

**THE POSSIBILITIES OF MOLECULAR DIAGNOSIS OF ALLERGIC DISEASES**

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**KEYWORDS:** allergic diseases, molecular diagnostics, allergens, antibodies.**SUMMARY**

Molecular diagnosis of allergy (MDA) is becoming more common in everyday clinical practice, and today more than 130 allergen molecules are commercially available for testing specific immunoglobulin E (sIgE) in vitro. The results of all sIgE tests related to the molecular diagnosis of allergy should be analyzed taking into account the clinical history, since sensitization to the allergen does not always imply clinical manifestations. Clinicians and immunologists specializing in the field of allergology should be aware of all the latest advances in the molecular diagnosis of allergies. The MDA strategy is based on the identification of sensitization to allergens at the molecular level using natural purified or recombinant allergen molecules. Thus, molecular diagnostics technologies are becoming an integral part of clinical practice, which allow improving the accuracy of diagnosing and predicting the course of allergies, playing an important role in three key aspects of allergy diagnostics: they allow differentiating true sensitization and sensitization due to cross-reactivity in multivalent sensitization and thereby identifying allergens -inductors.

In recent years, immunological laboratory tests have become increasingly important in allergology, which can be divided into 2 large groups: non-specific (aimed at identifying common changes in the immune system in case of AD); specific (identification of antibodies and cells involved in the immunological phase of AR (Allergic reactions)).<sup>[4,8,9,12]</sup>

In diagnosing the causes of AD (Allergic diseases), the diagnostic value of specific IgE (sIgE) in a patient becomes more diagnostic in allergology. Methods for determining sIgE are primarily divided into those performed in vivo and in vitro. In vivo tests are used to determine the hypersensitivity (HS) to individual AI (allergens), but, unfortunately, have a number of not only analytical, but also serious clinical limitations. It is also possible to obtain false-negative results due to the development of specific anti-IgE antibodies of the IgG class, the possibility of binding part of the general level of IgE by cross-section AI, and binding of sIgE to mast cells before their detection 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 determining the list of allergens necessary for research, which depends on the competence of the doctor. Currently, there are not just several methods, but several fundamentally different approaches to the definition of sIgE, the rational choice of which is determined both by the clinical task and the declared advantages of one method or another, and by

some specific limitations that exist for most of them.<sup>[7,12]</sup> Until recently, the fact that they are aimed at registering allergen-specific IgE antibodies circulating in the blood has served as a significant limitation of the use of in vitro methods. Since the life expectancy of free IgE does not exceed several days, the greatest reliability of the results of in vitro studies is achieved when analyzing samples taken in the acute phase of AD. Currently, there are methods aimed at registering allergen-specific IgG, IgG4, IgA antibodies, which significantly increases the ability to identify cause-significant AI, regardless of the time of exacerbation of AD. Diagnosis of the causative factor is the basis of effective life-saving therapy. One common and inexpensive method is enzyme-linked immunosorbent assay (ELISA). The method has significant drawbacks, depending on the apparatus on which the ELISA is carried out: the test can have very low sensitivity, and then gives positive tests only for very severe allergies (i.e., the child or adult actually has an allergy, and the test will not show will be false negative); The test may have a high sensitivity, but low specificity, which gives a lot of false positive reactions (i.e., the test will show the presence of an allergy when it really is not). The method of multiple chemiluminescence (MAST) is a modern and very sensitive method for the determination of allergen-specific antibodies of the classes IgE, IgG, IgG4 in blood serum.<sup>[2,6,12,15]</sup> Allergy tests are carried out in the form of panels (sets of allergens) using the immunofluorescence method on the CLA-1TM HITACHI automatic analyzer

apparatus (Japan). Advantages of the method: quality reliability (allergens are standardized and undergo several levels of quality control); convenience of the method for the patient (a wide range of single allergens and mixes (panels for multiple screening studies); high accuracy and sensitivity of the method (specific IgE and IgG are determined even in minimal concentrations); ability to reliably diagnose different forms of allergy: polyvalent (when allergies to several allergens); latent (hidden); cross-reactions between different groups of allergens. Immunofluorescent method on the apparatus ImmunoCAP. "Gold" standard of allergic diagnosis throughout the world *in vitro* is an immunofluorescent method carried out on the ImmunoCAP apparatus of the Swedish company Phadia. According to statistics, in Europe out of 10 laboratories in 7 have the ImmunoCAP apparatus, which indicates high quality of results and confidence in this method. ImmunoCAP is a tool for accurate Measuring IgE antibodies ImmunoCAP *in vitro* test for the detection of IgE antibodies to specific allergens is a reliable tool for confirming or excluding the diagnosis of true allergy, prescribing adequate therapy and predicting the development of the disease. The correct and accurate result is the necessary basis for clinical diagnosis.<sup>[3,4,9,17]</sup> Traditionally, tests for the detection of IgE antibodies have been qualitative (positive or negative response) or semi-quantitative (by grade). A wide range of sensitization requires very high accuracy for tests in order to reliably establish the limits of permissible values or the limits of a particular class. In order to understand the cause of an allergic disease, it is necessary to obtain more detailed information on the development of the patient's sensitization process. Such information can only be obtained by quantitative measurement of the level of IgE antibodies to various allergens in the blood serum. ImmunoCAP is a true quantitative test for the determination of IgE antibodies. For a reliable quantitative test for the detection of IgE antibodies, higher accuracy is required. ImmunoCAP measures the result in units (Ku / l), which is much more accurate than in classes. Methodological results confirmed by clinical data prove that ImmunoCAP (Phadia) is an excellent test system for accurate and correct measurement of the level of IgE antibodies, correlating with clinical symptoms.<sup>[9,14]</sup> Phadia has developed its own quality program that allows consumers to control the accuracy of the results. The test for detecting allergen specific IgE antibodies is a complex immune assay. Detecting IgE antibodies is a much more complicated test compared to other tests and requires a special approach in this specific area of technology. Such factors as the concentration of IgE antibodies in the blood is very low compared to other substances, even in highly sensitized patients, are complicated by the implementation of these methods. Most sources of allergens are a mixture of components in a biological material.<sup>[12,15,17]</sup> The composition of the allergen affects the source of receipt. Depending on geographical and seasonal differences, the composition of proteins can vary considerably. Therefore, to achieve

accuracy and reproducibility of the test system, it is necessary to control the source of the allergen, its composition and allergenic activity. Only with careful development of each component in the system, a high level of accuracy of immune analysis can be achieved as a comprehensive measurement of IgE antibodies. On the ImmunoCAP device, more than 500 of the most diverse allergy tests are determined, including for the diagnosis of food and drug allergies. The high confidence of clinicians in ImmunoCAP™ in the international scientific community is best demonstrated by the enormous amount of scientific research publications using the ImmunoCAP™ test system. More than 3000 scientific publications are devoted to the main clinical issues in the field of allergology and using a large number of allergens. The reliability of this method reaches 98%: due to the following characteristics: high accuracy and quality of results, due to the presence of the three-dimensional binding capacity of the solid phase in ImmunoCAP® technology (other methods use just the surface); high specificity of technology (no interaction with other human immunoglobulins (antibodies)); the hypersensitivity of the method - the lower limit of detection of the device is much smaller values than the indicator at which the patient's allergy is determined (this is 0.35 Ku / l). Unique Allergy Tests with ImmunoCAP - Molecular Allergy Diagnosis.<sup>[8,11]</sup> For MDA, single-plex and multiplex panels are used. The scope of molecular diagnostics of allergy is expanding, there is a need for large-scale population-based research aimed at formulating practical recommendations, identifying new allergen molecules and developing a strategy for interpreting the results. Allergists should gain access to the MDA evidence base as quickly as possible. This consensus serves as a practical guide for practitioners, which contains information about the indications for the molecular diagnosis of allergy and the interpretation of its results, as well as terminological concepts. In conclusion, we hope for practical relevance of this manual for the effective diagnosis of AD. It is indisputable that correct analysis and adequate assessment of allergological, pharmacological and nutritional history, clinical picture of the disease, results of skin and other provocative tests with allergens, specific laboratory, as well as general laboratory and instrumental methods of research, are essential. Only a comprehensive examination of the patient can help the doctor in the diagnosis of allergopathology and identify those responsible for the development of allergens.

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