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INNOVATIVE METHODS OF LABORATORY DIAGNOSTICS OF AUTOIMMUNE DISEASES

(guide for residents)

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The proposed guide contains material on innovative methods for diagnosis of autoimmune diseases, development and implementation of which became possible due to the receipt of new data on pathogenesis of human autoimmune diseases. In the introduction and in the first chapter of the teaching manual, modern data on pathogenesis of autoimmune diseases are presented, based on the fundamental data of the scientific literature on this problem. The guidel shows an understanding of the close relationship between progress in the study of pathogenetic mechanisms and the diagnosis of autoimmune diseases. The guide is intended for residents of all specialties.

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List of abbreviations

NSMC - National Scientific Medical Center
AID/AD - autoimmune diseases
ARD - autoimmune rheumatic diseases
DM –diabetes mellitus
Treg - T-regulatory cells
TCR - antigen - recognizing receptor
nTreg –natural T-regulatory cells
γδ-Т-клетки - a small population of T cells whose T-cell receptor is formed by γ and δ subunits .
APC - antigen - presenting cell
RA –rheumatoid arthritis
TNFα - tumor necrosis factor α
GEBP - genetically engineered biological preparation
mAB - monoclonal antibodies
CRP - C- reactive protein
SAA –serum amyloid protein A
rIL6R- soluble receptor IL6
VEGF - vascular endothelial growth factor
MCP1 - monocytic chemoattractant protein 1
MMP - matrix metalloproteinases
MHC II class - molecules of main histocompatibility complex of class II
PGE2 - prostaglandin E2
RF - rheumatoid factor
ACP - antibodies to citrullinated proteins
Th - T helpers
PsA - psoriatic arthritis
SLE - systemic lupus erythematosus
TGF β - transformative growth factor β
TCZ – tocilizumab
RD - rheumatic diseases
JAK - Janus- associated kinases
TLR –(Toll)- similar receptors
NLR - Nod- similar receptors
NLRP3 – Nod- similar receptors NLR families that recognize pathogen patterns or damage-related patterns.
ACV/ACPV - antibodies to the citrulinated protein vimentin
IEM - immunoenzyme method
PCR - polymerase chain reaction
iRNA - informational ribonucleic acid
mRNA - matrix ribonucleic acid
GMCSF - granulocyte-macrophage colony stimulating factor

IFNγ - interferon γ
YKL1 - cytoskeletal protein
IIR - indirect immunofluorescence reaction
ELISA - enzyme-linked immunosorbent assay
IB – immunoblotting
RIA – radioimmunoanalysis
AS - ankylosing spondylitis
CARD – systemic autoimmune rheumatic diseases
AHA (Antinuclear antibodies)-
APL/ APLA -
(ANCA) - antineutrophil cytoplasmic antibodies.
cANCA - cytoplasmic antineutrophil cytoplasmic antibodies
pANCA perinuclear antineutrophil cytoplasmic antibodies
ESR – erythrocyte sedimentation rate
CPK - creatine phosphokinase
EULAR (European League Against Rheumatism) - **ACR** (American College of Rheumatology) -
SLICC/ACR – damage index developed by International Organization for Clinic Cooperation SLE with assistance of the American College of Rheumatology
Anti- nDNA (anti-nDNA) antibodies to double-spiral (ds) DNA
Anti-Sm - – antibodies to the antigen Sm (Smith)
Anti-SSA/Ro - antibodies to the antigen Ro/SSA
Anti -SSB/La - antibodies to the antigen La/SS-B
ACL - antibodies to cardiolipin
aβ2-GP I - antibodies to β2- glycoprotein I
LA- lupus anticoagulant
SSD - системная склеродермия systemic scleroderma
Scl-70 (anti- Scl-70) - antibodies to topoisomerase I
ACA- anticentromeric antibodies CENP-A, CENP-B, CENP-C
Anti-RNA polymerase III - antibodies to RNA polymerase III
SS - Sjogren 's syndrome
MCTD - mixed connective tissue disease
Anti-PR3 - antibodies to proteinase 3
Anti-MPO - antibodies to myeloperoxidase
ACPA (anti-CCP) - antibodies to cyclic citrullinated peptide
ANCA-CP - autoantibodies to neutrophil cytoplasm components
PCT – прокальцитонин procalcitonin
MAS - macrophage activation syndrome
RTM –rituximab
ABC – abatacept
DCTD - diffuse connective tissue diseases
ANF - антинуклеарный фактор antinuclear factor
ANA-screen–antinuclear antibodies, screening
HLA - human leukocyte antigen

Anti-RNP - antibodies to ribonucleoprotein
Jo-1 – antibodies to histidyl- - 1RNC- synthetase
BMG (Glomerular Basement Membrane IgA&IgM& IgG antibody, anti-GBM) - basement membrane of glomeruli of the kidneys
GIT - gastrointestinal tract
AMA (M2) - antimitochondrial antibodies (subtype 2)
ASMA - antibodies to smooth muscles
APCA - antibodies to parietal cells of stomach
LKM1 - antibodies to type 1 liver microsomes
sp100 – antibodies to soluble nuclear protein (to Sp100-antigen)
gp210 - antibodies to integral membrane glycoprotein
LC-1 - antibodies to cytosolic liver antigen type 1
SLA - antibodies to soluble liver antigen
F- actin - antibodies to smooth muscles, including fibrillar F-actin
U1-sn RNP - antibodies to U1 ribonucleoprotein
Anti-GM1,GM2, GM3, GD1a, GD1b, GQ1b, GT1b class IgG - antibodies to gangliosides
AxP (AchR) - antibodies to acetylcholine receptors
ICA - antibodies to islet cells
DCTD - diffuse connective tissue diseases
ssDNA (ssDNA) - antibodies to single-chiral (denatured) DNA
RNA - ribonucleic acid
(DNA) - deoxyribonucleic acid
Ag- anti-gene
Ab –antibody
PCNA - antibodies to the nuclear antigen of proliferating cells
CREST-syndrome - symptom complex including symptoms: calcification of tissues (C), Raynaud's syndrome (R), esophagitis (E), sclerodactyly (S), telangiectasia (T)
NUMA 1 - antibodies to the mitotic apparatus of cell
CIC –circulating immune complexes
C3, C4 – complement
DM –dermatomyositis
PhD - photosensitive dermatitis
HIV - human immunodeficiency virus
AKA (Anti-keratin antibody) - antibodies to keratin
SV –systemic vasculitis
PBC - primary biliary cholangitic
(PBC) - primary biliary cirrhosis
AIH - autoimmune hepatitis
PSC - primary sclerosing cholangitis
Reticulin Antibody IgA&IgG, ARA - antibodies to reticulin IgA and IgG

RI - type of antireticulin antibodies

Anti-Endomisial Antibody IgA&IgG, EMA - antibodies to endomysium IgA and IgG
Anti-tissue transglutaminase IgG, tTG IgG - IgG class antibodies to tissue transglutaminase
Anti- tissue transglutaminase IgA, tTG IgA - антитела класса IgA к тканевой трансглютаминазе
CP - coefficient of positivity
Ig - immunoglobulin
IgA - immunoglobulin A
IgG – immunoglobulin G
IgM – immunoglobulin M
Fecal Calprotectin - fecal calprotectin
NAID - nonsteroidal anti-inflammatory drugs
Alpha-1-Antitrypsin, Feces (A1AT) - alpha-1-antitrypsin in feces
Autoantibodies against Exocrine Pancreas, Pancreatic Antibodies, PAB - antibodies to pancreatic acinar cells, IgG and IgA in total (antibodies to the exocrine part of pancreas)
GP2 - type 2 glycoprotein
Antibodies to GP2 (Anti-GP2) - antigen of centroacinar cells of pancreas **ASCA (antibodies to Saccharomyces cervisiae)** - antibodies to saccharomycetes
Anti-Intestinal Goblet Cells Antibodies, GAB, IgA, IgG, Total - antibodies of the IgA and IgG classes to goblet cells of the intestine, in total
CNS –central nervous system
PNS –peripheral nervous system
CMV –cytomegalovirus
GBS - Guillain–Barre syndrome
MFS - Miller-Fisher syndrome
MMN - multifocal motor neuropathy
CANOMAD - chronic toxic neuropathy
AMAN - acute motor axonal neuropathy
AchR - antibodies to acetylcholine receptors
TTH - thyroid - stimulating hormone
GAD - antibodies to glutamic acid decarboxylase

Introduction

Autoimmune diseases (AID) is a common name for immuno—mediated diseases caused by a specific immuno-inflammatory reaction against foreign antigens. AID are among the most severe chronic human immuno-inflammatory diseases, are widespread in the population and are represented by more than 100 nosological forms in 8% of the world's population. Autoimmune diseases, especially with cross-overlap syndromes, are caused by a high frequency of spread, difficulty of early diagnosis, progressive course and often unfavorable life prognosis [1]. Autoimmune rheumatic diseases (ARD) account for a significant share in the structure of AID: rheumatoid arthritis, systemic lupus erythematosus, seronegative arthritis, systemic vasculitis, systemic sclerosis, as well as other systemic connective tissue diseases.

The importance of understanding the fundamental mechanisms of immunopathogenesis of AID is necessary for development of innovative methods of early diagnosis, as well as to improve the basic pathogenetic treatment and predict the effectiveness of therapy for these pathological conditions.

The specificity of ARD diagnostics is associated with development of immunological and molecular biological research methods, including autoantibodies, acute-phase inflammatory proteins, cytokines, chemokines, markers of vascular endothelial activation, components of complement system, lymphocyte subpopulations, etc. Currently, worldwide emphasis is placed on the possibility of early diagnosis of autoimmune diseases.

The success of scientific medicine of XXI century is development and implementation of innovative molecular and cellular technologies, which increases the diagnostic sensitivity and specificity of laboratory tests, is a prerequisite for development of a complex of immunological and molecular biological methods for diagnosis of autoimmune diseases.

1. Problems of immunopathology of autoimmune diseases

1. 1 Classification of autoimmune diseases

Biomedical criteria are used to classify autoimmune processes (table.1).

Table №1. Biomedical criteria of autoimmune diseases Rose-Bona

Direct evidence	<u>Antibody-mediated</u> Autoantibodies in the blood that affect organ functions Autoantibodies fixed in tissue Immune complex localized in the tissue Reproduction of disease with passive transfer of Ig <u>Cell-mediated</u>

	Transfer of T cells to a SCID mouse with a tissue implant Cytotoxicity of autoaggressive T cells in vitro
Indirect evidence	Experimental immunization Immunization with an anti-idiotypic antibody Spontaneous disease in animals Experimental dysregulation of immune system in animals
Additional evidence	Autoantibodies in the blood Association with other autoimmune diseases Association with HLA genotype Lymphocytic infiltration of organ Response to immunosuppressive therapy

According to the classification, AID is divided into organ-nonspecific and organ-specific autoimmune diseases (Table 2). Organ-specific AID is characterized by damage to a specific organ and tissue. In organ-nonspecific diseases, several systems and organs can be affected, as in rheumatic diseases.

Table №2. Classification of autoimmune diseases

Organ-specific	Multiple sclerosis Hashimoto 's Craw Primary mixedema Thyrotoxicosis (Graves' disease) Pernicious anemia Primary biliary cirrhosis Autoimmune hepatitis Type 1 diabetes mellitus Crohn's disease Ulcerative colitis Autoimmune hemolytic anemia Idiopathic thrombocytopenic purpura Pemphigoid
Organ-nonspecific	Systemic lupus erythematosus Granulomatosis with polyangiitis (formerly c-m Wegener) Scleroderma Mixed connective tissue disease Dermatomyositis Rheumatoid arthritis

1.2 Immunological foundations of the pathogenesis of autoimmune diseases.

Genetically determined and acquired defects of immune system are of crucial importance in the pathogenesis of immuno-inflammatory rheumatic diseases.

The immune system normally does not recognize and does not respond to its own antigens, which is characterized by the presence of immunological tolerance to its own antigens or *auto-tolerance*. *Autoimmune* is the immune response to the antigens of its own cells and tissues, which leads to the development of *autoimmune diseases* (AD).

Regulatory T cells. T-regulatory cells (Treg) are involved in the development of non-response to their own antigens on the periphery. In the thymus, as a result of central selection, lymphocytes, antigen-recognizing receptor (TCR) have a high affinity for autoantigens, are subject to apoptosis or turn into regulatory cells. *Regulatory tolerance*, tolerance caused by regulatory cells, and the cells themselves are *natural or physical (nTreg).* There are two types of regulatory cells, and common to both types of regulatory cells is the expression of CD4 and CD25 on their surface, the transcription factor FoxP3. Recognition of autoantigens on periphery by special antigen-presenting cells (APC) or somatic cells (which in certain situations can also express antigens in combination with class II MHC) nTreg produce TGF-β and IL-10.These cytokines inhibit activation and differentiation of autoreactive T-lymphocytes into effector cells, their recognition of the autoantigen.

The source of autoantibodies is autoreactive B-lymphocyte clones, if their concentration is low. B-cells cannot effectively proliferate and produce antibodies with a lack of T-lymphocyte assistance. Therefore, autoantibodies to the spectrum of autoantigens can be detected in any healthy person, but in small, diagnostically insignificant amounts.

The synthesis of autoantibodies with defects in B-cell tolerance that support inflammation and destruction of human body tissues, contribute to the disruption of T-cell immune response, which plays an important role in the pathogenesis of immuno-inflammatory rheumatic diseases. It has been studied that plasma cells of the synovial tissue of one knee joint of RA patients produce as many immunoglobulins as the entire lymphoid tissue of a healthy person.

A particularly important role in the pathogenesis of autoimmune diseases belongs to proinflammatory cytokines – tumor necrosis factor α (TNFa) and interleukin 6 (IL6), as well as IL12, IL23, IL17, etc., which take part in the development of chronic inflammation, lead to the destruction of joints and other organs and systems (Fig.1)

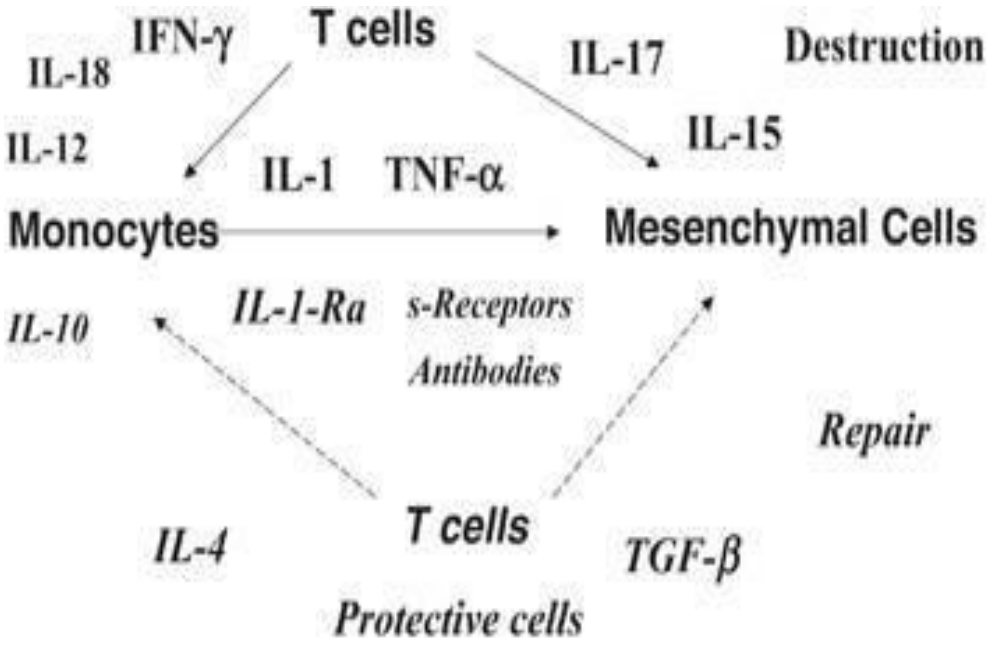


Fig.1 Contribution of TNF and interleukin IL-1 to systemic manifestations of chronic inflammation

Genetic risk factors in the development of most autoimmune RD do not exceed 12-67%

Genetic polymorphisms of RD include:

- monogenic mutations (AIRE, TNFRSF6, FOXP3, CD25),

- genes of predisposition to the development of the disease associated with HLA alleles of classes I, II, III (SLE is associated with HLA-II: DR3, DR2, DR8, HLA-III: TNF, C2, C4, C4B; PA – c HLA-II: DR4, HLA-III: TNF;

- ankylosing spondylitis (AS) – with HLA-I:B27) and genes unrelated to HLA loci detected by genome-wide association studies GWAS (PTPN22, IRF5-TNPO3, STAT4, CTLA4, PAD14 и др.) [20].

The pathogenesis of RD is based on the impact of many factors, with the loss of immunological tolerance towards their own antigens and the development of autoimmunity with a violation of the balance of cell populations (T reg and Tr1) and effector T helper cells (Th1, Th2, Th17, Tfh), stimulating the activation of B cells, maturation of plasma cells, the production of autoantibodies, Cytokines: TNFa, IL1, IL2, IL4, IL6, IL10, IL12, IL15, IL17, IL18, IL21, IL23, IFNy, B-cell activation factor/Lymphocytic stimulator (BAFF/BLyS), APRIL, CD40L – and formation of cytotoxic T-lymphocytes.

Autologous hematopoietic stem cells, mesenchymal stromal cells, autologous tolerant dendritic cells, T- and B-regulatory cells, genetically engineered biological therapy, peptide antigens are used for pathogenetic therapy of SLE, RA, SSD and other CARDs, correction of autoimmune disorders and restoration of immunological tolerance

Autologous hematopoietic stem cells, mesenchymal stromal cells, autologous tolerant dendritic cells, T- and B-regulatory cells, genetically engineered biological therapy, peptide antigens are used for pathogenetic therapy of SLE, RA, SSD and other CARDs, correction of autoimmune disorders and restoration of immunological tolerance.

Very often, autoimmune diseases can be preceded by acute infections. Proinflammatory cytokines released in response to infection contribute to the release of immature cells not only from the bone marrow, but also from the thymus. This is where the probability of T-lymphocytes that have not been selected entering the circulation increases, and there is a high probability of autoreactive diseases (Fig. 2).

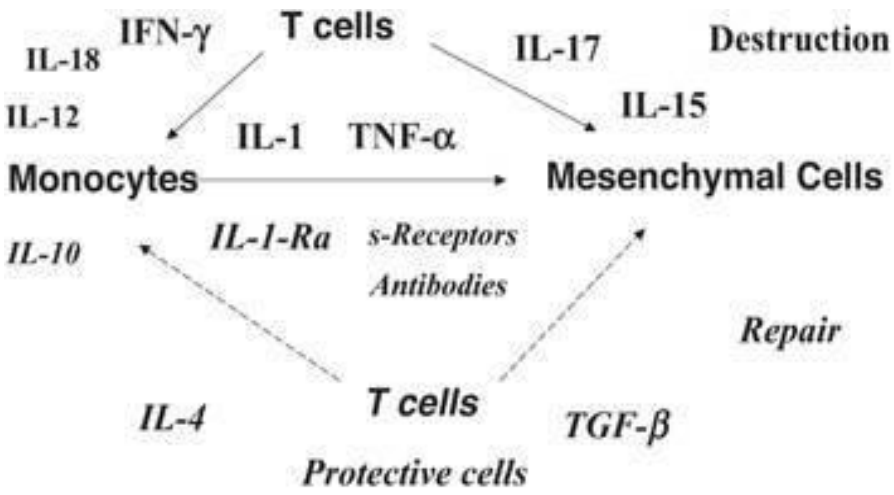


Fig.2 Interaction between regulatory cytokines, matrix destruction and repair defects

Pro-inflammatory cytokines, B- and T-cells are promising therapeutic targets for treatment of autoimmune rheumatic diseases [2], clinical and immunological effects of genetically engineered biological drugs (GEBP) allow us to obtain new data on the pathogenesis of rheumatic and other immuno-inflammatory human diseases.

At the present stage, more than 10 innovative genetically engineered biological drugs (GEBP) - monoclonal antibodies (mAT) and recombinant proteins inhibiting the activity of the most important pro–inflammatory cytokines – TNFa [2], IL6 [3], IL1 [4], IL17 [5] are developed for treatment of common forms of non-communicable diseases at the beginning of the XXI century., IL12/23 [6], as well as pathological activation of T-lymphocytes [2] and B-lymphocytes [7,8,9], many of which are successfully used in clinical practice all over the world, including in Russia and Kazakhstan.

One of the most characteristic systemic pro–inflammatory effects of IL6 is stimulation of an acute-phase inflammatory response, which is associated with an increase in the expression of the IL6 gene in the liver and is manifested in an increase in the concentration of proteins of the acute phase of inflammation (C–reactive protein - CRP, fibrinogen, serum amyloid protein A – SAA).

Under the action of IL6, synthesis of another acute–phase protein in the liver, hepcidin, increases, when bound, release of iron by macrophages is inhibited and iron absorption in the duodenum decreases, which leads to the development of anemia of chronic disease in patients with RA [10]. IL6 stimulates the production of leptin, the hormone of anorexia, characteristic of chronic inflammatory diseases. Manifestations of systemic action of IL6 – fever and morning stiffness, are associated with the daily rhythm of the secretion of this cytokine, maximum of which falls in the early morning hours. As already established, development of arthritis is characterized by neovascularization of synovial tissue followed by leukocyte infiltration and hyperplasia of synoviocytes, which ultimately leads to the formation of pannus.

IL6 in the presence of a soluble IL6 receptor (rIL6R) stimulates production of vascular endothelial growth factor (VEGF) by synovial fibroblasts of RA patients, activates the synthesis of chemokines such as MCP1 and IL8 by endothelial cells, mononuclear cells and synovial fibroblasts, enhances and promotes the migration of inflammatory cells into the joint cavity. In combination with IL-1, it stimulates the production of matrix metalloproteinases (MMP) 1, 3, 13 by synovial cells involved in the destruction of cartilage tissue in RA [11], increased osteoclastogenesis and bone resorption, which are central to the progression of erosive joint damage in RA, maturation of osteoclasts from hematopoietic stem cells of the granulocyte-macrophage series, activates the synthesis of prostaglandin E2 (PGE2) in interaction with rIL6R.

Cytokine IL-6 in the pathogenesis of RA affects the adaptive immune response. IL6 leads to proliferation and differentiation of B-lymphocytes into mature plasma cells secreting autoantibodies (rheumatoid factor – RF, antibodies to citrullinated proteins – ACP) and immunoglobulins, activates IL21 production in CD4+Th lymphocytes.

Th17 cells are involved in the immunopathogenesis of immuno-inflammatory diseases, including RA, psoriasis, PsA, inflammatory bowel diseases, SLE, Sjogren's disease, etc. Members of IL12 cytokine family - IL12 and IL23, TGF – participate in the formation of Th17. Differentiation of naive CD4+Th cells into Th17 lymphocytes also occurs under the influence of IL1ß and IL6 together with transforming growth factor β (TGF-β), accompanied by the synthesis of cytokines, primarily IL17, IL12, IL22, suppressing the influence of T-regulatory cells.It was found that a representative of TNFa-TWEAK superfamily acts synergistically with IL23 and IL21 in inducing Th17 cell differentiation and IL17A synthesis.

Many scientific studies have shown the role of IL17A in the immunopathogenesis of RA and other inflammatory diseases of the joints. It is known that IL17A is also involved in the secretion of proinflammatory cytokines and chemokines, MMP 1, 2, 9, 13.

Th17 IL17A synthesis is carried out by immunocompetent cells, including mast cells, neutrophils, dendritic cells, γδ-T cells, macrophages, natural killer cells.And the targets for IL 17A are cells expressing IL 17 P, including keratinocytes, synoviocytes, fibroblasts, epithelial cells.

Deciphering of key pathogenetic mechanisms of RD made it possible to identify molecular and cellular biomarkers that can be used as therapeutic "targets". The influence of IL-6 in the pathogenesis of rheumatic diseases has determined the importance of inhibition of IL6 using humanized mAT to IL6–tocilizumab receptors (TCZ, Actemra; "F. Hoffman-la Roche Ltd"), in the treatment of RA and other immuno-inflammatory rheumatic diseases, and has become an important achievement in the pharmacotherapy of inflammatory diseases than the creation of TNFa inhibitors.

Low-molecular chemically synthesized substances - a new class of drugs representing (small molecules – small molecules), allowed us to obtain new data on the pathogenesis of rheumatic and other immuno-inflammatory human diseases. Tyrosine kinases, primarily Janus-associated kinases, play an important role in the regulation of cytokine activity (JAK) [12].

The pathogenesis of autoimmune diseases does not fit into the framework of classical ideas about the mechanisms of development of this pathology, which is primarily associated with activation of acquired immunity and hyperproduction of pathogenic autoantibodies [13]. Ilya Mechnikov, the founder of the concept of auto-inflammation, demonstrated the role of macrophages in the development of inflammation in the absence of serum factors (autoantibodies) — phagocytic theory of immunity. The main role in this process is played by Toll- and NOD-like receptors that recognize certain sequences (patterns) of microorganisms, core components released from cells subjected to apoptosis (or necrosis), uric acid crystals, cholesterol, etc. A representative of the NLRP3 family of NOD-like molecules (Nucleotide-binding oligomerization domain, leucine-rich repeat and pyrin domain containing 3) regulates the activation of caspase 1— an enzyme that converts inactive pro-inflammatory interleukins (IL), such as pro-IL 1, pro-IL 18 and pro-IL 33, into active forms. Hyperproduction of IL 1 associated with NLRP3 activation is the leading mechanism combining autoimmune and autoinflammatory diseases [14]. The imbalance of innate immunity contributes to increased inflammation and destruction of body tissues, activation of monocytes/macrophages, dendritic cells, neutrophils, natural killer cells, mast cells, hyperproduction of IL1, IL18, IL33, IFNa/β, other pro-inflammatory cytokines and local tissue factors (enzymes, costimulating molecules.

Therefore, the prognosis and outcome of autoimmune rheumatic diseases depend on early diagnosis, pathogenetic therapy in the onset of diseases.

2. Modern approaches to laboratory diagnostics of autoimmune diseases

The solution of clinical problems involves the differentiated use of laboratory verification AID (Table.3).

Table 3. Modern laboratory diagnostic methods AID

task of survey	method used immunochemistry
Autoantibody screening for early diagnosis	IIR
Differential diagnosis	Immunoblot
Monitoring the effectiveness of therapy	ELISA, иммунохимия

The development and introduction into clinical practice of innovative molecular and cellular technologies have significantly increased the diagnostic sensitivity and specificity of new laboratory. biomarkers in rheumatology [15].

2.1. Autoantibodies - a criterion for the diagnosis of autoimmune rheumatic diseases

Biomarkers of rheumatic diseases (RD) are determined in various biological substrates:

- in the blood,
- synovial fluid,
- urine,
- biopsies of synovial membrane, kidneys and other affected tissues.

Currently, high-tech automated analytical systems are used using methods of immunochemical analysis:

- (indirect immunofluorescence reaction – IIR,
- enzyme immunoassay – ELISA,
- immunoblotting – IB,
- immunoblot,
- immunonephelometry,
- chemiluminescent immune analysis,
- radioimmunoanalysis – RIA),

On the basis of:

- DNA-,
- RNA-,
- protein and cellular microchips,
- polymerase chain reaction (PCR),
- flow cytometry.

Modern generation of molecular and cellular biomarkers RD includes:

- **pathogenetic biomarkers** - to obtain objective information about the nature of immunopathological disorders in RD;

- **diagnostic biomarkers** - to indicate classification and diagnostic criteria RD;
- **predictive biomarkers** – to make diagnosis of RD at an early, preclinical stage.

Pathogenetically significant RD biomarkers:

- (T- and B-lymphocytes, dendritic cells, macrophages),
- proinflammatory cytokines and their receptors,
- autoantibodies, products of bone and cartilage tissue metabolism,
- indicators of vascular endothelial activation,
- components of complement system,
- intracellular signaling molecules,
- prostaglandins,
- proteases,
- vasoactive amines,
- oxygen free radicals, etc. [18, 19, 20, 21].

Diagnostic biomarkers AID

Circulating antibodies – main serological marker of AID. Characteristic signs of CARD are pathological activation of B cells and hyperproduction of organon-specific autoantibodies [23].

Determination of titer of autoantibodies is the main share of immunological studies in rheumatology (63.6%), determined by serological tests. Basic Diagnostic Laboratory Markers of CARD:

- antinuclear antibodies (ANA),
- rheumatoid factor (RF),
- antibodies to citrullinated proteins (ACP),
- antiphospholipid antibodies (APL),
- antineutrophil cytoplasmic antibodies (ANCA).

We record positive results of these and a number of other antibodies, and an increase in the levels of inflammatory markers

- (ESR, C- reactive protein – СРБ),
- reducing the concentration of components of complement system (CH50, C3, C4),
- hematological disorders (hemolytic anemia, leukopenia, lymphopenia, thrombocytopenia, eosinophilia),
- biochemical changes (increased activity of creatine phosphokinase – CPK, aldolase),
- cryoglobulinemia and hyperimmunoglobulinemia

they are among the diagnostic and classification criteria of CARD and vasculitis [24,25,26,27].

2.2 Predictive biomarkers

Immunopathological disorders develop on average 5 years before the onset of the first clinical symptoms of CARD, associated with the loss of immunological tolerance to its own antigens and the initiation of systemic autoimmunity and inflammation, such as a decrease in the functional activity of T-regulatory cells, the formation of pathogenic autoantibodies (ANA, APL, IgM RF, ACP), increased levels of acute phase proteins, proinflammatory cytokines and chemokines, [30, 31]. At the same time, in the preclinical period of SLE, a number of autoantibodies (ANA, ADC, APL) are detected earlier than others.

Concentration of ANA and ACP increases at the clinical stage of SLE and RA, when there is already a breakdown of immunological tolerance and acceleration of autoimmune process, due to the expansion of epitopes recognized by autoantibodies.

Concentration of ANA and ACP increases at the clinical stage of SLE and RA, when there is already a breakdown of immunological tolerance and acceleration of the autoimmune process, due to the expansion of epitopes recognized by autoantibodies [32, 31].

Serum biomarkers - predictors of lupus nephritis exacerbation (increased concentrations of a-dsDNA and aC1q, decreased levels of C3 and C4) and ANCA-CB with kidney damage [33, 34].

Prognostic RD biomarkers are useful in clinical practice. These are acute-phase indicators (ESR, CRP, SAA, PCT, ferritin, calprotectin, hepsidin, haptoglobin, fibrinogen, etc.) [35,36,37]. CRP synthesis occurs in hepatocytes under the action of pro-inflammatory cytokines, and is a more stable, validated, reproducible and specific marker of inflammation than ESR.

It was established that calprotectin is a serum protein (S100A8/A9, MRP8/MRP 14) released by activated neutrophils and synovial monocytes. It is used as a promising marker of RA activity and the severity of synovial inflammation, prediction of radiological progression of the disease, evaluation of the effectiveness of basic anti-inflammatory drugs.

Marker and mediator of hyperferritinemic syndrome (increased serum ferritin levels >500-1000 ng/ml). The increase is observed in macrophage activation syndrome (MAS), adult Still's disease, catastrophic APHS and septic shock.

High activity of the pathological process in SLE patients is observed with a decrease in the concentration of C3 and C4 components of the complement system and an increase in the levels of complement activation products (C3d, C3a, C4a, C5a, iC3, C4d, Bb, C5b-9) in the blood [38].

The studied literature data and the conducted meta-analysis of clinical studies, seropositivity for RF and/or ADC, and high levels of these autoantibodies

in blood serum before treatment are predictors of a good response to RTM therapy in RA [39,40,4].

A high basal level of IgM RF in the sera of RA patients is achieved by a good clinical effect in the treatment of TCZ [42]. RA patients who are highly ACPA-positive respond better to ABC therapy than ACPA-negative patients [42, 43].

In early RA, compared with the advanced stage of disease, a significant increase in the concentration of Th1- (interferon γ, IFN γ) and Th17 (IL 17) - cytokines, chemokines (IP-10, MIF-1), colony stimulating factors (IL 7, G-CSF).

For prevention, early diagnosis, evaluation of activity, prognosis and effectiveness of RD therapy, modern molecular and cellular biomarkers are needed, which also help to uncover the pathogenetic mechanisms of RD. Most people always have a small amount of autoantibodies in their blood, which are not a manifestation of the disease.

Synthesis of low-affinity autoantibodies is normally controlled by a population of B-1 (CD5+) cells. Only in the case of a serious breakdown in immunity, the level becomes elevated and sufficient for diagnosis. The use of a specific type of autoantibodies as a diagnostic indicator is determined by their occurrence in autoimmune disease.

Autoantibodies that occur exclusively in this disease are called highly specific serological markers. The high content of antibodies implies their high affinity, reflects the specificity and severity of immune response.

It is believed that the content of autoantibodies does not correlate with the activity of disease, nevertheless, a special course of the disease is observed in patients with a specific set of antibodies in the serum, unlike the symptoms in patients without these antibodies.

In autoimmune diseases, inflammation can affect all tissues and organs of the body, which displays the so-called spectrum of autoimmune diseases and divides them into organ-specific and organ-specific. Table 2 presents diagnostic screening and confirmatory tests for various autoimmune diseases: diffuse connective tissue diseases (DCTD) and Antiphospholipid syndrome (APHS); rheumatoid arthritis and joint lesions, vasculitis and autoimmune kidney lesions, autoimmune liver and GIT lesions, autoimmune neurological diseases and endocrinopathy.

Currently, clinical informativeness of measuring the serum concentration of IgG4, the ratio of IgG4/IgG, the number of tissue and circulating IgG4-positive plasmoblasts and feasibility of using these indicators as laboratory criteria for diagnosis of immunoproliferative IgG-related diseases are studied [28].

The primary screening method for determining ANA in blood serum is IIR (indirect immunofluorescence reaction) using HEp-2 (IIR-HEp-2) cells as a substrate, "gold standard" according to the recommendations [29].

Autoantibodies – key criteria for diagnosis of autoimmune rheumatic diseases

Autoantibodies included in the diagnostic and/or classification criteria of autoimmune rheumatic diseases are presented in the table 4.

Table 4. Autoantibodies included in diagnostic and/or classification criteria of autoimmune rheumatic diseases

Disease	Autoantibodies	Diagnostic/or classification criteria ARD
Rheumatoid arthritis(RA)	Rheumatoid factor(RF). Antibodies to citrullinated proteins (ACP)	Classification criteria ACR/EULAR(2010)
Systemic lupus erythematosus (SLE)	Antinuclear antibodies (ANA) Antibodies to double-helical (ds)DNA (anti-dna)Anti-Sm Anti-SSA/Ru Anti-SSB/La Antiphospholipid antibodies(APL): Antibodies to cardiolipin (ACL) - Antibodies to β2-glycoprotein I(aß2-GPI) - Lupus anticoagulant (VA) - False positive Wasserman reaction Direct Coombs test (in the absence of hemolytic anemia)	Классификационные критерии SLICC(2012)
Systemic scleroderma (SSD)	AHA Topoisomerase antibodies I (Scl-70) (anti - Scl-70) Anticetromeric antibodies (ACA) K CENP-A,CENP-B,CENP-C antibodies K RNA-polymerase III (anti-RNA-polymerase III)	Classification criteria ACR/EULAR(2013)
Sjogren's syndrome (SS)	Anti-SSA/Ro Anti-SSB/La RF ANA	Classification criteriaACR(2012)

Mixed connective tissue disease (MCTD)	Anti-U1RNP	Diagnostic criteria (1996)
Undifferentiated connective tissue disease	AHA	Preliminary classification criteria (1997)
Antiphospholipid syndrome (APHS)	BAACLaβ_2-ГПІ	Classification criteria (consensus;2006)
Systemic vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA-CB)	ANCA Antibodies to proteinase 3 (anti-PR3) Antibodies to myeloperoxidase (anti-MPO)	Classification criteria (consensus;2007)

The complex of immunological and molecular biological methods of early diagnosis of RA is presented in the table 4.

Table №5. Complex of immunological and molecular biological methods of early diagnosis of RA

№	Research	Method
1	determination of autoantibodies (ACP, antibodies to citrullinated vimentin – ACV, IgM и IgA РФ)	enzyme immunoassay (IEM);
2	determination of proteins of the acute phase of inflammation (CRP, etc.)	highly sensitive immunonephelometric method
3	oligotyping of genes (HLA-DRB1 and its alleles, so–called shared epitope - SE)	using polymerase chain reaction (PCR);
4	evaluation of expression of informational RNA (iRNA) cytokines in mononuclear cells of peripheral blood and human synovial fluid	real-time PCR method
5	determination of cytokine concentration in biological fluids	using IEM and multiplex analysis (xMAP)

Out of 36 biomarkers, the most "strong" predictors of **early RA were identified, namely:**

- increased concentration of IL-6,
- CRP,

- granulocyte-macrophage colony stimulating factor (GM CSF),
- interferon γ (IFNy),
- IFNy-inducible protein,
- ACPV antibodies to citrulinated protein vimentin.

Table №6. Biomarkers reflecting pathogenetic mechanisms of RA

1	Proinflammatory cytokines/receptors (IL6, TNFa receptor type I),
2	growth factors (epidermal growth factor, vascular endothelial growth factor A),
3	MMP 1, MMP3 matrix metalloproteinases
4	cytoskeletal protein (YKL1),
5	vascular adhesion molecule 1
6	acute-phase proteins (CRP and SAA),
7	hormones (leptin and resistin).

2.3 Diagnostic screening and confirmatory tests for various autoimmune diseases at the Center for Autoimmune Diagnostics of JSC "NSMC"

Taking into account modern achievements of laboratory medicine, pathogenetic mechanisms of AID, immunological and molecular biological methods of diagnosis of autoimmune diseases using high-tech automated systems are introduced at National Scientific Medical Center (NSMC, Nur-Sultan).

There are about 200 varieties of antibodies to nucleoproteins and ribonucleic acids, which are called antinuclear factor (ANF). The study of the antinuclear factor (ANF) is the main method for detecting antinuclear antibodies, allowing detection of autoantibodies to nucleic acids (dsDNA, ssDNA, RNA), to soluble components of cell nucleus ribonucleoproteins, as well as most conformational and insoluble antigens.

Determination of ANF by indirect fluorescence is "gold standard" for detection of antinuclear antibodies (ANA) and the diagnosis of autoimmune diseases .

Normally, ANA is absent in the body. In autoimmune pathology, the immune system begins to produce specific immunoglobulins to its own cells and their components .

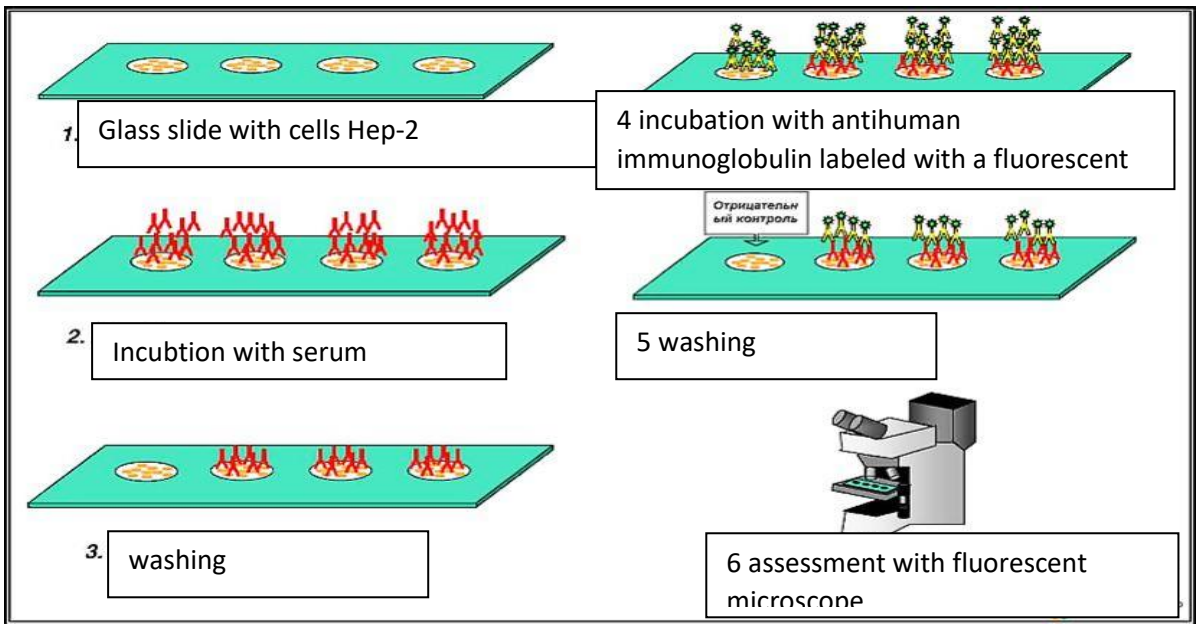
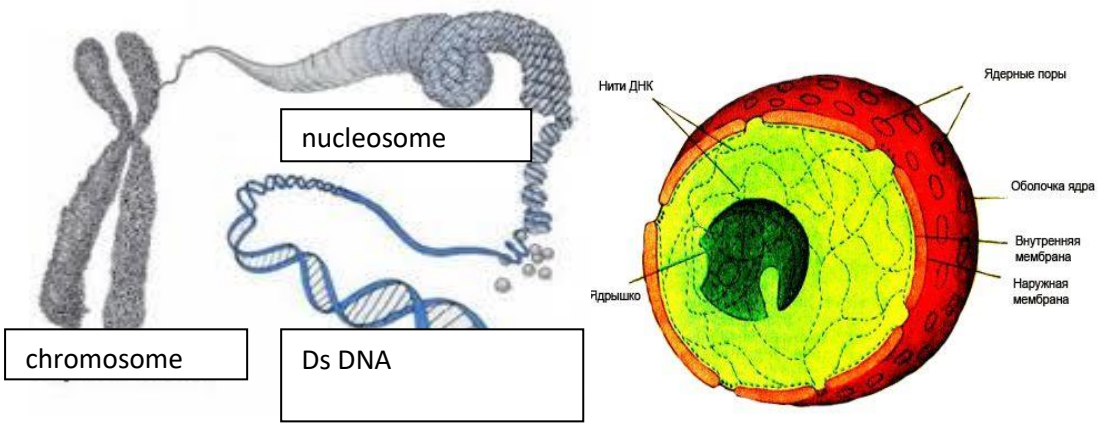


Fig. 3 Scheme of indirect immunofluorescence reaction



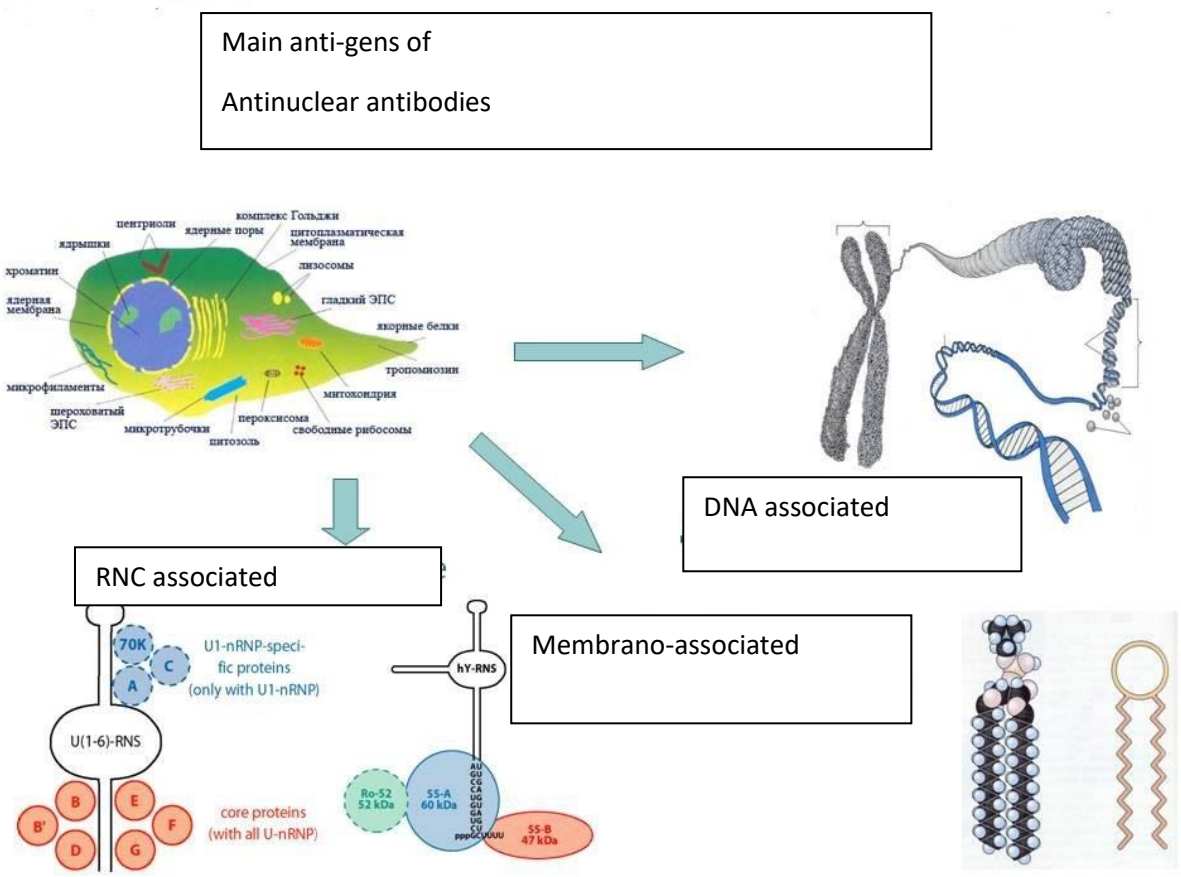


Fig.4 main antigens of antinuclear antibodies

Modern nomenclature of IUPAC 2014
types of glow of the antinuclear factor

International Conciliatory Group on Glow Types was founded on a section within the framework of the International Workshop on Autoimmunity and Autoantibodies (IWAA) in Sao Paolo, Brazil, in 2014.

The result of this work was formation of a generalized nomenclature and description of ANF glow types, a database of microphotographs, as well as a classification with a division into levels of competence in assessing glow types.

To popularize the results of the work of ICAP Conciliation Group, an Internet site was created **https://www.anapatterns.org** , which presented a nomenclature with a detailed description of the types of glow, which today has 30 types of glow, including negative (#AC-0).

Determination of *antinuclear factor (ANF) on HEp-2 cells*

HEp-2 cell line is recognized as the best object for NIF, which significantly improves the sensitivity of the test due to bright fluorescence even with significant dilutions of the patient's serum, and a large, euchromatin-rich nucleus allows you to accurately describe the type of glow.

The result is evaluated using a fluorescent microscope.

Upon detection of antinuclear factor (ANF), cell nuclei are stained, upon detection of antineutrophilic AT, glow is localized in the cytoplasm of neutrophils

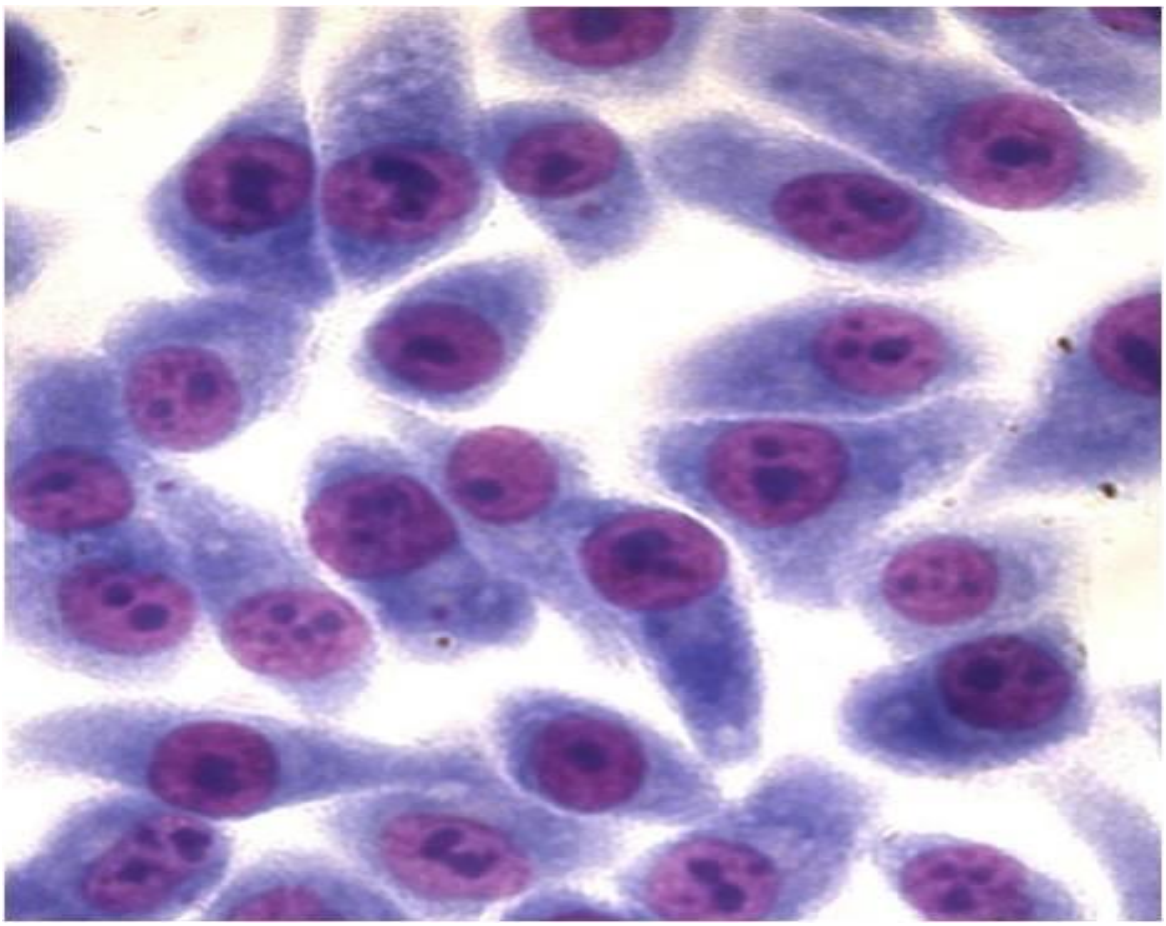


Fig. 5 Cells of HEp-2 cell line stained with hematological dye.

Table No 7. Comparative characteristics of IIR and ELISA

Method	IIR	ELISA
Defined parameter	Affine-dependent	Concentration-dependent
Content of IgG	Does not depend	depends
Number of targets	A lot of antigens	One antigen
Denaturation of antigen	Rarely	Frequently
Reactions with blocking substances	Rarely	Frequently
Soluble antigen	Not used	Used
Automation	Bad	Good

Table 8. List of studies conducted at Center for Autoimmune Diagnostics of JSC «NSMC»

Diffuse Connective Tissue Diseases (DCTD) and Antiphospholipid Syndrome (APHS)	
1	Antinuclear factor on the cell line HEp-2 (ANF)
2	Determination of high-affinity antibodies to dsDNA on C cells. Luciliae
3	Screening of connective tissue diseases ANF and ANA -screening: dsDNA, Sm, ribosomes, histones, RNP, SS-A 60 kDa, SS-A 52 kDa, SS-B, Scl-70, CENP-B и Jo-1
4	Detailed diagnosis of antiphospholipid syndrome: ANF, Antiphospholipid antibodies separately IgG and IgM (10 antigens-cardiolipin, phosphatidylic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, annexin V, β2-GP-1 and prothrombin)
5	Examination with SLE: dsDNA, ANF and antiphospholipid antibodies IgG and IgM (10 antigens: cardiolipin, phosphatidylic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, annexin V, β2-GP-1 and prothrombin)
Rheumatoid arthritis and joint lesions	
6	Screening for rheumatoid arthritis (anti-CCP and RF)
7	Detailed serology of rheumatoid arthritis (ANF, anti-CCP/ ACPA and RF)
8	Antibodies to Cyclic Citrulline-containing peptide (anti-CCP/ACPA)
9	Determination of IgM rheumatoid factor (RF)
Vasculitis and autoimmune kidney lesions	
10	Differential diagnosis of rapidly progressing glomerulonephritis and vasculitis: antibodies to the glomerular basement membrane (BMG), anti-PR3, anti-MPO
11	Antibodies to myeloperoxidase (anti–MPO)
12	Antibodies to proteinase -3 (anti PR-3)

13	1	Diagnosis of autoimmune kidney damage: ANF, BMG and anti-PR3, anti-MPO
Autoimmune liver damage and GIT		
14	1	Screening of autoimmune diseases GIT: ANF and determination of anti-nuclear, antimitochondrial, anti-smooth muscle, antiparietal antibodies (ANA/AMA/ASMA/APCA) indirect immunofluorescence method
15	1	Screening of autoimmune liver damage: ANF, determination of IgG antibodies to M2, gp 210, sp 100, LM1, LC1, SLA and F-actin
Neurological diseases		
16	1	Detailed examination for polyneuritis (ANF, ANA-screen: dsDNA, nucleosomes, Sm, PO, histones, U1-sn RNP, SS-A 60 kDa, SS-A 52 kDa, SS-B, Scl-70, CENP-B and Jo-1; antibodies to gangliosides: anti-GM1,GM2, GM3, GD1a, GD1b, GQ1b, GT1b of IgG class)
17	1	Test for the diagnosis of polyneuropathies: antibodies to gangliosides of IgG/IgM classes (анти-GM1,GM2, GM3, GD1a, GD1b, GQ1b, GT1b)
18	1	Test for the diagnosis of myasthenia gravis. Antibodies to acetylcholine receptors AxP (AchR)
Autoimmune endocrinopathy		
19	1	Diagnosis of type 1 diabetes. Screening of antibodies to islet cells (ICA)

2.4 Diffuse Connective Tissue Diseases (DCTD) and Antiphospholipid Syndrome (APHS)

1) Antinuclear factor on the cell line HEp-2 (ANF)

General information about research

The immune response is directed against nucleoprotein antigens, i.e. complexes of nucleic acids and proteins, which is seen in systemic lupus erythematosus (SLE) and other systemic rheumatic diseases. These endogenous nucleoprotein autoantigens can be formed during epithelial cell apoptosis aimed at destroying and removing damaged or defective cells. Acceleration of apoptosis processes under influence of ultraviolet irradiation, viral infections or medications, simultaneously with impaired or delayed removal of apoptosis products, triggers autoimmune responses in SLE. Nucleoprotein antigens condense in apoptotic bodies, which become a target for autoantibodies.

When determining ANF, epithelial cells of human laryngeal adenocarcinoma HEp-2 are used. HEp-2 cells have a large nucleus and grow in a single layer on the glasses, a very convenient substrate for laboratory research. When the serum of a patient with ANA is added to the glass, the fluorescence (glow) of the Ag + At complex is detected, which is recorded by the device. Antinuclear antibodies are detected due to their binding to intracellular antigens of transferable human epithelial cell line (HEp-2). The nucleus and cytoplasm of HEp-2 cells contain all antigens characteristic of a human cell, which makes it possible to detect all the main antinuclear antibodies in one test.

The method of indirect immunofluorescence on HEp 2 cell line is recommended as the gold standard for the detection of antinuclear antibodies by leading experts, including European (EASIgroup 2010) and American expert groups (ACRANATaskforce 2008).

ANF positive result is observed in more than 90% of patients with diffuse connective tissue diseases, such as SLE and cutaneous forms of this disease, scleroderma and its varieties, mixed connective tissue disease, Sjogren's syndrome.

Definition of ANF is of great importance in the diagnosis of juvenile rheumatoid arthritis and autoimmune liver diseases.

Autoantibodies are also detected in many other autoimmune (thyroiditis, diabetes), infectious (viral hepatitis), inflammatory and oncological diseases.

ANF occurs up to 1-3% in clinically healthy people and increases slightly in people over 65 years of age.

Increased risk of developing autoimmune diseases is observed in individuals with high ANF titers.

Table №9. Indications for research ANF

1	Systemic lupus erythematosus
2	Subacute cutaneous lupus and other varieties of cutaneous lupus
3	Mixed connective tissue disease
4	Sjogren's syndrome and associated diseases
5	Diffuse and localized scleroderma, CREST syndrome
6	Inflammatory myopathies (polymyositis and dermatomyositis)
7	Juvenile chronic arthritis
8	Autoimmune hepatitis
9	Primary biliary cirrhosis and sclerosing cholangitis
10	Polyneuropathies and myelitis

The most important criteria for the diagnostic evaluation of autoimmune markers are determination of antibody titer and the type of antibody glow

Antibody titer **greater than 1:160** is diagnostic

During the exacerbation of rheumatic diseases, titer exceeds **1:640**, and during remission it decreases to **1:160-1:320**. And the more antibodies, the higher the titer.

By the type of glow, targets of antinuclear antibodies are determined, which is of important clinical importance and determines the tactics of further examination of the patient.

The main types of glow: **homogeneous (or diffuse), peripheral, granular or speckled (small-/large-), centromeric, nucleolar (or nucleolar), fusiform apparatus and cytoplasmic types** of core staining. Each type of glow has characteristic features that allow to distinguish one option from another.

Determination of different types of glow indicates the presence of different types of antibodies.

Table 10. Types of antibody glow

Types of glow	What it is characteristic	antigens
Homogeneous (diffuse)	For SLE (high tatras), and low titers for: LV, SD, chronic active hepatitis, RA chromosomal region of mitotic cells – pronounced glow	**DNA. histones**
2) Peripheral	For SLE (high titers) is due to peripheral distribution of chromatin in the nucleus, is associated with **AT to DNA** and it is characteristic for SLE. Peripheral type of glow must be differentiated from staining of nuclear membrane, which is inherent in autoimmune liver diseases. Chromosomal area of mitotic cells is a pronounced glow	**DNA. histones**
3) Granular/ speckled/ in some sources reticulated	Granular type of staining is very common and it is least specific, characteristic of many autoimmune diseases. Autoantigens are nucleoprotein complexes in the nucleus: **Sm (SLE), nRNP (MCTD) -** rough-speckled glow, chromosomal region of mycotic cells is negative. For SLE, Sjogren's syndrome .	**Sm, nRNP, SS-A, SS-B -Scl-70, PCNA**

	SS-A, SS-B – mottled glow with uniform distribution, chromosomal region of mycotic cells is negative. Very often with primary Sjogren's syndrome, less often with SLE, **SS-A** – very often with neonatal lupus and congenital heart block. **Scl-70 –** finely speckled glow, chromosomal area of mycotic cells is positive. PSS Marker. **PCNA –** small-/large-speckled glow, the chromosomal region of mycotic cells is positive or negative. It is found in a small percentage with SLE. Very high ANF titers with a **large-granular type** of glow are characteristic for **mixed connective tissue disease .**	
4) Centromeric	chromosomal area of mycotic cells is negative. For form of scleroderma – CREST syndrome or Thibault-Weissenbach syndrome, less often with diffuse scleroderma and Raynaud's syndrome.	**Centromere of chromosome proteins, NSP-1(SP100)**
5) Nucleolar / Nucleolar	High titers are detected in PSS, polymyositis/dermatomyositis, low titers in Raynaud's syndrome, primary Sjogren's syndrome.	**RNA polymerase - 1, NOR, U_3RNP, PMScl, fibrillarin**
6) Spindle - shaped apparatus	A network of thin threads that connect centrosomes to each other in motor cells. A rare type of glow is characteristic of a number of autoimmune and other diseases: RA, SLE, RVS, carpal tunnel syndrome.	**Fusiform filaments in motor cells**
7) Cytoplasmic	Granular or filamentous glow in the cytoplasm. **Ribosomal RNP antibodies**	**Ribosomal antibodies RNP, Jo-1 (PL-7,PL-12),**

	– typical in some cases with SLE, confirmation is recommended – conducting a test on other sections of tissue **Jo-1 (PL-7,PL-12) -** dermatomyositis/polymyositis **Mitochondria** - marker of autoimmune liver disease, primary biliary cirrhosis **Cytoskeleton (actin, vimetin, tubulin) –** various AID, often in autoimmune hepatitis and infectious diseases.	**mitochondria , cytoskeleton (actin, vimetin, tubulin**)

Types of glow

NUCLEAR GLOW TYPES

Large - granular type of core glow (AC-5) indicates the presence of antinuclear antibodies against U1/RNP, Sm. Characteristic of mixed connective tissue disease, SLE, systemic scleroderma (SSD).

Homogeneous type of core glow (AC-1) indicates the presence of antinuclear antibodies against nucleosomes, double-stranded DNA and histones. Characteristic of SLE, drug-induced lupus, systemic scleroderma, chronic active hepatitis.

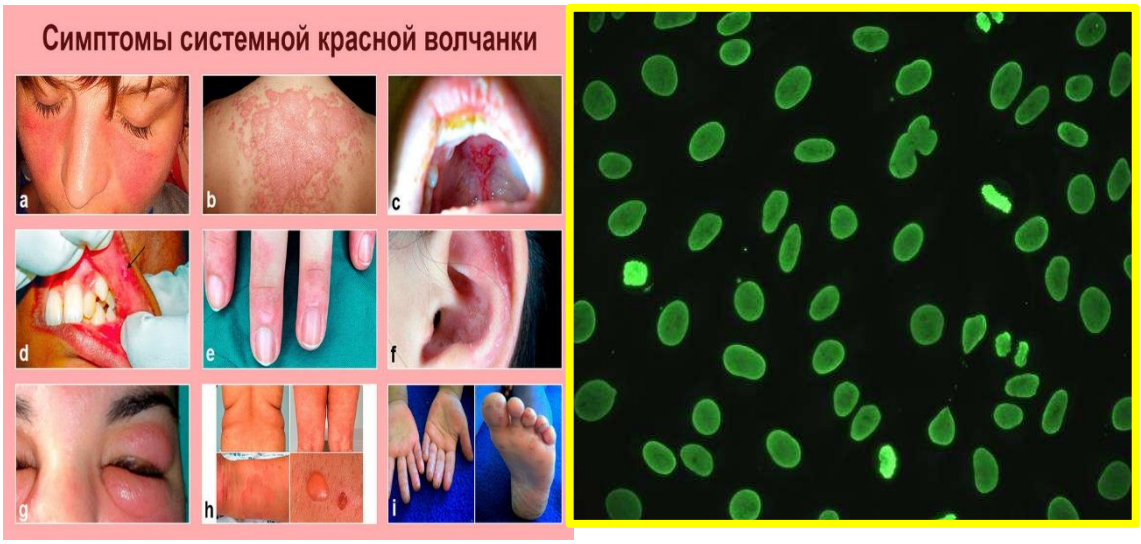


Fig. 6 Homogeneous type of glow ANF

Immunoblot ANA -Antibodies to dsDNA Sm-antigen

Antibodies to nucleosomes
Antibodies to cardiolipin

Main structural units of chromatin are nucleosomes - complexes of DNA and histones. Therefore, homogeneous type of glow suggests the presence of antibodies against nucleosomes, double-stranded DNA and histones.

Usually, detection of a high ANF titer with a homogeneous type of glow indicates the diagnosis of "systemic lupus erythematosus”.

Table No 11. Homogeneous type of glow ANF

Disease	**Main antigens**	**Antigens**
SLE	dsDNA 50%, Histones 30% Nucleosomes 60%	RNP/Sm 10% SSA 60 20% SSA 52 (protein ligase) 25%
Medicinal lupus/vasculitis		
Autoimmune hepatitis		
Scleroderma		
Juvenile idiopathic arthritis		

Fine-grained / homogeneous type of core glow (partially positive nucleolus) indicates the presence of antinuclear antibodies against Scl-70. Characteristic of systemic scleroderma (SSD), systemic sclerosis with diffuse lesions of the skin and internal organs.

Fine - grained type of core glow (AC-4) indicates the presence of antinuclear antibodies against SS-A (Ro), SS-B (La). Characteristic of Sjogren's syndrome, SLE, dermatomyositis, rheumatoid arthritis, systemic scleroderma (SSD), subacute cutaneous lupus erythematosus.

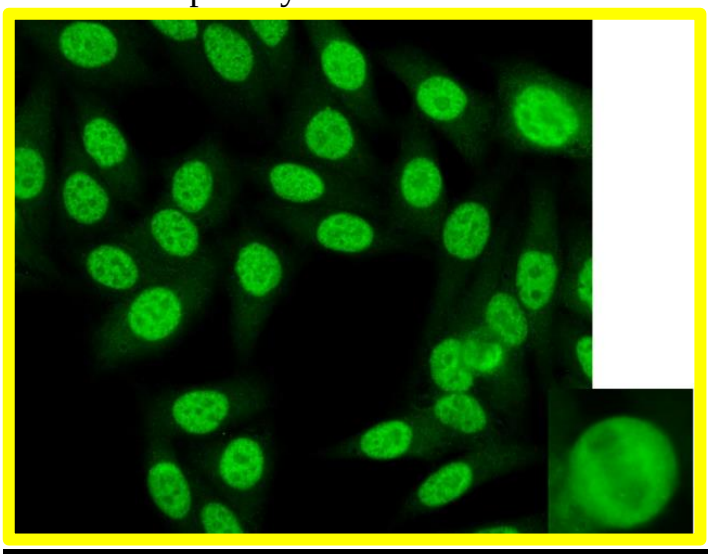


Fig. 7 Granular type of glow

Granular type is the most common and, at the same time, the most nonspecific. Sometimes this type of glow is called "speckled" or "reticulated" in domestic literature of diseases.
Identifying very high titles ANF (1:2560-1:10000) with a ***large-granular type*** of core glow, it usually indicates a diagnosis of a mixed connective tissue disease and requires further examination to identify RNP antigen, which is the main serological marker of this disease.

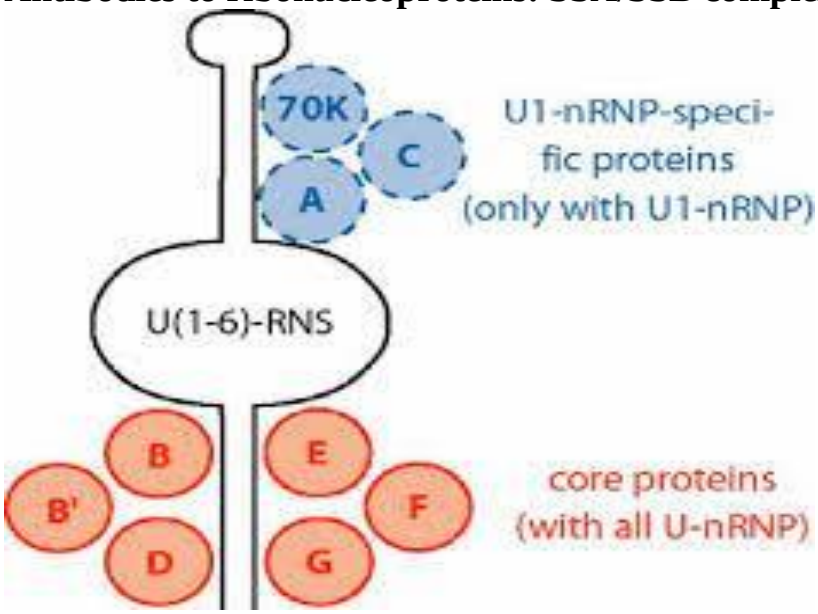
Table No 12. Granular type of glow

Disease	**Main antigens**
SLE, derma	RNP 60% Sm 30% SSA-60 20% SSA-52 40% SSB 20%
MCTD	
Sjogren syndrome	
RA	
Systemic scleroderma	
Dermatomyositis	
Polymyositis	

Antibodies to ribonucleoprotein
(RNP-Sm complex)

Large nuclear granules — a large-granular type of core glow
It consists of a complex of nucleic acid (RNA) and proteins that make up RNP (U1-RNP) antigens in the shell and Sm—antigen in the core
Sm — main SLE marker is met in isolation
U1-RNP — together with Sm —main serological marker of mixed connective tissue disease

Antibodies to ribonucleoproteins: SSA/SSB complex



Fine—grained type of glow is the most frequent

Antigens are soluble— they are lost from NEr2 cells
3 antigens:
zSSA 52 kDa —Ro 52
zSSA 60 kDa —Ro 60
zSSB -La
SSA 52 — most common ANA, non-specific, marker of most autoimmune diseases
SSA 60 — isolated—SLE, together with SSB —Sjogren's syndrome

Table No 13. Other ribonucleoproteins

Ribonucleoproteins	Diseases
Scl-70— antisclerodermal antibodies fragment of topoisomerase -1	marker of diffuse scleroderma
CENT A,B,C - centrometers	CREST syndrome
PCNA- CISlin p32 protein is a nuclear antigen protein of proliferating cells (PCNA).	SLE marker
RiboP — ribosome protein	Neurolupus
Белок Ku — nuclear matrix proteins	SLE
Nucleolar proteins—PM (polymyositis)- Scl, fibrillarin, NOR	Scleroderma
Jo-1 -тRNA- synthetases (antibodies to histidine-t NO-synthetase)	Polymyositis
Mi2 —240 кДа белок ядра	Dermatomyositis

Antibodies to histones and nucleosomes

Nucleosome nucleus—H1, H2A/B, H3, H4 —are the main targets of H1 and H 2B
Antibodies to histones and nucleosomes are most common in drug-induced lupus, scleroderma, autoimmune hepatitis

With medicinal lupus, they appear against the background of treatment and disappear within six months after cancellation

Nucleosomes represent the main immunological target in SLE
Antibodies to nucleosomes are highly specific, observed in 50-60% of SLE patients, can be used together with antibodies to dsDNA in the diagnosis of SLE

Table №14. Nuclear glow types

Type of glow	**Antibody targets (examples)**	**Diseases**
Homogeneous	Chromatin (DNA, histones	SLE, scleroderma
Granular (small- ; large -)	Nucleoproteins (RNP, Sm, SS-A, SS-B)	SLE, mixed connective tissue disease, discoid and subacute cutaneous lupus erythematosus, rheumatoid arthritis, juvenile rheumatoid arthritis, Sjogren's syndrome
Nucleolar	Nucleolar antigens (fibrillarin)	Diffuse scleroderma
Centromeric	Centromere in the chromosome (CENT-B)	Scleroderma
Cytoplasmic	Cytoplasmic antigens (tRNA synthetases, ribosomes, organelles)	Autoimmune liver diseases, systemic lupus erythematosus, polymyositis
Cytoplasmic (mitochondrial)	Mitochondrial antigens (antigens of the pyruvate decarboxylase complex)	Primary biliary cirrhosis
Points in the core	Nucleoproteins	Autoimmune liver diseases

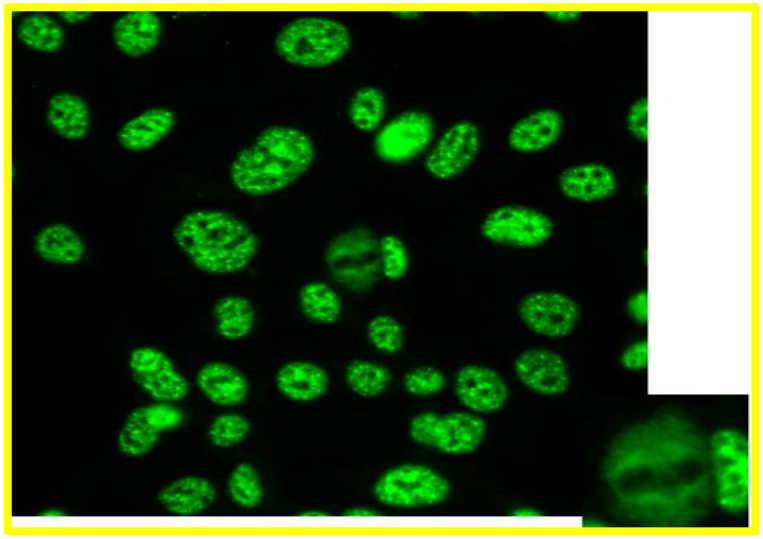


Fig.8 Nuclear type of glow is granular (large speckled)

Table №15. Nuclear type of glow is granular (large and small speckled)

Nuclear type of glow is granular		**Main antigens**	**Antigens**
Diseases (large speckled glow)	**Diseases (small speckled glow)**		
Systemic lupus erythematosus	Systemic lupus erythematosus	dsDNA 50%, Histones 30% Nucleosomes 60%	RNP/Sm 10% SSA 60 20% SSA 52 (protein ligase) 25%
Mixed connective tissue disease	Sjogren 's syndrome		
Raynaud 's syndrome	Mixed connective tissue disease		
Systemic scleroderma	Inflammatory myopathies		
Sjogren 's syndrome			
Undifferentiated connective tissue disease			

Nucleolar homogeneous homogeneous type of core glow (AC-8) indicates the presence of antinuclear antibodies against PM-Scl. It is characteristic of systemic scleroderma (SSD), dermatomyositis, subacute cutaneous lupus erythematosus.

Nucleolar lumpy type of core glow (AC-9) indicates the presence of antinuclear antibodies against U3-snoRNP/fibrillarin. Characteristic of systemic scleroderma (SSD).

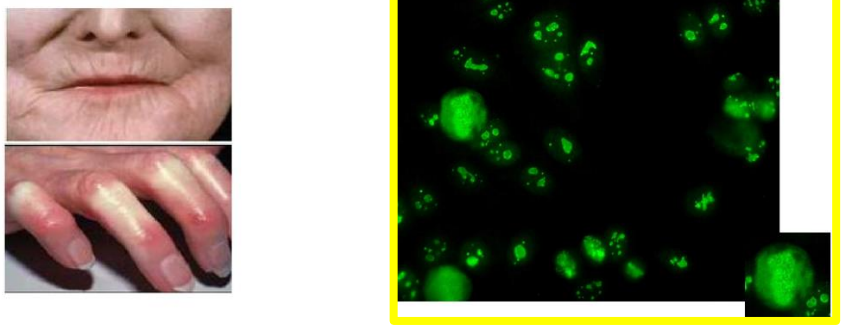


Fig. 9 Nucleolar type of glow

Table №16. Nucleolar type of glow

Nucleolar type of glow		
Diseases	**Main antigens**	**Others**
Diffuse scleroderma	Scl70 4/10	SSA52 –3/10
Dermatomyositis	PM-Scl 1/10	

Centromeric type of core glow (AC-3) (Dots>30) indicates the presence of antinuclear antibodies against CENP-A, B. Characteristic of systemic scleroderma (SSD), primary biliary cirrhosis (PBC), Sjogren's syndrome.

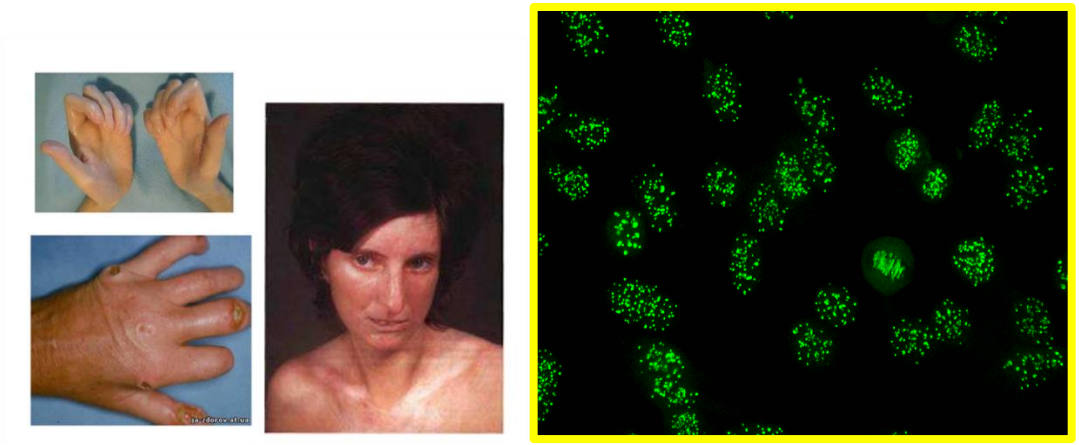


Fig.10 Centromeric type of glow

Table №17. Centromeric type of glow

Centromeric type of glow		
Diseases	**Main antigens**	**Others**
Diffuse scleroderma	Cent B 95%	PM-Scl 1/12
CREST syndrome	Scl70 20%	SSA 52 3/12

Multiple core glow type (points in the core, AC-6 Dots 6-20) indicates the presence of antinuclear antibodies against Sp-100. Characteristic of primary biliary cirrhosis (PBC), diffuse connective tissue disease (DCTD), dermatomyositis.

Homogeneous type of core glow with a thin linear glow of the nuclear membrane (AC-11) indicates the presence of antinuclear antibodies against Lamin (lamins A, B, C, or lamin-associated proteins). Typical for SLE, Sjogren's syndrome, seronegative arthritis.

Pleomorphic granular type of core glow, S-phase – positive (AC-13) indicates the presence of antinuclear antibodies against PCNA (Antibodies to the nuclear antigen of proliferating cells). Typical for SLE, other states.

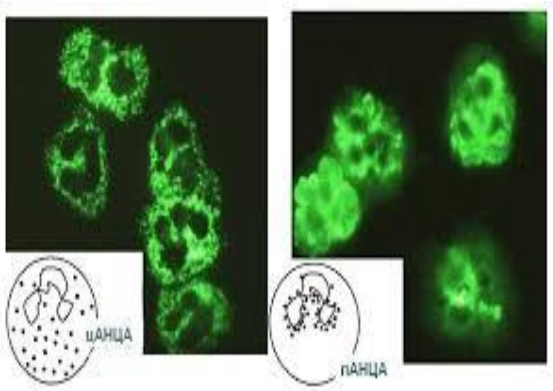
Pleomorphic fine-grained type of core glow, G-2 phase positive (AC-14) indicates the presence of antinuclear antibodies against CENP-F. It is typical for oncological diseases and other conditions.

Fine-grained type of core glow (AC-26, chromatin negative, spindie (mitotic spindle) positive) indicates the presence of antinuclear antibodies against NUMA 1 (antibodies to the mitotic apparatus of the cell). It is characteristic of SLE, systemic scleroderma (SSD), Sjogren's syndrome, mixed connective tissue diseases, rheumatoid arthritis, primary biliary cirrhosis (PBC).

Cytoplasmic types of glow

Cytoplasmic fine-grained, diffuse staining of the cytoplasm, superposition of large dots (AC-20) indicates the presence of antinuclear antibodies against Jo-1. Characteristic of "antisynthetic syndrome", polymyositis, dermatomyositis, local SSD, idiopathic pleural effusion.

Polar cytoplasmic staining on the one hand, Golgi complex in the cytoplasm (AC-22) indicates the presence of antinuclear antibodies against giantin/macrogolgin, goldin-95/GM130, golgi-160, golgi-97, golgin-245. It is characteristic of Sjogren's syndrome (RARELY), SLE, rheumatoid arthritis, diffuse connective tissue disease (DCTD), Wegener's granulomatosis, idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, viral infections.



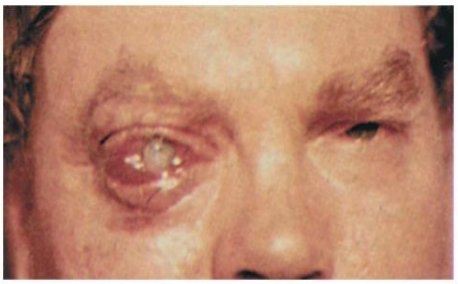


Рис. 19.2. Гранулематоз Вегенера правой орбиты

Fig.11 Wegener's granulomatosis and ANCA-associated vasculitis

Table №18. Antibodies to MRO (myeloperoxidase) are found in a number of vasculitis

Disease	**Antobodies**
GPA - granulomatosis with polyangiitis (formerly Wegener's granulomatosis)	cAHCA pAHCA MPA-pAHCA
Eosinophilic granulomatosis with polyangiitis (EGPA, formerly Charg-Strauss syndrome	
Micropolyangiopathies	
Nodular polyarteritis	
Antibodies are also found in glomerulonephritis, for example, in rapidly progressing glomerulonephritis	

Cytoplasmic glow from fine-grained to homogeneous, dots in the nucleus (mixed type) ndicates the presence of antinuclear antibodies against Ribosomal phosphoproteins, Coilin. Typical for SLE, systemic scleroderma (SSD), Sjogren's syndrome, primary biliary cirrhosis (PBC).

Differential diagnosis of rapidly progressive glomerulonephritis and vasculitis:

antibodies to the glomerular basement membrane (BMG), anti-PR3, anti-MPO

Antibodies to the basement membrane of the glomeruli of the kidneys IgA, IgM, IgG (anti-BMG, Glomerular Basement Membrane IgA&IgM&Ig Gantibody, anti-GBM**)**

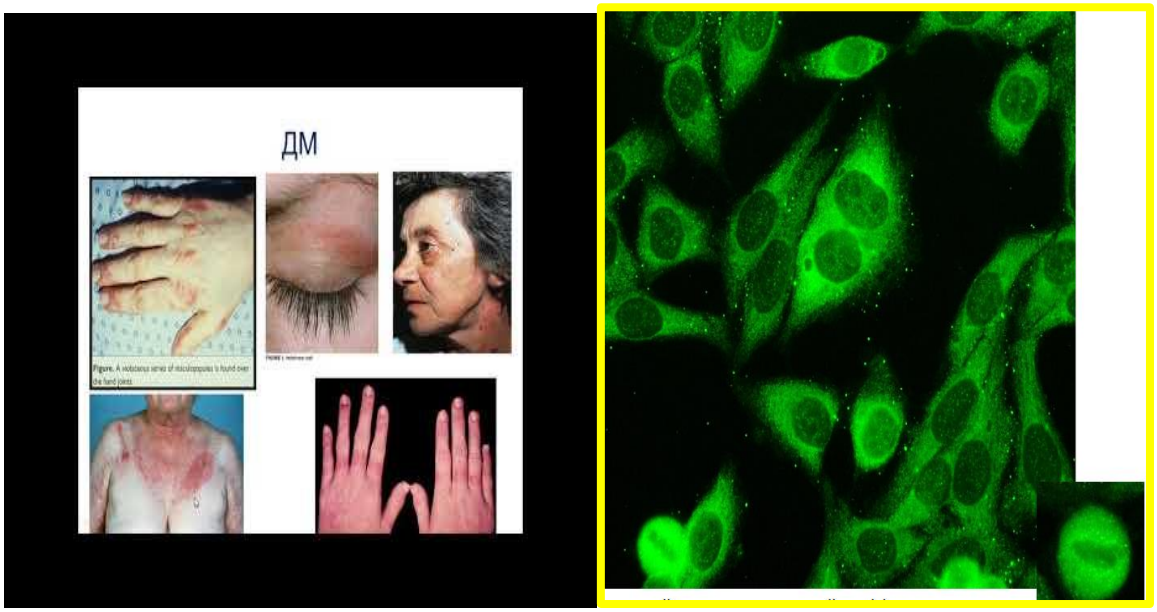


Fig.12 Cytoplasmic small speckled glow. Dermatomyositis

Table №19. Cytoplasmic fine speckled glow

Clinical options	**Diseases**	**Increased muscle enzymes**	**Diagnostics**
Symmetrical weakness of proximal muscles of the upper and lower extremities	Systemic lupus erythematosus	CPK, LDH, aldolases	**ANF with diffuse cytoplasmic glow**
Symmetrical weakness of proximal muscles of upper and lower extremities	inflammatory myopathies	ENMG data	Immunoblot ANA for polymyositis
Defeat of muscles of pharynx, larynx, esophagus	Primary biliary cholangitic		Jo1 antibodies (antibodies to histidine),
Defeat of muscles of pharynx, larynx, esophagus			SRP (signal recognition particle), Mi-2
Myalgia, muscle edema			
Skin manifestations			

When receiving a negative result in 90% excludes the diagnosis of autoimmune diseases: SLE, diffuse scleroderma, Sjogren's syndrome, CREST syndrome, MCTD, secondary APHS; and in 80% excludes the most common forms of autoimmune liver damage. ANF is found negative in isolated patients with SLE, cutaneous forms of lupus erythematosus, polymyositis, APHS, and of course requires an additional examination. The absence of antibodies to nucleosomes significantly reduces the likelihood of SLE, including such clinical manifestations as lupus nephritis, but does not exclude the presence of cutaneous forms of lupus in the patient.

The mitochondrial/reticular type of cytoplasmic glow (large cytoplasmic dots located in a filamentous network) (AC-21) indicates the presence of antinuclear antibodies against AMA-M2. Characteristic of primary biliary cirrhosis (PBC**).**

ANA increased is observed in endocrine diseases (type 1 diabetes mellitus, thyroiditis, thyrotoxicosis, polyendocrine syndrome), skin diseases (psoriasis, pemphigus), during pregnancy, after organ and tissue transplantation, in patients on hemodialysis.

The most important criteria for diagnostic evaluation of autoimmune markers are determination of antibody titer and the type of antibody glow

The antibody titer of more than **1:160 is considered to be diagnostically significant.**

With exacerbation of rheumatic diseases, it exceeds 1:640, 1: 1280, and during remission it decreases to 1:160-1:320. The more antibodies, the higher the titer.

By the type of glow, it is possible to identify targets of antinuclear antibodies, which is of important clinical importance and determines the tactics of further examination of the patient**.**

Table No 20. The reasons for the increase in ANF titer on HEp-2 cells are

№	**Diseases**
1	Systemic lupus erythematosus (in 95 % cases)
2	Dermatomyositis/polymyositis
3	Systemic scleroderma (in 60-90 % cases)
4	Sjogren 's syndrome (in 40-70 %cases)
5	Mixed connective tissue disease (Sharpe syndrome)
6	Raynaud 's syndrome
7	Discoid lupus
8	Medicinal lupus
9	Rheumatoid arthritis
10	Necrotizing vasculitis
11	Infectious mononucleosis
12	Leukemia
13	Malignant neoplasms (mainly lymphoma)

14	Severe myasthenia gravis
15	Infectious endocarditis
16	Chronic autoimmune hepatitis
17	Primary biliary cirrhosis of liver
18	Tuberculosis
19	Pneumoconiosis
20	Interstitial pulmonary fibrosis

Table №21. A false positive result is promoted by:

№	Reasons	
	Age over 60-65 years	in 10-37 % cases
	The use of medications that can lead to medicinal lupus	acetazolamide, carbidopa, chlorothiazide, chlorpromazine, clofibrate, ethosuximide, gold salts, griseofulvin, hydralazine, isoniazide, lithium salts, methyldopa, oral contraceptives, penicillin, phenylbutazone, phenytoin, primidone, procainamide, propyluracil, quinidine, reserpine, streptomycin, sulfonamide, tetracycline, thiazide diuretics
	Taking glucocorticosteroids	prednisone, dexamethasone, metipred

Important notes

- With SLE, a negative result of this study is found in 5% of cases (ANF-negative SLE). Then, to clarify the diagnosis, it is necessary to determine antibodies to SS-antigens (Ro).
- A positive test result may not always be an absolute proof of the presence of an autoimmune disease. In healthy people, in 3-13% of cases, the ANF titer is elevated and reaches **1:320**.
- Therefore, the test results must be evaluated in conjunction with clinical data and other laboratory indicators.
- To verify the diagnosis with a positive result of the study, it is recommended to determine the specificity of ANA with an **immunoblot.**

With an increase in the titer of serum antibodies, with a positive result of the analysis, the type of glow of the nucleus is characteristic. This is due to the wide range of antibodies that find their targets inside the cell. There are more than 40 types of luminescence, but 6 of them are used in clinical practice: homogeneous, peripheral, granular, nucleolar, centromeric and cytoplasmic

Reference values: < 1:160

There is a description of 2 types of glow, in which one in low credits masks the other. Then, if a positive ANF result is detected, it is necessary to establish antigenic targets of antinuclear antibodies, using additional tests, such as the determination of antibodies to double-stranded DNA and antibodies to nucleosomes, an immunoblot of antinuclear antibodies, an immunoblot of antibodies in scleroderma, an immunoblot of antibodies in polymyositis and the determination of antibodies to cardiolipin.

Autoimmune antibodies in diagnosis SLE

Systematic rheumatic-inflammatory diseases, such as SLE and its variants, are multisystem diseases. **Autoantibodies to native, double-helical DNA** are a pathognomonic sign of SLE and, in addition to **Sm** autoantibodies, belong to the classification criteria of American College of Rheumatology.

Determination of high-affinity antibodies to dsDNA on cells of C.Luciliae

Determined by indirect immunofluorescence on glass. For this research, protozoa of the flagellated microorganism Crithidia luciliae are used, they contain a kinetoplast - an organelle containing ring DNA, which, **with a positive test result, turns into a bright apple-green color.**

Determination of anti-nDNA by indirect immunofluorescence using C. lucilia is advisable to combine with the enzyme immunoassay (ELISA). However, ELISA gives a large number of false positive results.

The frequency of a positive result of antibodies to nDNA when determined by indirect immunofluorescence on C. luciliae cells using this kit was **68%** for patients with SLE, 0% with scleroderma, 0% with RA, control group – 0%.

At to nDNA are specific to SLE and are rarely found in patients suffering from RA, SSD or other autoimmune diseases. There is a direct involvement of anti-dsDNA IgG antibodies in the pathogenesis of vasculitis and lupus nephritis.

They can be detected in approximately 40-70% of patients in the active phase (sensitivity – 91%, specificity – 96%). The occurrence and titer of these antibodies varies depending on the activity of disease with a tendency to disappear during immunosuppressive therapy and the period of remission.

When conducting monthly monitoring of concentration of autoantibodies to nDNA, it will allow predicting an impending relapse of the disease. Antibody titers closely correlate with concentration of IgG-containing CIC in the blood serum of SLE patients. Anti-nDNA and hypocomplementemia tests are a diagnostic tool for identifying a category of patients at high risk of developing lupus glomerulonephritis. There is a direct correlation between the increase in anti- nDNA titer, severity of hypocomplementemia and severity of lupus nephritis.

dsDNA has the ability to bind to the basement membrane of renal glomeruli, and leads to the formation of immune complexes directly in the glomeruli. In this pathogenesis, accumulation of immune complexes leads to the activation of complement (with consumption of its serum reserves) and development of inflammation and tissue damage.

There is a correlation between the severity of glomerulonephritis and the level of anti-dsDNA IgG antibodies in patients with SLE. The concentration of these antibodies varies with changes in SLE activity. An increase in the level of anti-dsDNA IgG antibodies for several weeks and a decrease in the content of complement (complement components C3, C4) in most cases are harbingers of clinical exacerbation. A decrease in the level of antibodies is detected immediately at the time of exacerbation of glomerulonephritis. It occurs when anti-dsDNA antibodies are not detected in some patients with SLE.

n such cases, a negative test result does not always exclude the disease.

Reference values: negative result - fluorescent glow (titer) less than 10.0 units, positive result: more than or equal to 10.0 units.

Units of measurement in Independent INVITRO Laboratory: IU /ml.

Table №22. Determination of high-affinity antibodies to dsDNA on cells of C.Luciliae

<table>
<tr><th colspan="2">Reference values</th><td rowspan="8">Raising the level > 25 IU/ml</td><th>Diseases</th></tr>
<tr><td>< 20 IU/ml</td><td>negative</td><td>Systemic lupus erythematosus (SLE)</td></tr>
<tr><td>20 - 25 IU/ml</td><td>doubtful</td><td>Rheumatoid arthritis</td></tr>
<tr><td colspan="2" rowspan="5">> 25 IU/ml
positive</td><td>Sjogren 's syndrome</td></tr>
<tr><td>Scleroderma</td></tr>
<tr><td>Chronic active hepatitis</td></tr>
<tr><td>Biliary cirrhosis</td></tr>
<tr><td>Epstein-Barr virus infection and cytomegalovirus infection.</td></tr>
</table>

Table №23. Qualitative determination of IgG class antibodies to nuclear and cytoplasmic antigens in human serum or plasma by immunoblot (test strips with applied antigens)

№	Autoantibodies
1	dsDNA
2	Nucleosomen
3	Sm (Smith)
4	PO
5	Histon
6	RNP (anti-ribonucleoprotein)

7	SS-A/Ro60 белки, связанные с RNA
8	SS-A/Ro52
9	PM – polymyositis
10	SS-B/La (antibodies to the protein associated with RNA polymerase-3
11	Scl-70 (antisclerodermal antibodies with a molecular weight of 70 kDa, antibodies to topoisomerase I)
12	Jo-1 Anti-Jo-1 antibodies (antibodies to histidine-no-synthetase) were first detected in the blood serum of patients with myositis
13	CENP-B Anti-centromeric antibodies (anti-CENT-B antigen of proliferating cells (PCNA)
14	AT to the ribosomal protein P (Ribo P)

Antibodies to nucleosomes.

Antibodies to NCS were detected mainly in patients with kidney damage (85%) and cytopenia (75%)

Use anti-NCS and anti-dsDNA to identify SLE patients with severe lupus

They can be used to diagnose SLE when detecting high ANF titers

There may also be a peripheral type of glow for SLE.

2) Screening of connective tissue diseases - a comprehensive study includes:

a) Antinuclear factor on the cell line HEp-2 (ANF)

b) ANA-screen, 12 antinuclear autoantibodies (dsDNA, Nucleosomen, Sm, PO, Histon, RNP, SS-A/Ro60, SS-A/Ro52, SS-B/La, Scl-70, CENP-B, Jo-1)- systemic inflammatory rheumatic diseases (SLC, SSD, RA, Sjogren's syndrome, DM, Sharpe's syndrome) are characterized by production of a large number of antibodies directed against cytoplasmic and/or anti-nuclear antigens.

Simultaneous detection of cytoplasmic and anti-nuclear antibodies using **ANA-12** allows to determine characteristic profiles for differential diagnosis of systemic rheumatic inflammatory diseases.

This qualitative definition is positive/negative) IgG class antibodies to nuclear and cytoplasmic antigens in human serum or plasma by immunoblot method (test strips with applied antigens)

Test sensitivity: dsDNA-100%, Nucleosomen-94%, Sm-100%, PO-100%, Histon-87%, RNP-100%, SS-A/Ro60-100%, SS-A/Ro52-100%, SS-B/La-98%, Scl-70-96%, CENP-B-96%, Jo-1-100%

Table №24. Determination of antibodies in rheumatic inflammatory diseases, their frequency

Frequency, % / Disease	Type of antibodies, antibodies to …								
	ds-DNA	**ss-DNA**	**Histone**	**SS-A (Ro)**	**SS-B (La)**	**Sm**	**RNP/Sm**	**Scl 70**	**Jo-1**
SLE	> 90	> 90	30-50	10-30	30-50	10-30	10-30		
LV*		30-50	50-90						
S. Sharpe /MCTD	10-30	10-30					> 90		
PA		30-	3	10-30					
S. Sjogren	10-30	10-30		> 90	> 90				
Scleroderma	10-30	10-30		10-30				> 90	
FD, DM**	10-30	10-30							50-90

true anti-nuclear antibodies (ANA):
dsDNA, ssDNA, histones, nuclear RNA and DNA
extractable nuclear antigens:
Sm (Smith), n-RNP, Scl 70 и PM-1
cytoplasmic antigens:
SS-A (Ro)*, SS-B (La)* и Jo-1

3) Detailed diagnosis of antiphospholipid syndrome is a comprehensive study, it includes:

1) Antinuclear factor on the cell line HEp-2 (ANF) -

2) 10 antiphospholipid antibodies IgM and IgG (cardiolipin, phosphatidylic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, annexin V, β2-GP-1 and prothrombin).

This is a qualitative definition (positive/negative) antibodies of IgM and IgG class to phospholipids (cardiolipin, phosphatidyl acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine) and their cofactors (plasma proteins) - β2-glycoprotein I, prothrombin, annexin V in human serum or plasma by immunoblot (test strips with applied antigens.

For antiphospholipid syndrome, detection of antiphospholipid antibodies (APLA) as a laboratory marker of APHS, it determines the pathogenesis of its clinical manifestations. APLA affect the processes of regulation of the blood coagulation system, shifting the balance towards hypercoagulation – that is, thrombosis. The process of thrombosis involves the interaction of APLA with phospholipids of platelet membranes, endothelium (cells lining blood vessels from the inside) and plasma proteins associated with phospholipids. With APHS, vessels of any caliber are potentially affected – from capillary bed to large arteries, and is manifested by an extremely diverse range of clinical manifestations of disease.

APLA - is a family of immunoglobulins of different classes (IgA, IgM and IgG), and recognize certain areas of phospholipid molecules. According to recent studies, it turned out that the main targets of APLA are not phospholipids themselves, but plasma proteins binding to them, called cofactors. This cofactor-phospholipid complex forms a new molecular sequence to which specific antibodies are produced. APLA reacts with a heterogeneous group of phospholipids and protein antigens of blood plasma, which include:

- phospholipids – cardiolipin, phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, phosphatidylcholine and phosphatidylic acid;
- plasma proteins – "cofactors" – β2-glycoprotein I, prothrombin, thrombin, protein S, protein C, annexin V.

Diagnostic criterion of antiphospholipid syndrome (APHS)

Antibodies to phospholipids (APL) are autoimmune, or autoantibodies of the IgG and IgM class, act against the main components of cell membranes - phospholipids and, accordingly, against the body's own cells and tissues. Phospholipids can be negatively charged (phosphatidylserine, cardiolipin), positively charged (phosphatidylinositol and phosphatidylic acid), neutral (phosphatidylcholine). Cell membranes play an important role in the blood clotting process.

APL phosphatidylserine, of the anionic (negatively charged), turned out to be the most antigenic. Phosphatidylserine is located on the inner surface of platelets and cell membranes of vascular endothelium.

Disruption of normal functioning of the endothelium of blood vessels is caused by antibodies to phospholipids (APL), leading to vasculopathy (vasoconstriction) and the formation of vascular thrombi. In the interaction of APL with phospholipids, cofactors such as beta-2-glycoprotein, present in normal plasma and circulating in association with lipoproteins (apolipoprotein H), take part. Beta-2-glycoprotenin has natural anticoagulant activity.

By binding antiphospholipid antibodies to the vascular endothelium in the presence of beta-2-glycoprotein in APHS, stimulate the synthesis of Willebrand factor, induces the activity of tissue factor by endothelial cells, stimulates the process of hemocoagulation.

Determination of **a high level of antiphospholipid antibodies is characteristic of antiphospholipid syndrome (APHS)**, which is characterized by **damage to the vessels of the heart, brain, kidneys, liver, adrenal glands**.

A high titer of antibodies to phospholipids is a high risk of venous thrombosis, myocardial infarction in men, and repeated miscarriages in women (more often in the 2nd and 3rd trimester of pregnancy).

Antibodies to phospholipids of vascular endothelial cells lead to an imbalance between the coagulation and anticoagulation systems towards the formation of blood clots.

Microcirculation disorders during pregnancy can lead to circulatory disorders also in the placenta and even to rejection of the fetus.

With APHS, there is often a violation of cerebral circulation with development of stroke, neurological pathology, skin lesions (mesh livedo, skin ulcers).

In 2-4%, antibodies to phospholipids are detected in healthy people, more often elderly than young.

Table №25. Raising the level of APHS

№	Diseases
1	Recurrent vascular thrombosis, thromboembolism
2	Thrombocytopenia
3	Habitual miscarriage of pregnancy (intrauterine fetal deaths, miscarriages, preeclampsia)
4	Wasserman's false positive reaction
5	Collagenoses (systemic lupus erythematosus, nodular periarteritis

Table №26. Raising the level of APHS

№	Primary APHS	
1	Vascular pathology	Strokes
		Infarctions of internal organs
		Gangrene of extremities
		Thrombophlebitis (deep vein thrombosis of extremities)

2	Habitual miscarriage of pregnancy	recurrent unexplained spontaneous abortions in the first trimester or intrauterine fetal death in the II - III trimester
		development of HELLP syndrome in pregnancy pathology (hemolysis, increased activity of liver enzymes, decreased platelet count)
	Secondary APHS	
3	It is characteristic of inflammatory, autoimmune and infectious diseases	HIV infection
		Viral hepatitis C
		Systemic lupus erythematosus
4	Malignant tumors	
5	Taking medications	oral contraceptives, psychotropic drugs

The presence of antibodies to cardiolipin, which are main fraction of APL A, characteristic of development of thrombosis and thrombocytopenia. The increased titer of antibodies to cardiolipin against the background of clinical picture of thrombosis makes it possible to establish the diagnosis of antipholipid syndrome "APHS".

Antibodies to annexin V. A special role is assigned to annexin V, which is necessary to prevent thrombosis in the vessels of the placenta. It is present in many tissues, but mainly on endothelial cells and in the placenta. Annexin V prevents blood clotting by competing with prothrombin (blood clotting factor) for binding to phosphatidylserine on the membrane of endothelial and trophoblast cells. And in patients with APHS, antibodies to the protein annexin V displace it from the surface of endotheliocytes and trophoblast cells, which is accompanied by placental vascular thrombosis and pregnancy loss. More often, antibodies to annexin V are found in secondary antiphospholipid syndrome (occurring against the background of other autoimmune diseases), respectively, in practice, the syndrome of habitual miscarriage is more common in the clinical picture of such patients

And in patients with APHS, antibodies to the protein annexin V displace it from the surface of endotheliocytes and trophoblast cells, which is accompanied by placental vascular thrombosis and pregnancy loss. More often, antibodies to annexin V are found in secondary antiphospholipid syndrome (occurring against the background of other autoimmune diseases), respectively, in practice, the syndrome of habitual miscarriage is more common in the clinical picture of such patients.

Antibodies to prothrombin. The most basic cause of thrombosis, primarily venous, is the detection of antibodies to thrombin. An increased level of IgM-class

antibodies to prothrombin is detected in patients with APHS in about 30%, and IgG - in 18% of cases of the disease.

Antibodies to prothrombin are prescribed if the clinical picture does not match the negative test results recommended by the Sydney laboratory criteria of APHS (lupus anticoagulant, IgM and IgG class antibodies to cardiolipin and β2-glycoprotein I).

It is observed that about 5% of healthy people have an elevated APLA titer, more often they are elderly people – with age, the percentage of APLA-positive people who do not suffer from APHS increases. The tendency to detect antiphospholipid antibodies also increases in patients with inflammatory, autoimmune and infectious diseases (syphilis, HIV infection, viral hepatitis C, herpes virus infection), malignant neoplasms, as well as against the background of taking certain medications (psychotropic drugs, oral contraceptives). All of the above indicates that any of the laboratory signs in the absence of clinical symptoms is not a sufficient condition for the diagnosis of "APHS".

Diagnostic criteria of antiphospholipid syndrome Sydney Consensus Workshop
(Miyakis et al., J Thrombosis & Haemostasis, 2006)

Clinical criteria

1. Vascular thrombosis
2. Obstetric and gynecological manifestations (frozen pregnancy, etc.)

Serological studies:

1. Lupus anticoagulant
2. Antibodies to cardiolipin (IgG/IgM)
3. Antibodies to beta2 glycoprotein 1(IgG/IgM)

1 criteria + 1 criteria ⇒APHS

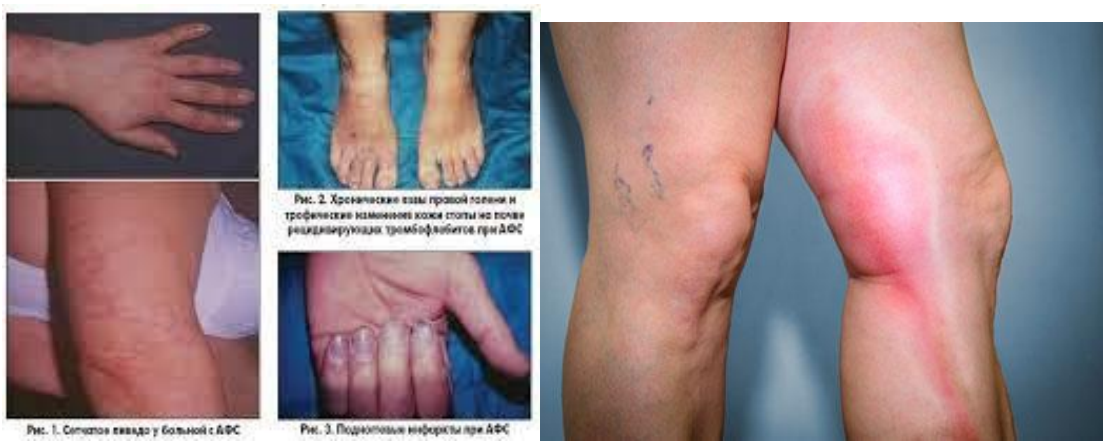


Fig. 13 Clinical manifestations of antiphospholipid syndrome

Table №27. Clinical manifestations of antiphospholipid syndrome

№	Target organs	Manifestations of diseases	%
1	Peripheral thrombosis	Deep vein thrombosis Arterial/venous thrombosis	64%
2	Miscarriage	Early/late miscarriage Early births	63%
3	Rheumatic complaints	Артралгия Артрит	68%
4	Neurological manifestations	Migraine Stroke	66%
5	Skin manifestations	Livedo reticularis Leg ulcers	40%
6	Hematology	Thrombocytopenia Hemolytic anemia	30%
7	Cardiac manifestations	Thickening of valves Myocardial infarction	27%
8	Pulmonary manifestations	TELA Pulmonary hypertension	20%

Table №28. Laboratory diagnostics APHS

№	Research	Reference values
1	Coagulological tests: APTT and lupus anticoagulant (LAC)	**<10 U/ml.**
2	Antibodies to cardiolipin IgGAM	
3	Antibodies to cardiolipin IgG/IgM	
4	Antibodies to beta 2-glycoprotein (IgGAM)	
5	Antinuclear factor and other antinuclear antibodies	

Antibodies to cardiolipin , β2glycoprotein and LAC may be transiently elevated after viral infections – repeated determination is required after 3-6 months

Examination with SLE- comprehensive study includes :

1) Determination of high-affinity antibodies to dsDNA on cells of C.Luciliae

2) Antinuclear factor on the cell line HEp-2 (ANF)

3) 10 antiphospholipid antibodies IgM and IgG (cardiolipin, phosphatidylic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine, annexin V, β2-GP-1 and prothrombin).

2.5 Rheumatoid arthritis

Screening of rheumatoid arthritis (anti-CCP and RF IgM) – a comprehensive study includes:

1) Antibodies to cyclic citrulline-containing peptide (anti-CCP) – despite the fact that RF IgM can be detected in the serum of patients with RA in 70-80% of cases, it is not strictly specific for rheumatoid arthritis and it is found in the serum of young healthy donors in 1-4%, and in elderly patients in 25%. Unlike antibodies to CCP, antibodies to this synthetic peptide are detected already in the early stages of disease and have high prognostic significance.

Anti-citrulline antibodies include ACPA. These autoantigens containing citrulline are characteristic of rheumatoid arthritis and they are an important discovery of recent rheumatology in the field of serological diagnostics. Citrulline does not belong to the standard amino acids included in proteins during their synthesis, it is formed as a result of subsequent modification of arginine.

The inducer of formation of antibodies to citrullinated peptides in the mechanism of development of rheumatoid arthritis is determined by citrullinated fibrin, which accumulates in large quantities in the inflamed synovial membrane. Citrullinated antigens of synovial tissues also include citrullinated vimentin. During development of methods for the determination of antibodies to citrullinated antigens, it was shown that the use of synthetic Cyclic forms of citrullinated peptides provides greater sensitivity of the test, compared with the use of linear peptides. Antibodies to Cyclic Citrullinated peptide are currently an informative serological marker of rheumatoid arthritis.

ACPA is often detected only in 30% of cases of seronegative rheumatoid arthritis (negative for rheumatoid factor). The expediency of using this test in the early diagnosis of arthritis and in order to predict recently developed rheumatoid arthritis has been determined (ACPA is more associated with progression and erosive arthritis than rheumatoid factor). The use of ACPA in order to monitor the activity of the process is not recommended (no correlation with activity markers, including ESR, CRP, is found).

Reference values for quantitative determination: negative – less than 30 units/ml, positive – more than or equal to 30 units/ml.

2) Determination of IgM rheumatoid factor (RF)

This is a quantitative ELISA method for determining class M antibodies.

Reference values:

1. negative (less than 10,0 IU/ml),
2. grey area (10-15 IU/ml),
3. positive (more than 15 IU/ml)

RF antibodies can belong to all classes of IgM, IgG, IgA, and IgM occur most often in patients with RA. Extraarticular manifestations, first of all, can be associated with IgA. A high concentration of IgG has a similar IgM manifestation

in patients with a severe course of the disease, expressed in progressive erosion of joints.

Table №29. Serological markers of rheumatoid arthritis

Autoantibodies	**Description**	**Frequency**
Rheumatoid factor	Waaler, 1940	80%
Antinuclear antibodies	Beck, 1964	30%
Antibodies to RA-33	Hassfeld et al. 1989	50%
Antiperinuclear factor	Nienhuis et al. 1964	50%
Antikeratin antibodies	Young et al. 1979	40%)
Antibodies to vimentin (Sa-antigen)	Despres N et al 1994	30%
Antibodies to Cyclic Citrulline Peptide	Schellekens GA et al. 1998, van Venrooj et al. 2000	70%
Antibodies to modified citrulline vimentin	**Bang et al. 2006**	**80%**
Other anticitrullinic antibodies (citrullinated filaggrin, citrullinated IgG, VEB peptides) – 2005-2008		50-80%

3) Antibodies to keratin (Anti-keratin antibody, AKA)

The presence of antibodies to keratin (AKA) was revealed as one of the specific serological markers of rheumatoid arthritis. This research is associated with a group of tests for the presence of antibodies to citrullinated antigens, which includes such studies as the determination of antiperinuclear factor and a test for antibodies to Cyclic citrulline-containing peptide.

Determination of antikeratin antibodies is important for early diagnosis of rheumatoid arthritis. Their presence may precede clinical manifestations of the disease – in retrospective studies on frozen serum samples, it is shown that in a quarter of cases AKA can be detected 5 years or less before onset of rheumatoid arthritis, in 10% of cases – for 5-9 years, in 8% of cases – for 10 years or more.

These antibodies may occur in patients with seronegative rheumatoid arthritis.

Unlike rheumatoid factor, AKA is characterized by higher specificity (88-99%), with lower sensitivity (40 - 60%).

It was revealed in patients with rheumatoid arthritis, presence of AKA is associated with the severity of erosive lesions, a high concentration of circulating immune complexes.

1) **Table №30. Determination of antibodies to keratin (Anti-keratin antibody, AKA)**

№	Indications	Reference values	Positive
1	Differential diagnosis of articular syndrome of unclear genesis	<1:10 (negative).	Rheumatoid arthritis
2	Early diagnosis of rheumatoid arthritis		Systemic lupus erythematosus
3	In order to assess the severity of pathology and prognosis in rheumatoid arthritis		Sjogren 's syndrome

Detailed serology of rheumatoid arthritis (ANF, anti-CCP and RF IgM) – a comprehensive research it includes:

1) Antinuclear factor on the cell line HEp-2 (ANF)

2) Antibodies to Cyclic citrulline-containing peptide (anti-CCP) by ELISA

3) Determination of IgM rheumatoid factor (RF) by ELISA

2.6. Vasculitis and autoimmune kidney lesions.

1) Differential diagnosis of rapidly progressing glomerulonephritis and vasculitis: antibodies to the glomerular basement membrane (BMG), anti-PR3, anti-MPO

2) Antibodies to the basement membrane of the glomeruli of the kidneys IgA, IgM, IgG (anti-BMG, glomerular membrane, antibodies to IgM and IgG, anti-GBM)

This test is used in the diagnosis of GPA granulomatosis with polyangiitis (formerly Wegener's granulomatosis). Diseases in which immunity is directed against the normal components of the basement membrane of the glomeruli of the kidneys and the alveolar epithelium associated with this type of antibodies are rare autoimmune diseases. It was revealed that the main antigenic target for antibodies of this type is the alpha-3 chain of NC1 domain of type IV collagen, present in the kidneys, lungs, lens, brain and testicles, but obviously more accessible at the level of the glomeruli of the kidneys due to the unique features of their structure.

Exposure to factors that violate the integrity of the alveolar epithelium (smoking, exposure to other inhaled toxic substances, respiratory infections) lead to lung damage.

As a result of activation under the action of antibodies of the local immunopathological process, clinical manifestations develop, including kidney pathology, up to rapidly progressive glomerulonephritis, pulmonary bleeding, signs of systemic pathology.

GPA -granulomatosis with polyangiitis (formerly Wegener's granulomatosis) includes a combination of glomerulonephritis and hemorrhagic alveolitis (renal-pulmonary syndrome). This disease is characterized by a genetic predisposition, and frequency of this pathology is higher among men.

When clinical symptoms appear, early diagnosis is extremely important for the appointment of adequate treatment and prognosis of the disease.

Diagnostic criteria determine the detection of antibodies to the basement membrane and allows differentiating the pathology associated with them from other causes of pulmonary hemorrhage and glomerulonephritis.

In a third of cases, antibodies to neutrophil cytoplasm are also detected in patients (ANCA).

Table №31. Diagnosis of vasculitis - antibodies to neutrophil cytoplasm (ANCA).

Indications	**Reference values**	**Positive**	**Negative**
GPA - granulomatosis with polyangiitis (renal-pulmonary syndrome; symptoms of general malaise, acute, usually rapidly progressive glomerulonephritis, pulmonary hemorrhages, often preceding nephritis)	Negative (<20).	GPA - granulomatosis with polyangiitis (formerly Wegener's granulomatosis) (renal-pulmonary syndrome);	1. Absence of antibodies to the glomerular basement membrane
		Rapidly progressing glomerulonephritis complicated by the formation of antibodies to the glomerular basement membrane;	
		Anti-GBM a disease that develops in some patients with Alport syndrome	2. Low antibody titers

		(hereditary type IV collagen anomaly) after kidney transplantation	
		Tubulointerstitial kidney pathology associated with antibodies to the glomerular basement membrane	

False positive results may be associated with the detection of antibodies binding to other collagen chains IV.

Qualitative determination of IgG antibodies to myeloperoxidase (MPO), proteinase 3 (PP3) and glomerular basement membrane (GBM – collagen IVa3) in human serum or plasma.

This research - immunoblot (test strip) is based on the principle of ELISA, gives a qualitative answer: **positive or negative**!

Antineutrophil cytoplasmic antibodies (ANCA) are a group of antibodies directed against cytoplasmic antigens of neutrophil granulocytes. They are mainly associated with inflammatory disease of blood vessels. There are cytoplasmic (cANCA) and perinuclear (p ANCA). Proteinase 3 has been identified as the main antigen for cytoplasmic. Antibodies to PR3 show greater specificity in the diagnosis of GPA -granulomatosis with polyangiitis (formerly Wegener's granulomatosis) of the nasopharynx, as well as kidney and lung. Antibodies to MRO are found in a number of vasculitis, for example, the microscopic form of polyangiitis, eosinophilic granulomatosis with polyangitis (EGPA, formerly Charg-Strauss syndrome), as well as in nodular polyarteritis, antibodies are also found in glomerudonephritis, for example, in rapidly progressing glomerulonephritis.

Autoimmune kidney diseases include GPA -granulomatosis with polyangiitis (formerly Wegener's granulomatosis), a combination of glomerulonephritis and pulmonary hemorrhage with high mortality in the absence of treatment. This disease is characterized by pathognomic autoantibodies that accumulate on the basement membrane of the glomerular apparatus (GBM) and are the direct cause of glomerulonephritis. The domain NC1 of the α3 chain of collagen – IV (a component of the basement membrane of the glomerular apparatus) was identified as the target antigens of autoantibodies. Thus, the confirmation of GBM antibodies (GSM) makes it possible to distinguish GPA granulomatosis with polyangiitis (formerly Wegener's granulomatosis) from other autoimmune kidney diseases.

3) Antibodies to myeloperoxidase (anti-MPO)

Quantitative determination of IgG antibodies to myeloperoxidase (MRO) – Nuclear autoantibodies (pANCA) are mainly associated with the reactivity of myeloperoxidase localized in primary neutrophil granules. Autoantibodies to MRO correlate with idiopathic vasculitis associated with rapidly progressive glomerulonephritis. They occur in 70% of patients with microscopic polyangiitis, and in 5-50% of patients with eosinophilic granulomatosis with polyangitis (EGPA, formerly Charg-Strauss syndrome).

. Reference values: positive – more than or equal to 10 units/ml, negative – less than 10 units/ml.

4) Antibodies to proteinase -3 (anti-PR3)

Quantitative determination of IgG antibodies to protease 3 (PR3) – the pathogenesis of systemic vasculitis (SV) is characterized by inflammatory processes of various walls of blood vessels as a result of morphological changes. Arteries and veins can be affected at the same time. The clinical picture is usually characterized by common symptoms: fever, exhaustion and weight loss. In the future, the picture varies depending on the affected types of vessels. The determination of cytoplasmic antibodies (ANCA) plays an important role in serological diagnostics. ANCA can be cytoplasmic (anca) or nuclear (pANCA). Proteinase 3 (PR3) is the most important autoantibody for cANCA (cytoplasmic) and it is these antibodies that are found in a wide range of vasculitis. This specific antibody is characteristic of GPA granulomatosis with polyangiitis (formerly Wegener's granulomatosis). Antibody titers are closely related to the activity of the disease.

Reference values: positive - more than or equal to 10 units/ml, negative – less than 10 units/ml.

GPA - granulomatosis with polyangiitis (formerly Wegener's granulomatosis) and ANCA-associated vasculitis, autoimmune kidney lesions.

Antineutrophil cytoplasmic antibodies (ANCA) are a group of antibodies directed against cytoplasmic antigens of neutrophil granulocytes.

They are mainly associated with inflammatory disease of blood vessels.

Cytoplasmic (anca) and perinuclear (hansa) are distinguished. Proteinase 3 has been identified as the main antigen for cytoplasmic cells. Antibodies to PR3 show greater specificity in the diagnosis of GPA granulomatosis with polyangiitis (formerly Wegener's granulomatosis) of the nasopharynx, as well as kidney and lung.

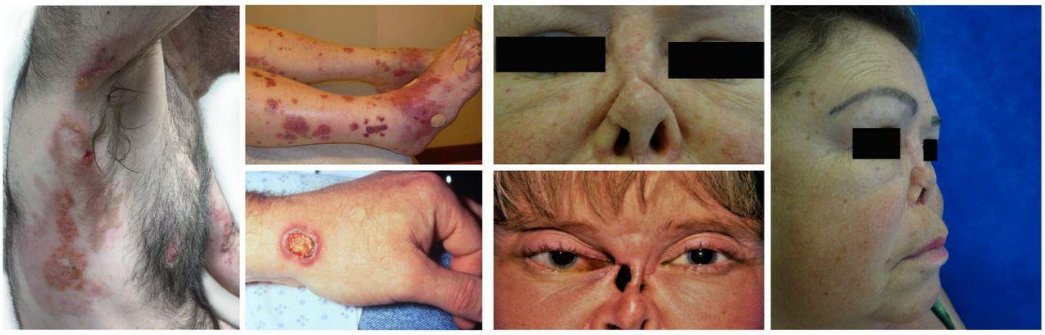


Fig.14 GPA - granulomatosis with polyangiitis (formerly Wegener's granulomatosis) and ANCA-associated vasculitis

Diagnosis of autoimmune kidney damage, a comprehensive study, it includes: ANF, BMG, anti-MPO, anti-PR3

1) Antinuclear factor on the cell line HEp-2 (ANF)
2) Qualitative determination of IgG antibodies to myeloperoxidase (MPO), peroxidase 3 (PR3) and the glomerular basement membrane (BMG).

2.7 Autoimmune liver damage and GIT

Screening of autoimmune diseases GIT, a comprehensive study it includes:

1) Antinuclear factor on the cell line HEp-2 (ANF)

2) Determination of anti-nuclear, antimitochondrial, anti-smooth muscle, antiparietal antibodies (**ANA, AMA, ASMA, APCA**) by indirect immunofluorescence on combined tissue sections from rat liver, stomach and kidneys in human blood serum.

ANA – antinuclear antibodies react with antigens in the cell nucleus. They are important markers of rheumatic diseases, such as, SLC, SSD, S.Sjogren or S.Sharpe. In addition, they are markers of autoimmune hepatitis. ANA may also be present in non-autoimmune diseases (infections, leukemia, lymphomas, melanomas). A low level of ANA can be caused by taking medications and is also found in healthy people in old age.

AMA – Antimitochondrial antibodies react predominantly with the phospholipid-rich inner membrane of mitochondria. AMA serve as a marker of primary biliary cholangitis, in which they are found in more than 90% of patients and even before clinical diagnosis. Low antibody titer is observed in scleroderma, S. Sjogren, RA and other AID, most often in this case, these diseases are associated with PBC.

ASMA – antibodies to smooth muscles are found in large numbers in autoimmune hepatitis. In this case, the antibodies are directed against the muscle

tissue protein actin. In small amounts and without specificity to actin, **ASMA** is found in many autoimmune and infectious diseases, as well as in breast and ovarian cancer, malignant melanomas.

APCA – circulating antibodies to the structures of the parietal cells of the gastric mucosa are a marker of malignant anemia. However, they can be found in other diseases, such as stomach diseases (chronic atopic gastritis, ulcer), thyroid gland (Hashimoto's thyroiditis, myxedema) less often in iron deficiency anemia, diabetes mellitus and in the elderly.

The combined tissue section makes it possible to differentiate various antibodies at the site for analysis and, as a result, can be effectively used as a search test for the following antibodies.

If the result is negative, the titer is less than 20 units, positive is more than or equal to 20 units.

Screening of autoimmune liver damage, a comprehensive study it includes:

1) Antinuclear factor on the cell line HEp-2 (ANF)
2) Qualitative determination of IgG to 7 antigens: **M2, gp210, sp100, LKM1, LC1, SLA, F-actin.**

The range of primary liver AIDS includes autoimmune hepatitis (AIG/ AIH), PBC (PBC) and primary sclerosing cholangitis (PSC/PSC). PAL clinic in most cases does not differ significantly from other chronic liver diseases. About 15% of cases of chronic liver diseases have an autoimmune pathogenesis. After the exclusion of infectious pathogenesis, in particular, viral infections, tests for determination of autoantibodies are used for differential diagnosis.

In PBC, antimitochondrial antibodies are considered pathognomic. Of the 9 subtypes described (M1-M9), only **M2** antibodies show high specificity to PBC. They react with cell epitopes in mitochondrial membranes. RVS is additionally characterized by specific OH, which react mainly with **gp210 and sp100** and indicate a different course of the disease. Liver failure, among other things, is associated with the detection of antibodies to gp 210. In patients with PBC with different reactions to ursodeoxycholic acid, confirmation of IT to gp210 or sp100 in the further course of the disease was associated with liver transplantation or death.

A patient with AIG/ AIH may have different reactions with antibodies. Based on the known antibodies, there is currently a discussion about the allocation of 3 subgroups. Characteristic of AIH type 1 is the detection of **ANA** and **ASMA**. In type 2, on the contrary, antibodies to liver and kidney microsomes (**LKM 1**) and **LC1** antibodies are detected. Samples of patients with type 3 detect exclusively antibodies to the soluble liver antigen **SLA.**

This research is a device-independent testing kit that allows to quickly and accurately determine the presence or absence of specific antibodies.

The sensitivity and specificity of the set is more than 99%.

Result: positive or negative for each antibody.

Autoimmune hepatitis

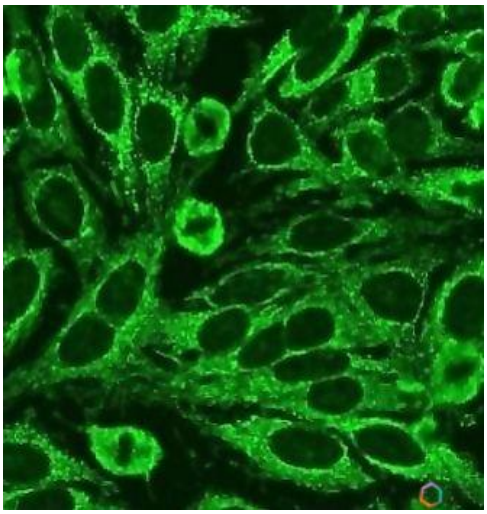
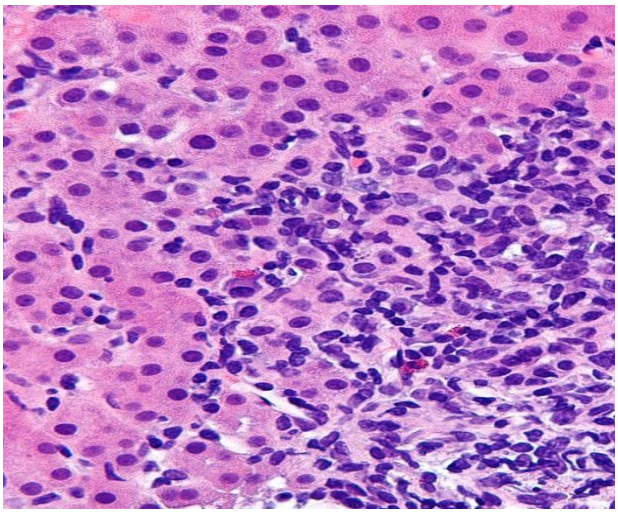



Fig.15 Cytoplasmic type of glow. Small speckled

The glow is divided into: -mitochondria -cytoskeleton –organelles

Table №32. Cytoplasmic type of glow

№	Indications	Main antigens
1	Autoimmune liver diseases	AMA
2	PBC (primary biliary cholangitic)	M2 –40%
3	Systemic lupus erythematosus	F-актин–25%
4	Inflammatory myopathies	RiboP –20%
		Jo **GPA - granulomatosis with polyangiitis (formerly Wegener's granulomatosis) and ANCA-associated ca vasculitis** -1 – 10%

Autoimmune lesions of the gastrointestinal tract. Celiac disease

Antibodies to reticulin IgA and IgG (ReticulinAntibodyIgA&IgG, ARA)

Method of determination: IIR

Autoimmune antibodies associated with celiac disease (gluten-sensitive enteropathy). See also the tests "Antigliadin antibodies" and "Antibodies to endomysium" Reticulin (histological term) – fibrils involved in the formation of

three-dimensional mesh structures - the reticulum forming the stroma of soft organs, mainly represent type III collagen.

RI type of antireticulin antibodies, highly pathognomonic for celiac disease, is characterized by binding to peritubular, periglomerular and perivascular fibers of renal sections.

The consumption of gluten (gluten protein of cereals) can cause the development of immune-mediated inflammatory bowel disease (celiac disease) or gluten-sensitive enteropathy, more often in genetically predisposed people. Celiac disease is characterized by appearance of autoimmune antibodies to extracellular matrix structures, including endomysium (connective tissue surrounding smooth muscle elements of intestinal crypts) and reticulin, and antibodies to gliadin (gluten fraction).

One of the factors in the development of such an autoimmune response is tissue transglutaminase (an enzyme involved in gluten metabolism).

Serological testing (detection of antibodies to endomysium, gliadin, and reticulin in blood serum) is necessary as a preliminary screening examination for clinical suspicions of celiac disease and deciding whether to send for a biopsy with histological examination, which is a decisive diagnostic test.

A combination of tests is needed that will increase the specificity and sensitivity of the serological **examination (gliadin antibody test is more sensitive, endomysium and reticulin antibody tests are more specific).**

In children under 2 years of age, testing is less informative, since antibodies characteristic of celiac disease may not yet develop in them.

Conditions of tests for celiac disease:

1. It is necessary to observe the rules when using the test in order to detect celiac disease (gluten-sensitive enteropathy). The main requirement, it should be carried out before switching to a gluten-free diet. If a gluten-free diet has already been started, it is advisable to return to a gluten-containing diet before conducting the study (to ensure proper sensitivity of the test at the time of examination, the patient should receive food containing gluten for at least a week).

2. The test is also used to monitor the treatment of celiac disease (in order to assess compliance with a gluten-free diet). In this case, the cancellation of a gluten-free diet is not required.

Table №33. Diagnosis of autoimmune lesions of the gastrointestinal tract. Celiac disease. Antibodies to gliadin

Indications (clinical manifestations)	Reference values	**Interpretation Positive**
flatulence		Celiac disease

bloating	Reference values: <1:10 (negative).	Herpetiform dermatitis
diarrhea		Crohn's disease
delayed growth and weight gain in children		Bullous dermatosis
weight loss in adults		**Negative**
unexplained anemia		healthy people
unexplained hypocalcemia or osteomalacia		effective treatment of celiac disease is a gluten-free diet for several months (antibodies reappear when the diet is violated and gluten is ingested from food)
selective IgA deficiency		
herpetiform dermatitis		

Antibodies to IgA endomysium and IgG (Anti-Endomisial Antibody IgA&IgG, EMA)

Endomysium – loose connective tissue surrounds smooth muscle cells. In genetically predisposed people, the use of gluten (gluten protein of cereals) can cause the development of immune-mediated inflammatory bowel disease (celiac disease, or gluten-sensitive enteropathy), characterized by the appearance of autoimmune antibodies to extracellular matrix structures, including endomysium and reticulin.

An important factor in the development of such an autoimmune response is tissue transglutaminase (an enzyme involved in gluten metabolism, apparently, being the main antigenic target of antibodies to endomysium and reticulin detected in studies on tissue preparations).

Serological testing is necessary (including a blood test for the presence of antibodies to endomysium, gliadin, reticulin) as a preliminary screening examination in case of clinical suspicion of celiac disease and deciding whether to send for a biopsy with histological examination, which is a decisive diagnostic test.

A combination of tests helps to make a diagnosis, which increases the specificity and sensitivity of serological examination (gliadin antibody test is more sensitive, endomysium and reticulin antibody tests are more specific).

The following algorithm of serological examination is also possible in case of suspected celiac disease: when detecting antibodies to gliadin, confirm the result by examining antibodies to endomysium and reticulin .

When choosing a single test, it is preferable to choose the study of antibodies to endomysium.

As mentioned above, testing of children under 2 years of age is less informative, since antibodies characteristic of celiac disease may not yet develop in them.

The research of antibodies associated with celiac disease can also be used in monitoring the treatment of celiac disease (exclusion of gluten from food). Antibodies to endomysium, gliadin, and reticulin disappear a few months after switching to a gluten-free diet.

1. It is necessary to comply with the requirements when using the test in order to detect celiac disease (gluten-sensitive enteropathy), it should be carried out before switching to a gluten-free diet. If a gluten-free diet has already been started, it is advisable to return to a gluten-containing diet before conducting the study (to ensure proper sensitivity of the test by the time of examination, the patient should receive food containing gluten for at least a week).
2. The test can also be used to monitor the treatment of celiac disease (in order to assess compliance with a gluten-free diet). In this case, the cancellation of a gluten-free diet is not required.

Table №34. Diagnosis of autoimmune lesions of gastrointestinal tract. Celiac disease. Antibodies to endomysium IgA and IgG (Anti-Endomisial Antibody IgA&IgG, EMA)

<table>
<tr><td>Indications
(clinical manifestations)</td><td>Reference values</td><td>Interpretation
Positive</td></tr>
<tr><td>flatulence</td><td rowspan="10">< 1:2,5 - negative

>\= 1:40 – positive</td><td>Celiac disease</td></tr>
<tr><td>bloating</td><td rowspan="3">Herpetiform dermatitis</td></tr>
<tr><td>diarrhea</td></tr>
<tr><td>delayed growth and weight gain in children</td></tr>
<tr><td>weight loss in adults</td><td>Negative</td></tr>
<tr><td>unexplained anemia</td><td>healthy people</td></tr>
<tr><td>unexplained hypocalcemia or osteomalacia</td><td rowspan="4">effective treatment of celiac disease is a gluten-free diet for several months (antibodies reappear when the diet is violated and</td></tr>
<tr><td>selective IgA deficiency</td></tr>
<tr><td>herpetiform dermatitis</td></tr>
<tr><td>It is used for screening examination of relatives of patients with celiac disease</td></tr>
</table>

It allows to monitor the effectiveness of treatment (disappear after a few months of a gluten-free diet)	1:2, 5 – 1:40 - doubtful	gluten is ingested from food)

IgG class antibodies to tissue transglutaminase (anti-tissuetransglutaminaseIgG, tTGIgG)

The test is important and is used in a complex of serological tests for the diagnosis of celiac disease. In the laboratory diagnosis of celiac disease (gluten-sensitive enteropathy), a test for antibodies of IgA class to tissue transglutaminase is usually used as the most sensitive and specific screening test. At the same time, since celiac disease can be associated with a deficiency of class A immunoglobulins, it is advisable to determine the overall level of IgA in parallel. If the concentration of total IgA is low, a study of IgG antibodies to transglutaminase should be added to laboratory screening. In the complex of serological tests for diagnosis of celiac disease, the research of antibodies to gliadin, antibodies to endomysium is also used. The gold standard for the diagnosis of celiac disease is the confirmation of histological changes in the mucous membrane of the small intestine during biopsy.

It is also necessary to comply with the requirements when using the test in order to detect celiac disease (gluten-sensitive enteropathy), it should be carried out before switching to a gluten-free diet. If a gluten-free diet has already been started, it is advisable to return to a gluten-containing diet before conducting the study (to ensure proper sensitivity of the test by the time of examination, the patient should receive food containing gluten for at least a week).

1. he test can also be used to monitor the treatment of celiac disease (in order to assess compliance with a gluten-free diet). In this case, the cancellation of a gluten-free diet is not required.

Table №35. Diagnosis of autoimmune lesions of the gastrointestinal tract. Celiac disease. IgG class antibodies to tissue transglutaminase (anti-tissuetransglutaminaseIgG, tTGIgG)

Indications	Reference values	**Interpretation Positive**
In the complex of serological tests for suspected celiac disease	< 1.0 – negative	Celiac disease

In the complex of laboratory studies in the diagnosis of herpetiform dermatitis	>1.0 - 2.0 - weakly positive; >2.0 - 5.0 - positive; > 5.0 – highly positive	Herpetiform dermatitis

The result should be evaluated in conjunction with clinical data and the results of other laboratory examinations

* The positivity coefficient (PC) is determined by the ratio of the optical density of the patient's sample to the threshold value.

PC - positivity coefficient is a universal indicator that is used in high-quality enzyme immunoassays.

PCdetermines the degree of positivity of the test sample and is necessary for the doctor to correctly interpret the result. This positivity coefficient does not correlate linearly with the concentration of antibodies in the sample, so it is not recommended to use PC for dynamic monitoring of patients, including monitoring the effectiveness of treatment.

Антитела класса IgA to tissue transglutaminase (anti-tissuetransglutaminaseIgA, tTGIgA)

This screening research is used for diagnosis of celiac disease. Gluten-sensitive enteropathy (celiac disease) is characterized by an inability to digest gluten, a gluten protein found in large quantities in some cereals (wheat, rye, barley), which leads to chronic inflammation and damage to the mucous membrane of the small intestine. This pathology is characteristic of genetically susceptible children and adults. It has been revealed that this pathological condition currently quite often remains undiagnosed.

The most common clinical manifestations include in adults

- diarrhea,
- iron deficiency anemia;

Among children

- diarrhea,
- bloating,
- lag in development.

Less common manifestations may include:

- repeated aphthous stomatitis,
- recurrent abdominal pain,
- steatorrhea,
- fallout-deficiency anemia,
- osteopenia or osteoporosis,
- vitamin K deficiency,

- increased liver enzymes,
- thrombocytosis (hypospenia),
- arthralgia or arthropathy,
- polyneuropathy,
- chronic fatigue,
- excitability or depression,
- alopecia,
- repeated miscarriage of pregnancy,
- infertility,
- growth retardation,
- puberty delay.

Pathological conditions such as herpetiform dermatitis, immunoglobulin A deficiency, autoimmune disorders (type 1 diabetes, autoimmune thyroid diseases, Sjogren's syndrome, microscopic colitis, rheumatoid arthritis), Down syndrome, IgA nephropathy are often observed in combination with gluten-sensitive enteropathy.

It is possible that associations of pathologies include an additional spectrum of pathological conditions. It has been proven that tissue transglutaminase is an enzyme that is widely distributed in many organs. It has been shown that this protein is the main antigenic target in the autoimmune reaction in celiac disease. Modification of gliadin (a component of gluten) by tissue transglutaminase of intestinal mucosa cells plays a key role in triggering the T-cell autoimmune response in this pathology. The study of IgA antibodies to tissue transglutaminase is a sensitive and specific screening test used in the laboratory diagnosis of celiac disease and herpetiform dermatitis. The concentration of IgA antibodies correlates with the activity of the disease, the test can be used for monitoring, including monitoring compliance with a gluten-free diet. It is estimated that in screening for celiac disease, the sensitivity of tests for antibodies to tissue transglutaminase is 85-98%, the specificity is 95-99%. Since celiac disease may be associated with IgA deficiency, the level of total immunoglobulins A should be investigated in parallel. If the concentration of total IgA is low, IgG antibodies to IgG tissue transglutaminase, IgA class antibodies, IgG to gliadin, test - total IgA and IgG antibodies to endomysium should be added to laboratory screening).

The gold standard for diagnosis of celiac disease is confirmation of histological changes in the mucous membrane of the small intestine during biopsy. Some patients may have positive results in the test for IgA antibodies to tissue transglutaminase, but negative results in the test for anti-endomysial or anti-gliadin IgA antibodies, which may be evidence of a false positive result or an early stage of the disease. Positive results of serological tests, but a negative biopsy result may indicate a gluten-free diet before the study, a latent or early stage of enteropathy. If a gluten-free diet has already been started, it is advisable to return to a gluten-free diet before the study. In case of negative results of serological

tests, on the remaining suspicion of celiac disease (diarrhea, steatorrhea, weight loss, etc.), a biopsy is justified.

Limits of determination: 0.6 rel. units/ml-200 rel. units/ml

1. When using the test to detect celiac disease (gluten-sensitive enteropathy), it should be carried out before switching to a gluten-free diet. If a gluten-free diet has already been started, it is advisable to return to a gluten-containing diet before conducting the study (to ensure proper sensitivity of the test by the time of examination, the patient should receive food containing gluten for at least a week).

2. The test can also be used to monitor the treatment of celiac disease (in order to assess compliance with a gluten-free diet). In this case, the cancellation of a gluten-free diet is not required.

Table №36. Diagnosis of autoimmune lesions of the gastrointestinal tract. Celiac disease. Antibodies of the IgA class to tissue transglutaminase (anti- tissuetransglutaminaseIgA, tTGIgA)

Indications	Reference values	**Interpretation Positive**
Screening study for suspected celiac disease	< 20 rel. units/ml (negative).	Celiac disease
Process activity monitoring, gluten-free diet control		Herpetiform dermatitis
Diagnosis of herpetiform dermatitis		

Interpretation of result. The specificity of tests for antibodies to tissue transglutaminase is estimated to be 95-99% (the probability of false positive results is 1-5%), sensitivity is 85-98% (the probability of false negative results is 2-15%).

Autoimmune lesions of gastrointestinal tract.

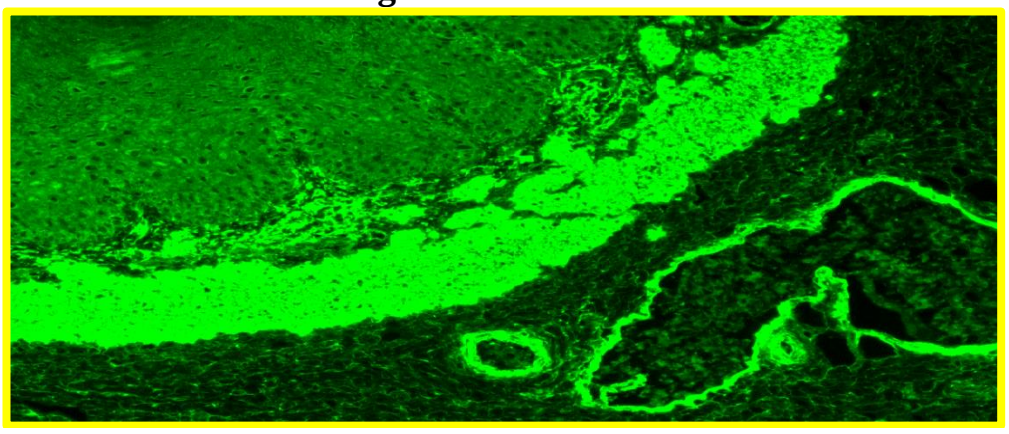


Fig. 16 Celiac disease

Antibodies to reticulin IgA and IgG (Reticulin Antibody IgA&IgG, ARA)

Autoimmune antibodies associated with celiac disease (gluten-sensitive enteropathy). Reticulin (histological term) – fibrils involved in the formation of three-dimensional mesh structures - the reticulum forming the stroma of soft organs, mainly represent type III collagen**.**

Highly pathognomonic for celiac disease RI type of antireticulin antibodies

The combination of tests increases the specificity and sensitivity of serological examination:

gliadin antibody test is more sensitive,

tests for antibodies to endomysium and reticulin are more specific

Antibodies to IgA endomysium and IgG (Anti-EndomisialAntibodyIgA&IgG, EMA

IgG class antibodies to tissue transglutaminase (anti-tissuetransglutaminaseIgG, tTGIgG)

Antibodies of the IgA class to tissue transglutaminase (anti-tissuetransglutaminaseIgA, tTGIgA)

Autoimmune lesions of the gastrointestinal tract.

Fecal calprotectin

(FecalCalprotectin)

Calprotectin, biomarkers of inflammatory bowel diseases, a product of neutrophilic granulocytes, the detection of which in feces indicates a lesion of the intestinal wall. Lactoferrin, lysozyme, elastase, myeloperoxidase and calprotectin are also among the proteins of this type. Among them, lactoferrin and calprotectin are the most stable and are slowly decomposed by microbial proteases, which makes it possible to use the study of their concentration in feces for diagnostic purposes. These proteins are referred to as biomarkers of "fecal inflammation".

Calprotectin is a protein with a molecular weight of 36 kDa, containing calcium and zinc ions in its composition and having a bacteriostatic and fungicidal effect of invitro. It makes up 60% of the protein contained in the cytoplasm of neutrophils, in low concentrations can be found in monocytes and tissue macrophages. The synthesis of calprotectin in feces reflects the influx of neutrophils into the intestinal lumen, which is confirmed by the high correlation of the results of the study of the concentration of fecal calprotectin with the assessment of the excretion of granulocytes labeled with indium-111. Bleeding from the intestinal wall has a slight effect on the concentration of calprotectin in the stool and increases its concentration by no more than 10 mcg/g. An increase in the concentration of fecal calprotectin over 120 mcg / g is noted in more than 90% of patients with inflammatory bowel diseases at the stage of primary diagnosis.

The determination of fecal calprotectin makes it possible to differentiate patients with irritable bowel syndrome from patients with organic causes of gastrointestinal tract damage.

Moderately elevated values of calprotectin were noted in mucosal lesions (including celiac disease, lactase deficiency, autoimmune gastritis), significantly increased concentrations were noted in inflammatory bowel diseases, bacterial infections of the gastrointestinal tract, diverticula and oncological diseases, constant intake of nonsteroidal anti-inflammatory drugs (NAID).

During the newborn period, as well as in young children, the concentration of calprotectin is on average higher than in adults.

Due to its low specificity, fecal calprotectin cannot replace instrumental methods for diagnosis of Crohn's disease. Histological examination is the "gold standard" of diagnostics, combination of endoscopic imaging methods allows you to clarify the localization of sites and the volume of intestinal lesions. The advantage of studying fecal calprotectin in Crohn's disease is that its increased concentration may reflect segmental lesions of small intestine, which is not available for endoscopic and /or gisotological studies.

Often, the concentration of fecal calprotectin in the stool directly correlates with the histological and endoscopic activity of the disease.

A persistently elevated level of fecal calprotectin may indicate the ineffectiveness of therapy, and an increase in the content of calprotectin in the dynamics of observation may indicate the likelihood of exacerbation of the disease.

In the diagnosis of intestinal diseases, the study of fecal calprotectin and the study of feces for hidden blood in patients with specific complaints indicate the need for colonoscopy.

Limits of determination: 10 – 1800 mcg/g

Table №37. Autoimmune lesions of the gastrointestinal tract. Fecal calprotectin (FecalCalprotectin)

Indications	Reference values	**Increase**
differential diagnosis of organic (inflammatory) changes in the intestinal wall and functional disorders	Up to 1 year - <500 mcg/g ; 1-4 years <150 mcg/g ;	Crohn's disease and ulcerative colitis

comprehensive diagnosis and assessment of the activity of inflammatory bowel diseases	4-65 years <50 mcg/g ; Over 65 years <100 mcg/g .	Bacterial infections of the gastrointestinal tract
monitoring of therapy for nonspecific ulcerative colitis and Crohn's disease		Diverticula and oncological diseases
in combination with the analysis of feces for latent blood when assessing the need for a colonoscopy		Taking nonsteroidal anti-inflammatory drugs
enteropathy associated with the use of nonsteroidal anti-inflammatory drugs		Inflammatory lesions of the gastric mucosa in celiac disease, autoimmune gastritis, diverticulitis, etc.

With a reference limit of <50 mcg/g, interpretation of increased results: 50-200 mcg/g is a moderate increase, which may indicate an organic lesion caused by nonsteroidal anti–inflammatory drugs, diverticulitis and inflammatory bowel disease in the remission phase. And also about a weak immune response, in this case it is recommended to monitor the dynamics;

more than 200 mcg / g – marked increase. Probably inflammatory bowel disease.

Protein-losing enteropathy
Alpha-1-antitrypsin in feces
1. (Alpha-1-Antitrypsin, Feces)

The test is used to assess **the loss of protein in the intestine in order to diagnose protein-losing enteropathy .**

Alpha-1-antitrypsin (A1AT) is the main component of the alpha–1 fraction of serum proteins, where its concentration is 1-2 g/l. A1AT is produced mainly by liver cells, but also by intestinal macrophages, monocytes and epithelial cells. It is the main inhibitor of serine proteases. Thus, A1AT is the main inhibitor of neutrophil elastase and is released during inflammatory processes to reduce the activity of this enzyme in areas of inflammation, it also inhibits other serine proteases (trypsin and chymotrypsin, plasma coagulation proteinases, etc.).

Determination of alpha-1-antitrypsin in feces is used to assess the condition of mucous membrane and loss of protein in the intestine. Most whey proteins, when ingested into the intestine, are quickly broken down by the action of digestive enzymes. A1AT molecules, according to its unique function of inhibiting proteolytic enzymes, are very resistant to the action of proteases and

remain intact in the intestinal contents, unlike other whey proteins. The residual concentration of A1AT in feces is a reliable marker of presence of blood proteins in the intestinal lumen in a number of pathological conditions combined by the symptom complex of enteropathy with protein loss (excessive loss of plasma proteins into intestinal lumen through lymphatic vessels or through the mucosa altered by the inflammatory process).

Enteropathy with protein loss may be associated with:

- with allergic enteritis,
- bacterial,
- viral,
- parasitic etiology,
- celiac disease,
- erosions and ulcers of the mucous membrane of the gastrointestinal tract,
- carcinoma,
- lymphatic obstruction,
- damage or abnormality of lymphatic vessels.

Table №38. Diagnosis of Alpha-1-antitrypsin in feces (Alpha-1-Antitrypsin, Feces) – protension - losing enteropathy

Indications	Reference values	**Interpretation**
Chronic diarrhea	**<250 mg/l.**	erosions and ulcers of the esophagus, stomach, duodenum, hypertrophic gastritis
Abdominal pain		enteritis, allergic enteritis, ulcerative junoileitis, Schenlein-Henoch purpura, celiac disease
Progressive weight loss		pseudomembranous colitis, ulcerative colitis, cytomegalovirus enteritis, worm infestation
Hypoalbuminemia with edematous syndrome		tumors of the mucosa, carcinoid, Kaposi's sarcoma
Hyponatremia		amyloidosis, lymphatic obstruction in tuberculosis, sarcoidosis, retroperitoneal fibrosis
Anemic syndrome		heart failure with ascites
Hypovitaminosis		enteropathy caused by nonsteroidal anti-inflammatory drugs or chemotherapy

Antibodies to pancreatic acinar cells, IgG and IgA in total (antibodies to the exocrine part of the pancreas, AutoantibodiesagainstExocrinePancreas, PancreaticAntibodies, PAB)

Detection of antibodies associated with Crohn's disease.

The acinar cells of pancreas take part in ensuring its exocrine function – formation of digestive enzymes secreted into the intestine. Antibodies to pancreatic acinar cells detected by indirect fluorescence are clinically associated with inflammatory bowel diseases and are most characteristic of Crohn's disease.

The causes of appearance of antibodies to antigens of the exocrine part of the pancreas in inflammatory bowel diseases are currently insufficiently studied. There is no connection between pancreatitis and the appearance of such antibodies, such antibodies are detected only rarely in patients with acute or chronic pancreatitis (their titer in such cases is significantly lower than in Crohn's disease). At the same time, it was proven that the antigens of pancreatic acinar cells are a normal component of the digestive juice of the intestine, getting into it during secretion of digestive enzymes by pancreas.

Among them, GP2 glycoprotein (membrane protein of secretory granules of acinar cells) is identified as the main antigen for antibodies to pancreatic acinar cells associated with Crohn's disease. Antibodies to GP2 antigen of the centroacinar cells of the pancreas). Relatively recently, it was discovered that the expression of this protein is inherent not only in the acinar cells of the pancreas. In studies using biopsy material, GP2 mRNA transcription and enhanced GP2 expression were demonstrated in areas of colon inflammation in patients with Crohn's disease, which aroused additional interest in studying the possible role of GP2-specific antibodies in the pathogenesis of this disease.

Antibodies to the exocrine part of pancreas are detected on average in 39% of patients with Crohn's disease (with a disease duration of more than two years – in 50% of patients), somewhat more often in relatively young patients. Much less often, antibodies to the centroacinar cells of the pancreas can be detected in other diseases of the gastrointestinal tract. Patients with Crohn's disease are also characterized by a loss of tolerance to normal intestinal flora and the presence of a constant inadequate immune response to antigens of normal intestinal flora (see tests No. 1335, 1336 Antibodies to saccharomyces, ASCA, IgG and IgA). The definition of ASCA and PAB is a useful addition in the diagnosis of Crohn's disease. However, the results of these serological tests in themselves are not the basis for a diagnosis and should be considered in conjunction with the results of clinical, endoscopic, radiological methods of examination and histological evaluation.

Table №39. Antibodies to pancreatic acinar cells, IgG and IgA in total

Indications	Reference values	**Interpretation Positive**

Examination of patients with inflammatory bowel diseases		
Suspected Crohn's disease		
Malabsorption, loss of body weight	title less than 1:10.	Crohn's disease

Interpretation of the results: detection of antibodies to the exocrine part of pancreas indicates a high probability of inflammatory bowel disease and requires in-depth clinical and instrumental examination. Detection of antibodies to pancreatic acinar cells in combination with other (clinical, endoscopic, histological, serological) signs allows to diagnose Crohn's disease.

IgG and IgA antibodies to GP2 antigen of pancreatic centroacinar cells (Anti-GP2, IgG, IgA)

Detection of antibodies associated with Crohn's disease.

Type 2 glycoprotein (GP2) is a quantitatively predominant membrane protein of excretory granules of pancreatic acinar cells. In a number of studies, it is noted that this protein is main antigen for pancreatic antibodies associated with Crohn's disease (Antibodies to pancreatic acinar cells).

The causes of appearance of antibodies to GP2 in inflammatory bowel diseases is not sufficiently studied. During secretion of pancreatic enzymes, GP2, which is a normal component of pancreatic juice, simultaneously enters the intestinal lumen. Having a high structural homology with the Tamma-Horsfall protein of urine, GP2, according to studies, performs a similar protective antibacterial function, binding to the fimbriae of bacteria in the intestine. Pancreatic acinar cells are not the exclusive site of GP2 expression. The recent detection of expression of this protein on the apical membrane of intestinal M cells, as well as the detection of GP2 mRNA transcription and enhanced GP2 expression in biopsies taken from intestinal inflammation sites in patients with Crohn's disease, provide a possible explanation for previously incomprehensible association of autoantibodies to pancreatic antigen with localization of pathological process in the intestine.

Antibodies to GP2 of IgG and IgA classes can be detected in 30-35% of patients with Crohn's disease, regardless of presence of antibodies to saccharomycetes characteristic of this pathology (Antibodies to saccharomycetes, ASCA, IgG and IgA). The combined use of these markers increases the reliability of laboratory testing and makes it possible to identify serological signs of this disease in 60-70% of patients.

Antibodies to GP2 are found in younger patients. The presence of these antibodies shows a connection with individual form of the disease. In Crohn's disease, antibodies to the GP2 antigen are noted in ileocolitis, a structuring form of disease with frequent perianal inflammation.

Unlike Crohn's disease, such antibodies are rarely found in patients with ulcerative colitis (less than 8% of cases). Anti-GP2 can be observed in some other diseases of gastrointestinal tract, including active celiac disease, as well as in 3% of healthy individuals.

Indications:

- Examination of patients with inflammatory bowel diseases.
- Suspected Crohn's disease.
- Malabsorption, weight loss, vitamin deficiency, iron deficiency.

Table №40. Interpretation of results:

Antibodies to GP2 class IgA	• <5 Units/ml – no antibodies detected • 5-10 Units/ml – borderline content of antibodies • > 10 Units/ml – diagnostic level of antibodies
IgG class GP2 antibodies	• <10 Units/ml – no antibodies detected • 10-15 Units/ml – borderline content of antibodies • > 15 Units/ml – diagnostic level of antibodies

Detection of IgG and/or IgA class GP2 antibodies indicates a high probability of inflammatory bowel disease and requires in-depth clinical and instrumental examination.

Detection of anti-GP2 in combination with other (clinical, endoscopic, histological, serological) signs makes it possible to diagnose Crohn's disease. In Crohn's disease, antibodies to GP2 antigen are more often found in young patients with ileocolitis, a structuring form of disease with frequent perianal inflammation.

ASCA (antibodies to Saccharomus cervisiae Ig A and IgG – differential diagnosis of Crohn's disease and ulcerative colitis.

Antibodies of IgA and IgG classes to goblet cells of intestine, in total (Anti-Intestinal Goblet Cells Antibodies, GAB, IgA, IgG, Total)

Detection of antibodies associated with nonspecific ulcerative colitis.

Goblet-shaped intestinal cells are present in all parts of intestinal tract, but their maximum number is in the rectum, especially in the crypts of large intestine. The main function of these cells is the production of mucins – high-molecular glycoproteins capable of forming gel. Intestinal mucins form a surface layer of mucus, which facilitates the promotion of the contents in the intestinal lumen and at the same time serves to protect its mucous membrane from both physical and

chemical factors of intestinal contents, and from the penetration of potential pathogens.

The presence of circulating antibodies to intestinal goblet cells is associated with inflammatory bowel diseases. These antibodies are most characteristic of nonspecific ulcerative colitis (15-28% of cases) and they are very rare in Crohn's disease and other intestinal diseases or in healthy individuals. The main target of these antibodies is mucin produced by goblet-shaped intestinal cells (antigenic epitopes are found both in the core protein and in the carbohydrate components of mucin).

Antibodies to goblet cells of intestine may be involved in pathogenetically significant autoimmune processes in nonspecific ulcerative colitis. The localization of these cells macro- and microscopically coincides with localization of affected areas in this disease, histologically there is a decrease in the number of goblet cells, there is a decrease in the amount of mucin. But the primary role of these antibodies in the development of pathological process in the large intestine is doubtful, given the relatively low frequency of their detection. Their appearance may be a secondary phenomenon.

Other antibodies associated with ulcerative colitis include antibodies to neutrophil cytoplasm (Antibodies to neutrophil cytoplasm, ANCA, IgA, IgG), but they are less specific. Serological tests are used in the diagnosis of inflammatory bowel diseases as a supplement, their results alone cannot serve as a basis for the diagnosis of ulcerative colitis and should be considered in conjunction with results of clinical, endoscopic, radiological examination methods and histological evaluation.

The presence of circulating antibodies to goblet cells is also noted in a rare disease – autoimmune enteropathy, which is characterized by untreatable diarrhea, inflammation and histological changes in the mucous membrane of the small intestine (with a decrease in the number of goblet cells and villi atrophy), the presence of circulating antibodies to enterocytes and predisposition to other autoimmune diseases and polyendocrinopathy.

It is preferable to withstand 4 hours after the last meal, there are no mandatory requirements.

Table №41. Antibodies of the IgA and IgG classes to goblet cells of the intestine, in total

Indications	Reference values	**Interpretation Positive**
Examination of patients with inflammatory bowel diseases		Nonspecific ulcerative colitis

Suspicion of nonspecific ulcerative colitis	**title less than 1:10**	Autoimmune enteropathy
Suspected autoimmune enteropathy		
Malabsorption, weight loss, vitamin deficiency, iron deficiency of unclear etiology		
Comprehensive examination of patients with autoimmune diseases, including autoimmune		

Interpretation of results: an increase in the titer of antibodies to goblet cells of intestine indicates a high probability of inflammatory bowel disease. Detection of antibodies to goblet cells of the intestine in combination with other (clinical, endoscopic, histological, serological) signs helps to diagnose nonspecific ulcerative colitis or autoimmune enteropathy.

According to the manufacturer's data, the clinical sensitivity of the test system used (established for a group of patients with ulcerative colitis) is 28%, the clinical specificity (established for a group of healthy donors) is approaching 100%.

Autoimmune lesions of the gastrointestinal tract
Detection method – a combination of substrates for IIR
Autoantibodies in Crohn's disease

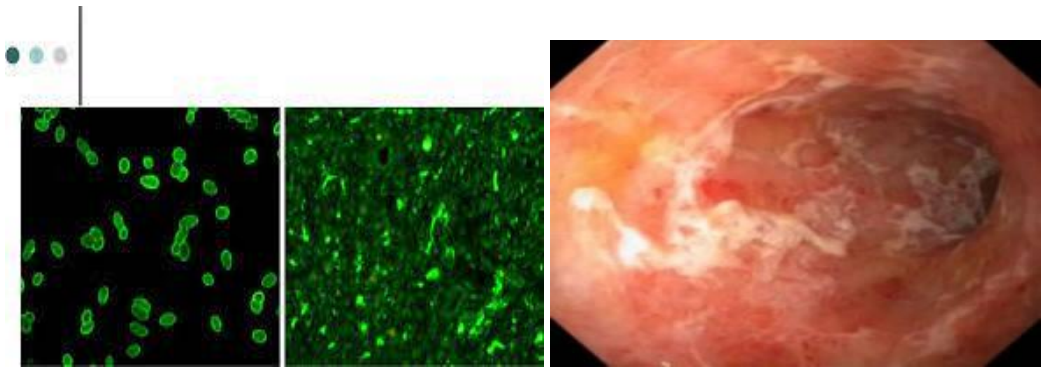


Fig.17 Crohn's disease

Table №42. Autoantibodies in Crohn's disease

№	Autoantibodies
1	Autoantibodies to Saccharomyces cerevisiae
2	Antibodies to the centroacinar cells of the pancreas

Table №43. Antibodies to Saccharomyces cerevisiae polysaccharides (ASCA) and Antibodies to pancreatic centroacinar cells

Indications	Reference values	**Interpretation Positive**
Examination of patients with inflammatory bowel diseases	title less than 1:10	Crohn's disease
Suspected Crohn's disease		
Malabsorption, weight loss, vitamin deficiency, iron deficiency		

Autoantibodies in nonspecific ulcerative colitis

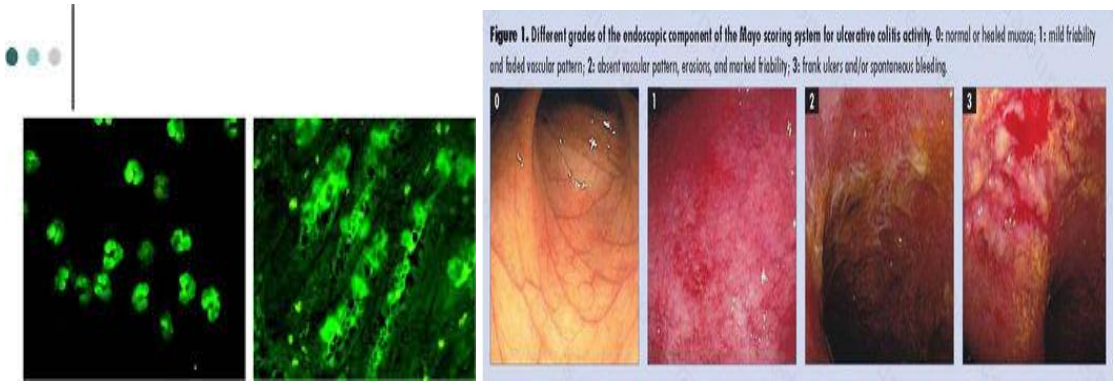


Fig.18 Nonspecific ulcerative colitis

Table №44. Autoantibodies in nonspecific ulcerative colitis

№	**Autoantibodies**
1	Antibodies to neutrophil cytoplasm, пANCA, neutrophils of the donor
2	ASCA (antibodies to Saccharomyces cervisiae Ig A and IgG – differential diagnosis of Crohn's disease and ulcerative colitis
3	Antibodies of IgA and IgG classes to goblet cells of the intestine
4	Detection of antibodies associated with nonspecific ulcerative colitis

Antibodies to neutrophil cytoplasm (ANCA) occur in PR3 and sclerosing cholangitis, but do not react with MPO (myeloperoxidase) and PR3 (proteinase 3)

2.8 Autoimmune neurological diseases.

Table №45. A detailed examination for polyneuritis, a comprehensive study it includes:

№	Autoantibodies
1	Antinuclear factor on the cell line HEp-2 (ANF)
2	ANA-screen, 12 antinuclear autoantibodies (dsDNA, Nucleosomen, Sm, PO, Histon, RNP, SS-A/Ro60, SS-A/Ro52, SS-B/La, Scl-70, CENP-B, Jo-1)
3	Qualitative determination of IgG and/or Ig M antibodies to gangliosides (anti-GM1, GM2, GM3, GD1a, GD1b, GQ1b,GT1b).

Inflammatory neuropathies of the peripheral nervous system are characterized by a large number of clinical symptoms, ranging from a slight feeling of fatigue to respiratory failure and cardiac arrest. In diseases of the peripheral nervous system, an increasing number of cases of detection of antibodies to gangliosides have recently been detected. Gangliosides are acidic glycolipids, they are part of the cell membrane and are found in CNS and PNS.Structures similar to gangliosides can be found on the surface of microorganisms, thus inflammatory polyneuropathies are often found as a result of past infections, such as infection caused by microorganisms Campylobacterjejuni, CMV, Epstein-Barr virus, mycoplasma, hemophilic bacillus, Pfeiffer's bacillus. Antibodies to pathogens of ganglion structures can cross-react with gangliosides of bone marrow or nerve fibers and contribute to inflammation with subsequent demyelination.

Table №46. The following antibodies have been described as characteristic of peripheral neuropathies

Guillain–Barre syndrome (GBS)	GM1, GD1a, GD1b, GT1a, GT1b, GQ1b	IgM (IgG)
Miller-Fisher syndrome (MFS)	GQ1b, GT1a	IgG
Multifocal motor neuropathy (MMN)	GM1, GM2, GM3, GD1a, GD1b	IgM

Chronic inflammatory demyelinating polyneuropathy	GM2, GM3, GD1a, GD1b	IgM
Chronic toxic neuropathy (CANOMAD)	GM3, GD1b, GD2, GD3, GT1b, GQ1b	IgM
Acute toxic sensory neuropathy	GD1b, GD3	IgG
Acute motor axonal neuropathy (AMAN)	GM1, GD1a	IgG
IgM- paraproteinemic demyelinating neuropathy	Сульфатиды	IgM (IgG)

Test for diagnosis of polyneuropathies, IgG and/or Igg antibodies to gangliosides (anti-GM1, GM2, GM3, GD1a, GD1b, GQ1b, GT1b,)

Test for diagnosis of myasthenia gravis, antibodies to acetylcholine receptors (AchR)

Quantitative determination of antibodies to the acetylcholine receptor (AChRAK) in human serum by solid-phase ELISA. Antibodies to the acetylcholine receptor are responsible for failure of neuromuscular transmission in patients with myasthenia gravis. The detection of antibodies is crucial for the diagnosis and treatment of this disease.

Reference values:
negative <0,45 nmol/l,
positive ≥ 0,45 nmol/l

2.9 Autoimmune endocrinopathy

Diagnosis of type 1 diabetes, screening of antibodies to islet cells (ICA)

Type 1 diabetes mellitus (insulin-dependent), occurs as a result of a chronic autoimmune process directed specifically against the beta cells of islets of Langerhans of the pancreas. The destruction of cells is determined by the interaction of CD4 and CD8 autoreactive T-lymphocytes. Already before the diagnosis of type 1 diabetes, autoantibodies directed against various autoantigens of islet cells are detected in the serum of patients. This process can last for many years and take place at any age.

These IgG autoantibodies are the most important markers for identifying people with an increased risk of diabetes even when the available metabolic tests still show a normal result. This is a semi-quantitative determination of antibodies

by solid-phase ELISA, the concentration of antibodies is determined by binding coefficient.

In laboratory practice, for the determination of antibodies to islet cells (ICA), the method of enzyme immunoassay (ELISA) is used - semi-quantitative enzyme immunoassay ICAscreen.

The detection of ICA has the greatest prognostic value in the development of type I diabetes. They appear 1-8 years before the clinical manifestation of the disease. The high prognostic significance of the definition of ICA is also determined by the fact that patients with the presence of ICA, even in the absence of signs of diabetes, eventually also develop type I diabetes. Therefore, the definition of ICA is useful for early diagnosis of this disease. Their detection allows the clinician to select a diet and conduct immunocorrective therapy. Depending on the immunological features of type I diabetes, type A1 is distinguished, in which the frequency of detection of autoantibodies after the development of the clinical picture reaches 90%, and after a year decreases to 20%, and type B1, in which the persistence of autoantibodies persists for a long time.

Table №47. Determination of antibodies to islet cells **(ICA)**

Indications	Reference values
Type I diabetes mellitus	negative <0 ,7, grey zone 0,7-1,0, positive ≥ 1,0.

Table №48. Diagnosis of thyroid AID

Indications	Antibodies
autoimmune thyroiditis	Antibodies to thyroglobulin Antibodies to thyroperoxidase Antibodies to TSH receptors Antibodies to the microsomal fraction of thyrocytes Antibodies to thyroid tissue
Graves' disease	
Hashimoto 's Craw	
Atrophic thyroiditis	
Primary thyrotoxicosis	

Table №49. Diagnosis of the pancreas (type 1 diabetes mellitus

Indications	Antibodies
Type 1 diabetes mellitus	Antibodies to insulin
	Antibodies to pancreatic islet cells
	Antibodies to glutamic acid decarboxylase (GAD)

During the asymptomatic development of diabetes, antibodies to these three antigens can be detected in patients 7 years before the clinical manifestation of the disease.

Conclusion

Therefore, the methodological guide presents the methodological foundations of laboratory diagnostics of various autoimmune diseases at the present stage of medical development. The manual discusses the general issues of pathogenesis underlying the development of AID. Innovative technologies of quantitative and qualitative analysis of AID biomarkers with analysis of research results, accuracy and specificity of individual diagnostic methods are presented.

The textbook can be useful for interns, residents, all specialties.

To date, the availability of a huge number of new tests for the diagnosis of pathological conditions requires clinicians to know not only the basics of clinical biochemistry, but also new algorithms for laboratory diagnostics.

The presented work recommends clinicians to choose the right approach in the laboratory diagnosis of a number of pathological conditions, based on the recommendations of the World Health Organization, the latest achievements of science and clinical practice, their own scientific and practical research.

The presented work will enable doctors to characterize pathological processes in the body, reach the correct diagnostic criteria and track the course of AID treatment.

Annex№1

Diagnostic and pathogenetic significance of autoantibodies

Autoantibodies	Diagnostic and pathogenetic significance
Autoantibodies to double - stranded DNA (anti-ds-DNA)	They are the most specific marker for SLE. With effective therapy, the titer of these antibodies is significantly reduced. With the help of ELISA in healthy individuals, anti-ds-DNA is detected in 2.5% of cases, with SLE – in 40-70%, with medicinal lupus are not detected, and with RA, JURA, SSD, Sjogren's disease are detected in 4-17% of cases.
Autoantibodies to single - stranded DNA (anti -ss-DNA)	They are nonspecific in relation to certain diseases, being present in the body with SLE, connective tissue diseases, rheumatoid arthritis, scleroderma and Sjogren's syndrome
Autoantibodies to the extracted nuclear antigen Sm	They occur in 10-30% of cases with SLE. Negative results do not exclude the presence of SLE. Clinical signs associated with the presence of Sm antibodies are aggressive course of the disease, CNS lesion, lupus psychosis and relative preservation of kidney function.
Autoantibodies to histones	They are one of the varieties of antinuclear antibodies and are determined mainly by SLE, being its early markers. They are detected in 80% of SLE patients, as well as in patients with primary biliary cirrhosis, RA (in 15-50%) and scleroderma. They are often detected in patients with medicinal lupus erythematosus after taking medications such as hydralazine and procainamide. However, such patients lack antibodies to ds-DNA.
Autoantibodies to nucleosomes	It is detected with SLE with a specificity close to 100%. They often indicate kidney damage (lupus nephritis)
Autoantibodies to ribosomal proteins P	Specific to SLE. They occur in 10-20% of patients with SLE. They interact with ribosome phosphoproteins and are found in SLE patients with central nervous system, kidney and liver damage. Their detection is specific for SLE occurring with lupus psychosis.
Autoantibodies to mitochondrial antigens AMA-2	Antibodies to mitochondrial antigens MA-2 located on the inner organelle. They are characteristic of primary biliary cirrhosis of the liver. They can also be found in other liver diseases (30%), with progressive systemic sclerosis (7-25%)
Autoantibodies to ribonucleoprotein	They are detected in 100% of patients with mixed connective tissue diseases (Sharpe syndrome) and almost half of SLE patients. The antibody titer correlates with the clinical activity of the disease.

Autoantibodies to centromeres	They are found with a limited form of scleroderma, with CREST syndrome (calcinosis, Raynaud's syndrome, esophagitis, telangiectasia) in 70% of patients. Their presence indicates a favorable outcome of the disease, a low probability of damage to internal organs.
Autoantibodies to cytoplasmic antigen Jo-1 (to histidine-тRNA -synthetase)	They are found in 25-35% of patients with dermatomyositis. Often associated with systemic manifestations: pulmonary fibrosis and Raynaud's syndrome.
Autoantibodies to the native nuclear antigen SS-A	They are most often detected in patients with Sjogren's syndrome (4-080% of cases), with SLE (30-40%), with primary biliary cirrhosis (20%), and in almost 100% of cases of lupus erythematosus in newborns. It is usually found in the population of SLE patients with pronounced symptoms of photosensitive skin manifestations.
Autoantibodies to Ro-52	They are detected in various AIDS, but are most characteristic of SLE and Sjogren's syndrome
Autoantibodies to SS-B	They are found almost exclusively in women (29:1) with Sjogren's syndrome (40-80%), but also with disseminated lupus erythematosus (10-20%). In Sjogren's syndrome, combinations of antibodies to SS-A and SS-B are usually found
Autoantibodies to Scl-70	Anti-sclerodermal antibodies (antibodies to topoisomerase) are detected with diffuse and less often with a limited form of scleroderma, CREST syndrome. They are highly specific for scleroderma and are a poor prognostic sign regarding the development of pulmonary fibrosis.
Autoantibodies to Pm-Scl	They are found in 50-70% of patients with a combination of symptoms of polymyositis, dermatomyositis and scleroderma. Prevalence is 3% in scleroderma (diffuse form) and 8% in DM and PM .
Rheumatoid factor (RF)	It is determined in 75-80% of RA patients with arthritis, in addition, it is found in Sjogren's syndrome, scleroderma, DM, hypergammaglobulinemia, B-cell lymphoproliferative diseases. By its nature, RF is an antibody against Fc fragment of IgG. More often (up to 90%) these antibodies belong to IgM, but there are also IgG, IgA and IgE = antibodies
Antibodies to Cyclic Citrullinated Peptide (anti-CCP)	It is shown that antibodies to a linear synthetic peptide containing the unusual amino acid citrulline are present in 79% of sera from RA patients with 97% RA specificity and are detected at a very early stage of RA. In addition, the test makes it possible to differentiate erosive and non-erosive forms of RA.

	In ACCP (+) patients, there is a greater degree of cartilage damage compared to ACCP (-) patients. The prognostic value of the method increases if I use it in combination with the Russian Federation. This test makes it possible to differentiate RA with other connective tissue diseases.

Test tasks

1. Antinuclear antibodies, IgG, screening, ELISA (Antinuclear antibodies, ANAs, EIA)

A. One of the common screening tests used to diagnose autoimmune diseases

B. One of the common screening tests used to diagnose autoimmune kidney diseases

C. One of the most common screening tests used to diagnose autoimmune diseases of the musculoskeletal system

D. One of the common screening tests used to diagnose autoimmune liver diseases

E. One of the common screening tests used to diagnose polyneuropathy

2. Antinuclear factor (ANA, HEp-2, titers. Antinuclear antibodies by indirect immunofluorescence on HEp-2 cell preparations; ANA IF, titers)

A. The test is indicated for differential diagnosis of diseases of the blood system

B. The test is indicated for differential diagnosis of autoimmune diseases

C. Antinuclear antibodies are a laboratory marker of systemic connective tissue diseases.

D. The test is indicated for differential diagnosis of respiratory diseases

E. The test is indicated for differential diagnosis of polyneuropathy

3. IgG class antibodies to double-chiral (native) DNA (анти-дсDNA IgG, anti-double-stranded (native) DNA IgG antibodies, anti-dsDNA IgG)

A. A highly specific marker of systemic lupus erythematosus

B. It is used to diagnose and monitor the course of systemic lupus erythematosus

C. The presence of anti-dsDNA IgG in a lower concentration is noted in other diffuse connective tissue diseases or drug-induced SLE

D. All of the above is true

E. It is used for diagnosis of polyneuropathy

4. Panel of antinuclear antibodies in scleroderma (SCLERODERMA ANTIBODIES PANEL) (Scl-70, CENP A,CENP B, RP 11, RP 155, fibrillarin, NOR 90, Th/To, PM-Sc100, PM-Scl 75, Ku, PDGFR, Ro-52)

A. The study is designed to determine antinuclear antibodies in scleroderma, to diagnose and severity of disease

B. The study is designed to determine antinuclear antibodies in scleroderma to assess the degree of damage to organs and systems

C. The study is intended for the determination of antinuclear antibodies, for diagnosis and severity of the course of antiphospholipid syndrome

D.The study is designed to determine antinuclear antibodies for diagnosis of the focal form of scleroderma

E. It is used for diagnosis of polyneuropathy

5. Antibodies to nucleosomes, Ig G (NUCLEOSOME, CHROMATIN ANTIBODY, IgG)

A. They are of great importance in the pathogenesis of kidney damage in lupus nephritis

B. It is indicated for diagnosis and differential diagnosis of SLE, lupus glomerulonephritis

C. It is indicated for the diagnosis and differential diagnosis of clinical signs of rheumatic disease

D. All of the above is true

E. It is indicated for diagnosis of autoimmune endocrine diseases

6. Immunoblot of antinuclear antibodies (ANTINUCLEAR ANTIBODY) (Sm, RNP/Sm, SS-A (60 кДа), SS-A (52 кДа), SS-B, Scl-70, PM-Scl, PCNA, CENP-B, dsDNA, Histone, Nucleosome, Rib P, AMA-M2, Jo-1 antigen)

A.Immunoblot provides differential diagnosis of major systemic rheumatic diseases

B. Immunoblot provides differential diagnosis of diseases of the blood system

C. Immunoblot provides differential diagnosis of lung diseases

D.Immunoblot provides differential diagnosis of diseases of the digestive system

E. Immunoblot provides differential diagnosis of autoimmune endocrine diseases

7. Antibodies to the extracted nuclear antigen IgG (ENA, Extractable Nuclear Antigen Antibodies, ENA) (mixture RNP/Sm, SS-A (Ro), SS-B(La), Scl-70, centromeric protein in and Jo-1)

A.This test makes it possible to carry out early diagnosis of systemic diseases

B. Provides clarification of the diagnosis of a systemic disease with an unclear clinical picture

C. Provides clarification of the diagnosis of a systemic disease during differential diagnosis

D.All of the above is true

E. Provides clarification of the diagnosis of autoimmune endocrine diseases

8. ANCA Antibodies to proteinase 3 (anti-PR3)Antibodies to myeloperoxidase (anti-MPO)

A. Provides clarification of the diagnosis of autoimmune endocrine diseases

B. Provides diagnostics of diseases of musculoskeletal system

C. Provides diagnostics of diseases of blood system

D. Provides diagnostics of diseases of the genitourinary system

E. Provides diagnostics of systemic vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA-CB)

9. Qualitative definition (positive/negative) IgM and IgG class antibodies to phospholipids (cardiolipin, phosphatidylic acid, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylserine) and their cofactors (plasma proteins) - β2-glycoprotein I, prothrombin, annexin V in human serum or plasma by immunoblot (test strips with applied antigens)

A. Characteristic of antiphospholipid syndrome

B. Typical for SLE, systemic scleroderma

C. Characteristic of diffuse connective tissue disease (DCTD).

D. Characteristic of dermatomyositis

E. Characteristic of autoimmune inflammatory bowel diseases

10. Rheumatoid factor(RF). Antibodies to citrullinated proteins (ACP)

A. This test makes it possible to diagnose Sjogren's syndrome

B. This test makes it possible to diagnose rheumatoid arthritis

C. This test makes it possible to diagnose Behcet's disease

D. This test makes it possible to diagnose ankylosing spondylitis

E. This test makes it possible to diagnose autoimmune inflammatory bowel diseases

11. Large-granular type of nuclear glow (AC-5) indicates the presence of antinuclear antibodies against U1/RNP, Sm.

A. Characteristic of mixed connective tissue disease (Sharpe syndrome)

B. Typical for SLE

C. Characteristic of systemic scleroderma (SSD)

D. All of the above is true

E. Characteristic of ankylosing spondylitis

12. The homogeneous type of nuclear glow (AC-1) indicates the presence of antinuclear antibodies against nucleosomes, double-chiral DNA and histones.

A. Characteristic of SLE, medicinal lupus
B. Characteristic of systemic scleroderma
C. Characterized by chronic active hepatitis
D. Characteristic of ankylosing spondylitis
E. All of the above is true

13. A fine-grained/homogeneous type of nuclear glow (partially positive nucleolus) indicates the presence of antinuclear antibodies against Scl-70.

A. Characteristic of systemic scleroderma (SSD).
B. Characteristic of systemic sclerosis with diffuse skin lesions
C. All of the above is true
D. Characteristic of systemic sclerosis with damage to internal organs
E. Characteristic of ankylosing spondylitis

14. The fine-grained type of nuclear glow (AC-4) indicates the presence of antinuclear antibodies against SS-A (Ro), SS-B (La).

A. Characteristic of Sjogren's Syndrome, SLE
B. Characteristic of dermatomyositis, rheumatoid arthritis.
C. Characteristic of systemic scleroderma (SSD), subacute cutaneous lupus erythematosus.
D. Characteristic of ankylosing spondylitis.
E. All of the above is true

15. Nucleolar homogeneous homogeneous type of nuclear glow (AC-8) indicates the presence of antinuclear antibodies against PM-Scl.

A. Characteristic of systemic scleroderma (SSD
B. Characteristic of dermatomyositis
F. All of the above is true
C. Characteristic of subacute cutaneous lupus erythematosus
D. Characteristic of ankylosing spondylitis

16. The nucleolar lumpy type of nuclear glow (AC-9) indicates the presence of antinuclear antibodies against U3-snoRNP/ fibrillarin.

A. Characteristic of systemic scleroderma (SSD)
B. Characteristic of Sjogren 's syndrome
C. Characteristic of Behcet's disease
D. Characteristic of ankylosing spondylitis
E. Characteristic of rheumatoid arthritis

17. The centromeric glow type of the nucleus (AC-3) (Dots>30) indicates the presence of antinuclear antibodies against CENP-A, B.

A. Characteristic of systemic scleroderma (SSD
B. All of the above is true

C. Characteristic of primary biliary cholangitis (PBC)
D. Characteristic of Sjogren 's Syndrome.
E. Characteristic of ankylosing spondylitis

18. The multiple type of glow of the nucleus (dots in the nucleus, AC-6 Dots 6-20) indicates the presence of antinuclear antibodies against Sp-100.

A. Characteristic of primary biliary cholangitis (PBC).
B. Characteristic of diffuse connective tissue disease (DCTD).
F. All of the above is true
C. Characteristic of dermatomyositis
D. Characteristic of ankylosing spondylitis

19. The homogeneous type of glow of the nucleus with a thin linear glow of nuclear membrane (AC-11) indicates the presence of antinuclear antibodies against Lamin (lamins A, B, C, or lamin-associated proteins).

A. Characteristic for SLE.
B. Characteristic of Sjogren 's Syndrome.
C. Characteristic of seronegative arthritis.
D. Characteristic of ankylosing spondylitis.
E. All of the above is true.

20. The fine-grained type of glow of the nucleus (AC26, chromatin negative, spindie (mitotic spindle) positive) indicates the presence of antinuclear antibodies against NUMA 1 (antibodies to the mitotic apparatus of cell).

A. Characteristic for SLE, systemic sclerodermaSSD)
B. Characteristic of Sjogren's syndrome, mixed connective tissue diseases, rheumatoid arthritis.
C. Characteristic of primary biliary cholangitis (PBC).
D. All of the above is true
E. Characteristic of ankylosing spondylitis

21. Cytoplasmic fine-grained, diffuse staining of the cytoplasm, superposition of large dots (AC-20) indicates the presence of antinuclear antibodies against Jo-1.

A. Characteristic of "antisynthetic syndrome", polymyositis dermatomyositis
F. All of the above is true

B. Characteristic for local SSD
C. Characteristic of idiopathic pleural effusion
D. Characteristic of ankylosing spondylitis

22. Polar cytoplasmic staining on the one hand, the Golgi complex in the cytoplasm (AC-22) indicates the presence of antinuclear antibodies against giantin/macrogolgin, golgin-95/GM130, golgin-160, golgin-97, golgin-245.

A. Characteristic of Sjogren's Syndrome (RARE), SLE, rheumatoid arthritis

B. Characteristic of diffuse connective tissue disease (DCTD), Wegener 's granulomatosis

G. All of the above is true

C. Characteristic of idiopathic cerebellar ataxia, paraneoplastic cerebellar degeneration, viral infections

D. Characteristic of ankylosing spondylitis

23. The mitochondrial/reticular type of cytoplasmic glow (large cytoplasmic dots located in a filamentous network) (AC-21) indicates the presence of antinuclear antibodies against AMA-M2.

A. Characteristic of primary biliary cholangitis (PBC).

B. Characteristic of Behcet's disease

C. Characteristic of Sjogren 's syndrome

D. Characteristic of diffuse connective tissue diseases

E. Characteristic of ankylosing spondylitis

24. Cytoplasmic glow from fine-grained to homogeneous, dots in the nucleus (mixed type) indicates the presence of antinuclear antibodies against Ribosomal phosphoproteins , Coilin.

A. Characteristic for SLE, systemic scleroderma (SSD)

B. Characteristic of Sjogren 's Syndrome

C. Characteristic of primary biliary cholangitis (PBC).

H. All of the above is true

D. Characteristic of ankylosing spondylitis

25. Calprotectin is a product of neutrophilic granulocytes

A. Characteristic of irritable bowel syndrome

B. Characteristic of inflammatory bowel diseases (Crohn's disease, nonspecific ulcerative colitis (NSUC)

C. Characteristic of primary biliary cholangitis (PBC)

D. Characteristic of autoimmune liver lesions

E. Characteristic of ankylosing spondylitis

26. Antibodies to pancreatic acinar cells, IgG and IgA in total (antibodies to the exocrine part of the pancreas, AutoantibodiesagainstExocrinePancreas, PancreaticAntibodies, PAB)

A. Characteristic of diseases of the pancreas

B. Characteristic of autoimmune liver diseases

C. Clinically associated with inflammatory bowel diseases and most characteristic of Crohn's disease
D. Characteristic of primary biliary cholangitis (PBC)
E. Characteristic of ankylosing spondylitis

27. Antibodies of IgA and IgG classes to goblet cells of the intestine, in total (Anti-Intestinal Goblet CellsAntibodies, GAB, IgA, IgG, Total)

A. Detection of antibodies associated with irritable bowel syndrome.
B. Detection of antibodies associated with Crohn's disease
C. Detection of antibodies associated with celiac disease
D. Detection of antibodies associated with nonspecific ulcerative colitis
E. Characteristic of autoimmune hepatitis

28. **IgG and/or IgM antibodies to gangliosides (anti-GM1, GM2, GM3, GD1a, GD1b, GQ1b, GT1b,)**

A. Test for diagnosis of polyneuropathies
B. Test for diagnosis of autoimmune inflammatory bowel diseases
C. Test for diagnosis of endocrine diseases
D. Test for diagnosis of mixed connective tissue disease
E. Test for diagnosis of autoimmune hepatitis

29. **Screening of antibodies to islet cells (ICA)**

A. Test for diagnosis of endocrine diseases
B. Test for diagnosis of autoimmune hepatitis
C. Test for diagnosis of inflammatory bowel diseases
D. Test for diagnosis of polyneuropathy
E. Test for diagnosis of type 1 diabetes mellitus

30. IgG and IgA antibodies to GP2 antigen of pancreatic centroacinar cells (Anti-GP2, IgG, IgA)

A. Detection of antibodies associated with nonspecific ulcerative colitis
B. Detection of antibodies associated with Crohn's disease
C. Detection of antibodies associated with autoimmune hepatitis
D. Detection of antibodies associated with vasculitis
E. Detection of antibodies associated with diabetes mellitus

Answers to test tasks

Question	*Answer*
1	A
2	C
3	D
4	B
5	D
6	A
7	D
8	E
9	A
10	B
11	D
12	E
13	C
14	E
15	D
16	A
17	B
18	C
19	E
20	D
21	B
22	C
23	A
24	D
25	B
26	C
27	D
28	A
29	E
30	B

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