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DIFFERENT METHODS OF DERMATOPHAGOIDES FARINAE SENSITIZATION DETERMINATION IN PATIENTS WITH RESPIRATORY ALLERGY

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Abstract. *Objective* was to study and compare the parameters of the specificity and sensitivity of skin testing and serologic determination of specific *Dermatophagoides farinae* IgE.
Materials and methods. 88 patients with allergic rhinitis and/or atopic bronchial asthma 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 and/or atopic bronchial asthma diagnosis (both intermittent and persistent) with proven sensitivity to domestic allergens. Skin prick test was carried out by 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 specific IgE to *D. Farinae* by methods Rida AllergyScreen and Euroline are consistent with each other, but there is a systematic difference in indicators (-0.71 kU/l). There is good agreement between the data of skin testing with allergens *D. Farinae* and the detection of specific IgE by the method of Rida AllergyScreen, but there is a satisfactory agreement between the results of skin testing with *D. Farinae* allergens and the detection of specific IgE by the Euroline method.
Key words: skin prick testing, allergy, western blotting, IgE, Dermatophagoides farinae.

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РІЗНІ МЕТОДИ ВИЗНАЧЕННЯ СЕНСИБІЛІЗАЦІЇ ДО DERMATOPHAGOIDES FARINAE У ПАЦІЄНТІВ З РЕСПІРАТОРНОЮ АЛЕРГІЄЮ

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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 та/або атопічною бронхіальною астмою 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 та/або атопічної бронхіальної астми (як інтермітуючих, так і персистуючих) 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 спе-

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цифічного IgE до *D. Farinae* методами Rida AllergyScreen та Euroline узгоджуються між собою, проте існує систематичне розходження показників (-0,71 kU/l). Між даними шкірного тестування з алергенами *D. Farinae* та виявленням специфічного IgE методом Rida AllergyScreen існує добра погодженість між результатами, між даними шкірного тестування з алергенами *D. Farinae* та виявленням специфічного IgE методом Euroline існує задовільна погодженість між результатами досліджень.
Ключові слова: прик-тест, алергія, імуноблотинг, IgE, Dermatophagoides farinae.

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РАЗЛИЧНЫЕ МЕТОДЫ ОПРЕДЕЛЕНИЯ СЕНСИБИЛИЗАЦИИ К DERMATOPHAGOIDES FARINAE У ПАЦИЕНТОВ С РЕСПИРАТОРНОЙ АЛЛЕРГИЕЙ

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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 и/или атопической бронхиальной астмой были обследованы тремя различными методами специфической аллергической диагностики (*in vivo* и *in vitro*). Критериями включения были диагноз аллергического ринита и/или атопической бронхиальной астмы (как интермиттирующих, так и персистирующих) 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. Farinae* по данным Rida AllergyScreen и Euroline имеют систематическую разницу в результатах (-0,71 кЕ/л). Между данными кожного тестирования с аллергенами *D. Farinae* 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. Farinae* и определением специфического IgE по методу Euroline.
Ключевые слова: прик-тест, аллергия, иммуноблоттинг, IgE, Dermatophagoides farinae.

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House dust mites are very important airborne allergens. The main pathogenic species of mites in allergic diseases are *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, which account for 90 % of house dust mites allergies [1]. The sensitization profiles to these house dust mites vary geographically, as *D. farinae* is present in drier areas and *D. pteronyssinus* predominates in humid areas of the central United States and central Europe [2, 3].

Different diagnostic tools are available in clinical practice to detect sensitization in patients with respiratory allergy. As we showed before [4], different diagnostic parameters of skin prick test and serological methods are typical for *D. pteronyssinus* sensitization. Below we present the data of our research in patients with allergic rhinitis and asthma in 2015–2018.

Materials and methods. During this research, 88 patients with allergic rhinitis and/or atopic bronchial asthma 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. Skin prick test (SPT) was carried out by classical testing procedure in accordance with regulatory

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T a b l e 1. Sensitization to *D. Farinae* by the results of skin testing and the detection of specific IgE by Rida AllergyScreen

Prick test	Specific IgE (ku/l)			Total
	< 0.35 (negative)	0.35–0.7 (questionable)	> 0.7 (positive)	
Induration 0 mm(negative result)	54	0	4	58
Induration 1–2 mm (questionable result)	0	0	0	0
Induration ≥ 3 mm (positive result)	2	2	26	30
Total	56	2	30	88

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 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. 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 process of this study, three different methods of specific allergic diagnosis (*in vivo* and *in vitro*) were used to examine 88 patients with asthma and/or allergic rhinitis. Among these patients, sensitization to the allergen *D. Farinae* was revealed in 34.1 % (30 patients) by skin prick test, specific IgE by Rida AllergyScreen was found in 34.1 % (30 patients) and the presence of specific IgE by Euroline was detected in 34.1 % (30 patients).

In Table 1 the results of the comparison of Rida AllergyScreen to the *D. Farinae* mite allergen with the data of prick test method are shown. Comparing two different types of specific allergic diagnosis by the method of establishing the correlation relations with *D. Farinae*, the dominance of the elements of the main diagonal is noted, indicating a close coincidence of the results of two different methods (validity coincidence of results was 90.9 % – 80 cases).

The results of two different methods of specific diagnostics to determine allergic sensitization to *D. Farinae* are almost similar, 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 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 analysis of harmony results of two different methods to determine the sensitization to *D. Farinae* through the construction of the confidence interval (Table 2) showed that the coefficient suggests a good agreement (r = 0.804) of this two different tests. The limits of the 95 % confidence interval (0.65–0.926) exclude zero, 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.

T a b l e 2. The results of statistical estimation of the consistency of results on the data of skin testing and the detection of specific IgE by Rida AllergyScreen to determine sensitization to *D. Farinae*

Kappa coefficient	0.804
Asymptotic kappa error	0.064
Lower border 95 % confidence interval	0.657
Upper border 95 % confidence interval	0.926

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine allergic sensitization to mites *D. Farinae* shown in Table 3.

T a b l e 3. The results of statistical estimation of the null hypothesis of the lack of consistency of the results of skin testing and the detection of specific IgE by Rida AllergyScreen for determination of sensitization to *D. Farinae*

Asymptotic kappa error for H_0,	0.0721
Z	3.8610
One-way testing $Pr > Z$	< 0.0001
Two-sided testing $Pr > \|Z\|$	< 0.0001

The hypothesis is rejected both in one-sided and bilateral tests, which testifies to the true consistency of both allergic tests.

Thus, skin testing with allergens *D. Farinae* and the detection of specific IgE by the Rida AllergyScreen have a good agreement between the results.

In Table 4 we showed the comparison of the presence of specific IgE to *D. Farinae* by Euroline with the data of skin prick test. Comparing two different types of specific diagnostics by setting correlative relationships to *D. Farinae,* it is noted the absence of the domination of the elements of the main diagonal, indicating a medium degree of coincidence of the results of two different methods (validity of the results was 68,2 % – 60 cases).

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T a b l e 4. Sensitization to D. farinae by the results of skin testing and the detection of specific IgE by Euroline

Prick test	Specific IgE (ku / l)			Total
	< 0.35 (negative)	0.35-0.7 (questionable)	> 0.7 (positive)	
Induration 0 mm (negative result)	40	8	10	58
Induration 1–2 mm (questionable result)	0	0	0	0
Induration ≥ 3 mm (positive result)	10	0	20	30
Total	50	8	30	88

The results of two different methods of specific allergic diagnosis to determine the sensitization to the *D. Farinae* are closely identical, but there is a certain asymmetry in the differences of 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 analysis of harmony results of two different methods to determine the diagnosis of allergic sensitization to mites *D. Farinae* through the construction of the confidence interval (Table 5) showed that the coefficient suggests satisfactory agreement (r = 0.375) of these two different tests. The limits of the 95 % confidence interval

T a b l e 5. The results of statistical estimation of the consistency of results on the results of skin testing and the detection of specific IgE by the Euroline to determine the sensitization to the allergen *D. Farinae*

Kappa coefficient	0.375
Asymptomatic kappa error	0.091
Lower border 95 % confidence interval	0.195
Upper border 95 % confidence interval	0.559

T a b l e 6. The results of statistical estimation of the null hypothesis of the lack of consistency of the results of skin testing and the detection of specific IgE by Euroline for determination of sensitization to *D. Farinae*

Asymptotic kappa error for H_0 ,	0.675
Z	7.7944
One-way testing $Pr > Z$	< 0.0001
Two-sided testing $Pr > \|Z\|$	< 0.0001

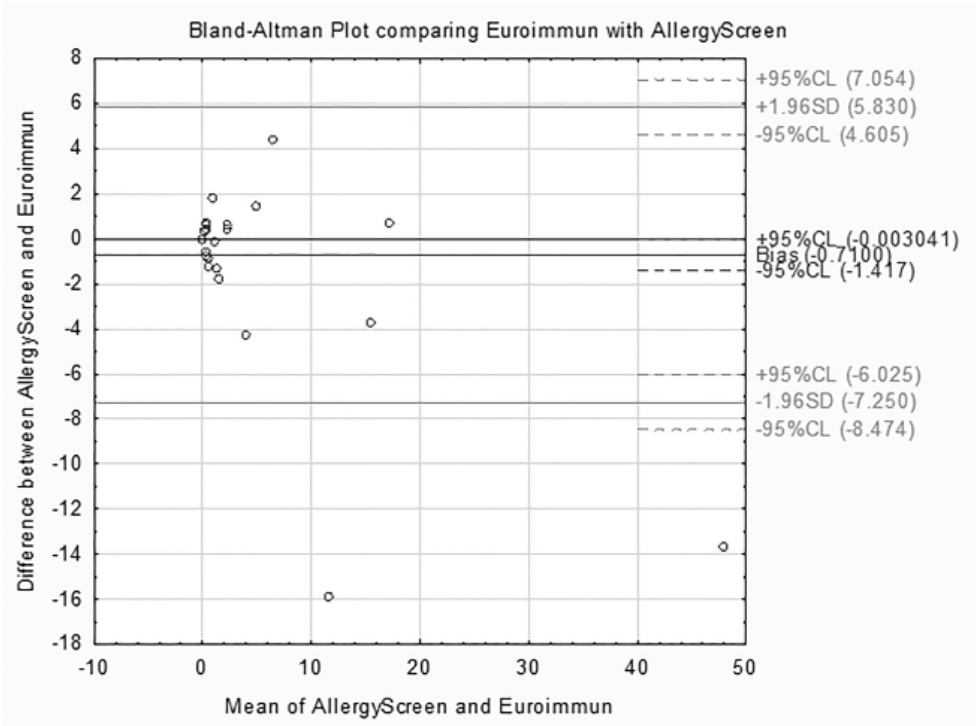
(0.195–0.559) exclude 0, which indicates the accuracy of the match. The lower limit lies in the range of poor consistency, and the upper one is in the area of moderate coherence.

A statistical evaluation of the null hypothesis lack of consistency of the results of two different methods of specific diagnostics to determine Allergic sensitization to mites *D. Farinae* shown in table 6.

The hypothesis is not accepted either by one-sided, or by double-sided testing.

Thus, according to the data of skin testing with allergens *D. Farinae* and the detection of specific IgE by the Euroline method, there is a satisfactory agreement between the results.

To evaluate a difference in the results of two systems for determining specific IgE to *D. Farinae* by Rida



Picture 1. The Blend-Altman plot for determining of specific IgE to *D. Farinae* by Rida AllergyScreen and Euroline.

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AllergyScreen and Euroline we conducted a comparative analysis according to the Blend- Altman plot. The comparison results are shown in Picture 1.

Firstly, the systematic error of measurement results is (-0.71) ku/l, which indicates the presence of systematic differences. 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 3.33, which is substantially compared with the values themselves. Thirdly, there is a certain dependence of the difference in measurements on the amount 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 lie into the confidence interval of ± 95 %.

Conclusions. The results of two systems for determining specific IgE to *D. Farinae* by Rida methods AllergyScreen and Euroline are consistent with each other, but there is a systematic difference in data (-0.71 ku/l).

There is good agreement between the data of skin testing with allergens *D. Farinae* and the detection of specific IgE by the method of Rida AllergyScreen, but there is a satisfactory agreement between the results of skin testing with *D. Farinae* allergens and the detection of specific IgE by the Euroline method.

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