Ministry of Health of Ukraine
Poltava State Medical University

Approved"
at the meeting of the Department of
of Internal Medicine No. 3, Phthisiology
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Minutes № from
Head of the Department
Associate Professor _____ PhD
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METHODOLOGICAL RECOMMENDATIONS FOR CONDUCTING AND PREPARING FOR PRACTICAL CLASSES

Academic discipline	Clinical immunology and allergology
*Module **4***	Clinical immunology and allergology
Content module	Clinical immunology and allergology
*Topic **2***	Immunological methods of research. The concept of an immunogram.
Course	5
Hours	2

Poltava 2023

Methodological recommendations for the practical training for independent work of students in preparation for the practical training and during the class were prepared by:
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Methodological recommendations were re-approved at the meeting of the Department of Internal Medicine of Internal Medicine №3 with Phthisiology_____
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1. Relevance of the topic.

The relevance of studying the research methods used in clinical immunology is due to the fact that knowledge of these methods, correct interpretation of the research results allows to identify defects in one or another link of the immune system (congenital and acquired immunodeficiencies); diagnose auto-aggression against the body's own substances (autoimmune diseases) and excessive accumulation of immune complexes (diseases of immune complexes); identify dysfunctions in which a particular immune system link develops signs of hyperfunction, damaging the functioning of other links (hypergammaglobulinaemia, heavy chain disease, myeloma, etc.); to monitor the effectiveness of immunosuppressive or immunostimulatory therapy; to type and select donors for organ transplantation and monitor immunosuppressive therapy during transplantation; to perform phenotyping of haemoblastosis; to diagnose genetic predisposition to diseases.

General objective: mastering the principles of clinical examination methods (taking an immunological history, identifying symptoms and syndromes of immunopathology), laboratory methods of studying the components of the immune system, the ability to identify major immunological disorders on an immunogram, knowledge of the basics of flow cytometry.

Specific objectives:

1. Knowledge of the features of immunological anamnesis in various types of immunopathology.

2. Use of clinical and instrumental methods for assessing the immune system.

3. Characteristics of laboratory methods for assessing the immune system.

4. Methods for assessing humoral factors of innate immunity.

5. Laboratory methods for assessing cellular immunity.

6. Comprehensive assessment of local immunity.

7. Mastering an integrated approach to assessing the immune status of a person.

8. Immunogram, interpretation of results.

Initial level of knowledge and skills:

1. Ability to collect an immunological history.

2. Knowledge of the main symptoms and syndromes of immunopathology.

3. Knowledge of the main indicators of the leukogram in the clinical blood test and understanding of their changes in the infectious process.

4. Knowledge of proteinogram indicators and understanding of its changes in the pathology of the immune system.

5. The concept of enzyme-linked immunosorbent assay.

6. Knowledge of the main indicators of the immunogram, their values in the main syndromes of immunopathology.

Theoretical questions for the practical session:

1. Immunological history.
2. Clinical examination data.
3. Typical clinical manifestations of immunopathological syndromes.
4. Tests performed on the patient (in vivo).
5. Immunological methods of examination.
6. Immunological tests that characterise cellular immunity (T-link).
7. Immunological tests that characterise humoral immunity (B-link).
8. The concept of immunogram, interpretation of results.

Approximate basis of action

Immunological history

When collecting immunological history, the following data should be established: 1) hereditary burden: the presence of allergic, oncological, chronic inflammatory, endocrine or immunoproliferative diseases in one or both parents, repetition of pathology in the family tree;

2) pathologies of development and formation: pathologies of childbirth, congenital anomalies, diathesis, rickets, artificial feeding, infections and other pathologies of early childhood;

3) harmful environmental factors: contact with physical, including radiation, chemical, biological factors (living, working conditions), medicines, biological products, exposure to magnetic fields, high or low temperatures, constant stressful situations;

4) past injuries, diseases: severe or complicated injuries, burns and frostbite; chronic inflammatory processes, intoxication, septic conditions;

5) chronicity of a somatic disease: fever of unknown etiology, unexplained weight loss, prolonged diarrhoea;

6) episodes of allergic reactions (seasonality, age, allergic factor);

7) reactions to blood transfusion and its products;

8) iatrogenic effects: surgical interventions (appendectomy, tonsillectomy, thymectomy in case of cardiac interventions, etc.), radiation and chemotherapy in oncological pathology, glucocorticoids and other hormonal agents, oral contraceptives, cytostatics, anti-inflammatory drugs (doses, duration of administration);

9) bad habits and lifestyle: smoking, drug and alcohol abuse, physical inactivity and sedentary lifestyle, sedentary work, hyperinsolation, unhealthy diet, stress;

10) pregnancy pathology (infertility, miscarriage).

Clinical examination data

1. Physical examination of the organs and tissues of the immune system: lymph nodes, spleen, tonsils (lymphadenopathy, splenomegaly, thymomegaly, local or generalised hyper- or aplasia of lymph nodes, tonsils).

2. Skin (turgor, pustular rash, eczema, dermatitis, neoplasms, haemorrhagic purpura, petechial rash).

3. Mucous membranes and sinuses (candidiasis, ulcers, dryness, inflammation, gingivitis, sinusitis, cyanotic macules or papules). 4. Бронхолегенева система (запальні, обструктивні процеси, бронхоектази, фіброз).

5. Digestive and excretory systems (inflammatory processes, dyskinesia, hepatomegaly, pathology of the biliary and genitourinary tracts).

6. Neuroendocrine system (inflammatory processes of the central and peripheral nervous system, endocrinopathies, developmental defects).

7. The apparatus of movement and support (inflammatory lesions of joints and bones, destruction, impaired motor function).

8. Cardiovascular system (bleeding, inflammation, atherosclerosis, thrombosis).

9. Malignant neoplasms.

10. The presence of chronic diseases.

11. Features of the course of infectious processes and specificity of microflora.

Typical clinical manifestations of immunopathological syndromes

The infectious syndrome is characterised by prolonged subfebrile illness, fever of unclear etiology; chronic infections of the ENT organs (sinusitis, otitis media), recurrent lymphadenitis; recurrent and chronic bronchitis, chronic obstructive pulmonary disease; recurrent pneumonia (in combination with an ENT infection); frequent ARVI (in adults more than 4 times and in children more than 6 times per year); bacterial diseases of the skin and subcutaneous tissue (pyoderma, furunculosis, abscesses, phlegmon, recurrent paraproctitis in adults); parasitic infections; aphthous stomatitis, periodontal diseases; recurrent purulent conjunctivitis; recurrent herpes; chronic urogenital infections (chronic purulent vulvitis, urethritis, frequently recurrent cystitis, chronic pyelonephritis); dysbiosis, chronic gastroenteropathy with diarrhoea of unclear etiology; generalised infections.

The allergic syndrome is characterised by: skin allergy (atopic and contact dermatitis, urticaria, Quincke's edema, Arthus' phenomenon, eczema); allergy of the ENT organs; bronchial asthma, pollinosis, allergy to food, drugs, chemical compounds.

The autoimmune syndrome is characterised by: inflammatory diseases of connective tissue, glands, joints (rheumatoid arthritis, Sjögren's syndrome, Felty's syndrome, etc.); SLE, dermatomyositis, scleroderma; systemic vasculitis (Wegener's granulomatosis, nodular periarteritis, etc.); glomerulonephritis; thyroid pathology, insulin-dependent diabetes mellitus, Adisson's disease and other hormonal disorders; neurological diseases (multiple sclerosis, myasthenia gravis,

etc.); ulcerative colitis; cytopenic blood diseases; autoimmune liver diseases; autoimmune forms of infertility, pregnancy pathologies, severe forms of menopausal syndrome; certain types of psychopathology (schizophrenia).

Primary immunodeficiency syndrome (mainly in children) is characterised by Louis-Bar syndrome - ataxia in combination with telangiectasia, hyper- and depigmentation spots; Wiskot-Aldrich syndrome - haemorrhagic symptom complex in combination with eczema and thrombocytopenia in boys; DiGeorge syndrome - seizures with hypocalcaemia, malformations of facial bones and cardiovascular system, thymic hypoplasia; hereditary angioedema (C1 complement inhibitor deficiency).

Secondary immunodeficiency syndrome is characterised by the following: prolonged torpid course of infectious syndrome, tendency to generalisation of the process; alopecia, de- and hyperpigmentation of the skin; AIDS; other cases of acquired immunological deficiency.

The lymphoproliferative syndrome is characterised by: tumours in the immune system (lympho-leukaemia, lymphosarcoma, Hodgkin's disease, lymphoma, Kaposi's sarcoma); X-dependent recessive lymphoproliferative syndrome in children: a) hyperplasia of all groups of lymph nodes with inflammatory processes in them in combination with frequent bacterial infections of other localisation; b) splenomegaly; c) history of mononucleosis.

Tests performed on a patient (in vivo)

Elimination test. The patient is prohibited from using certain items or means of cosmetics, hygiene, staying in certain rooms or conditions, eating certain foods. In case of suspected occupational hazards, a leave of absence is used.

Provocative test. A potential allergen is injected directly into the target organ (conjunctival, bronchial or nasal mucosa) or contact with provocative factors (insolation, hot water bottle, ice cube) is provided. The reaction is assessed clinically: hyperaemia, edema, impaired function (e.g., nasal breathing) and by laboratory methods: leukaemia and thrombocytopenia in food allergy. Ado test is a test of inhibition of leukocyte migration in the oral cavity. Before and after rinsing the oral mucosa with an allergen solution, the number of leukocytes in the rinsing fluid is measured. If the number of leukocytes in the rinsing fluid increases by more than 30%, the test is considered positive. To increase the informative value of the test, instrumental control is often used (rhinopneumotachometry, spirometry, fibrogastroscopy, etc.).

Skin tests (a type of provocative tests). There are skin drip tests, application tests, scarification tests, prick tests, intradermal tests and subcutaneous tests. When assessing skin tests, the general reaction of the body (fever, chills, facial flushing, myalgia, headache), local (flushing, swelling, itching at the site of allergen injection) and organ reaction (target organ reaction: bronchospasm, rhinorrhoea or lacrimation) are taken into account. Contraindications for skin and provocative tests include exacerbation of allergic diseases; acute intercurrent infectious diseases, tuberculosis, AIDS; decompensated diseases of vital organs and systems (cardiovascular, respiratory, endocrine systems, liver, kidneys); rheumatism, collagenosis; malignant tumours; severe mental and neurological diseases; long-term treatment with cytostatics and glucocorticoids; anaphylactic shock or anaphylactoid reactions in history.

Cutaneous tests, scarification and prick tests are evaluated 10-20 minutes after the test. Intradermal tests are evaluated after 24 hours (72 hours is the optimal time for Mantoux test), subcutaneous tests - after 24-72 hours (Koch test - after 48 hours), 44 application tests (allergen is applied to filter paper attached to the skin for 12 hours) - after 12-24 hours after termination of contact with the allergen.

Immunological tests that characterise cellular immunity (T-cell)

T-cell immunity is assessed by the following indicators: - the number of T-lymphocytes; - determination of T-lymphocyte subpopulations.

Flow cytometry is performed using monoclonal antibodies bound to fluorescent dyes. Staining of blood cell elements is performed.

The method of rosette formation. The rosette test with sheep erythrocytes (E-ROC, rosette cells) is used to detect T lymphocytes. It has been shown that E-receptors are identical to CD2, which are detected by monoclonal antibodies and are present on T cells.

Load tests with drugs and other substances. Usually, cells are incubated for a certain period of time with or without small doses close to physiological amounts of drugs. Tests are performed with drugs, in particular immunocorrective drugs (thymalin, levomycin, etc.), in order to determine the effect of the drug on cells and predict the effectiveness of its use in treatment.

Lymphocyte blast transformation reaction (LBTR). The following substances are used to stimulate T lymphocytes in vitro. 1. Mitogens - phytohaemagglutinin, concanavalin A, etc. - cause nonspecific (not caused by binding to antigen-recognising receptors) activation of T-lymphocytes. 2. Soluble antigens - antigens of Candida albicans, tetanus toxoid - binding to antigen-recognition receptors of memory T-lymphocytes cause specific activation of these cells. 3. Allogeneic cells (in a mixed lymphocyte culture) activate T-lymphocytes because they carry HLA class II antigens on their surface. 4. Antibodies to T-lymphocyte surface antigens involved in their activation are CD2, CD3, CD43. 5. Chemicals, for example, forbolmyristat acetate (activates protein kinase C) and ionomycin (increases intracellular calcium). T-lymphocyte activation is usually assessed by the following indicators. 1. Proliferation. 2. Production of cytokines - interleukins-2-4, -5, interferon γ, tumour necrosis factor. 3. Expression of activation markers - CD25 and HLA class II antigens. 4. cytotoxicity.

Immunological tests that characterise humoral immunity (B-link)

The humoral link of the immune system is assessed by the following indicators: - the number of B lymphocytes; - the number of serum immunoglobulins (classes A, M, G, E). Today, 3 groups of methods are used to identify lymphocytes: immunofluorescence - flow cytometry, rosette formation, enzyme-linked immunosorbent assay.

Flow cytometry method. The cell membrane of lymphocytes contains many glycoproteins that can be detected by flow cytometry using monoclonal antibodies. Some of these glycoproteins are specific for a particular cell type, e.g: T-, B- and NK-lymphocytes, various subpopulations of T-lymphocytes, monocytes and even for certain stages of their maturation and differentiation. These molecules are commonly referred to as CDs. Determination of B-lymphocytes by flow cytometry is based on the detection of immunoglobulins fixed on the cell surface, CD19 and CD20. When evaluating the results of the test, the patient's age should be taken into account. In older children and adults, B-lymphocytes make up 10-20% of all blood lymphocytes, in younger children, there are more of them.

The rosette formation method is based on the presence of a receptor for the Fc fragment of immunoglobulins and the third component of the complement system (C3) on the membrane of a B lymphocyte. They are able to form rosettes only with antibody-treated mouse erythrocytes and in the presence of complement (EM-

ROSET). It has been shown that EM receptors are identical to those detected by CD22 monoclonal antibodies and are present on mature B-lymphocytes. Thus, the mouse erythrocyte rosette test (EM-ROC) is used to detect B-lymphocytes with CD22 receptors.

Determination of the absolute number of B-lymphocytes. The absolute number of B-lymphocytes is normally 0.28-0.31-106 /L. An increase in the absolute number of B-lymphocytes is observed in acute bacterial, fungal, parasitic diseases, AIDS (initial period), chronic liver diseases (cirrhosis, viral hepatitis), autoimmune diseases (rheumatoid arthritis, SLE, rheumatism, collagenosis), sarcoidosis, cystic fibrosis, Crohn's disease, Waldenström's disease, monoclonal gammopathy, infectious mononucleosis, chronic lymphocytic leukaemia, in the acute period of repeated infection.

Determination of IgE by enzyme-linked immunosorbent assay (ELISA test). The allergen, for example, pollen extract, is immobilised on a sorbent, which is then incubated with the patient's serum. The amount of specific IgE 53 bound to the sorbent is determined using IgE antibodies immobilised in wells on special plates.

Interpretation of immunograms

Recommendations for the interpretation of immunograms (K. A. Lebedev):

1. Complete information can be obtained by analysing the immunogram in conjunction with the assessment of the clinical picture of the patient.

2. A comprehensive analysis of immunograms is more informative than the assessment of each indicator separately.

3. The real information in the immunogram is provided only by persistent shifts in indicators.

4. Analysis of immunograms in dynamics is more informative both in diagnostic and prognostic terms than a single immunogram.

5. In the vast majority of cases, the analysis of only one immunogram makes it possible to draw only indicative, not unconditional, diagnostic and prognostic conclusions.

6. In conclusions based on the clinical picture and immunogram analysis, the clinical diagnosis should be the leading one.

7. The absence of immunogram changes in the presence of a clinical picture of the inflammatory process should be interpreted as an atypical reaction of the immune system and is an aggravating sign of the process.

8. Assessment of immune status is not the only, but one of the most important stages of detection of diseases based on disorders in the human immune system.

The development of any inflammatory process is accompanied by a decrease in the content of T-lymphocytes throughout its duration. The high sensitivity of the blood T-cell count is due to the fact that the most active T-cells are quickly sent to the inflammatory site along with granulocytes, while T-cells with low metabolic activity (young, old or defective cells, as well as cells with blocked receptors, i.e. temporarily inactive) that remain in the bloodstream are poorly detected by conventional laboratory methods and therefore fall into the category of null cells. Therefore, in the analysis, we have a sharp decrease in the content of T-lymphocytes and an increase in the number of null cells.

The appearance of plasma cells in the peripheral blood is a sign of a sharp irritation of the lymph node tissue, which leads to their hyperproduction, which leads to an increased release of plasma cells into the bloodstream. The detection of plasma cells in the blood of an adult (usually in the amount of 1-3%) is associated with the presence of one of the following diseases: measles, rubella (up to 20% of cases), cholera (late stages), bacterial dysentery, as well as severe forms of malaria, typhoid fever and typhoid fever. Severe forms of influenza in children can also be accompanied by the appearance of a significant number of plasma cells in the blood. Plasma cells can be found in the blood of patients with severe anaemia.

Normal values of the number of T-helper cells (%), T-suppressors (%) and their ratio (according to the rosette test with theophylline) in the blood of healthy people are characterised by the following values. Middle-aged adults: T-helpers - 70% (40-62%); 90% (35-70%); 95% (28-76%); T-suppressors - 70% (8-25%); 90% (6-35%); 95% (4-45%); Tx/Ts - 70% (2.5-5.0); 90% (1.8-6.0); 95% (1.3-7.5). Younger children: T-helper - 70% (30-56%); 90% (24-65%); 95% (21-70%), T-suppressors - 70% (7-20%); 90% (5-30%); 95% (3-40%); Tx/Tc - 70% (2.0-4.4); 90% (1.5-5.5); 95% (1.2-6.6). At different stages of the normal inflammatory process, the number of T-helper and T-suppressor cells in the blood changes, but in such a way that T-suppressors do not significantly outnumber T-helper cells.

Тестові завдання для перевірки початкового рівня знань

Test tasks to check the initial level of knowledge

1. The diagnosis of an infectious disease is confirmed:

A. Decrease in titres of specific antibodies in the dynamics of the disease.

B. Increase in titres of specific antibodies in the dynamics of the disease.

2. Antibody titer is:

A. The highest dilution of the test serum that provides a specific immunological reaction (agglutination, precipitation, lysis, etc.).

B. The smallest dilution of serum that provides a specific immunological reaction.

3. For the quantitative determination of the content of serum immunoglobulins of different classes are most often used:

A. The radial immunodiffusion reaction.

B. Agglutination reaction.

C. Immunoelectrophoresis method.

4. To assess the state of neutrophils is determined:

A. Their number in the leukogram.

B. The efficiency of nitroblue tetrazol recovery (NST test).

5. Assessment of the functional state of T-lymphocytes can be based on:

A. Calculation of leuko-T-cell index.

B. Determination of differentiated antigens.

C. Blast transformation reactions with FGA.

D. Calculation of the leukaemia B-cell index.

6. The Coombs' direct test is used to:

A. Determination of antibodies to erythrocytes in neonatal haemolytic disease syndromes.

B. Determination of antibodies in autoimmune haemolytic diseases.

C. Determination of antibodies to erythrocytes in haemolytic disease of the newborn.

D. For the determination of haemolysins.

7. The indirect Coombs test is used to:

A. Determination of circulating non-agglutinating antibodies in the serum of women sensitised to Rp-antigen.

B. Determination of circulating haemolysins.

8. The Waaler-Rose reaction is a reaction designed to:

A. Diagnosis of rheumatism.

B. Determination of rheumatoid factor in the serum (anti-IgG antibodies).

C. Determination of histiocyte function.

9. Enzyme-linked immunosorbent assays are used for quantitative determination:

A. Hormones (insulin, growth hormone, ACTH, thyroxine, estrogen).

B. Tumour markers.

C. Neurohumoral factors.

D. Creatinine and urea.

E. Blood electrolytes.

10. Enzyme-linked immunosorbent assays (ELISA, IFA) are methods based on:

A. Determination of the content of antigens or haptens in the sample under study by determining the ratio of the enzymatic activity of free and bound antibodies.

B. Determination of antibody content by enzymatic reaction.

Correct answers to the questions: 1 - B; 2 - A; 3 - A; 4 - B; 5 - C; 6 - ABC; 7 - A; 8 - B; 9 - ABCD; 10 - A.

Sources of educational information

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