УДК 616-097:616-056.3:57.083.322 DOI: http://doi.org/10.30978/TB2019-2-72

A.Ye. Bogomolov
National Pirogov Memorial Medical University, Vinnytsya

Diagnostic value of methods for alder (Alnus incana) allergen sensitization determination in people with respiratory allergy

Objective — to study the parameters of diagnostic value (specificity and sensitivity) of skin prick testing with alder allergen and serologic determination of specific IgE to alder allergen among patients with respiratory allergy.

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 alder allergen. 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 (level 0 — negative), 0.36—0.69 IU/mL (level 1 — boundary), 0.7—3.49 IU/mL (level 2 — slightly elevated), 3.50—17.4 IU/mL (level 3 — moderately elevated), 17.5—49, 9 IU/mL (level 4 — high levels), 50—100 IU/mL (level 5 — very high levels), and > 100 IU/mL (level 6 — extremely high levels).

Results and discussion. In the examined patients, the sensitization to the alder allergen was 25.0 % (22 cases) by the presence of specific IgE by Rida Allergy Screen, 13.6 % (12 cases) by the presence of specific IgE by Euroline and 27.3 % (24 persons) according to the data of skin test by a blind test method with an appropriate allergen. 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 alder through the construction of the confidence interval showed that the coefficient indicates a satisfactory agreement (r = 0.409) of the findings of the two different tests.

Conclusions. The results of two serologic tests for determining the specific IgE to alder by Rida AllergyScreen and Euroline have a systematic difference in rates (1.4 kU/mL). Between the data of skin testing with alder allergen and detection of specific IgE by Rida AllergyScreen test, there is good agreement between the results, there is satisfactory agreement between the results of the research between the data of skin testing with alder allergen and the detection of specific IgE by the Euroline method.

Key words

Skin prick testing, allergy, western-blotting, IgE, alder.

The advantages of methods for the determination of specific IgE (sIgE) for the diagnosis of allergic diseases include: safety, lack of influence on the skin of pharmacopoeia, independence from cooperation with the patient (especially with children), good reproducibility, elimination of false-positive and false-negative skin test results, single invasiveness at the intake blood, the possibility of remote examination of the patient [1].

However, these methods have drawbacks: expensiveness and lower sensitivity compared to skin

Table 1. **Sensitization to alder allergen based on skin testing and the detection of specific IgE method Rida Allergy Screen**

Prick test	Specific IgE (ku/l)			Total
	< 0,35 (negative)	0,35—0,7 (doubtful)	> 0,7 (positive)	
Papula 0 mm (negative)	60	0	4	64
Papula 1—2 mm (doubtful result)	0	0	0	0
Papula ≥ 3 mm (positive result)	2	4	18	24
Total	62	4	22	88

tests, the absence of conditions in a number of laboratories for a sufficiently long time to obtain results, the possibility of fixing only circulating IgE, the presence of cross-reactions between inhalation and food allergens, inability to recognize non-protein allergens [2, 3]. In addition, determining the level of specific IgE is usually less sensitive than conducting skin prick tests with an allergen, titre sIgE is not always associated with the severity of allergy symptoms, the assessment of the significance of increased serum IgE concentration depends on the method of research, the type of allergen, patient age and nature of the disease. In some cases, patients have false positives (due to increased levels of total IgE, formation of IgG-IgE immune complexes and generation of false IgE binding) or false negatives (due to the development of specific anti-IgE antibodies of the IgG class, the possibility of binding part of the total IgE level by cross-allergens, binding 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 an allergen [4, 5].

This article is a continuation of our study on the evaluation of diagnostic parameters of *in vitro* and *in vitro* tests to determine the sensitization of patients with respiratory allergies.

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 and/or atopic bronchial asthma diagnosis (both intermittent and persistent) with proven sensitivity to alder allergen. Skin prick test 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 negative (sodium chloride, disodium phosphate dodecahydrate (sodium phosphate dibasic), potassium dihydrogen phosphate (potassium phosphate monosubent phenol, tween 80, water for injection) controls (Immunolog, Vinnytsya, 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 (level 0 — negative), 0.36—0.69 IU/mL (level 1 — boundary), 0.7—3.49 IU/mL (level 2 — slightly elevated), 3.50—17.4 IU/mL (level 3 — moderately elevated), 17.5—49, 9 IU/mL (level 4 — high levels), 50—100 IU/mL (level 5 — very high levels), and > 100 IU/mL (level 6 — extremely high levels).

Results and discusion

In the examined patients, the sensitization to the alder allergen was 25.0% (22 cases) by the presence of specific IgE by Rida Allergy Screen, 13.6% (12 cases) by the presence of specific IgE by Euroline and 27.3% (24 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 alder allergen 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, 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 88.6% — 78 cases).

The results of two different methods of specific allergic diagnosis to determine the sensitization to the

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 Allergy Screen method to determine the sensitization to the alder allergen**

Kappa coefficient	0.729
Asymptotic error kappa ($\sqrt{}$ *var*)	0.074
Lower border 95% confidence interval	0.564
Upper border 95% confidence interval	0.869

Table 3. **Results of «null hypothesis» checking between the results of skin testing and Rida Allergy Screen to alder alergen**

Asymptotic error kappa H_0, ($\sqrt{}$ var_0(κ))	0.734
Z	3.8610
One-way test $Pr > Z$	< 0.001
Two-way test $Pr > \|Z\|$	< 0.001

Table 4. **Sensitization to alder based on skin prick tests and detection of specific IgE by Euroline**

Specific IgE (ku/l) < 0,35 (negative)	**Specific IgE (ku/l)**			
	< 0,35 (negative)	**0,35—0,7 (doubtful)**	**< 0,35 (negative)**	**Total 0,35—0,7 (doubtful)**
Specific IgE (ku/l)	58	4	2	64
< 0,35 (negative)	0	0	0	0
Specific IgE (ku/l)	12	2	10	24
< 0,35 (negative)	70	6	12	88

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 test for the determination of sensitization to the alder allergen**

Kappa coefficient	0.409
Asymptotic error kappa ($\sqrt{}$ *var*)	0.098
Lower border 95% confidence interval	0.218
Upper border 95% confidence interval	0.587

alder allergen.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 alder allergen through the construction of the confidence interval (table 2) showed that the coefficient indicates a good agreement (r = 0.729) of the findings of the two different tests. The limits of the 95% confidence interval (0.564—0.869) 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.

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 alder allergen 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 with alder allergens and the detection of specific IgE by the Rida Allergy Screen method.

In table 4 the results of the comparison of the determination of the presence of specific IgE to alder according to the Euroline test system with the data of skin testing by the blind test method are presented. Comparing two different types of specific allergic diagnosis by the method of establishing correlation relations to alder, 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 77.3% — 68 cases).

The results of two different methods of specific allergic diagnosis for the determination of sensitization to alder allergen are in part identical, but a certain asymmetry of the differences in the results of skin testing by the blind 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 alder through the construction of the confidence interval (table 5) showed that the coefficient indicates a satisfactory agreement (r = 0.409) of the findings of the two different tests. The limits of the 95% confidence interval (0.218—0.587)

exclude 0, which indicates the accuracy of the correspondence. The lower limit lies in the range of moderate coherence, and the upper one — in the area of good 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 alder allergen are shown in table 6.

Table 6. **Results of «null hypothesis» checking between the results of skin testing and Euroline to alder**

Asymptotic error kappa H_0, ($\sqrt{var_0(\kappa)}$)	0.113
Z	3.744
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.

Thus, between the data of skin testing with alder allergen and of specific IgE detection 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 alder by Rida Allergy Screen and Euroline, we conducted a comparative analysis according to the Blend-Altman charts. The comparison results are shown in Figure.

First, the systematic error of measurement results is 1.4 ku/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 7.23, which is significantly compared with the values themselves. Thirdly, there is a certain dependence of the difference in measurements on the 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 Allergy Screen and Euroline have a systematic difference in rates (1.4 kU/l).

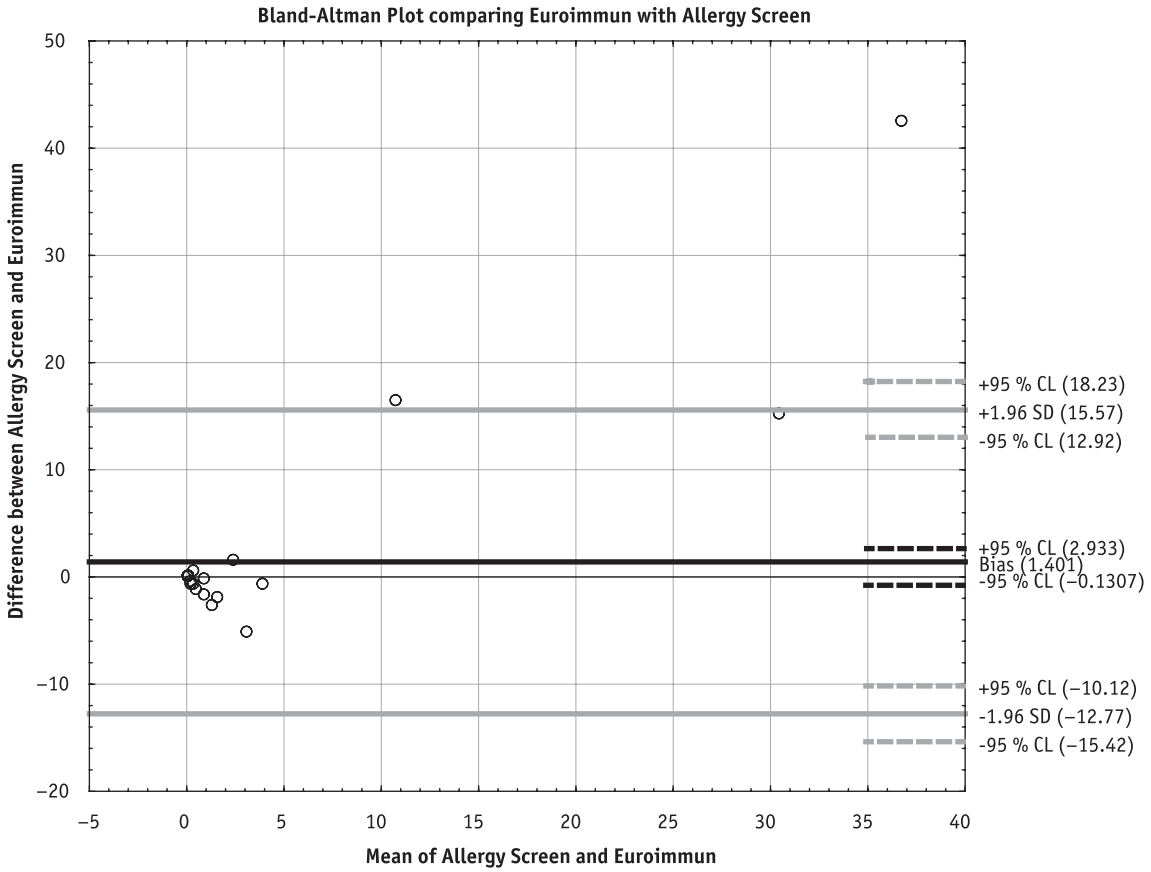

Figure. **Bland — Altman plot to determine the specific IgE to alder by tests Rida AllergyScreen and Euroline**

Conclusions

Between the data of skin testing with alder allergen and detection of specific IgE by the Rida Allergy Screen method, there is good overall agreement between the results, there is satisfactory agreement between the results of the research between the data of skin testing with alder allergen and the detection of specific IgE by the Euroline method.

No conflicts of interest.

References

1. Зайков С.В., Богомолов А.Е. Современные подходы к лабораторной диагностике аллергических заболеваний // Новости медицины и фармации.— 2013, № 14. — 25—34.
2. Heinzerling L., Mari A. et al. The skin prick test — European standarts // Clin. Transl. Allergy.— 2013.— Vol. 3.— P. 3.
3. Burbach G., Heinzerling L. et al. GA(2) LEN skin test study II: clinical relevance of inhalant allergen sensitizations in Europe // Allergy.— 2009.— Vol. 64 (10).— P. 1507—1515.
4. Maeda Y., Ono E. Correlations between alder specific IgE and alder-related tree pollen specific IgE by RAST method // Allergol. Int.— 2008.— Vol. 57 (1).— P. 79—81.
5. Kalogjera L. Allergic rhinitis in adults // Acta Med. Croatica.— 2011.— Vol. 65 (2).— P. 181—187.

А.Є. Богомолов
Вінницький національний медичний університет імені М.І. Пирогова

Діагностична цінність методів визначення сенсибілізації до алергену вільхи (Alnus incana) у осіб з респіраторною алергією

Мета роботи — вивчити параметри діагностичної цінності (специфічності і чутливості) шкірного тестування і серологічного визначення специфічного IgE до алергену вільхи у пацієнтів з респіраторною алергією.

Матеріали та методи. 88 пацієнтів з алергійним ринітом та/або атопічною бронхіальною астмою обстежено за трьома методами специфічної алергійної діагностики (*in vivo* та *in vitro*) відповідно до рекомендацій комітету з етики Вінницького національного медичного університету імені М.І. Пирогова. Причому всі обстеження проведено поза періодом загострення. Критеріями введення були діагноз алергійного риніту та/або атопічної бронхіальної астми (як інтермітуючі, так і персистуючі) з доведеною чутливістю до алергену вільхи. Прик-тест виконували 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 таких правил: < 0,35 МО/мл (0-й рівень — негативний), 0,36—0,69 МО/мл (1-й рівень — граничний), 0,7—3,49 МО/мл (2-й рівень — дещо підвищений), 3,50—17,4 МО/мл (3-й рівень — помірно підвищений), 17,5—49,9 МО/мл (4-й рівень — високий), 50—100 МО/мл (5-й рівень — дуже високий) і > 100 МО/мл (6-й рівень — дуже високий).

Результати та обговорення. У обстежених пацієнтів сенсибілізація до алергену вільхи склала 25,0 % (22 випадки) за специфічним IgE Rida Allergy Screen, 13,6 % (12 випадків) за специфічним IgE по Euroline і 27,3 % (24 особи) за даними шкірного прик-тесту методом сліпого тесту з відповідним алергеном. Результати аналізу узгодженості результатів двох різних методів алергійної діагностики для визначення сенсибілізації до вільхи через побудову довірчого інтервалу показали, що коефіцієнт вказує на задовільну узгодженість (r = 0,409) результатів двох різних тестів.

Висновки. Результати двох систем визначення специфічного IgE до алергену вільхи, за даними Rida Allergy Screen і Euroline, мають систематичну різницю (1,4 кОд/л). Між результатами шкірного тестування з алергеном вільхи та визначенням специфічного IgE за тестом Rida Allergy Screen існує хороше узгодження, а між результатами шкірного тестування з алергеном вільхи та визначенням специфічного IgE за тестом Euroline — задовільне.

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

А.Е. Богомолов
Винницкий национальный медицинский университет имени Н.И. Пирогова

Диагностическая ценность методов определения сенсибилизации к аллергену ольхи (Alnus incana) у лиц с респираторной аллергией

Цель работы — изучить параметры диагностической ценности (специфичности и чувствительности) кожного тестирования и серологического определения специфического IgE к аллергену ольхи у пациентов с респираторной аллергией.

Материалы и методы. 88 пациентов с аллергическим ринитом и/или атопической бронхиальной астмой 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 Н.И. Пирогова, причем все вне острого периода. Критериями включения были диагноз аллергического ринита и/или атопической бронхиальной астмы (как интермиттирующего, так и персистирующего) с доказанной чувствительностью к аллергену ольхи. 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) и > 100 МЕ мл-1-уровня 6 (очень высокие).

Результаты и обсуждение. У обследованных пациентов сенсибилизация к аллергену ольхи составила 25,0 % (22 случая) по специфическому IgE Rida Allergy Screen, 13,6 % (12 случаев) по специфическому IgE по Euroline и 27,3 % (24 человека) по данным кожного прик-теста методом слепого теста с соответствующим аллергеном. Результаты анализа согласованности результатов двух разных методов аллергической диагностики для определения сенсибилизации к ольхе через построение доверительного интервала показали, что коэффициент указывает на удовлетворительную согласованность (r = 0,409) результатов двух разных тестов.

Выводы. Результаты двух систем определения специфического IgE к аллергену ольхи, по данным Rida Allergy Screen и Euroline, имеют систематическую разницу в результатах (1,4 кЕд/л). Между результатами кожного тестирования с аллергеном ольхи и определением специфического IgE тестом Rida Allergy Screen существует хорошее согласие, между результатами кожного тестирования с аллергеном ольхи и определением специфического IgE тестом Euroline — удовлетворительное.

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

Контактна інформація:

Богомолов Артемій Євгенович, к. мед. н., доцент кафедри фтизіатрії з курсом клінічної імунології та алергології
orcid.org/0000-0002-5336-4858
21000, м. Вінниця, вул. М. Пирогова, 56
E-mail: art.bogomolov@gmail.com

Стаття надійшла до редакції 18 травня 2019 р.