

UDC: 612.017.3:616-079

A. E. Bogomolov

SI «Vinnitsa national medical university named after N. I. Pyrogov»

Correlation between skin prick test and MAST-immunoblot results in patients with airway allergies

Key words: allergy, diagnostics, skin prick test, western blot, sensitivity, specificity, allergic rhinitis, bronchial asthma.

According to World Allergy Organization, the prevalence of allergic diseases worldwide is rising dramatically in both developed and developing countries. About 30 % of adults and 35 % of the child's population of the planet suffer from allergies, and 40 % of cases occur in countries with high economy. These diseases include asthma; rhinitis; anaphylaxis; drug, food, and insect allergy; eczema; and urticaria (hives) and angioedema. This increase is especially problematic in children, who are bearing the greatest burden of the rising trend, which has occurred over the last two decades. In spite of this increase, even in the developed world, the care of patients with allergic diseases is fragmented and far from ideal.

This increase has been observed in both industrialized and developing countries [1]. World Health Organization estimates that 400 million people suffer from AR and 300 million from asthma. By 2025, additional 100 million will be affected by asthma [2]. This sharp increase in asthma and other allergic diseases between early 1960's and late 1980's is perceived to be a consequence of an intense migration from rural to urban regions, from poor, developing countries to rich, but heavily industrialized regions of Europe, Asia and Americas.

Respiratory allergies among the all possible forms of allergy occupy the top places and are the most common. The works of many local researchers from Ukraine showed

great difference when comparing official statistics of respiratory allergy forms in our country with prevalence of similar diseases on the territory of the other world countries. For example, in Ukraine we diagnose only every 15–60 patient with allergic rhinitis and 10–20 with asthma [3]. According to other authors [4], promptly identified patients with respiratory allergy are only 20–25 % for allergic rhinitis and 5–10 % for asthma.

Objectively difficulties with detection of respiratory allergy are largely related to diagnostic reasons. Optimal schemes of the patient examination from many methods of instrumental and laboratory diagnostics always represent a difficulty in each individual case.

Diagnosis of allergic diseases in most is based on the sequential (pass) application of the following research methods:

- anamnestic (collection of complaints and anamnesis data);
- physical;
- skin tests with allergens;
- provocative (bronchial, nasal, conjunctival, sublingual and other) tests with allergens;
- laboratory tests (non-specific general laboratory diagnostic methods and specific laboratory tests with allergens);

- functional tests (spirometry, peak flow measurement, nasal and tracheobronchial tree endoscopy, radiography and tomography of the paranasal sinuses and chest organs etc.);
- consultations of other specialists.

According to many authors [5], the «gold standard» diagnostic method for IgE – dependent reactions is a skin prick testing. However, the main contraindications for skin prick testing are:

- severe or uncontrolled allergic disease;
- acute intercurrent infections;
- chronic diseases in decompensation phase;
- exacerbation of chronic infection foci;
- active phase of chronic infectious diseases (tuberculosis, syphilis, brucellosis, and others);
- malignant and systemic autoimmune diseases;
- nervous and mental diseases, convulsions;
- pregnancy, lactation, menstruation;
- acquired immunodeficiency syndrome;
- anamnesis data of an acute reaction to a particular allergen;
- anaphylactic shock, Lyell syndrome, Stevens-Johnson syndrome history;
- currently therapy by antihistamines, mast cell membranes stabilizers, systemic or local administration of glucocorticoid drugs (inhaled and nasal glucocorticosteroid drugs do not affect the test results);
- early childhood.

In such cases, as well as in several other cases, the patient needs the usage of different methods of laboratory diagnostics. Laboratory diagnostics of IgE – dependent allergic reactions provides usage of the modern effective methods, characterized by a high (acceptable) standardization and reproducibility, which is especially important in the following situations:

- inability to cancel usage of an antiallergic drugs;
- severe reactions, anaphylactic reactions in anamnesis;
- examination of the patient during an exacerbation of disease;
- diagnostics in early childhood;
- multiple sensitization by inability to conduct in vivo tests with all prospective hypertension and limited terms of examination;
- changes in the reactivity of the skin (false-negative and false-positive results of skin testing);
- a number of differential diagnosis cases;
- the period of an allergen-specific immunotherapy.

This study was designed to confirm whether a MAST-immunoblot assay can reliably diagnose allergic rhinitis and / or asthma.

Materials and Methods

45 patients with allergic rhinitis and/or asthma were included in the study to investigate the sensitivity of western-blot methods comparing with skin prick testing. The study was open, prospective, comparative. All patients included in the supervision group, were interviewed and signed a letter of the study participation. All the women were interviewed for possible pregnancy.

The main criteria for patient inclusion in the study were:

- 1) age – 18 to 60 years;

2) controlled intermittent asthma and mild persistent asthma according to criteria specified in GINA 2010 and Adopted clinical guidelines based on evidence «Bronchial asthma», approved by the Ministry of Health of Ukraine № 868 of 08.10.2013;

3) allergic origin of asthma (increased total IgE level in the patient's blood);

4) seasonal or perennial allergic rhinitis;

5) clinically significant mono- or polysensitization to allergens by results of skin prick testing with these allergens;

Exclusion criteria were:

1) age less than 18 and older than 60 years;

1) uncontrolled asthma, including persistent moderate and severe;

2) the presence of concomitant severe allergic disease and severe allergic reactions in history;

3) the presence of concomitant severe acute and chronic somatic pathologies;

5) pregnancy and breastfeeding;

6) taking simultaneous participation in another clinical trial;

7) patient's refusal to participate in this study.

The group of patients who were participated in this study consist of 45 patients. Among them were 24 patients (53,3 %) with allergic rhinitis and 21 patient (46,7 %) with asthma, 35,5 % of them were men and 64,5 % were women aged 18–53 years (mean age $38,3 \pm 6,5$ year).

Complaints and medical history collection, physical examination, functional tests and patients skin tests were performed in the office of an allergist and functional dia in the Private clinics «Allergoimmunocentr KPP».

Collection of allergic history was conducted by the standard scheme predicted by determining the presence of a history of atopic dermatitis, allergic rhinitis, including «atopic march» and other possible clinical manifestations of allergic disease, details of complaints for preliminary determination of cause and significant allergen establishment of preliminary diagnosis for further laboratory and functional test. There were also collecting and detailing of hereditary anamnesis of allergic patients.

As a diagnostic solutions of allergens in our study we used the original Ukrainian manufacture Vinnitsa allergens MP «Immunolog» – *Dermatophagoides pteronissynus*, *Dermatophagoides farinae*, alder, birch, hazel, oak, rye, wormwood, plantain, hair cats, dogs, hamster, rabbit, *Alternaria alternata*, *Cladosporium*, *Aspergillus mxt* (*A. fumigatus*, *A. niger*), *Penicillium sp. div.*, which are a water-salt solutions of protein-polysaccharide complexes isolated from the appropriate raw materials by liquid Evans-Koch extraction. Form of issue of allergens performed in liquid form 2 ml allergen (mixed allergen) in a brown glass bottles. Selected dosage and method of use of drugs were followed by instructions for medical use.

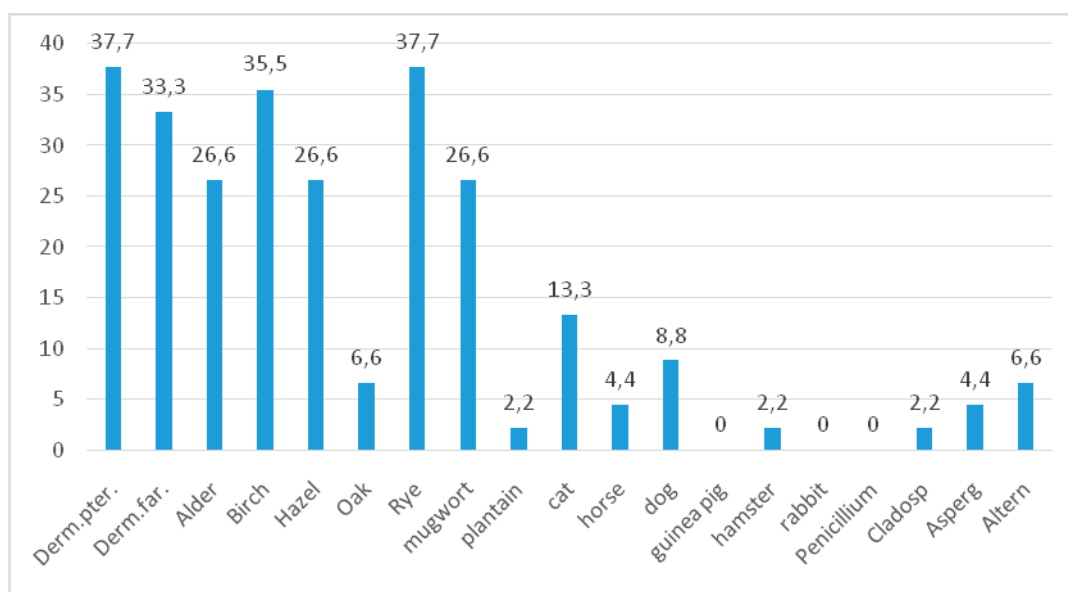
Performance technology of skin tests and evaluation of results were performed according to the order of the Ministry of Health and Medical Sciences of Ukraine № 127/18 of 02.04.2002 «On the organization to implement of modern technologies for diagnosis and treatment of allergic diseases». After dropping positive or negative control allergen extracts,

epicutaneous pricks were performed. After 20 minutes, using histamine as a positive contrast solution, we compared the average value of the longest diameter and the longest diameter in the perpendicular plane of the former longest diameter with the positive contrast solution.

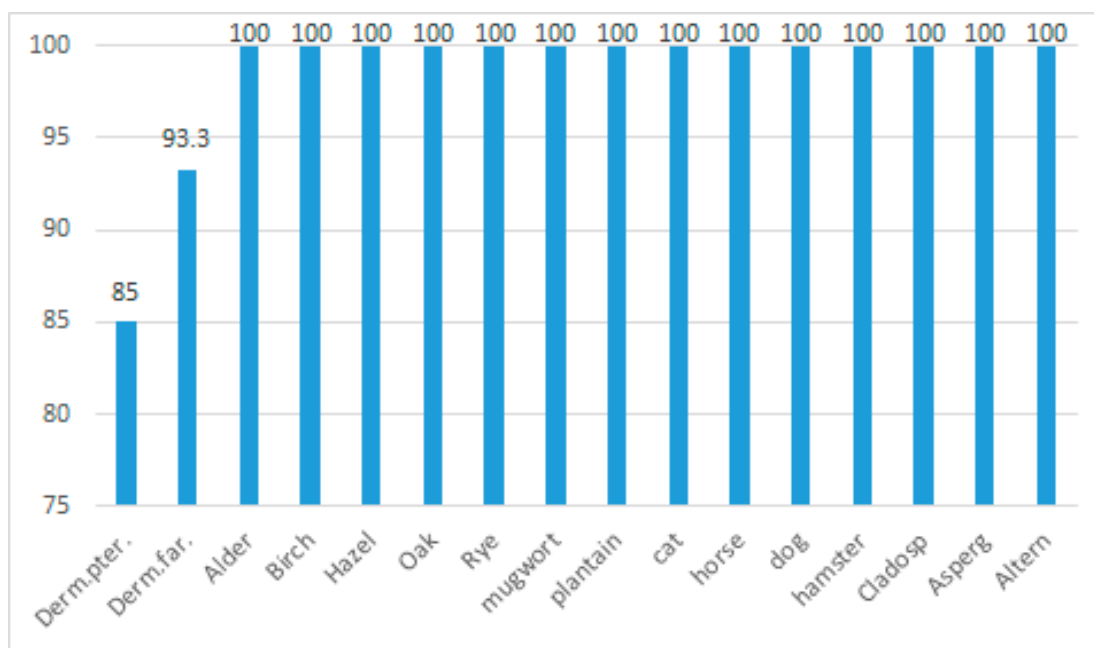
Quantitative determination of specific IgE in serum was carried out by immunoblot «RIDA® AllergyScreen» (R-Biopharm AG, Germany) on the basis of private laboratory inPrivate clinics «Allergoimmunocentr KPP».

Standards of specific IgE concentrations ranged from 0,35 to 100 kU/l. Two hundred and fifty uL of patient serum were added to reaction wells of inhalant panel which contain 20 kinds of allergens («Respiratory allergens» – Art. No.: A2242 – positive control, *Dermatophagoides pteronyssinus*, *Dermatophagoides*

farinae, alder, birch, hazel, oak (pollens), mixed grass, rye (pollen), mugwort, plantain, cat, horse, dog, guinea pig, golden hamster, rabbit, *Penicillium notatum*, *Cladosporium herbarum*, *Aspergillus fumigatus*, *Alternaria alternata*). After 45 min of incubation at room temperature and wash, 250 uL of Biotin tagged anti-IgE were added. After 45 minutes of incubation at room temperature and wash, 250 uL of streptavidin conjugate were added. Twenty minutes of incubation at room temperature and wash, 250 uL of luminescent reagent were added. After 20 minutes of incubation, results were scanned with CCD camera (RIDA X-Screen Reader) and interpreted as class 0–6. Class ≥ 1 was interpreted as positive. In clinical practice, allergens with results greater than that of class 2 (sIgE $\geq 0,7$ kU/L) were considered positive.



Picture 1. Sensitization frequency by skin prick testing, %



Picture 2. Western-blot sensitivity in comparison with skin-prick testing results, %

After counting patients who were true positive (TP; positive results in both tests), true negative (TN; negative results in both tests), false positive (FP; positive in AS but negative in SPT), and false negative (FN; vice versa), the sensitivity, specificity, and efficacy of AS were also calculated according to the following formula using the results of SPT as the standard; sensitivity = $TP/(TP+FN)$, specificity = $TN/(TN+FP)$, and efficacy = $(TP+TN)/(TP+TN+FP+FN)$. The agreement rate was calculated as the ratio of the number of patients in whom the difference between classes of AS and SPT was less than 2 over the total number of patients. Statistical analysis was done using Statistica base 12,0 (Dell Software Company; Aliso Viejo, CA, USA).

Results and conclusions

According to the results of skin prick testing monosensitization was found in 6 patients (13,3 % of cases). The most frequently observed sensitization was to pollen allergens (rye – 17 patients (37,7 % of cases), birch – 16 patients (35,5 % of cases), alder – 12 patients (26,6 % of cases)) and household mites allergens (*Dermatophagoides pteronissynus* – 17 patients (37,7 % of cases) and *Dermatophagoides farinae* – 15 patients (33,3 % of cases)). The results of skin testing of patients are shown in picture 1.

The most frequently in patients of the supervision group we observed sensitization to allergens of dust mites and pollen groups, less frequently – to allergens of opportunistic fungi. This result was probably due to the peculiarities of the immune system response to fungal allergens (in this case reaction can take place by the mechanisms of immediate hypersensitivity and delayed hypersensitivity).

Since immunoblot panel did not contain allergens decryption accurate proportions in mixed pollen allergen, comparing the diagnostic efficiency of the method for testing results of this allergen is not calculated for objective reasons. The data of western-blot sensitivity calculation are shown in picture 2.

As can be seen from the data, sensitivity compared with testing method prick test is high for all investigated allergens. Reduced sensitivity indices registered for dust mites allergens (85,0 % and 93,3 %). Average sensitivity of western-blot over skin prick testing was 98,6 %. The test is highly sensitive for the determination of specific IgE to pollen and epidermal middle-sensitive for dust mites allergens.

The data obtained by the calculation of western-blot specificity in comparison over skin prick testing are shown in picture 3.

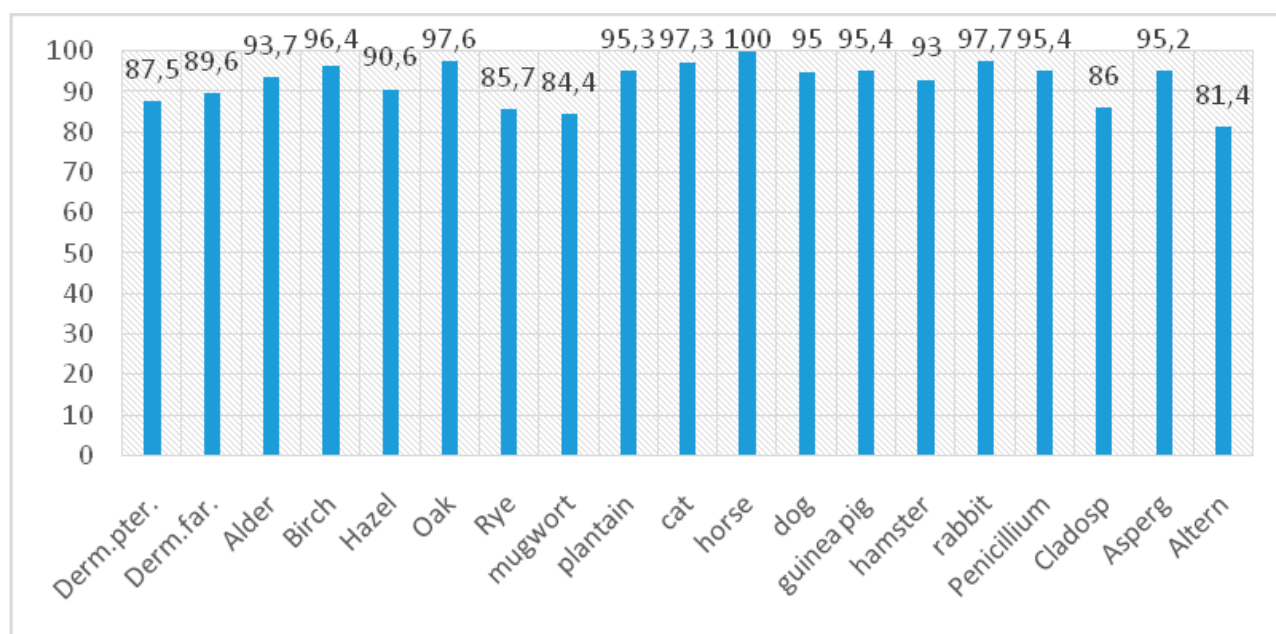
The specificity of the method was high for some pollen allergens (oak – 97,6 % birch – 96,4 %) and epidermal allergens (cat – 97,3 %), the average for some micro-fungi (*Penicillium* – 95,4 %, *Aspergillus* – 95,2 %), epidermal (95,0 coat dogs %), but for most pollen allergens and dust mites allergens it is low (84,4 % – 93,0 %).

Data of used method accuracy shown on picture 4.

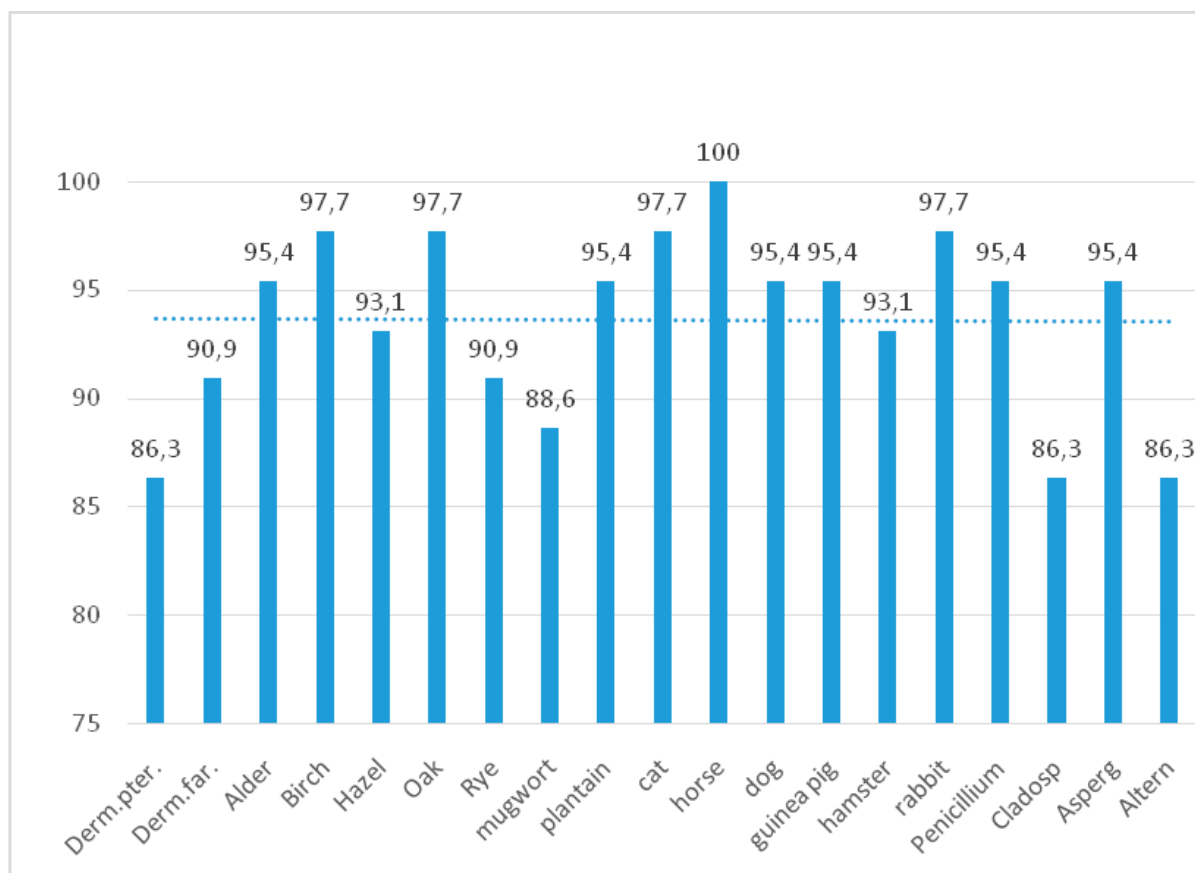
The accuracy of the method was near 100 % only for certain allergens, while most pollen allergens accuracy was at least 95 %.

Conclusions

The allergens that cause the allergy are the most important factors in selecting SIT as the main treatment method. The standard method for allergy diagnosis is the skin prick test (SPT), which has high sensitivity and good reproducibility. However, it requires multiple skin pricks and the results can be influenced by recent medications. Various methods to measure serum-specific IgE (sIgE) have been developed to overcome these limitations, and upon development were confirmed to have good reliability and correlation with SPT. But still specificity of this methods are not very high, so future scientific thinking must present some ideas to increase this parameter.



Picture 3. Western-blot specificity in comparison with skin-prick testing results, %



Picture 4. Western-blot accuracy in comparison with skin-prick testing results, %.

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КОРЕЛЯЦІЯ МІЖ РЕЗУЛЬТАТАМИ ШКІРНОГО ТЕСТУВАННЯ МЕТОДОМ ПРИК-ТЕСТУ ТА ІМУНОБЛОТТИНГОМ У ПАЦІЄНТІВ З АЛЕРГІЧНИМИ ЗАХВОРЮВАННЯМИ ДИХАЛЬНИХ ШЛЯХІВ

А. Є. Богомолов

Резюме

Мета дослідження — порівняльний аналіз діагностичної цінності методів виявлення сенсibilізації (шкірного тестування та імуноблоттингу) у пацієнтів з алергічними захворюваннями дихальних шляхів.

Об'єкт дослідження: 45 пацієнтів з алергічним ринітом та / або atopічною бронхіальною астмою з клінічно значимою та під-

твердженою результатами прик-тестів сенсibilізацією до досліджуваних алергенів, віком від 18 до 60 років.

Матеріали та методи дослідження

Клініко-анамнестичний, фізикальний, функціональний, шкірне тестування методом постановки прик-тестів, лабораторний, статистичний.

Результати та їх обговорення

За результатами шкірного тестування методом прик-тесту найбільш часто спостерігалась сенсibilізація до пилоквих алергенів (жито — 17 пацієнтів (37,7 % випадків), береза — 16 пацієнтів (35,5 % випадків) та вільха — 12 пацієнтів (26,6 % випадків) та алергенів побутових кліщів (*Dermatophagoides pteronissynus* — 17 пацієнтів (37,7 % випадків) та *Dermatophagoides farinae* — 15 пацієнтів (33,3 % випадків)).

Специфічність методу імуноблоттингу є високою для деяких пилоквих алергенів (дуб — 97,6 %, береза — 96,4 %) та епідермальних алергенів (кішка — 97,3 %), середньою для деяких мікрогрибів (*Penicillium* — 95,4 %, *Aspergillus* — 95,2 %), епідермальних (шерсть собаки 95,0 %), проте для більшості пилоквих алергенів та алергенів кліщів домашнього пилу вона є низькою (84,4 % — 93,0 %).

Ключові слова: алергія, діагностика, шкірний прик-тест, імуноблоттинг, чутливість, специфічність, алергічний риніт, бронхіальна астма.

Науково-практичний журнал «Астма та алергія», 2015, №1

А. Є. Богомолов

канд. мед. наук,

ДУ «Вінницький національний медичний університет

імені М. І. Пирогова»

21018, Україна, Вінниця, вул. Пирогова, 56

тел.: +3 8(043) 256 6641; +3 8(097) 066 3555

e-mail: art.bogomolov@gmail.com

**КОРРЕЛЯЦИЯ МЕЖДУ РЕЗУЛЬТАТАМИ КОЖНОГО
ТЕСТИРОВАНИЯ МЕТОДОМ ПРИК-ТЕСТА
И ИМУНОБЛОТТИНГОМ У ПАЦИЕНТОВ
С АЛЛЕРГИЧЕСКИМИ ЗАБОЛЕВАНИЯМИ
ДЫХАТЕЛЬНЫХ ПУТЕЙ**

А. Е. Богомолов

Резюме

Цель исследования — сравнительный анализ диагностической ценности методов определения сенсибилизации (кожного тестирования и иммуноблоттинга) у пациентов с аллергическими заболеваниями дыхательных путей.

Объект исследования: 45 пациентов с аллергическим ринитом и/или atopической бронхиальной астмой с клинически значимой и подтвержденной результатами прик-тестов сенсибилизацией к рассматриваемым аллергенам, в возрасте от 18 до 60 лет.

Материалы и методы исследования

Клинико-anamnestический, физикальный, функциональный, кожное тестирование методом постановки прик-тестов, лабораторный, статистический.

Результаты и их обсуждение

По результатам кожного тестирования методом прик-теста наиболее часто наблюдалась сенсибилизация к пыльцевым аллергенам (рожь — 17 пациентов (37,7 % случаев), береза — 16 пациентов

(35,5 % случаев) и ольха — 12 пациентов (26,6 % случаев) и аллергенов бытовых клещей (*Dermatophagoides pteronissynus* — 17 пациентов (37,7 % случаев) и *Dermatophagoides farinae* — 15 пациентов (33,3 % случаев)).

Специфичность метода иммуноблоттинга является высокой для некоторых пыльцевых аллергенов (дуб — 97,6 %, береза — 96,4 %) и эпидермальных аллергенов (кошка — 97,3 %), средней для некоторых микроорганизмов (*Penicillium* — 95,4 %, *Aspergillus* — 95,2 %), эпидермальных аллергенов (шерсть собаки 95,0 %), однако для большинства пыльцевых аллергенов и аллергенов клещей домашней пыли она была низкой (84,4— 93,0 %).

Ключевые слова: аллергия, диагностика, кожный прик-тест, иммуноблоттинг, чувствительность, специфичность, аллергический ринит, бронхиальная астма.

Научно-практический журнал «Астма та аллергия», 2015, №1

А. Е. Богомолов

канд. мед. наук,

ГУ «Винницкий национальный медицинский университет

им. Н. И. Пирогова»

21018, Украина, Винница, ул. Пирогова, 56

тел.: +38(043)256-66-41; +38(097)066-35-55

e-mail: art.bogomolov@gmail.com