DEFINITION OF SENSITIZATION TO POLLEN ALLERGENS WORMWOOD AND HAZEL IN PATIENTS WITH RESPIRATORY ALLERGIC DISEASES BY IMMUNOBLOT AND MULTIPLEX COMPONENT TEST

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Specific diagnosis of respiratory allergic diseases is an indispensable component of allergology, which must be used at the stage of diagnostic search in a patient with allergopathology [1-3].

The place of each of these methods in the patient’s itinerary, however, is being actively discussed with the advent of new and new methods available for routine practice [4,5].

The aim of our study was to evaluate the diagnostic parameters of immunoblot and ImmunoCAP ISAC methods to determine allergic sensitization to wormwood and hazel in patients with respiratory allergic diseases – allergic rhinitis and bronchial asthma.

Materials and methods

In this study, 40 patients with bronchial asthma and/or allergic rhinitis were examined by two different methods of specific allergic diagnosis (in vitro). The study was open-ended, comparative. All the patients were interviewed and signed a letter of informed consent to participate in the study. All women were interviewed for possible pregnancy.

Quantitative determination of specific IgE in the serum was performed using the RIDA® AllergyScreen immunoblot method (R-Biopharm AG, Germany) at the private laboratory of “Allergo-Immunological Center KPP”.

The RIDA® AllergyScreen test (panels 1, 2, 3, 4) is based on the immunoblot principle. Specific allergens, corresponding to the composition of the panel, are deposited on the surface of nitrocellulose membranes (strips). IgE antibodies specific to these allergens present in the blood samples of patients react with antigens, thereby providing at the second stage of incubation the attachment of antibodies to human IgE conjugated with antibodies (exhibiting antibodies) to strips of allergens on strips. For the testing, we used panel 2 (respiratory), which contained the following allergens: house dust (Derm. Pteronyssinus, Derm. Farinae), alder, birch, hazel, oak, herb mixture, rye, wormwood, plantain, epidermal allergens of horse, dog, guinea pig, hamster, rabbit, Alternaria alternata, Penicillium notatum, Cladospor. herbarum, Aspergillus fumigatus.

According to the existing criteria, the results were interpreted depending on the concentration of specific IgE as a class 0–6. Class ≥1 was interpreted as positive. In clinical practice, allergens with results greater than grade 2 (sIgE ≥ 0.7 Ku/l) were considered positive. After receiving the results, all data were converted to nominal scales.

ImmunoCAP Allergy Chip (Phadia AB, Thermo Fisher Scientific, Sweden) – a method for quantitative measurement of IgE-antibodies to various allergens in the serum, developed by Phadia, examination by the ImmunoCAP ISAC method was performed at the Clinic of Allergology and Immunology. The method allows one to determine at the same time the presence of class E antibodies to 112 allergen components from 51 sources of allergens – food, pollen, epidermal allergens of animals, allergens of fungi and insects, house dust mites on the immune solid-phase allergy chip (ISAC). After the results were obtained, all data were also converted to nominal scales.

Statistical processing of the results, plotting and distribution of statistical analysis values was performed using IBM SPSS Statistics 21 software package.

Results
In the main phase of the study 40 people aged 19-42 years were selected, the average age of the group was 31.6 years (95% CI: 26.6; 41.6), gender distribution –60.0% of men and 40.0% of women.

Among the patients, allergic sensitization to wormwood was 27.5% (11 people) by the presence of specific IgE by the Rida AllergyScreen, 25.0% (10 people) by the presence of specific IgE by the ImmunoCAP ISAC; sensitization to hazel allergen was 27.5% (11 people) by the presence of specific IgE by the Rida AllergyScreen, 30.0% (12 people) by the presence of specific IgE by the ImmunoCAP ISAC.

Table 1 shows the results of constructing the affinity table of the results of determining specific IgE to wormwood.

Table 1
Summary table of the affinity of the results of determination of specific IgE to allergen wormwood

<table>
<thead>
<tr>
<th>AllergyScreen</th>
<th>«negative»</th>
<th>«positive»</th>
</tr>
</thead>
<tbody>
<tr>
<td>«positive»</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 2 shows the results of constructing the affinity table for the determination of specific IgE to hazel.

Table 2
Summary table of the affinity of the results of determination of specific IgE to hazel allergen

<table>
<thead>
<tr>
<th>AllergyScreen</th>
<th>«negative»</th>
<th>«positive»</th>
</tr>
</thead>
<tbody>
<tr>
<td>«positive»</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>12</td>
</tr>
</tbody>
</table>

The expected frequency of the sign in one of the cells (25%) of the summary tables below is 5 and we used the Fisher exact two-way criterion method to analyze the relationship. As a result of statistical data processing, it is determined that the absolute value of the exact two-sided Fisher criterion for the wormwood allergen corresponds to the two-sided asymptotic significance of 0.024, which accepts the null hypothesis. At such levels of asymptotic significance, the difference between the measurement groups is the null hypothesis, and, therefore, no statistically significant difference was found between survey methods for the determination of specific IgE for allergen wormwood.

In the case of hazel allergy sensitization by two different methods, the expected symptom frequency in one of the cells (25%) of the summary tables below 5 was also applied to analyze the relationship of the Fisher exact two-sided criterion. As a result of the statistical data processing, it is determined that the absolute value of the Fisher’s exact two-sided criterion for hazel allergen corresponds to a two-sided asymptotic significance of 0.001, which rejects the null hypothesis. At such levels of asymptotic significance, the difference between the measurement groups is null and void, and therefore a statistically significant difference was found between survey methods for the determination of specific hazelnut IgE.

For a complete analysis of the diagnostic parameters of the methods we calculated the sensitivity, specificity, accuracy, positive and negative predictive value of the immunoblot against ImmunoCap ISAC. The results of these parameters for birch allergen are shown in table 3.
Table 3  
Diagnostic value of immunoblot versus Immunocap ISAC to determine specific IgE for allergen wormwood

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100.00%</td>
<td>69.15% to 100.00%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.67%</td>
<td>82.78% to 99.92%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>90.91%</td>
<td>59.28% to 98.57%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100,00%</td>
<td>---</td>
</tr>
<tr>
<td>Accuracy</td>
<td>97.50%</td>
<td>86.84% to 99.94%</td>
</tr>
</tbody>
</table>

As we can see, the immunoblot has a high sensitivity and negative predictive value (100.00% in both cases) compared to ISAC, a high specificity of 96.67% (95% CI: 82.78; 99.92), but a positive predictive value was 90.91% (95% CI: 59.28; 98.57) and accuracy was 97.50% (95% CI: 86.84; 99.94).

Sensitivity is the ability of the diagnostic method to produce the correct result, which is defined as the proportion of true positive results among all tests performed. Specificity is the ability of the diagnostic method not to give in the absence of the disease false positive results, which is defined as the proportion of true negative results among healthy individuals in the group of subjects. Therefore, immunoblotting for the determination of specific IgE for allergen wormwood compared to Immunocap ISAC is highly sensitive, highly specific and highly accurate. Special attention should be paid to the positive predictive value, which is defined as the percentage of true positive tests among all positive tests obtained as a result of the examination. In this case, it was 90.91%, which indicates the possibility of a false positive result in every tenth case. The results of these parameters for hazelnut allergen are given in table 4.

Table 4  
Diagnostic value of immunoblot versus Immunocap ISAC for determination of specific IgE for hazel allergen

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>75.00%</td>
<td>42.81% to 94.51%</td>
</tr>
<tr>
<td>Specificity</td>
<td>92.86%</td>
<td>76.50% to 99.12%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>81.82%</td>
<td>53.23% to 94.68%</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>89.66%</td>
<td>76.39% to 95.87%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>87.50%</td>
<td>73.20% to 95.81%</td>
</tr>
</tbody>
</table>

As we can see, immunoblot compared to ISAC for the determination of specific hazards of hazel allergen has a relatively high specificity and negative predictive value (92.86% and 89.66%), but the sensitivity and positive predictive value have a value of 75.00% (95 % CI: 42.81; 94.51) and 81.82% (95% CI: 53.23; 94.68), respectively, and the accuracy of the method is 87.50% (95% CI: 73.20; 95.81).

Therefore, the immunoblotting method for the determination of specific IgE for hazel allergen compared to Immunocap ISAC is highly specific, but medium-sensitive. Special attention should be paid to the predictability of negative and positive results, which amounted to 89.66% and 75.00%, which indicates a significant possibility of false negative and false positive result.
Discussion

The higher the sensitivity of the test, the more often it will detect the disease, so it is more effective. At the same time, if such a highly sensitive test proves negative, then the presence of the disease is unlikely. Therefore, they should be used to exclude diseases. Because of this, highly sensitive methods are often called identifiers, and it is recommended to use them in the early stages of the diagnostic process, when you need to narrow down the range of suspected diseases. On the other hand, the higher the specificity of the method, the more reliable it is to confirm the disease, and therefore, it is more effective. Highly specific methods are called discriminators in diagnosis. Such studies are effective in the second stage of diagnosis, when the range of suspected diseases is narrowed and it is necessary to prove with great certainty the presence of the disease.

The results of the analysis showed high sensitivity, specificity and accuracy of immunoblotting for the determination of IgE specific for allergen wormwood compared to Immunocap ISAC, as well as high specificity, moderate sensitivity and accuracy for determination of specific IgE for the allergen of hazel.

In our opinion, there may be a number of factors that may cause such differences. The main point is that the increase in the sensitivity of the test is inevitably accompanied by the loss of its specificity, and vice versa, the increase in specificity is associated with a decrease in its sensitivity. Diagnosis techniques with high sensitivity rarely «miss» patients who have the disease, and techniques with high specificity do not qualify as healthy patients. On the other hand, the reduced sensitivity of the method may be directly related to the immunoblotting technique itself, since photometric analysis depends in practice on many factors – the quality of the scan, the correct preparation procedures, the presence or absence of calibration, and the like.

The negative predictive value is defined as the frequency of its coincidence with the absence of the disease. This criterion thus shows how likely the patient is to be healthy if the results of the study are negative. In our case, the negative predictive value for the immunoblot method is generally related to the sensitivity of the method.

Summary

As we can see, for the determination of specific IgE to wormwood allergen the immunoblot has a high sensitivity and negative predictive value (100.00% in both cases) compared to ISAC, a high specificity of 96.67% (95% CI: 82.78; 99.92), but a positive predictive value was 90.91% (95% CI: 59.28; 98.57) and accuracy was 97.50% (95% CI: 86.84; 99.94).

Immunoblot compared to ISAC for the determination of specific IgE to hazel allergen has a relatively high specificity and negative predictive value (92.86% and 89.66%), but the sensitivity and positive predictive value were 75.00% (95% CI: 42.81; 94.51) and 81.82% (95% CI: 53.23; 94.68), respectively, and the accuracy of the method was 87.50% (95% CI: 73.20; 95.81).

РЕЗЮМЕ

ВИЗНАЧЕННЯ СЕНСИБІЛІЗАЦІЇ ДО ПІЛКОВИХ АЛЕРГЕНІВ ПОЛИНУ ТА ЛІЩИНИ У ХВОРИХ НА РЕСПІРАТОРНІ АЛЕРГІЧНІ ЗАХВОРЮВАННЯ ЗА ДОПОМОГОЮ ІМУНОБЛОТУ ТА МУЛЬТИПЛЕКСНОГО КОМПОНЕНТНОГО ТЕСТУВАННЯ

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Метою нашого дослідження було оцінити діагностичні параметри методів імуноблоту та Immunocap ISAC для визначення сенсібілізації до алергенів полину та ліщини у пацієнтів з респіраторними алергічними захворюваннями – алергічним ринітом та бронхіальною астмою.

Матеріали та методи. У процесі цього дослідження двома різними методами специфічної алергологічної діагностики (in vitro) було обстежено 40 пацієнтів, хворих на бронхіальну астму та / або алергічний риніт. Дослідження було відкритим, порівняльним. Кількісне визначення специфічних IgE в сироватці крові проводили за допомогою методу імуноблоту «RIDA® AllergyScreen» (R-Biopharm AG, Німеччина) на базі приватної лабораторії ТОВ «Алерго-імунологічний центр КПП». Обстеження методом Immunocap ISAC було виконане у «Клініці
Результаты и обсуждение. Серед пациентов аллергическая сенсибилизация до полыни составила 27,5% (11 человек) при наличии специфического IgE методом Rida AllergyScreen, 25,0% (10 человек) при наличии специфического IgE методом Immunocap ISAC; сенсибилизация к аллергену орешника составила 27,5% (11 человек) при наличии специфического IgE на Rida AllergyScreen, 30,0% (12 человек) при наличии специфического IgE от Immunocap ISAC.

Определение специфического IgE к аллергену полыни иммуноблот имеет высокую чувствительность и прогнозируемость отрицательного результата (100.00% в обоих случаях) по сравнению с ISAC, высокую специфичность 96,67% (95% ДИ: 82,78; 99,92), но прогнозируемость положительного результата составила 90,91% (95% ДИ: 59,28; 98,57), а точность – 97,50% (95% ДИ: 86,84; 99,94).

Выводы. Иммуноблот, по сравнению с ISAC для определения специфического IgE к аллергену орешника, имеет относительно высокую специфичность и прогнозируемость отрицательного результата (92,86% и 89,66%), но чувствительность и прогнозируемость положительного результата составляли 75,00% (95% ДИ: 42,81; 94,51) и 81,82% (95% ДИ: 53,23; 94,68), соответственно, а точность метода составила 87,50% (95% ДИ: 73,20; 95,81).

Ключевые слова: аллергия, иммуноблотинг, IgE, молекулярная аллергология.

**SUMMARY**

**DEFINITION OF SENSITIZATION TO POLLEN ALLERGENS WORMWOOD AND HAZEL IN PATIENTS WITH RESPIRATORY ALLERGIC DISEASES BY IMMUNOBLOT AND MULTIPLEX COMPONENT TEST**

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The aim of our study was to evaluate the diagnostic parameters of immunoblot and Immunocap ISAC methods to determine allergic sensitization to wormwood and hazel in patients with respiratory allergic diseases – allergic rhinitis and bronchial asthma.
**Materials and methods.** In this study, 40 patients with bronchial asthma and / or allergic rhinitis were examined with two different methods of specific allergic diagnosis (*in vitro*). The study was open-ended, comparative. Quantitative determination of specific IgE in the serum was performed using the RIDA® AllergyScreen immunoblot method (R-Biopharm AG, Germany) on the basis of the private laboratory of LLC “Allergy-Immunological Center KPP”. Immunocap ISAC testing was performed at “Forpost Allergy and Immunology Clinic”.

**Results and Discussion.** Among patients, allergic sensitization to wormwood was 27.5% (11 people) in the presence of specific IgE by the Rida AllergyScreen method, 25.0% (10 people) in the presence of specific IgE using ImmunoCAP ISAC; sensitization to hazel allergen was 27.5% (11 people) in the presence of specific IgE on Rida AllergyScreen, 30.0% (12 people) in the presence of specific IgE from ImmunoCAP ISAC.

For the determination of specific IgE to wormwood allergen the immunoblot has a high sensitivity and negative predictive value (100.00% in both cases) compared to ISAC, a high specificity of 96.67% (95% CI: 82.78; 99.92), but a positive predictive value was 90.91% (95% CI: 59.28; 98.57) and accuracy was 97.50% (95% CI: 86.84; 99.94).

**Conclusions.** Immunoblot compared to ISAC for the determination of specific IgE to hazel allergen has a relatively high specificity and negative predictive value (92.86% and 89.66%), but the sensitivity and positive predictive value were 75.00% (95 % CI: 42.81; 94.51) and 81.82% (95% CI: 53.23; 94.68), respectively, and the accuracy of the method was 87.50% (95% CI: 73.20; 95.81).

**Keywords:** allergy, immunoblotting, IgE, molecular allergy.

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