тестирования с аллергенами *D. Pteronissinus* и определением специфического IgE методом Rida AllergyScreen, существует хорошее согласие между результатами, существует удовлетворительное согласие между результатами исследования между данными кожного тестирования с аллергенами *D. Pteronissinus* и определение специфического IgE по методу Euroline.

Ключевые слова: прик-тест, аллергия, иммуноблоттинг, IgE.

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ДІАГНОСТИЧНА ЦІННІСТЬ МЕТОДІВ ВИЗНАЧЕННЯ СЕНСИБІЛІЗАЦІЇ У ОСІБ З АЛЕРГІЧНИМ РИНІТОМ
(Огляд літератури та власні дані)

А. Є . Богомолов

Резюме

Метою дослідження було провести огляд літератури за тематикою діагностичної цінності різних методів визначення сенсибілізації у пацієнтів з алергією, вивчити параметри специфічності і чутливості шкірного тестування і лабораторного визначення специфічного IgE.

Матеріали та методи. В ході дослідження 88 пацієнтів з алергічним ринітом були обстежені трьома різними методами специфічної алергічної діагностики (in vivo та in vitro) відповідно до рекомендацій комітету з етики Вінницького національного медичного університету імені Пирогова, причому всі вони були поза гострого періоду. Критеріями включення були діагноз алергічного риніту (як інтермітуючого, так і персистуючого) з доведеною чутливістю до побутових алергенів. Прик-тест проводився за класичною методикою тестування відповідно до нормативних документів з комерційними екстрактами алергенів.

Вестерн-блот для визначення рівнів IgE проводили з використанням тест-систем RIDA qLine (R-Biopharm AG, Дармштадт, Німеччина) і Euroline (Euroimmun). Концентрацію sIgE переводили в номінальну шкалу (оцінки) відповідно до наступних правил: < 0,35 МО мл-1-рівень 0 (негативний), (0,36-0,69 МО) мл-1-рівень 1 (граничні рівні), (0,7–3,49) IU mL-1-level 2 (злегка підвищений), (3,50–17,4) IU mL-1-level 3 (помірно підвищений), (17,5–49,9) IU mL-1-level 4 (високі рівні), (50–100) МО мл-1-рівня 5 (дуже високі рівні) і > 100 МО мл-1-рівня 6 (дуже високі рівні).

Результати та обговорення. Результати двох систем визначення специфічного IgE до D. Pteronissinus за даними Rida AllergyScreen і Euroline мають систематичну різницю в результатах (-1,27 кЕ/л).

Між даними шкірного тестування з алергенами *D. Pteronissinus* і визначенням специфічного IgE методом Rida AllergyScreen, існує гарна згода між результатами, існує задовільний згоду між результатами дослідження між даними шкірного тестування з алергенами *D. Pteronissinus* і визначення специфічного IgE за методом Euroline.

Ключові слова: прик-тест, алергія, імуноблотинг, IgE.

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