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Federal State Budgetary Educational Institution of Higher Education
"North Ossetian State Medical Academy"
of the Ministry of Health of the Russian Federation (FGBOU VO SOGMA of the
Ministry of Health of the Russian Federation)

Department of Internal Medicine No. 3

GUIDELINES FOR PERFORMING INDEPENDENT (EXTRACURRICULAR)
WORK) WORKS

in clinical immunology

the main professional educational program of higher education – the specialty
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The guidelines are intended for extracurricular independent work of students of the 3rd year (5th semester) of the Medical Faculty of the Federal State Budgetary Educational Institution of the Ministry of Health of the Russian Federation in the discipline of Clinical Immunology

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Topic: Immunodiagnostics. Assessment of the immune system. Immune status. Principles of immune status assessment. Methods of immunodiagnostics.

Methodological development includes the following tasks:

I. Introduction to the purpose and objectives of the practical lesson; II. Restoration of basic knowledge, control of the initial level of knowledge;
III. Study of the literature on the topic of the lesson, the main provisions of the topic; IV. Control of the assimilation of methodological materials;

Task I. Introduction to the purpose and objectives of the lesson.

The purpose of the lesson: to give basic ideas about modern immunological methods of diagnosis, assessment of the immune status.

The student should know:

1. What is the immune status?
2. What is an immune diagnosis?
3. The main indications, characteristics and differences of the tests of the 1st and 2nd levels. 4. Biological materials used for immunodiagnostics.

The student should be able to:

Indicate the main differences and characterize the immunodiagnostic methods of the 1st and 2nd levels.

Relevance of the topic.

The assessment of the immune status is necessary for determining quantitative and qualitative changes in the immune system, analyzing the development of post-vaccination immunity, diagnosing primary and secondary forms of immunological insufficiency, for immunological monitoring, dispensary monitoring of groups of people with various forms of immunological insufficiency, collecting epidemiological data on the prevalence of immune system disorders, identifying adverse factors of the internal and external environment and taking measures to

stop their effects on the body, to select methods of immunoprophylaxis and immunotherapy, to make forecasts of the course and outcome of the disease.

Task II. Restoration of basic knowledge, control of the initial level of knowledge.

You are offered a list of questions to determine the adequacy of your basic knowledge. Check yourself if you are able to answer them:

Questions for determining the initial level of knowledge:

1. What is the immune status?
2. Formulate the main approaches to assessing the human immune system.
3. What is the two-step principle of assessing the immune status? List the tests of levels 1 and 2.
4. What biological materials are used to assess the state of the human immune system?
5. For what purposes are serological reactions used
6. What are the reactions of agglutination, precipitation, and ELISA used for?
7. Indications and contraindications for skin tests with allergens.

Task III. Study of literature on the topic of the lesson.

2.. Medical microbiology, virology and immunology: textbook edited by V. V. Zverev M.: GEOTAR-Media, 2016 Vol. 1-240T. 2-236

"Student Consultant"<http://www.studmedlib.ru/book/ISBN9785970436417.html>4.

Additional information:

1. Immunology. Atlas: ucheb.manual Khaitov R. M., Yarilin A. A., Pinegin B. V. M.: GEOTAR-Media, 2011 "Student's consultant"<http://www.studmedlib.ru/book/ISBN9785970418581.html>
2. Allergology and Immunology National Guidelines \\»GEOTAR-Media " 2009
3. Allergology: Clinical recommendations, edited by P. M. Khaitov, N. I. Ilyina. - m.: GEOTAR-Media, 2016.
4. Kurbacheva O. M. Principles of therapy of allergic diseases. ConsiliumMedicum, J. 34, vol. 4, 2016

Main provisions of the topic

The spectrum of immunological laboratory tests performed on the patient is his immunogram. All the basic principles of interpretation of immunograms are developed on the basis of the accumulated experience of using a general blood test in everyday medical practice since the 20s of the last century and the experience of using an immunogram by clinicians over the past 30 years. The diagnosis and prognosis should always be based on the totality of changes in all indicators of the leukogram and immunogram. The same end result of the immune response under the same conditions can be obtained by a different quantitative and qualitative combination of components of the immune system. Fundamental principles of immunogram interpretation: 1. A full-fledged clinical analysis of the immunogram can be carried out only in conjunction with the assessment of the clinical picture of the disease in this patient and the data of his anamnesis. It is impossible to make a clinical conclusion based only on the immunogram, since the same changes in the immunogram parameters can be observed in fundamentally different pathological processes. 2. A comprehensive analysis of the immunogram is more informative than an assessment of any indicator individually. The same changes in a certain indicator in different phases of the acute inflammatory process can be considered as a favorable and unfavorable sign. 3. Real information about changes in the

immunogram is provided by significant violations of the indicators in the immunogram (40-50% of the norm or more). Due to the lability of the immunogram parameters, their slight fluctuations are possible in completely healthy individuals. 4. Clinical data play a crucial role, and the immunogram has an auxiliary diagnostic and prognostic value. The absence of changes in the immunogram in the presence of a clinical picture of pathology requires studying the function of the components of individual parts of the immune system. 5. The analysis of the immunogram in dynamics (especially in comparison with clinical dynamics) is more informative from the point of view of both diagnosis and prognosis of the course of the disease, helps to avoid erroneous interpretation. 6. Diagnostic and prognostic value are the individual indicators of the norm in this patient (taking into account age and the presence of concomitant chronic diseases, the effects of harmful factors, drug therapy). 7. The primary importance in the evaluation of the immunogram is the ratio of the immunogram indicators, and not their absolute values. 8. When assessing the immunogram indicators, it is necessary to take into account the possibility of their fluctuations, in connection with food intake, physical exertion, fear, time of day. 9. The discrepancy between the shifts in the immunogram and the clinical picture of the disease (dissociation syndrome) indicates an unfavorable development of the process. 10. The higher the antigenicity of the foreign factor and the larger the zone of its penetration, the brighter the inflammatory process will be. Therefore, changes in the immunogram should also be more pronounced, which will indicate in favor of the adequacy of the immune system response. The absence of these changes in the leuco-and immunogram is an unfavorable symptom, which indicates the inadequacy of the immune system. Timely recognition of the signs of such a discrepancy is the main task of the clinician-immunologist.

Before proceeding to the analysis of the structure of the immunogram, it is necessary to focus on several fundamental points. First, it is important to note that all laboratory immunological data must be interpreted in comparison with the data of the general blood test, the clinical picture of the disease and the stage of the immune response. Thus, the change of the neutrophil phase of the immune response to the lymphocytic phase in bacterial infections is accompanied by a gradual regression of the symptoms of inflammation in the clinical picture. If there is no such pattern, the immunogram data helps to find out the cause of the imbalance. The low content of lymphocytic cytokines (see below) against the background of increasing lymphocytosis in the general blood test may indicate insufficient functional activity of immunocompetent cells, which, in fact, explains the long-term persistence of inflammatory symptoms mediated by the activity of

innate resistance factors. On the other hand, an immunogram can demonstrate the source of the formation of lymphocytosis in a general blood test. Thus, with a high content of so-called null lymphocytes (insufficiently mature or defective immunocompetent cells), even high lymphocytosis is not accompanied by proper performance of the functions of lymphocytes. Secondly, the immunogram data should be evaluated by comparing the absolute and relative (percentage) values of immunological indicators. The normal percentage of one or the other subpopulation of lymphocytes does not yet indicate a sufficient absolute number of the corresponding immunocompetent cells.

What disorders in the immunogram are subject to correction? Not all changes detected in the immunogram are indications for immunotherapy. First of all, it is necessary to focus on clinical data. At the same time, we recommend using the screening clinical and laboratory criteria for the diagnosis of immunodeficiency diseases, given in this manual. If we talk about the results of immunological studies, then the following violations of immunity indicators are subject to correction: 1. Reliable (there is a decrease in the indicator by at least 30-40% from the lower limit of the age norm).

2. Persistent (persist, at least, with a double examination, with an interval of at least 2 weeks). 3. Clinically manifest (i.e., the change in the indicator can be associated with certain clinical data, based on modern ideas about the functional purpose of various components of the immune system; for example, it is known that phagocytosis defects lead mainly to the development of chronic bacterial and fungal infections, and a deficiency in interferon production predisposes to chronic viral pathology). 4. Not only quantitative, but also qualitative (i.e. it is desirable to conduct a parallel determination of the quantitative and qualitative characteristics of the studied immune defense factors).

Structure of the immunogram. Immunogram indicators are divided into certain groups, depending on which part of the immune system they characterize.

The innate immune system is characterized by: the number of neutrophils and blood monocytes, the value of phagocytosis assessment indicators, the value of spontaneous and induced NST tests, the level of natural killers and large granular lymphocytes, the serum titer of complement, the concentration of individual complement components in the blood serum, the concentration of lysozyme in secretions. At the same time, the phagocytosis assessment indicators allow us to assess mainly the absorption activity of phagocytes, and the NST test (test with nitrosine tetrazolium)-the intensity of the "oxygen explosion" that occurs inside the phagocytic cells. Indicators for assessing the phagocytic activity of neutrophils: -

phagocytic number – the average number of microbes absorbed by one blood neutrophil; - phagocytic index – the percentage of neutrophils involved in phagocytosis; - phagocytic capacity of blood – the number of microbes that can absorb neutrophils contained in 1 liter of blood; - the number of active phagocytes – the absolute number of phagocytic neutrophils in 1 liter of blood; - phagocytosis completion index-characterizes the digesting ability of phagocytes and the completion of the phagocytosis process. Similar indicators can be calculated for monocytes.

The cellular link of immunity is characterized by the content of CD3+ T-lymphocytes (an integral indicator of the cellular link), CD3+CD4+ T-lymphocytes (so-called T-helpers), CD3+CD8+ T-lymphocytes (so-called T-killers, or cytotoxic T-lymphocytes), CD4+CD25+ T-lymphocytes (regulatory T-cells), CD3-CD16+CD56+ cells (so-called natural killers), CD3+CD16+CD56+cells (natural killer T-lymphocytes). The cellular link is predominant in viral, fungal pathogens, atypical pathogens (mycoplasma, chlamydia), bacterial infections with intracellular presence of the pathogen (mycobacteria), as well as in the immune response to tumors and tissue forms of helminths (for example, larvae of ascaris or Trichinella). The humoral link of immunity is characterized by the levels of CD3-CD19+, CD3-CD20+, CD3-CD21+ and CD3-CD22+cells (B-lymphocytes in different maturation phases), as well as the levels of immunoglobulins of different classes (IdM, IgG, IgE, serum and secretory IgA). Since the synthesis of antibodies is a T-dependent process, the level of T-helper cells (CD3+CD4+ T-lymphocytes) should be taken into account for a proper assessment of the humoral link of immunity, which once again confirms the feasibility of a comprehensive approach to the interpretation of the immunogram. The humoral link is predominant in bacterial infections with extracellular presence of the pathogen (streptococci, staphylococci, Escherichia, Pseudomonas aeruginosa, proteus, etc.), as well as in cavity protozoan and helminth infestations.

IdM is an antibody of the acute period of the immune response, which is synthesized by plasma cells at the first contact with a certain pathogen. IdM has 10 antigen binding centers at once, which is especially important in the acute period of infection, when there is a need for rapid recognition and destruction of a large number of pathogens. This requirement is also met by the strongest among all immunoglobulins, the ability of IdM to activate complement, which ensures the implementation of complement-dependent cytotoxicity. On average, high concentrations of specific IdM are recorded from 6-7 days after infection, later the level of IdM significantly decreases against the background of an increase in IgG content, that is, there is a switch from IgM synthesis to IgG. There is a hereditary

form of IDZ associated with a violation of the switching of antibody isotypes. In such patients, very high levels of IdM are recorded against the background of a deep deficiency of antibodies of other classes. In the clinic, this is manifested by a tendency to develop chronic infections.

The diagnostic value of high levels of specific IdM is the possibility of establishing the fact of acute infection, in which there was a primary infection with a certain pathogen. However, it should be borne in mind that in patients with IDZ, the formation of immune memory is disrupted, and therefore there may be cases when, with repeated infection with the same pathogen, the phase of predominant IdM production takes place again. This feature can be a laboratory criterion for the diagnosis of IDZ.

IgG is a late-phase antibody of the immune response that begins to be synthesized after a period of predominance of IdM. The properties of IgG take into account the conditions of the periods of regression of clinical manifestations and convalescence of the inflammatory process, during which the number of pathogens decreases and the quality of antigen recognition is of primary importance for the cure. In this regard, IgG is a more specific antibody than IdM. On the other hand, the properties of IgG take into account the disadvantages of the IdM molecule, which, due to their large size, have a rather limited ability to penetrate into tissues. For successful pathogen eradication, it is necessary to ensure reliable control of peripheral tissues by immunoglobulins for the presence of the pathogen. IgGs, which have only 2 antigen binding centers and a smaller molecular weight, have a better ability to penetrate peripheral tissues. High levels of specific IgG are recorded during periods of regression of clinical manifestations and convalescence in acute inflammatory process. Specific IgGs can be produced and circulated in the blood serum for a long time after treatment, since this class of antibodies is synthesized by immune memory cells. The choice of IgG to provide immune memory is not accidental, since it is both the most economical and the most specific antibodies. After the infection, either a stable concentration of specific IgG may be provided, or there may be a gradual decrease in their titers. The increase in the titers of specific IgG after a long period of time after an acute inflammatory process indicates not the maintenance of immune memory, but an incomplete cure and chronization of the infection, since IgG are antibodies of the secondary immune response, which is realized upon contact with an already familiar antigen. Thus, with repeated acute infection or exacerbation of chronic infection, the phase of predominance of IdM is absent, since IgG is immediately synthesized. Violation of such a pattern can be a criterion of IDZ. IgG deficiency is most often manifested in the form of chronic purulent bronchitis, sinusitis and otitis media, pneumonia,

which are resistant to treatment with antibiotics, as well as in the form of pustular skin diseases (pustules, furunculosis, carbuncles, abscesses, etc.) with a chronic or recurrent course. IdA are immunoglobulins of the mucous membranes and skin. There are serum and secretory forms of IdA. The deficiency of sIdA can be associated with a decrease in the concentration of the serum form, which is the precursor of the secretory form, and with a violation of the activity of the epithelium, where a secretory component is synthesized for IdA, which protects the immunoglobulin molecule from being broken down by digestive enzymes. Thus, for an adequate assessment of the exchange of IdA, it is necessary to conduct a parallel study of the levels of its serum and secretory forms. SIDA plays an important role in maintaining the immune memory of the mucous membranes and ensuring the phenomenon of immune solidarity of the mucous membranes. With a deficiency of SIDA, the clinic has a high susceptibility to infections (especially viral ones), the entrance gates of which are formed on the mucous membranes. Often, a deficiency of this immunoglobulin is the cause of chronic viral lymphadenitis and thymomegaly. In addition, sIdA deficiency may be the basis of combined inflammatory processes on the mucous membranes of various organs (for example, chronic sinusitis and gastroduodenitis), which is the result of a violation of the maintenance of the immune solidarity of the mucous membranes.

IdE are second-level antibodies that protect the mucous membranes. If the pathogen overcomes the protective barrier of sIdA, it is recognized by IDEs, which are produced in the tonsils, lymph nodes, and solitary lymphatic follicles, which leads to the degranulation of mast cells and the development of mucosal inflammation. In other words, the mechanism associated with IdE activity is an alternative to the neutralizing effect of SIDA. In addition, IDEs play a key role in antiprotozoal and anthelmintic immunity. The IdE received a bad reputation among clinicians due to their participation in atopic reactions.

IgD-immunoglobulins with an unknown function. Only a doctor can correctly assess the number of antibodies, the variant of class detection of immunoglobulins, and, consequently, set the stage of the disease and the need for treatment.

We must not forget that for any test system, its own "reference values" (variants of the norm) are developed, and if they are exceeded, a particular disease (variants of pathology) is diagnosed. The "reference values" are different for different test systems.

The correct comparison of the ELISA results taken in dynamics is possible only if they are made in the same laboratory.

Content of independent work:

1. study of the main and additional literature from the recommended list;
2. notes and extracts from the text;
3. preparation of the plan and theses of the answers;
6. answers to questions for self-training;
7. use of internet resources.

Methodological and visual support of the lesson:

1. guidelines for students on independent work;
2. informing students about the purpose of studying this topic;

Task IV. Check the assimilation of the methodological materials for the proposed tests. Choose one or more correct answers.

1. Which type of immunodiagnostic tests is most informative? 1. Level 1 tests
2. Level 2 tests 3. Level 3 tests 4. Level 4 tests
2. Select the tests related to the Level 1 tests

1. determination of the relative and absolute number of white blood cells 2. determination of the serum IgE concentration

3. determination of the immunoregulatory index

4. determination of the expression of markers: CD25, CD69

3. What biological materials can be used for level 1 immunodiagnostic tests:

1. whole peripheral blood; 2. cerebrospinal fluid;

3. synovial fluid;

4. bronchoalveolar fluid; 5. all options.

4. What biological materials can be used for level 2 immunodiagnostic tests:

1. whole peripheral blood; 2. cerebrospinal fluid;

3. synovial fluid;

4. bronchoalveolar fluid; 5. all options.

5. Serological reactions include

Answers:

question #1, answer 2. Question #2, Answer 1, 3. Question #3, answer 1. Question #4, Answer 5. Question #5, answer1, 2