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«ГРОДНЕНСКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ»

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КЛИНИЧЕСКАЯ ЛАБОРАТОРНАЯ ДИАГНОСТИКА

Пособие
для студентов факультета иностранных учащихся
с английским языком обучения

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CLINICAL LABORATORY DIAGNOSTICS

Study guide
for the Medical Faculty for International Students

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This manual provides information on some issues of clinical laboratory diagnostics. Data on laboratory evaluation of renal functional state are given; questions of clinical hematology, laboratory hemostasis system evaluation are discussed as well as a number of clinical studies.

The manual is intended for the students of the Medical Faculty for International Students.

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INTRODUCTION

Diversity of nosologycal forms, peculiarities of their manifestations in different patients makes diagnosing difficult; subjective means depending on doctor's skills and knowledge are not enough. Clinical medicine constantly seeks modern objective footholds in scientific achievements of biology, physics, chemistry, technology and electronics. As a result the sphere of laboratory diagnostics in modern evidentiary medicine is steadily widening.

Major responsibility for quality of diagnostics and treatment is layed on a clinician, but adequate laboratory-diagnostic service makes his job considerably easier.

Laboratory studies a number of biological material samples of a patient and detects diagnostically significant characteristics:

1) *Qualitative* (structural) – form and structure of the cells, presence of chemical compounds of specific structure (for example, hemoglobin variants, enzymes in urine, etc.);

2) Q*uantitative* – size and correlation of structural components; chemical compounds concentration; number of certain cellular elements; balance of structurally close elements (for example, albumin-globulin coefficient);

3) *Functional* – realization of transformation cycle (glucose tolerance test) and circulation of substances in an organism, development cycle, cell maturation.

The main function of clinical diagnostic laboratory is to present authentic information on composition of biological materials samples, taken from a patient and on a degree of correspondence of this composition indices to a generally accepted “norm”.

Resources of laboratory diagnostics include early (preclinical) pathology verification, confirmation and differential diagnostics of clinically manifested nosological forms as well as therapy efficiency evaluation.

Clinical laboratory diagnostics includes study of organic and non-organic chemical substances and biochemical processes in blood and other human’s biofluids in order to diagnose, monitor a disease course, detect prognosis and screen a disease.

Clinical laboratory diagnostics is a discipline on clinical diagnostics which deals with the development and use of standard diagnostic and disease control methods. Clinical laboratory diagnostics allows to make scientifically based diagnosing easier, it helps to chose the methods of adequate treatment and disease prevention.

Clinical laboratory diagnostics includes such parts as clinical biochemistry, clinical laboratory hematology, immunology, clinical serology and microbiology, clinical toxicology and others. It disposes a wide range of diagnostic tests, information and development prospective, which enables a clinicist to solve the questions of pathogenesis and etiology of a pathological process. Information received due to laboratory research methods allows to asses dynamics of pathological process development at molecular, cellular and organ levels, which is essential for modern disease diagnostics and for evaluation of therapy efficiency.

Clinical laboratory diagnostics is mostly based on theoretical basis and is closely connected with such sciences as general and bioorganic chemistry, biologic chemistry, histology, normal and pathologic physiology and pathologic anatomy.

GENERAL ANALYTICAL CLINICAL LABORATORY DIAGNOSTICS

Stages of laboratory examination

A patient's laboratory examination is usually divided into three stages: **pre-analytic, analytic and post-analytic**. Analytic stage takes place in laboratory; the other two stages require significant extra laboratory work.

It is important to understand that, as in any sphere of human activity, it is impossible to avoid some mistakes in clinical diagnostic laboratories. The aim of each laboratory is, using quality control system, to develop reliable set of tools, which allows to detect the mistakes and to make arrangements in order to minimize these mistakes.

Quality assurance is a complex of planned and systematically performed activities, necessary to be confident that diagnostic information in the authorized report corresponds to certain quality requirements.

Undoubtedly, it is a patient who is interested in high quality of laboratory attendance. Specialists in clinical laboratory diagnostics are interested in quality of their product as well. As any professional, they need to be confident that their work is well done. Quality of work as motivation is one of the components of professionalism. Another group of concerned persons are clinicians as main users of diagnostic information, including laboratory examination data. Part of laboratory indices in the whole flow of diagnostic information, used by a clinician comprises 70-80%.

The question of laboratory tests standardization in general, and at this stage particularly, is rather acute. Laboratory examination starts with appointment of tests list, necessary for diagnosing and observing a patient's condition. One of the widespread reasons of mistakes is inadequate laboratory examination. A valuable test will have no use if the analysis results are not used. There are following reasons of inadequate laboratory tests appointment:

- diagnostic test value
- variability of reference values

- use of negative results and assessment of tests complex (comparison of tests results)
- disease course
- doubling of laboratory tests
- sufficiency of diagnostic information.

A doctor should understand that laboratory tests, appointed to approve or eliminate the diagnosis, can be more harmful than useful.

It is evident, that there is no a unified standard on pre-analytic stage performance and hardly it will ever appear (due to broad specificity of medical institutions and clinical diagnostic laboratories). There are differences in work organization of centralized laboratory and hospital laboratory. In this case each medical institution needs to develop and approve the standard of this stage of laboratory examination performance taking into consideration recognized international and national standards and recommendations.

Preparing the patient for laboratory tests is one of the most important components of pre-analytical stage. Certain actions should be obligatory performed:

- a clinician should explain the necessity of laboratory examination to a patient
- a nurse should inform a patient on how to get prepared for examination/

Laboratory part of pre-analytical stage starts from the moment of sample delivery and application to laboratory. This part has following steps:

- organization of test performance and applications (registration of patient's tests);
- samples identification, centrifugation;
- conditions and terms of samples preservation if necessary;
- detection of influences (hemolysis, lipemia) and admixtures (drugs metabolites, impurity);
- distribution of samples to laboratory departments (laboratory assistants), examination performance.

In the laboratory it is recommended to arrange the place where biological material will be collected. On receiving the material a receptionist checks correspondence of samples to applications, samples state, date of sampling and delivering the material. Specialists

in clinical laboratory diagnostics should determine and confirm criteria for refusal in material examination (for example, divergence of data in application and on a tube label, impossibility to read an application, the material was taken with the wrong anticoagulant or preservative, expiring of delivery terms, clots in whole blood with coagulant, etc.). After centrifugation the most common refusal criteria are: hemolysis, sample turbidity.

Various kinds of material are centrifugated and then used for laboratory tests. Assessment of centrifugation time is, first of all, a certain correspondence of time and conditions of centrifugation to this or that kind of material (visual centrifugation control).

The basic control form of pre-analytic stage is periodic internal and external checkups. But this control form can not be considered an effective one. The problem of control over that laboratory examination stage remains one of the major problems of modern laboratory medicine today.

In modern clinics and clinical diagnostic laboratories about 90-95% of blood and other biological samples are collected by vacuum systems. Comparison of costs of open blood sampling (syringe blood sampling), secondary tubes for analyzers, syringes, needles, detergents, disinfectants, electricity, additional equipment for cleaning test tubes, glass breakage accounting, purchasing the necessary reagents (anticoagulants: EDTA, citrate, heparin) and wages for the personnel on the one hand, and the acquisition of advanced systems for the collection of biological material (blood, urine), on the other hand, shows that the economic costs are comparable. But we can not ignore the factor of a significant risk reduction of personnel contamination with hepatitis and HIV when using blood collection systems (while blood sampling the sterility is guaranteed, the contact with the blood of a patient is excluded, so medical personnel and patients are safe as well as transportation of biological material).

Without the introduction of vacuum or other systems for blood and body fluids collection into general practice we can’t expect the improvement of the pre-analytic stage and quality of laboratory examination.

Biological material, used in laboratory examinations

The most widespread materials for clinical biological examination are blood, urine and some other biological fluids. The blood is recommended to be collected in the morning (between 8 and 10 a.m.) before physical activity and other diagnostic procedures. On a day before blood sampling meals can be normal, but alcohol should be excluded. Healthy and ambulatory patients are forbidden to smoke (after 2 a.m.), eat and drink (a glass of water is allowed at 10 p.m. – 5 a.m.) Most blood tests require 8-12 hours' fasting, but to detect triacylglycerols there should be a 10-12-hour interval after a meal. Just prior to blood sampling a patient should take a sit for 10-30 minutes (the procedure is performed with the hand placed at an angle of 45 degrees). The blood in patients with a strict bed rest should be collected at 7-9 a.m., and a patient's hand should be placed horizontally. It should be noted that when people stay in a horizontal position for a few hours plasma volume in blood is 10-15% higher than that of a patient with normal motor activity. So the concentration of substances in the blood of a patient lying more than an hour is always lower than of a patient after a walk. Body position influences the concentration of whole protein, albumin, creatinine, cholesterol, triacylglycerol, alkaline phosphatase activity, aspartate aminotransferase and other plasma components. Content of these substances and enzyme activity are considerably increased in an upright patient's position and decreased in a horizontal position. The maximum change is characteristic of the total protein level, enzyme activity (11%) and calcium content (3-4%). When collecting the blood by venipuncture the time of vascular embarrassment with tourniquet should be minimal. A patient shouldn't bend and straighten the finger as it induces local stasis, hypoxia and shifts in distribution of some substances (cholesterol, potassium, sodium, calcium etc.) between blood corpuscles and its fluid part. If it is impossible to use a single-use syringe one should boil a reusable syringe in distilled water only, and it shouldn't be washed by isotonic sodium chloride solution. To avoid hemolysis blood should be collected by a dry syringe, dry needle, into a dry test tube under sterile conditions. If the blood is transferred from a syringe to a test tube this procedure should be performed slowly (to avoid blood foaming). In hemostasis system

examination there is a number of additional requirements for blood sampling. So, it is recommended to use a *wide lumen* needle without a syringe (it is used for blood collecting in children, adults with hypotension or in terminal states; a syringe should be plastic or siliconized). Skin disinfection (skin area above the puncture site in the bend of elbow) should be performed by 70% ethyl alcohol. As a needle passes through the skin tissue fluid and tissue fragments can be found in a needle lumen. These substances can significantly affect coagulation tests, so the first (after a tourniquet application and vein puncture) 0,5-1,0 ml of blood should not be used for coagulogram. But this blood portion can be used for all the other biochemical tests. To exclude the influence of passive venous congestion on coagulation, it is recommended to loose a tourniquet for 2-3 seconds while venous blood collection. The blood should flow down the walls of a tube. If it is necessary to receive plasma the corresponding coagulant (lithium citrate, sodium or potassium oxalate, etc.) should be put into a test tube beforehand to avoid blood from clotting. The blood is carefully mixed with anticoagulant (without foaming), a test tube is covered with a piece of polyfilm and left on a rack for 20-25 min. Intensive shaking causes erythrocytes hemolysis, which distorts the parameters of coagulogram.That is why plasma with hemolysis signs is not suitable for such kind of examination.

Table 1. Types of biological materials, examined in clinical diagnostic laboratories

Venous whole blood	Is collected from the major veins (usually from the ulnar vein) into a test tube, containing anticoagulants (EDTA) for hematologic tests.
Arterial whole blood	Is collected from the major arteries (usually femoral or subclavicular) into a test tube, containing anticoagulant. Arterial blood collection requires special care and should be performed by a doctor. Most often heparin is used as anticoagulant in that case. Usually arterial blood is used for acid-base balance analysis.

Capillary whole blood	Is collected by heparinized capillaries for gasometric and morphologic tests as well as for glucose and electrolytes detection.
Plasma	Is received after blood centrifugation and cellular elements separation. Depending on anticoagulant type heparinized, citrate plasma and plasma with EDTA is distinguished.
Serum	It is plasma without fibrinogen. To obtain serum the blood is collected into a dry glass or plastic tube, containing coagulation activators. After blood clotting the blood should be centrifuged and serum should be removed from a blood clot.
Urine for "clinical analysis"	Morning urine should be delivered to a laboratory as fast as possible.
«24-hour urine»	Urine, collected during a day. The first urine portion is not considered, though all the other portions during the day are collected including a next morning portion.

Table 2. Changes of laboratory indices in various mistakes of pre-analytic stage

Index	Reason of the mistake
Albumin ↑	Prolonged tourniquet application in venipuncture
Bilirubin ↓	Prolonged exposure of a sample to light
Bicarbonate ↓	Uncovered tube with a sample
Calcium ↑	Prolonged tourniquet application in venipuncture
Cholesterol ↑	A patient did not fast 12-16 hours before a test

γ- glutamyltrans-ferase activity↑	Alcohol, barbiturates or phenytoin intake
Glucose ↓	Fluorid or another glycolysis inhibitor is not added.
Potassium ↑	Long blood storage
Complete protein ↑	Prolonged tourniquet application in venipuncture

Methods of clinical laboratory diagnostics

Among all the known methods of analysis in clinical laboratory diagnosis physicochemical methods are the basic ones. These are methods allowing the study of correlation between chemical, physical and physicochemical properties of biological material, received from a patient.

Depending on the properties of the examined system physicochemical methods are divided into:

1. Optical
2. Electrochemical
3. Chromatographic
4. Kinetic

In clinical diagnostic laboratories optical methods of analysis are used most often:

- Refractometry
- Polarimetry
- Photometry:

А. Absorptive:

- Spectrophotometry
- Nephelometry
- Atomic absorption photometry

Б. Emissive:

- Fluorimetry
- Flame photometry

- Atomic emission spectral analysis

Optical quantitative analysis is based on a detection of changes in a light beam passing through an examined solution.

Absorption intensity (Absorptive photometry)

Glow of molecules and atoms of a substance (fluorimetry, flame photometry)

- The values of deviation of monochromatic light flux from the original direction of propagation (refractometry)
- Change of the angle of plane-polarized light rotation (polarimetry)

The following kinds of optical instruments are used in the work of clinical diagnostic laboratories:

1. Photometers and spectrophotometers
2. Densitometers (scanning of various sample fractures (proteins, lipoproteins, etc.).
3. Nephelometers and turbidimeters (detection of particles suspended in liquid volume according to light scattering intensity)
4. Fluorimeters (Detection of concentration of complex organic substances (hormones, vitamins, etc) by changing the fluorescence intensity)
5. Flame photometers (detection of light emission of metal ions in the flame)
6. Luminometers (measurement of the emitted light quantity)
7. Atomic absorptiometers (measurement of monochromatic light flux by atoms of a substance in incandescent gas).

In modern clinical diagnostic laboratories besides optical methods of analysis methods of fractionating of biological fluids and tissues components (chromatography and electrophoresis) are widely used as well as immunologic tests (immunoenzymatic, radioimmunological and ummunofluorescent tests).

LABORATORY ASSESSMENT OF RENAL FUNCTION

Complete urinalysis

Complete urinalysis is one of the oldest types of analysis used in clinical practice. National Committee for Clinical Laboratory Standards (NCCLS) defines routine urinalysis as the testing of urine with procedures commonly performed in an expeditous, reliable, accurate, safe and cost-effective manner. Though it may vary in different laboratories, usually it includes physicochemical tests with determination of colour, clarity and specific gravity, semiquantitative analysis by using test strips (plastic strips with fixed chemically impregnated sections of porous material). Chemical substances may react with different urine components. After chemical analysis the urine is centrifuged, and concentrate is used for microscopic examination, for measurement of cylinders, cells and fibers.

The term ‘complete urinalysis’ includes the following tests:

1. Macroscopic examination (colour, transparency)
2. Assessment of physical and chemical properties of urine
3. Microscopic examination of urinary sediment

Objectives of the complete urinalysis

1. Diagnosing of diseases
2. Monitoring of disease development
3. Monitoring of specific therapy
4. Screening among population groups to detect congenital, inherited pathology or diseases proceeding asymptomatically

Sample collection

Biological reference material for urinalysis is the first voided in the morning urine. The first voided in the morning urine reflects the ability of the kidneys to concentrate urine most adequately. Urine should be collected in special clean and dry containers, if necessary sterile containers are used (if you are going to inoculate urine on medium to detect causative agent). The optimum amount of urine to bring to the laboratory is 50 ml, the minimum amount for sediment

microscopy is 12 ml. Time interval between urine collection and examination should not exceed 2 hours.

Incorrectly collected or stored urine samples should not be used for analysis.

Macroscopic examination

Colour and transparency

To determine the colour of urine adequate source of light should be used and the sample should be examined against white background.

Normal urine is usually transparent, pale yellow, yellow or amber. Intensity of the yellow colour is changed with depending on the level of urine concentration.

Orange colour of the urine may indicate presence of bile pigments. Bilirubin may oxidize into biliverdin and turn urine into the green colour. Red colour of the urine can be caused by presence of erythrocytes, hemoglobin and myoglobin. In this case strip test on the presence of blood in the urine will be positive. Occasionally porphyrines may colour the urine into red.

Urine opacity may be the result of presence in the urine of erythrocytes, leukocytes, epithelium, bacteria, fat drops, salts precipitation (urates, phosphates, oxalates), pH and temperature of urine storage (low temperature leads to salts precipitation). Urine that is stored for a long time can become turbid because of bacteria reproduction. Normally insignificant opacity can be caused by epithelium and mucus.

Assessment of physical and chemical properties of urine

This examination consists of two stages:

1. Analysis by using strip tests (Table 21)
2. Validation of the pathology that was detected at the first stage with reference or quantative method.

Specific Gravity

Specific gravity (relative density) indicates the weight of certain urine volume related to the same water volume. Specific gravity increases alongwith the concentration of substances dissolved in the urine. Normal components which comprise specific gravity of the urine are sodium and chlorine ions, as well as urea and creatinine.

In pathological situations presence of glucose, ketones, protein in the urine can affect its specific gravity. The normal level of specific gravity in a person depends on the watering schedule and it can vary from 1.003 to 1.040. Specific gravity of morning urine sample is to be more than 1.020 reflecting the kidneys' ability to concentrate.

High specific gravity can be observed in diabetes, when the urine contains excess amounts of glucose. Urine with high specific gravity may be observed as well in syndrome of inappropriate secretion of antidiuretic hormone. Proteinuria is also a major reason for the increase of urine specific gravity of more than 1.015.

Impaired renal concentrating ability and following low specific gravity of the urine may be the result of renal pathologies (tubular necrosis, tubulopathy) or violation of anti-diuretic hormone secretion (diabetes insipidus). In renal failure urine is of constant density ≈ 1,010, which corresponds to the density of the primary filtrate. The urine with low specific gravity may as well be common for people taking diuretics and those who are on a low-protein diet.

pH

Usually urine pH reflects blood pH.

Normally, urine is slightly acidic (pH 4.8 - 7.5; 6.0 on the average). Acidic urine may be released in patients with metabolic acidosis. Urine with a pH more than 5.5 accompanied by metabolic acidosis with normal anionic gap may indicate distal tubular acidosis. Alkaline urine is observed in patients with metabolic alkalosis; in this case the body traps H+ and releases bases. In infections of the urinary tract, microorganisms in the urine can convert urea to ammonia, with pH of the urine increased. In alkaline urine the destruction of erythrocytes and casts may occur, especially at low urine specific gravity. Moreover, evaluation of pH is useful for identification of crystals at microscopic analysis, as well as for determination of the treatment tactics.

Protein

Glomerular membrane has elective penetrating ability for proteins of plasma that depends on molecular weight and concentration of certain proteins. Each 24 hours 150 mg protein is released with urine. The urine may also contain proteins from vagina, prostate, seminal fluid.

Depending on the etiology of proteinuria it can be of four types: prerenal, renal (glomerular, tubular), postrenal and asymptomatic (transient).

1. Prerenal proteinuria is caused by non-renal diseases.
2. Renal proteinuria can be connected with affection of renal glomerular apparatus (glomerular proteinuria) and of tubules of nephron (tubular proteinuria).
3. Postrenal proteinuria is observed in urinary tract infections with exudation of protein through the mucosa of the urinary tract. This form of proteinuria is secondary to infection of the bladder or urinary tract.
4. Asymptomatic (transient) proteinuria is commonly associated with traumas. Its most frequent variant is orthostatic proteinuria. Such patients do not have proteinuria in recumbent position; however, after 2 or more hours in standing position mild proteinuria is developed. About 50% of such patients have insignificant changes in glomeruli which can be confirmed by biopsy data.

Strip test is performed for screening assessment. When interpreting the results of the test the fact should be kept in mind that the method is sensitive, primarily, to albumin and does not detect pathological protein as Bence Jones protein (immunoglobulin light chain), as well as gamma globulin.

The most popular laboratory method of proteinuria quantative assessement is sulfosalicylic test followed by turbidimetric detection. This method enables to detect protein level in concentrations of more than 200 mg/L.

Microalbuminuria

Immunochemical methods enabled to detect small concentrations of albumin (<200 mg/L), which is important for monitoring development of vascular complications in diabetis.

The term “microalbuminuria” is defined as the excretion of 30 – 300 mg/24 hours of albumin with urine or 20 – 200 μg of albumin per minute or 2.8 – 28 μg/mmol of creatinine. Microalbuminuria is the earliest sign of diabetic nephropathy (prior to proteinuria). At present diabetic nephropathy is the leading cause of morbidity and mortality among patients with diabetes melitus. The frequency of diabetic

nephropathy in patients with type 1 diabetes is 40-50% and 15-30% in patients with type 2 diabetes. Besides, diabetes mellitus is the main cause of kidney disease leading to the patient's death in Europe, as well as in the USA.

Constant microalbuminuria indicates forthcoming development of advanced stage of diabetic nephropathy (within the following 5-7 years).

Glucose

Glycosuria can be defined as presence of glucose in the urine in the concentrations that can be detected by routine screening semiquantitative tests.

Cells of proximal parts of renal tubules reabsorb the most of glucose from glomerular filtrate. Glycosuria develops only when glucose content in glomerular filtrate exceedes reabsorptive capacity of renal tubules.

Such a situation can occur when:

1. Glucose concentration in blood plasma and in glomerular filtrate is more than 10 mmole/L (renal threshold), which significantly exceeds reabsorption capacity of healthy renal tubules

2. Reabsorption capacity of renal tubules is lowered, which leads to glycosuria at glycemia levels below the renal threshold (renal glycosuria)

Strip analyzer enables to make a semiquantitative estimation of glucose level in urine. The result can be totaled in the following ways: negative, 6 – 15 mmol/L, 5-30 mmol/L, 30-100 mmol/L, > 100 mmol/L.

Ketone bodies

Ketone bodis are catabolite products of free fatty acids and they include acetoacetate, acetone and beta-hydroxybutyrate. In healthy people ketone bodies are found in blood and urine at low concentration (ketonemia level < 0,5 mmol/L). Increase in the level of ketone bodies in a patient with diabetes mellitus or without antecedent diabetis, but accompanied by hyperglycemia (diabetes mellitus may appear as ketoacidosis) indicates development of diabetic ketoacidosis (DKA).

The level of ketone bodies in urine and blood is often estimated in patients with diabetes for diagnosing and evaluating treatment of diabetic ketoacidosis.

At high urine concentration degree or during administration of some drugs (e.g. blockers of angiotensin converting ferment) false-positive results of test for ketonuria may be obtained.

Hemoglobin (hematuria)

Hematuria is defined as detection of three or more erythrocytes per high-power field on microscopic evaluation of urinary sediment from two of three urinalysis specimens of a patient.

Strip test for blood in urine can be positive in the following cases: hemoglobinuria (intravascular hemolysis), myoglobinuria (destruction of muscular tissue following hard physical load, cramps, coma, or different myopathies), erythrocyturia (damaging of glomecular renal apparatus, urinary tract bleeding).

Glomerular hematuria is a result of pathology of renal glomerule and is often accompanied by proteinuria and development of erythrocyte tubular casts. On sediment microscopy erythrocytes with morphological changes (dysmorphic erythrocytes) can be detected. It should be noted that only in 20% of cases glomerulonephrites manifest only as hematuria.

Postrenal hematuria is caused by urinary tract bleeding following tumor, trauma (e.g. by bladder stone), infection. The main reason (20% of cases) of massive postrenal hematuria is oncological diseases of urinary tract.

Bilirubin

Normally urine contains minimal amount of conjugated bilirubin (7 - 20 μg/L) which cannot be detected by qualitative methods.

Bilirubin is quickly destroyed in urine so it has to be measured within a few hours. Bilirubin is assessed with indicator test-strip which changes colour depending on its concentration. The test is specific for conjugated bilirubin. Presence of bilirubin is assessed qualitatively, i.e. the result is either positive or negative.

Urobilinogen

Urobilinogen is a product of bilirubin. There are known several urobilinogen compounds (urobilinogen, stercobilinogen) and urobilin compounds (urobilin, stercobilin). Urobilinogen is colorless substance, urobilin is yellowish brown. It is difficult to distinguish analytically urobilinogen from stercobilinogen, so the term

"urobilinogen" combines both these substances. Concentration of 17 mmol/L (i.e. < 1 mg/dl) is considered to be the upper limit of the physiological concentration of urobilinogen in urine. Increased urobilinogen amount is called urobilinogenuria, it is typical for hemolytic states, parenchymal liver and intestinal diseases.

In intestinal diseases intensive stercobilinogen reabsorption of mucous membrane of the colon occurs, resulting in increasing its concentration in the urine. This type of stercobilinogenuria is more common in children with the following symptoms: colitises, constipation, intestinal obstruction.

Urobilinogen level in the urine also increases after "liver shunt," which is considered to be one of the most sensitive tests of liver functional state, after liver cirrhosis with portal hypertension, portal vein thrombosis, etc. If a patient has no hemolysis and bowel disease, it is a sign of damage of urobilinogenuria liver parenchyma. Urobilinogenuria is considered to be one of the most sensitive tests of functional liver analysis. In liver tumor, abscess, hydatid cyst urobilinogenuriya is observed only during generalization period, when the disease process captures most of the body, disrupting the function of the liver.

Leukocytes

Urine usually contains neutrophils. At low osmolality and alkaline (pH 8.0 - 9.0) reaction leukocytes increase in size, the Brownian motion of neutrophil granules starts in the cytoplasm. Neutrophils are destroyed when staying for a long time in the urine containing bacteria. Normally, one μL of morning urine contains up to four white blood cells. Approximate examination of urinary sediment enables to detect 0-2 leukocytes in men and up to 4-6 leukocytes in women per high-power field. Differentiation of leukocytes in urine stained preparations is carried out by microscopy, expressing the number of different forms in percentages.

Pyuria (leukocyturia) is the most common symptom of infectious inflammatory process in kidneys and urinary tract. Pyuria unlike bacteriuria is typical for chronic and nonchronic inflammations. Pyuria is more common in women than in men, which is connected with larger number of diseases of the urinary tract and with a high

possibility of urine contamination with leukocytes in vaginal discharge.

Leukocyturia and bacteriuria are common for acute and chronic pyelonephritis. This is especially important when diagnosing chronic pyelonephritis, which often occurs without evident clinical signs. Leukocyturia is the main symptom of inflammatory diseases of the urinary tract (pyelitis, cystitis, urethritis). It can occur alongwith congenital and acquired disorders of urinary outflow, including structural abnormalities and urolithiasis.

Lymphocyturia is typical for kidney diseases of immune origin: chronic glomerulonephritis, lupus nephritis, late stage of chronic lymphatic leukemia. Eosinophils appear in the urine in chronic pyelonephritis of tuberculous origin, pyelonephritis, cystitis, urethritis of allergic etiology.

Nitrites

Normally, people excrete small number of nitrates which are turned into nitrites by certain types of bacteria. Indicator methods enable to detect nitrites presence in urine. Nitrites development is connected with consumption of vegetables, which should be taken itno consideration at examination.

Escherichia coli, Proteus, Klebsiella, Enterobacter, Citrobacter, Salmonella have an expressed ability to produce nitrites. Enterococcus, Stafilococcus Psevdomonas have a limited ability. Other microorganisms do not lead to this transformation.

At urine examination the following principles should be taken into account:

- The first morning urine should be examined, as it needs to stay for a long time in the urinary bladder so that bacteria can reduce NO_3 to $N0_2$.
- The day before the examination the patient should eat sufficient amount of vegetables (spinach, cabbage, carrot).
- To cancel antibacterial therapy or interrupt it at least for 3 days fefore the examination.

Microscopic examination of urinary sediment

Microscopic examination of urinary sediment is an integral part of urinalysis. This examination includes:

1. Specimen preparation: centrifugation of urine sample for a certain period of time, decantation of supernatant, sediment dissolution in 1 ml of urine
2. Microscopic examination.

Urinary sediment is commonly classified as:

- Organized sediment: cells (erythrocytes, leukocytes, epithelial cells), casts, causative agents (bacteria, protozoa, fungi, helminth eggs, etc.).
- Unprganized sediment: salts, crystals.

Table 3. Characteristics of main types of urinary casts

Type of the cast	**Composition**	**Clinical significance**
Hyaline	Tamm-Horsfall protein	Glomerulonephritis, pyelonephritis, congestive cardiac failure
Erythrocyte	Erythrocytes	Glomerulonephritis
Leukocyte	Leukocytes	Pyelonephritis
Epithelial	Tubular epithelium	Tubular necrosis
Granular	Remains of leukocytes, bacteria, urates, protein aggregate, tubular epithelium	Urinary retention, urinary tract infection
Waxy	Are formed by hyaline and granular casts	Urinary retention, chronic kidney disease
Lipid	Epithelial casts, contain fatty droplets	Nephrotic syndrome
Coarse casts	Are formed in collecting tubules	Marked urinary retention
Pseudo casts	Mucin, fibrin, impurities	Can be taken as tubular casts

Laboratory assessment of fractional renal functions

Separate quantative assessment of filtration level in glomerulus, reabsorption and secretion in tubulus as well as renal plasma and blood flow is diagnostically important. Modern methods of

assessment of renal functions are based on the the measurement of ***clearance*** (sieving coefficient).

Clearance is volume of blood plasma in mL, which going through kidneys is fully eliminated (cleared) from any endogenous or exogenous substance per one minute. Clearance of any substance excreted with urine is calculated by using the formula:

$$\mathbf{C = U / P \times V} \text{ (mL/min)},$$

C — clearance; U — concentration of the examined substance in urine; P – concentration of the examined substance in blood plasma; V — volume of the urine excreted per one minute (minute diuresis)

Estimation of glomerular filtration rate

Glomerular filtration (GF) is a volume (in mL) of liquid part of blood passed through glomerular filter into the cavity of Bowman capsule per one minute.

Glomerular filtration rate (GFR) is one of the best indicators of renal function. GFR is a reliable parameter indicating chronic renal disease. Since general GFR equals to the summed filtration rates in every functioning nephrons, this indicator may be used as a marker of functioning renal masses. A decrease in GFR precedes kidney failure in all forms of progressive kidney disease. The level of GFR is a reliable predictor of the time to onset of kidney failure as well as the risk of complications of chronic kidney disease. Additionally, estimation of GFR in clinical practice allows proper dosing of drugs excreted by glomerular filtration to avoid potential drug toxicity.

State of glomerular filtration reflects most clearance of inulin, which is considered to be a standard for comparing GFR, determined by clearance of other substances. The reason is that molecules of inulin are smaller in size than pore diameter in basal membranes of the glomerular capillaries, so they can easily penetrate into the capsule cavity. Amount of inulin filtered in glomerulus amounts to GFR multiplied by inulin concentration in plasma ($GFR \times P_{in}$). Amount of the excreted inulin equals to inulin concentration in the urine (U_{in}), multiplied by the rate of urine formation (V, urine volume excreted per unit time).

As the amount of filtered inulin equals to the amount of excreted inulin ($GFR \times P_{in} = U_{in} \times V$), GFR can be calculated using the following formula:

$$\mathbf{GFR = (U_{in} \times V)/ P_{in}}$$

The expression $(U_{in} \times V)/P_{in}$ is defined as inulin clearance and it is an accurate measurement of GFR. Inulin clearance (ml/min) refers to volume of plasma per unit time that is cleared of inulin by renal excretion.

As a result, inulin concentration in glomerular filtrate fully corresponds to inulin concentration in blood plasma. On average, inulin clearance in healthy young men is **127 ml/min/1.73 m^2** and in women it amounts to **118 ml/min/1.73 m^2** with a standard deviation of 20 ml/min/1.73 m^2. GFR decreases approximately by 1.0 ml/min/1.73 $^{m2.}$in people older than 20.

However, in clinical practice, inulin clearance is not implemented widely because of intravenous drip of this substance all the time throughout the study, as well as the strong possibility of allergic reactions. This fact was the reason for the development of alternative methods for GFR assessment.

Urinary clearance of exogenous radioactive tracers (^{125}I-iothalamate and ^{99}mTc-DTPA) is a reliable method of GFR measuring, but it is currently not used in routine practice. Plasma clearance of such exogenous substances as iohexol and ^{51}Cr-EDTA was used, but it requires calculating body sizes, which reduces the accuracy of the method. Capillary electrophoresis enabled to measure the content of iothalamate without radioactive tracers in blood and urine with promising results. Measurement of cystatin C was used for GFR assessment, but contradictory data was obtained whether this method can give reliable results to ensure its widespread clinical use.

Currently in clinical practice the most popular method for GFR assessment is based on the measuring daily endogenous creatinine clearance. This method was proposed by P. Rehberg in 1926. Rehberg intravenously injected exogenous creatinine and determined its clearance. In 1936 E.M. Tareev modified the method by suggesting the use of concentration of endogenous creatinine, since it had been found that concentration of creatinine in blood plasma does not change during the day.

Control of time when the urine is collected is an important factor of the study reliability. The most reliable results of GF are obtained when diuresis is 1.5-2 ml/min. GF values may be lowered, if diuresis is less than 1 ml/min., and increased at rates of more than 2.5 ml/min. The main object of the study is to collect daily urine, estimate creatinine concentration in blood and urine, calculate minute diuresis and calculate clearance using the formula:

C= Urine Creatinine / Serum Creatinine x V (ml/min)

Normal: men 80-120 ml/min;
women 70-110 ml/min

Recalculation of C per body surface area:

$$C_{1,73} = (C \times 1,73) / S\ m^2$$

1,73 m^2 – standard body surface area;
S m^2 – body surface area of the patient.

Using clearance of endogenous createnine as an indicator estimating GFR causes some problems. The most important of them: correct sample collection of daily urine which is especially topical for out-patients and newborns; attaining necessary quality if creatinine in blood and urine.

Creatinine is produced mainly in the muscles from the creatine metabolism, therefore, its production is proportional to total muscle mass. As a result, average rate of creatinine formation in men is higher than in women, in younger is higher than in the elderly. This fact leads to different concentrations of serum creatinine depending on age and gender.

Muscle exhaustion causes decreased creatinine formation, which in its turn leads to lower concentrations of serum creatinine, if compared to the expected one (based on GFR) in patients with protein-energy malnutrition. Creatinine generation is also influenced by the consumption of meat, because during the process of preparation its creatine turns into creatinine. Therefore, patients on a low-protein diet, have lower serum creatinine than could be expected from the

level of GFR. These effects particularly influence inadequacy of values of serum creatinine and GFR in patients with chronic kidney disease. Besides, though in patients with normal renal function, extrarenal creatinine excretion is minimal, in patients with chronic kidney disease it increases due to creatinine degradation caused by bacterial overgrowth in small intestine. In patients with severe renal impairment up to two-thirds of total daily creatinine excretion can occur by extrarenal elimination.

As a result, decrease in excretion of creatinine and urine, activation of extrarenal creatinine loss in chronic kidney disease leads to its decrease in the blood and thus, to the systematic overassessment of GFR, calculated by creatinine serum. So, the increase in serum creatinine is not a sensitive indicator of GFR decrease. Only 60% of patients with decreased GFR have increased serum creatinine level. In other words, 40% of people with decreased GFR have serum creatinine level within normal limits.

Another problem of this blood creatinine measurement for GFR estimation is the analytical features of its laboratorial assessment. Thus, young people in norm have serum creatinine level of 0.088 mmol/L approximately. The traditional method of creatinine determination is Jaffe reaction with picric acid, which enables not only to determine GFR level, but a number of non-creatinine chromogens as well (approximately 20% of the concentration in serum creatinine). At the same time, normally urine does not contain non-creatinine chromogens, but in chronic kidney disease these substances appear there. Thus, measured creatinine clearance systematically lowers true creatinine clearance.

Modern biochemical autoanalyzers use methods for creatinine determination that are less dependent on the level of non-creatinine chromogens (e.g., modified kinetic Jaffe reaction or methods based on enzymatic reactions). In addition to non-creatinine chromogens, there are a number of substances that can affect the results of the serum creatinine determination. These substances include ketone bodies and some drugs. The presence of these substances in blood can lead to false elevation of serum creatinine concentration and consequently to underestimated GFR.

Thus, serum creatinine level is influenced by GFR as well as other factors, including age, sex, race, body surface area, diet, certain drugs, laboratory analytical methods (Table 23).

Table 4. Factors affecting creatinine level in serum

Factor	Effect	Effect
Renal pathology	Increase	Decreased GFR, with the increase in tubular creatinine secretion and decrease in the intensivity of its formation, which in turn reduces the level of serum creatinine growth
Decrease in muscle mass	Decrease	Creatinine formation is reduced (observed in children, women, elderly and people with protein-calorie deficiency
Meat consumption	Increase	Transient increase in creatinine formation
Trimethoprim, cinamet	Increase	Inhibition of tubular creatinine secretion
Flucytosine and certain cephalosporins	Increase	Interference with analytical methods of serum creatinine determination
Ketoacidosis	Increase	

On the basis of mentioned above we can conclude that creatinine clearance is not an accurate indicator of renal function, and creatinine should not be used to determine the stage of chronic kidney disease.

In 2006, National Kidney Disease Education Program USA (NKDEP) together with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and European Community Confederation of Clinical Chemistry offered standardization program for serum creatinine measurment. Within NKDEP and a number of funds for patients older than 18 years so-called MDRD-formula (Modification of Diet in Renal Disease) was developed for GFR determination:

GFR (mL/min/1,73 m^2) = 11,33 x $[creatinine]_{plasma}$ x (Age, years)$^{-0,203}$ x (0,742 for women) x (1,210 for afro-americans)

The advantages of this formula include its derivation from the following data:

- GFR measured using the method of determining urinary clearance of ^{125}I-iothalamate;
- analysis of a large group of patients (>500) with a wide range of renal diseases;
- inclusion in the study patients of different races

This formula enables to determine levels of GFR standardized for body surface area. Calculations are performed using the calculators available online and for download in the Internet.

A number of formulas for determining GFR in infants were developed. Schwartz formula is the most common. It employs the product of the constant and child's height divided by the serum creatinine:

GFR= (4,3 x Height, m)/ [creatinine, mmol/l]

Schwartz formula was developed during measurement of inulin clearance and creatinine measured by modified Jaffe method, which may overassess true level of creatinine. Schwartz formula is reported to have an average difference between calculated and measured GFR values. It ranges from -0.4 to 10 mL/min/1, 73 m^2, with the standard deviation (SD) from 2 to 20 mL/min/1, 73 m^2. Studies evaluating the accuracy of calculations by Schwartz formula show that about 75% of GFR estimates are in the range of ± 30% of GFR measured by inulin clearance.

Despite some inaccuracy, Schwartz formula for GFR estimation in children good and efficient, it employs height as it is proportional to muscle mass.

GFR in infants differs quantitatively from GFR in older children and adults. GFR increases during the first 12-18 months. (Table 5).

Таблица 5. GFR in children and teenagers

Age (gender)	**Average GFR ±SD (mL/min/1,73 m^2)**
1 week of life (boys and girls)	40,6 ± 14,8
2-8 weeks of life (boys and girls)	65,8 ± 24,8
> 8 weeks of life (boys and girls)	95,7 ± 21,7
2-12 years (boys and girls)	133,0 ± 27,0
13-21год (boys)	140,0 ± 30,0
13-21 год (girls)	126,0 ± 22,0

Note: SD - standard deviation

Clinical Applications of GFR

GFR calculations based on the serum creatinine determination using corresponding formulas in adults and children provide the basis for classification of chronic kidney disease and diagnostics of its progression. Depending on the severity of decrease in GFR a particular stage of disease can be established (Table 6).

Table 6. Stages of chronic renal disease

Stage	**Description**	**GFR (mL/min/1,73 м^2)**
1	Injury of kidney with normal GFR	≥ 90
2	Injury of kidney with a slight decrease in GFR	60-89
3	Moderate decrease in GFR	30-59
4	Severe decrease in GFR	15-29
5	Renal insufficiency	< 15 (or dialysis)

Chronic kidney disease is diagnosed either by direct detection of organ impairment (e.g., using methods of instrumental diagnostics) or by reduction of GFR level below 60 mL/min/1.73 m^2 during three months.

People with GFR level of 60 to 89 mL/min/1.73 m^2 without identified kidney impairment are classified as having "decreased GFR". Reasons of chronically decreased GFR without kidney damage

in adults may include vegetarian diet, unilateral nephrectomy, reduction in extracellular fluid, systemic pathology associated with decreased renal perfusion, such as heart failure and cirrhosis. Currently there are debates, whether people with chronically decreased GFR (60-89 mL/min/1.73 m^2) without kidney impairment are at higher risk group of adverse outcomes such as toxicity of drugs removed by kidneys or acute kidney injury. Numerous studies were followed by the conclusion that there are no enough premises.

Decreased GFR is diagnosed at values <90 mL/min/1.73 m^2. The lower limit of normal GFR depends on age. E.g., GFR <90 mL/min/1.73 m^2 is subnormal in young people (Table 2). On the other hand, GFR 60-89 mL/min/1.73 m^2 may be normal at the age of about 8 weeks to 1 year, and in the elderly. GFR of 30-59 mL/min/1.73 m^2 can be normal in patients in the extreme age groups, vegetarians, after unilateral nephrectomy. GFR <30 mL/min/1.73 m^2 is definitely abnormal for people of all ages, except infants. For this reason, when diagnosing chronic kidney disease it is recommended to use GFR level below 60 mL/min/1.73 m^2 as a key criterion, while those with GFR of 60-89 mL/min/1.73 m^2 are diagnosed as having chronic kidney disease, only if there is an indicator of kidney impairment.

GFR decrease may be acute or chronic. Acute decrease does not necessarily indicate kidney failure. E.g., it is well known that a short period of slight decline in renal blood flow or transient obstruction of the urinary tract can cause a decrease in GFR without kidney damage. However, significant decreases in blood flow or prolonged obstruction are more often associated with kidney impairment. Chronic GFR decrease is often related to kidney damage. NKDEP specialists determined period of GFR decrease of more than 3 months as a critical period in determining chronic kidney disease. People with reduced GFR should be examined for markers of kidney impairment in order to determine whether they have chronic kidney disease, and what is the reason for the deterioration of renal function. Even if there are no signs of kidney damage, those with decreased GFR are at high risk of adverse effects of such factors as the toxicity of drugs removed by kidneys, and development acute kidney injury.

GFR decrease is connected with a wide range of different complications in other organs and systems (Table 7).

Table 7. Relations of GFR to different complications

Complication	**Relation to GFR**	**Medical diagnostic solutions**
Hypertension	Is both a cause and a complication of chronic kidney disease. As a complication, arterial hypertension may develop in the early stages of the disease	• Monitoring of arterial pressure • Treatment of hypertension using non-pharmacological correction and specific drugs to prevent the progression of kidney disease and cardiovascular pathology
Anaemia	Develops with the progression of chronic kidney disease	• All patients with GFR<60 mL/min/1.73 m^2 should be diagnosed for anemia
Nutritional status	Protein-energy malnutrition develops with the progression of chronic kidney disease. Low protein intake is an important cause of protein-energy malnutrition in chronic kidney disease	• All patients with GFR<60 mL/min/1.73 m^2 should be assessed for protein intake, caloric value of food and nutritional status (body weight, total protein, albumin, nitrogenous bases) • Patients with low intake of protein and calories or with protein-energy deficiency should correct their diet, undergo diet training or they should be applied specialized nutritional therapy • For patients with CRI (GFR<25 mL /min), not undergoing dialysis treatment should be administered low-protein diet with a daily amount of protein of 0.6 g/kg

Bone pathology associated with the violation of calcium and phosphorus metabolism	Develops with the progression of chronic kidney disease	• Patients with GFR<60 mL/min/1.73 m^2 should be examined for bone pathology and disorders in calcium and phosphorus metabolism
Neurological disorders	Develops with the progression of chronic kidney disease	• Patients with chronic kidney disease should be occasionally examined for disorders of central and peripheral nervous systems • Special laboratory tests for diagnosing neurological disorders in patients with chronic kidney disease are indicated only if there are corresponding symptoms.

Urea and plasma creatinine

Urea is one of the end products of protein decompensation and is produced in the liver. It is not a good indicator of glomerular lesions, as creatinine. This is due to the fact that the level of urea is determined by glomerular filtration rate as well as by: food protein intake, non-food protein intake in the gastrointestinal tract (in bleeding), formation of urea from protein in the liver. The process of transformation of protein to urea intencifies in catabolic conditions, acidosis. On the other hand, liver pathology may reduce the synthesis of urea. Urea reabsorption in renal tubules is intensified by vasopressin, so in dehydration or in other conditions with increasing vasopressin concentrations, urea increases regardless of GF.

Creatinine of blood, as mentioned above, is formed from creatine - muscle protein, therefore mass of muscle tissue directly influences creatininemia. 15-25 mg creatinine/kg is produced in men and 10-20 mg/kg in women. Severe muscle injury leads to hypercreatininemia regardless of GF level. It should be kept in mind

that in a healthy person 90% of creatinine is excreted by glomerular filtration and 10% - by tubular secretion. In this regard, drugs suppressing the secretory activity of the tubular cells (histamine receptor blockers, a number of antibiotics, etc.), raise the level of creatinine in the blood that does not depend on GFR.

Norm: **Creatinine –** men 60-120 μmol/L;
women 44-97 μmol/L

Urea – 2,5- 8,3 mmol/L

Criteria of the functional state of kidneys on the regulation of osmotic homeostasis

The kidneys are important in the regulation of extracellular fluid volume and its osmotic pressure. Osmolarity is concentration of osmotically active compounds (OAC) in biological fluids. Mostly they are ions of sodium, potassium and chloride, to a lesser extent glucose and urea. In healthy people blood is 275-300 mOsm/L, urine osmolality 50-1500 mOsm /L.

Osmolality is the concentration of OAC per kg of water. Morning urine sample of a healthy person should have osmolality of 600 mOsm/kg of water.

Less accurately content of OAC can be assessed by urine specific gravity. Morning sample should have gravity of at least 1020. If we multiply the last two figures of the specific gravity value by 26, we will get approximate urine osmolality.

Zimnitskiy test is one of the most common clinical tests for assessment of kidney concentration ability: eight of three-hour urine samples are taken for assessment of specific gravity and volume, ratio of diurnal to nocturnal diuresis (normal 3:1, 4:1) and changes in specific gravity throughout the day (normally not less than 8-12). Sample collection begins at 9 a.m. and it is performed until 9:00 a.m. the next morning.

Osmolality index (osmolality ratio) is the ratio of urine osmolality to plasma osmolality.

Norm: 2.8 ± 0.1

The index is used to identify causes of disorders of concentrational renal function:

• = 1 osmotic diuresis (diabetes mellitus);

• 0.5-1 disorder associated with kidney pathology (CRI, pyelonephritis, etc.);

• <0.5 diabetes insipidus (decreased ADH secretion e.g. after traumatic brain injury)

Osmolality clearance is the volume of plasma which is completely cleared of all OAC in 1 minute passing through kidneys:

$$\mathbf{C\ osm = (U_{OSM} \times V) / P_{osm},\ mL/min}$$

Norm: up to 3 mL/min.

Water eliminated by kidneys, may be divided into two factions:

1. Osmotically bound water. It contains the OAC in similar to plasma concentrations.

2. Osmotically free water. It stays in tubules after OAC reabsorption through wall of renal tubules which are impervious.

Clearance of osmotically bound water characterizes the ability of kidneys to clear plasma from OAC, and clearance of osmotically free water characterizes the ability of kidneys to clear plasma from excess water:

$$\mathbf{Minute\ diuresis\ (V) = C_{OSM.} + C_{OFW};\ C_{OBW} = V - C_{OSM}}$$

A number of functional clinical studies are carried out to assess the ability of the kidneys to concentrate urine:

1. Water loading test (dilution of urine).

2. Water deprivation test (in concentration)

Water loading test is performed to assess urinary concentration ability in hyperhydration: in the morning patient drinks 20-22 mg/kg of fluid (water, tea without sugar) and urinates in the toilet. Next 2 hours urine is collected. A healthy people eliminates at least 1 liter of urine with specific gravity less than 1002-1005, Osmolality clearance should be about 10 mL/min.

Water deprivation test is performed to assess urinary concentrating ability in dehydration: for 16-18 hours patient is forbidden to take any fluid and fluid food. During this period of time urine is collected (the same as per Zimnitskiy test). Necessary condition is the prescription of high-protein diet. *When concentration*

ability of kidneys is normal, the total volume of eliminated urine is 500-600 ml, and its specific gravity is at least 1028. When *loss of the concentration function is moderate*, specific gravity value is less than 1028. If the value falls below 1020 it indicates *severely impaired urinary concentrating ability*.

Determination of tubular reabsorption

Tubular reabsorption can be calculated with the following formula:

$$\mathbf{R = F - V/F \times 100\%}$$

R – tubular reabsorption; F – glomerular filtration; V – minute diuresis

Normally, tubular reabsorption is 98-99%.

This indicator is used mainly for differential diagnosing of diseases of glomerular and tubular kidney apparatus. In *tubulointerstitial disorders* (pyelonephritis, hydronephrosis, polycystic renal disease, etc.) decreased reabsorption occurs in the early stages, and in *glomerular diseases* (glomerulonephritis, diabetic glomerulosclerosis, etc.) it can be observed later.

Evaluation of renal secretory function

For evaluation of secretory renal function they use techniques for determination of clearance of substances excreted from the body mainly by tubular secretion. Most often this is done using exogenously administered phenol red, 95% of which is excreted with urine by tubular secretion.

Technique of phenol red clearance: in the morning patient drinks 400 mL of water fasting and 15-20 minutes he urinates in the toilet. Then he administered 1 mL of 6% phenol red solution intramuscularly and 2-hour urine samples are collected. Each of these samples is analyzed colorimetrically for concentration of colouring agent.

Normally, 40-60% of phenol red should be excreted within the first hour, and 20-25% within the second one (60-85% in total).

If tubular secretory function is reduced, removal of colouring agent is slow and it’s more intensive in the second urine sample.

This function of kidneys may be studied using anumber of other substances which are actively secreted by tubular epithelium (diodrast, para-aminohippuric acid).

Evaluation of effective renal plasma and blood flow

Evaluation of effective renal plasma flow is performed by determining clearance of substances which are not only filtered in glomerulus, but also are secreted in renal tubules (diodrast, para-aminohippuric acid). Using clearance of these substances we can calculate the amount of plasma that flows through glomeruli and tubules per unit of time (1 min), i.e., ***effective renal plasma flow***.

Normal - 550-680 mL/min.

After determining effective renal plasma flow and hematocrit, ***renal blood flow*** can be calculated.

Normal - 1000-1300 mL/min.

Evaluation of these parameters is important for objective evaluation of the effectiveness of treatment of patients with various renal diseases.

Assessment of urine sodium excretion

In physiological conditions kidneys save sodium effectively, and its ecretion entirely depends on tubular reabsorption. In healthy people limitation of sodium to 10 mmol is followed by decrease in its urine excretion of 10 mmol/day for 7 days. If sodium intake is the same but the excretion is more than 10 mmol/day, it means that ability of the renal tubules to reabsorb is impaired.

In patients with oliguria, but with the normal tubular function, urine should be concentrated, and excretion of sodium should be equal to its intake. If oliguria is an effect of renal insufficiency, urinary osmolality is almost the same as plasma osmolality and sodium excretion exceeds its intake. In clinics fractional excretion is used for evaluation of urine sodium excretion rate:

$$\mathbf{Fe_{Na} = ([Na^{+}_{urine}]: [Na^{+}_{plasma}]) \times ([Creatinine_{serum}] / [creatinine_{urine}]) \times 100\%}$$

Norm – 1%. Diagnostically significant increase – more than 1.5%.

The role of kidneys in the regulation of acid-base balance (ABB)

The kidneys are involved in maintaining hydrogen ions homeostasis through three main mechanisms:

- hydrocarbonate reabsorption in the proximal tubules;
- hydrocarbonate regeneration in the distal tubules;
- urine elimination of hydrogen ions

In physiological conditions the urine contains almost no hydrocarbonate ions (<1 mmol/L), as it is almost completely reabsorbed in renal tubules. Amount of reabsorbed HCO_3^- in the renal tubules is 26 mmol per liter of primary urine. This value depends directly on pCO_2 and is inversely related to chloride and potassium content in blood serum. About 90% of hydrocarbonate is reabsorbed in proximal tubules.

Energy accumulated as gradient of electrochemical potential of sodium ions between the tubular lumen and the cell is used for hydrocarbonate transportation. This gradient is maintained by Na, K-ATPases located on antiluminal cytoplasmic membrane of tubular cells. The process when sodium ions get into the cell from the tubular lumen is associated with hydrogen ions release in the primary filtrate. This process is catalyzed by sodium-hydrogen exchanger.

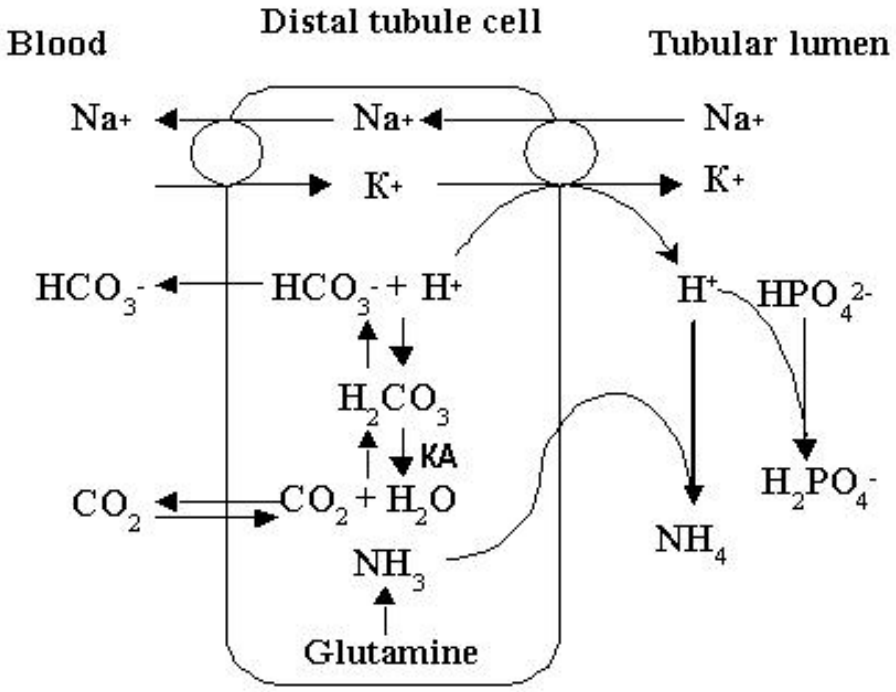

Fig. 1. Involvement of distal tubule in ABB regulation

In the tubular lumen released hydrogen ion reacts with hydrocarbonate ion thus producing carbonic acid. As a result of carbonic acid dehydration, carbon dioxide forms on the luminal membrane with involvement of carbonic anhydrase. CO_2 diffuses into tubular cells, where hydrocarbonate is produced, which later penetrates into the blood. Electroneutrality law is implemented at the level of proximal tubules in the following way: total positive charge of sodium nephron reabsorbed in the proximal tubule is equivalent to the total negative charge of the Cl^- anions and hydrocarbonate reabsorbed here.

Bicarbonate ion regeneration occurs at the level of the distal tubule and is associated with the process of hydrogen ions secretion into urine. Carbon dioxide diffuses in the cells of the distal tubules; carbonic acid is generated from this carbon dioxide and carbonic anhydrase. Carbonic acid dissociates producing hydrocarbonate ion, which gets into blood stream, and hydrogen ion, which is secreted into the urine. Passage of all hydrogen ions in the tubular lumen is associated with the formation and transition in the blood of one hydrocarbonate ion. This process depends on the activity of carbonic anhydrase (Fig. 1).

Hydrogen ions are excreted with the urine primarily in the form of ***diphosphate anions*** (formation of titratable acidity) and ***ammonium*** ions (amoniogenesis).

Emitted in the tubular lumen hydrogen ion binds to HPO_4^{2-} forming diphosphate anion, which is excreted in the urine. The number of H^+ reacting with mono-phosphate anion corresponds to the number of equivalents of sodium hydroxide to be added to the urine in order to bring its pH to pH of primary filtrate (pH 7.4). This indicator is called ***titratable acidity***:

$$\mathbf{H^+ + HPO_4^- => H_2PO_4}$$

Ammoniogenesis: glutamine, glutaminase and glutamate dehydrogenase in the distal tubules converse into α-ketoglutarate and ammonia. The latter diffuses into the tubular lumen, which ammonium ionis produced:

$$\mathbf{H^+ + NH_3 => NH_4^+}$$

At the level of the distal tubule total positive charge of reabsorbed sodium is equivalent to the total negative charge of regenerated hydrocarbonate and positive charge of potassium and hydrogen, secreted into the urine.

As a result, the kidney releases hydrogen ions in three forms:

• free hydrogen ion (pH_{urine});

• buffered form as titratable acidity ($H_2PO_4^-$);

• ammonium ion (NH_4^+).

Urine normally is slightly acid, with pH around 6.2 (although it can range from 4.5 to 7.5). The total daily excretion of H^+ is ***40-80 mmol***, titratable acidity is ***10-30 mmol***, ammonium excretion is ***30-50 mmol***.

Urine excretion of titratable acid and ammonium ion in people with normal renal function is closely associated with the acid-base balance of the body: intracellular acidosis stimulates H^+ production; increases the activity of enzymes (glutaminase) which are involved in ammonia formation; stimulates reabsorption of bicarbonate and increases urine excretion of phosphate. When there are disorders in H^+secretion, especially in distal tubular acidosis, kidney ability to urine acidification and excretion of titratable acidity and ammonium ion is limited. The level of titratable acidity values correspond to the number of sodium hydrocarbonate, which is necessary to make urine pH and blood pH equel. This value will be reduced by the amount of bicarbonate ion contained in the urine.

Disorders of acid base balance in kidney pathology

Disorders of acid-base balance of the body, which develop due to pathological processes in kidneys, are called renal acidosis. This is pathological conditions caused by insufficient hydrogen ion excretion by the kidneys. They may be caused by reduction in the total number of functioning nephrons (***uremic acidosis***) or by disorders in hydrogen ion excretion by renal tubules with normal amount of kidney nephrons (***renal tubular acidosis***).

Depending on activity of its development can be of two types:

• renal nitric acidosis caused by chronic renal failure;

• renal nitric acidosis caused by acute renal failure.

Nitric acidosis is one of the syndromes of chronic renal insufficiency and it proceeds with increased anion gap. Anion gap in

patients with uremia is usually <25 mEq/L. Increase in this rate indicates concomitant pathology. In this case the main reason for acidosis is reduction of normally functioning kidney nephrons. This leads to the decrease of hydrogen ions eliminated with urine, mainly by lesser intensity of ammoniagenesis in distal tubules. Decrease in titratable urine acidity occurs at lesser extent, because the body tries to compensate the volume of eliminated hydrogen ions, thus increases the excretion of phosphates with urine, which is provided by an increase in parathyroid hormone secretion. Chronic renal insufficiency is usually accompanied by secondary hyperparathyroidism. Parathyroid hormone activates buffering function of bone tissue, by reducing bicarbonate reabsorption in proximal tubules, it causes an increase in urine pH in the distal tubule, which in turn inhibits ammoniagenes and aggravates acidosis. Disorders of endocrine function of kidneys is followed by decreased intensity of formation of 1,25-hydroxycholecalciferol, which, alongwith hyperphosphatemia leads to hypocalcemia. To measure adaptive mechanisms of kidneys method of calculation of titratable acidity and ammonia per 1 mL of glomerular filtrate. The results show, that in the early stages of disease intact nephrons significantly intensify formation of ammonia and titratable acidity, thus compensating this function of the dead ones. However, with progression of disease compensatory mechanisms get exhausted and buffer effect of bone tissue is stimulated. Due to rather large buffer capacity of bones main parameters of acid-base balance of the body remain stable in nitrogen acidosis for a long time, despite positive balance of hydrogen ions.

Tubular acidosis is a medical condition when alongwith normal GFR failure of the kidney to secrete hydrogen ions and/or reabsorb bicarbonate is observed. These disorders may be classified into two main types:

1. hypokalemic tubular acidosis:
• distal tubular acidosis;
• proximal tubular acidosis.
2. hyperkalemic tubular acidosis:
• tubular acidosis sensitive to mineralocorticoids;
• tubular acidosis insensitive to mineralocorticoids.

According to their etiology these disorders can be **primary** (genetically determined) and **secondary**.

Primary distal tubular acidosis is characterized by failure of normal hydrogen ion concentration gradient between the blood and tubular fluid. This pathology is determined by genetic defect in tubules cell membrane. The disease is inherited in an autosomal dominant pattern.

Pathological processes observed in primary and secondary distal tubular acidosis are similar. Disorders in hydrogen ions excretion in the distal tubules causes inhibited production of hydrogen in this part of nephron. Therefore, with normal bicarbonate reabsorption reduction in HCO3-concentration is observed in the proximal tubule which leads to excessive retention of chloride ions in the body and to hyperchloremia. In addition, reabsorption of sodium ions and excretion of potassium and hydrogen ions occurs in the distal tubule. Charge of reabsorbed sodium is compensated primarily by increasing potassium excretion, which results in hypokalemia and acidosis. With the disease progression the following diseases develop: hyperphosphaturia and hypophosphatemia, as well as hypercalciuria and later in nephrocalcinosis.

The main and probably the only reason of ***primary proximal tubular acidosis*** is disorder in hydrocarbonate reabsorption in the proximal part of nephron. This type of disorders is observed primarily in male newborns and it accompanied by developmental disorders and acidosis.

For a long time it was generally considered that normal hydrocarbonate reabsorption in proximal tubules of nephron is fully related to the activity of carbonic anhydrase. Recent studies, however, demonstrated involvement of active ATP-dependent transport of hydrogen carbonate from primary renal filtrate into blood.

Disorders in hydrocarbonate reabsorption increase its urine excretion and increased chloride reabsorption.

Main diagnostic method, allowing to differentiate distal tubular acidosis from proximal one, is to determine the fractional excretion of bicarbonate. Normally and in distal tubular acidosis fractional bicarbonate excretion is not more than 5%, and in proximal tubular acidosis, this value exceeds 15%.

A number of functional loading tests are performed for assessment of renal tubular function:

• ***Ammonium chloride acidification test***: a patient drinks solution of ammonium chloride (0.1 g/kg of body weight). Then urine is collected in 4 and 8 hours. In these samples urine is analyzed for pH, titratable acid, concentration of ammonium ions. Normally, after ammonium chloride loading urinary pH drops below 5.5; excretion of hydrogen ions in the form of titratable acidity, and ammonium ions increases twice or thrice. In distal tubular acidosis pH is > 5.5, and the excretion of hydrogen ions does not change significantly.

• ***Sodium hydrocarbonate loading test***: a patient is administered intravenously solution of sodium hydrocarbonate until level of HCO_3 reaches 24 mmol/L. Blood is analyzed for creatinine and hydrocarbonate concentration. Then urine sample (collected in two hours) is also analyzed for creatinine and hydrocarbonate concentration. Then fractional hydrocarbonate excretion (***Fe нсо$_3$-***) is calculated with the following formula:

$$\mathbf{Fe\ нсо_3^- = (нсо_3^-{}_{urine}] : [нсо_3^-{}_{plasma}]) \times ([Creatinine\ _{plasma}] : [Creatinine\ _{urine}]) \times 100\ \%}$$

In a healthy person Fe $нсо_3$- is less than 3%. When distal tubular acidosis develops fractional excretion of hydrocarbonate increases slightly and equals to 3-5%. Significant increase in Fe $нсо_3$- (up to 15% or more) can be observed in proximal tubular acidosis.

Evaluation of the activity of urinary enzyme ***N-acetyl-beta-D-glucosaminidase (β -NAG)*** is performed for assessment of structural integrity of renal tubules. The enzyme appears in urine in acute tubular necrosis.

Normal - <5 U/L.

Laboratory diagnosis of acute kidney injury

Acute kidney injury (AKI), or acute renal failure is a sudden, potentially reversible failure of kidney function accompanied by hyperazotemia, in some patients with oliguria (with urine output per hour less than 20 mL) and hyperkalemia (plasma potassium level above 5,2 mmol/L).

ARF is diagnosed in 5% of patients appealing for therapeutic or surgical aid and in 30% of patients of intensive care departments.

According to the cause of the development AKI may be classified into 3 categories, as follows:

- Prerenal (70% of AKI)
- Renal (25%)
- Postrenal (5%)

1. About half of the patients have AKI of combined type. AKI associated with CKI is diagnosed in 13%.

Prerenal AKI is a physiological response to reduced renal perfusion characterized by decrease in glomerular filtration, hyperazotemia, oliguria.

The main causes of AKI are:

- hypovolemia (dehydration, bleeding, excessive diuresis stimulation, vomiting, diarrhea, redistribution into the third space)
- low cardiac output (congestive heart failure)
- systemic vasodilation (sepsis, neurogenic shock)
- drug intake (NSAID in patients with reduced blood volume, angiotensin-converting enzyme inhibitors in renal artery stenosis or small blood volume, other antihypertensive drugs, cyclosporine, amphotericin B)

Thus, compensatory dilation (myogenic by prostaglandins and nitric oxide) of preglomerular vessels and constriction (influenced by angiotensin II) of post-glomerular arterioles does not occur.

Table 8. Diagnostics of prerenal AKI

Clinical picture	**Urinalysis**
Symptoms of AKI: (thirst, orthostatic or constant arterial hypotension and tachycardia, collapse of cervical veins, fluid intake increases dieresis), intake of NSAID and ACE inhibitors	• hyaline casts • $Fe_{Na} < 1\%$ • Na < 20 mmol/L • Specific gravity> 1018

In some cases diagnostic value of determination of fractional excretion decreases.

False increase in **Fe_{Na}** ***of more than by 1%:***

- Prerenal AKI accompanied by CRI;
- When diuretics are taken;
- acute increase in blood volume

False decrease in **Fe_{Na}** ***of less than by 1%:***

- acute glomerulonephritis and oliguria;
- vasculitis retaining tubular function;
- in some cases of acute tubular interstitial nephritis;
- AKI following the exposure of radiocontrast agents, heme pigments;
- First 48 hours after conversion of prerenal AKI into acute tubular necrosis

Diagnostics of renal AKI

Renal AKI follows parenchymatous kidney disease (acute glomerulonephritis, acute tubular interstitial nephritis) and/or tubular necrosis (ischemic, nephrotoxic).

Main causes of ***ischemic tubular necrosis*** are:

- Prolonged kidney hypoperfusion (outcome of prerenal AKI);
- Reperfusion injury (oxygen radical formation, cell membrane ruptures, inflow of calcium and other cations in cell, decrease in content of macroergs, phosphates, mitochondrial dysfunction).

Nephrotoxic tubular necrosis develops:

- Affected by ectotoxins (X-ray contrast agents, aminoglycosides, antitumoral drugs, salts of fluorine-containing anesthetics, heavy metal salts, organic solvents);
- Affected by endotoxins (myoglobin in rhabdomyolysis, hemoglobin, tumor lysis syndrome, myeloma, hypercalcemia).

Table 9. Differential diagnostics of prerenal and renal AKI

Indicators	Prerenal AKI	Renal AKI
Specific gravity	1,018 un. and >	Approximately 1010 un.
Urine sodium	< 20 mmol/L	>40 mmol/L
Fractional sodium excretion	<1 %	>1,5 %
Urinary osmolality	> 500 mOsm/kg of water	< 300 mOsm /kg of water
Urinary sediment	Normal, or with hyaline casts	Cells of renal tubules and «muddy brown» granular casts

Mortality in acute tubular necrosis is 50 - 70%. The main reasons:

- infections (30-70%);
- cardiovascular complications (5-30%);
- gastrointestinal, pulmonary, neurological complications (7-30%);
- hyperkalemia or technical dialysis problems (1-2%).

Postrenal (obturation) AKI

Development of postrenal AKI:

- obstruction of urethra or ureter is followed by more than 40% increase of renal blood flow, then blood flow decreases after vasoconstriction;
- intratubular pressure increases more proximal to the obstruction which causes decrease in filtration pressure and glomerular filtration rate place

Table 10. Diagnostics of postrenal AKI

Causes of AKI	**Clinical data**	**Urinalysis**
• urolithiasis; • tumours of ureter, small pellvis; • fibrosis of retroperitoneal space (Ormond's disease)	Medical history may include: renal colic attacks, evidence of tumour, alternation of anuria and polyuria. Pain in stomach, loin, specific symptomatology of organs of small pelvis (incl. palpable bladder)	Is often normal; hematuria in urolithiasis, bleeding, cancer and prostate adenoma

CLINICAL HEMATOLOGY

Investigation of various changes in the blood system has important clinical and diagnostic value. In the medical practice the most common of all hematological tests is complete blood cell count, which includes: detection of hemoglobin concentration, red blood cell count, color index detection, white blood cell and leukogram count, erythrocyte sedimentation rate. This test is performed in most cases of primary examination of a patient under outpatient conditions and in many types of medical examination, as well as in all hospital patients. In hematologic pathology the in-depth study of peripheral blood and bone marrow cells has primary diagnostic importance.

Pre-analytical and main principles of analytical stage in hematological tests

The results of hematologic tests can be influenced by individual peculiarities and physiological state of a patient. Changes in cellular composition of peripheral blood are observed not only in various diseases; they depend on age, sex, diet, smoking and alcohol intake, menstrual cycle, pregnancy, physical activity, emotional state, circadian and seasonal rhythms; climatic and meteorological conditions; patient's position during blood collection, pharmacological preparations intake and others. In analysis of hematological changes it is necessary to consider that various treatment and diagnostic activities have a significant impact on the correctness of the tests. These activities include pharmacotherapy, surgeries, physiotherapy, radiation therapy, diagnostic procedures. Usually the blood for hematologic tests is collected in the morning between 7 and 10 hours on an empty stomach (8-hour fasting). A time interval between blood collection and analysis itself should be minimal. (Table 11)

Table 11. Influence of a long-term storage and transportation of blood samples on some parameters of hematologic analysis

Character of the canges	Effect
Swelling of erythrocytes	MCV increase; Change of erythrocytes shape (aculeiform and spherical); ESR decrease
Vacuolization and loss of normal shape of leukocyte nucleus	Difficulties in cellular differentiation in leukogram count
Swelling and aggregation of platelets	Falsely elevated platelet count

Modern methods of blood cell analysis

To count and analyze blood cells manual microscopic methods are used as well as hematologic calculators with different levels of automatization. Over the last 15-20 years the development of technology and equipment for automatic cell analysis according to flow cytometry principle has significantly improved. In some countries of the world an automated blood test system almost completely has replaced manual and semi-automated methods.

At present hematologic autoanalyzer is an integral part of modern clinical diagnostic laboratory. Numerous electronic calculators are able to detect not only the number of cells but some other hematological parameters. Electronic calculators can quickly analyze a great number of blood samples; moreover they minimize technical errors common for manual calculation as a device calculates a much greater number of cells.

Hematological parameters

Hemoglobin

Hemoglobin (Hb) is one of the main parameters, used for assessment of erythropoiesis. Hemoglobin level in the blood reflects its oxygen capacity. In a healthy person hemoglobin is represented by several types (hemoglobin A_1, A_2, A_3 and F), different in

physicochemical properties. Hemoglobin A_1 comprises 96–99% of total hemoglobin level. In some congenital pathologies (haemoglobinopathies) a person can have other (pathological) hemoglobin types (more than 200).

Reference ranges:

140-180 g/l for men;

120-160 g/L for women.

The decrease of hemoglobin level is characteristic of anemias of different etiology. The increase of hemoglobin concentration is observed in erythremia (polycythemia) and symptomatic reactive polycythemia. It should be borne in mind that in blood thickening (dehydration in intractable vomiting, polyuria, diarrhea, etc.) a relative increase of hemoglobin concentration may occur.

Hematocrit

Hematokrit (Ht) is a parameter that shows the ratio of blood cells to the total blood volume, expressed in percentage or as an index.

Reference ranges:

for men 40-54 %;

for women 36-47 %.

Hematocrit parameter is widely used in detection of anemia stage, in which this parameter is decreased, sometimes even to 20-25%. Hematocrit increase (55-65% and higher) is characteristic of erythremia, a less dramatic increase (50-55%) is observed in symptomatic polycythemia associated with congenital heart disease, pulmonary insufficiency, some hemoglobinopathies.

Erythrocytes

Erythrocytes (RBC) make up the bulk of blood cells. Mature human red blood cells lack nuclei and have a biconcave form, which increases the contact area of hemoglobin and plasma and facilitates the transfer of oxygen and carbon dioxide.

Reference ranges:

for men 4,5-6,0 x 10^{12}/L;

for women 4,0-5,5 x 10^{12}/L.

Changes in RBC count

Reduction of RBC count is one of the major characteristic of anemia (see below). Erythropenia, besides anemia, is common in the increase of circulating blood volume (pregnancy, hyperhydration, hyperproteinemia).

Increase of RBC count i.e. polycythemia can be primary (erythremia) or secondary. **Erythremia** (polycythemia, Vaquez's disease), absolute polycythemia is the disease, based on the myeloproliferative process in the bone marrow. The disease belongs to hemoblastoses and is usually accompanied by proliferation of other haematopoietic lineages. In these cases evident Polycythemia is usually combined with leukocytosis and thrombocytosis.

Secondary symptomatic reactive polycythemia can be absolute and relative (Table 12).

Table 12. Secondary polycythemia

polycythemia	**Pathogenetic mechanisms**	**Clinical situationa**
Absolute	Due to hypoxia	Chronic pulmonary diseases, postinfarction period, congenital cyanotic (blue) heart defects, increased physical activity, stay at a high altitude or in the areas with low partial oxygen pressure, pickwickian syndrome.
	Due to erythropoietin hyperproduction	Kidney cancer, hydronephrosis and cystic kidney disease, benign familial polycythemia.
	Due to excess of hormones (adreno-corticosteroids or androgenic hormones)	Cushing's syndrome, pheochromocytoma, hyperaldosteronism.
	Increased leucemic production of erythro-cytoses	Erythremia
Relative	Due to hemoconcentration	Dehydration, stress.
	Active physiological erythrogenesis	Physiological polycythemia of newborn babies.

Erythrocytic index

In laboratory and clinical practice various indices are used. They can quantitatively characterize the average volume of red blood cells, the degree of their saturation by hemoglobin, anisocytosis.

Mean corpuscular volume (MCV)

MCV is expressed in cubic microns (mcm^3), or in femtoliters (fL).

MCV reference ranges are: for men - 80 - 94 fL, for women - 81 – 99 fL.

1. Normocytic (MCV 80 - 100 fL);
2. Microcytic (MCV less than 80 fL)
3. macroocytic (MCV more than 100 fL).

MCV is an important parameter in anemia differential diagnosis (tables 1.4. and 1.5.).

Mean corpuscular hemoglobin (MCH)

MCH index characterizes average hemoglobin concentration per red blood cell in absolute units. Detection of this index is based on determination of total hemoglobin and correlation between this value and RBC level. The result is expressed in picograms (pg), reference ranges are 27 - 31 pg.

Color index reference ranges are 0,86-1,05.

According to MCH changes there is the following anemia classification:

- Normochromic (MCH - 27 - 31 pg);
- Hypochromic (MCH less than 27 pg);
- Hyperchromic (MCH more that 31 pg).

Mean corpuscular hemoglobin concentration (MCHC)

Differences between two latter indices lie in the fact that MCH indicates hemoglobin mass in one erythrocyte and is expressed in pictograms. MCHC indicates hemoglobin concentration in one erythrocyte, i.e. correlation between hemoglobin content and a cell volume. It reflects saturation of erythrocyte with hemoglobin. Normally MCHC makes up 32- 38 g/dl or %. MCHC does not depend on cellular volume (as opposed to MCH) and is a sensitive index of disturbance in hemoglobin synthesis.

Red cell distribution width, anisocytosis (RDW)

RDW is calculated as a coefficient of variation of MCV (norm is 11.5 – 14.5%).

Anisocytosis is an increase of RDW> 14,5%.

Pathology of RBC morphology

In addition to hemoglobin concentration indices and total RBCs number the important information on erythrogenesis can be obtained by RBC morphology test. It is performed by a light microscope in the stained blood smears.

Change of RBC size

Macrocytosis is a presence of RBC more than 9,0 mcm in diameter in the blood smears.

Macrocytosis is detected in so called macrocytic anemia, anemia in pregnancy, vitamin B_{12} and folic acid deficiency. In these cases macrocytosis is accompanied by the decrease of RBC and hemoglobin level. It is necessary to remember that regardless presence or absence of anemia symptoms macrocytisis can occur in many liver diseases, alcoholism, malignant neoplasms, decreased thyroid function, myeloproliferative disorders and after splenectomy, etc.

Microcytes is a prevalence of small red blood cells (5.0-6.5 mm) in blood smears. This symptom is observed in hereditary spherocytosis, iron-deficiency anemia, thalassemia and other diseases.

Megalocyte. Hyperchromic oval red blood cells 11.0-12.0 mcm in diameter. Megalocytosis is characterized not only by significant red blood cells increase, but by increased hemoglobin concentration in the cells too. Megalocytosis is observed in vitamin B_{12} and folic acid deficiency anemia, anemia in pregnancy, helminthic invasion, etc.

Anisocytosis. Red blood cells of different diameter found in the peripheral blood. Usually it indicates the presence of pathologically changed and normal RBC pool in an organism. Anisocytosis is observed in iron deficiency anemia, hypoplastic anemia, paroxysmal nocturnal hemoglobinuria, myeloproliferative diseases, thalassemia. Microcytes, normocytes and macrocytes are present.

Shape changes in RBC (**poikilocytosis**)

Shape changes of RBC of various severity may occur in any anemia. Normally some red blood cells can have a shape different from a flattened disc shape. (Table 13).

Table 13. RBC poikilocytosis

Variant of a shape change in RBC	**Clinical situation**
Microspherocyte	Hereditary microspherocytosis, immune hemolytic anemia, G-6-PD-enzymopathy of RBC, microangiopathic hemolytic anemia.
Target cell	Thalassemia, hemoglobinopathy, liver diseases, iron deficiency, post-splenectomy condition.
Elliptocyte	Hereditary elliptocytosis, megaloblastic anemia, iron deficiency anemia.
Stomatocyte	Hereditary stomatocytosis, hemolytic anemia, other anemias.
Sickle cell	sickle-cell anemia.
Spur cell	Artefact, uremia.
Acanthocyte	Severe liver diseases.
Schizocyte	Disseminated intravascular coagulation (DIC), uremia, mechanical damage to red blood cells, hemolytic-uremic syndrome, drugs, toxines.
Teardrop cell	Extramedullary erythropoiesis (myeloproliferative disorders, myelofibrosis), thalassemia, severe iron deficiency, megaloblastic anemia.
Burr cell	Children (healthy), liver diseases, uremia, DIC, carcinomatosis.

Color changes in RBC

Hypochromia is a decrease of RBC color intensity. Hypochromia occurs due to low saturation of red blood cells by hemoglobin and is characteristic of many iron-deficiency anemias, thalassemia, lead poisoning and some hereditary hemolytic anemias. **Hyperchromia** of RBC occurs due to increased saturation of red

blood cells by hemoglobin. It is characteristic of conditions and diseases associated with vitamin B_{12} and folic acid deficiency (Addison–Biermer anemia, diphyllobothriasis, chronic stomach and bowel diseases, alcoholism, pregnancy).

Red cell inclusions

Howell-Jolly bodies are common in anemias caused by vitamin B_{12} and folic acid deficiency anemia as well as after splenectomy.

Cabot rings are common in severe vitamin B_{12} and folic acid deficiency, in polycythemia, and in heavy metal salts poisoning.

Heinz-Ehrlich bodies indicate hemoglobin destruction, leading to RBC membrane damage, accompanied by RBC increased hemolysis in the spleen. Significant number of Heinz-Ehrlich bodies in RBC occurs in hemolytic crises in patients with hereditary glucose-6-phosphate dehydrogenase deficiency.

Basophilic stippling of RBC occurs in lead or heavy metals intoxication, thalassemia, alcohol intoxication, cytoxic action of drugs, severe anemias.

Siderotic (ferric) granules. Their increase is detected in hemolytic anemia, sideroblastic anemia, after splenectomy, lead poisoning, more seldom, in pernicious anemia and thalassemia. Decrease of siderocytes and sideroblasts is observed in iron deficiency anemia.

Reticulocytes

Reticulocytes are immature red blood cells, formed after loss of nuclei by normoblasts.

Normally there is 0,2 – 1% of reticulocytes (2-10‰) in the peripheral blood.

To count reticulocytes hematology analyzers are used. They allow to detect relative and absolute reticulocytes count as well as reticulocyte maturation index and volume indices.

Volume indces

MCVr (Mean Cell Volume Reticulocytes) – mean reticulocyte volume (fL);

MSRV (Mean Sphered Reticulocyte Volume) – mean volume of spherical reticulocytes (fl)

Reticulocyte count investigation is used to:

– detect erythropoiesis activity in the conditions associated with hemolysis or blood loss;

– detect disorders of bone marrow regeneration capacity in iron deficiency, vitamin B_{12} , B6, folates, copper deficiency; to monitor corresponding therapy;

– evaluate erythropoiesis during treatment with erythropoietin;

– evaluate bone marrow regeneration capacity after cytotoxic therapy and bone marrow transplantation;

– evaluate restoration of erythropoietin synthesis after kidney transplantation;

– control doping in athletes (erythropoietin intake).

Platelets

Platelets (**PLT, thrombocytes**) are blood corpuscles, participating in hemostasis. Platelets are small oval or round cells without nuclei. Their diameter is 2-4 mcm

Reference ranges for manual method: 180 – 320 x 10^9/L.

Reference ranges for automatic method depend on the analyzer type.

Hemostatic minimum of platelets in the blood is 50 x 10^9/L.

Platelet indices

Mean platelet volume (MPV) is expressed in femtoliters (fL) or in cubic microns (mcm^3).

Normally this index varies from 7,4 to 10,4 fL. Platelet distribution width (PDW) is expressed in per cents (coefficient of thrombocytometric curve variation) and quantitively reflects size heterogeneity of these cells population (platelets anisocytosis stage). Normally this index is 10—20%. Platelet crit (PCT) is a parameter reflecting, proportion of the volume of whole blood occupied by platelets. Normally platelet crit makes 0,15-0,40%.

Changes in platelet count

Thrombocytosis is the increase of platelet counts to more than 320 x 10^9/л. There are reactive and clonal thrombocytoses. (Table 14).

Table 14. Thrombocytosis

Thrombocytosis	Diseases and syndromes
Reactive	Splenectomy, acute blood loss and acute hemolysis, post-operative period, malignant neoplasms, atrophic arthritis, ulcerative colitis, osteomyelitis and others.
Clonal	Myeloproliferative diseases (chronic myeloleukemia, myelofibrosis, erythremia, megakaryocytic acute and chronic leukosis, idiopathic hemorrhagic thrombocythemia).

Thrombocytopenia is a disease or syndrome characterized by a decreased platelets count. Thrombocytopenia is a result of insufficient formation, increased destruction of platelets. Thrombocytopenias can be inherited or acquired.

Acquired thrombocytopenia is observed in hypersplenism, infectious diseases, chronic intoxication of any genesis, hyper- and metaplastic bone marrow lesions, radiation and cytotoxic therapy. Acquired thrombocytopenia may be accompanied by hemorrhagic syndrome. Immune and autoimmune forms (forms, in which platelets are destroyed by antibodies) are most common among acquired thrombocytopenias.

The most famous and widespread disease of this group is **idiopathic thrombocytopenic purpura.** In autoimmune thrombocytopenia antibodies are produced against unchanged thrombocytic antigens, i. e. the reason of their production is not the change of platelet antigenic structure, but violation of patient's immune system tolerance to his/her own antigens. Most often autoantibodies are directed against the main and most immunogenic proteins of platelets (membrane glycoprotein IIb-IIIa and Ib complex). The incidence of idiopathic thrombocytopenic purpura is 1/10000, women are affected twice more often than men (in childhood incidence is the same in boys and girls). Autoimmune thrombocytopenia is observed in other pathologies such as systemic lupus erythematosus and lymphoproliferative diseases.

Thrombocytopathy is a large group of syndromes and diseases, based on hemostatic disorders due to qualitative platelet deficiency or dysfunction. As a matter of fact this is a group of hemorrhagic diathesis with hemorrhagic manifestations at the microcirculatory level.

Thrombocytopathy pathogenesis is not sufficiently studied. Shortening of platelets life span is explained by defects in the structure of their membranes or cellular energetic, caused by enzymes deficiency.

Differential diagnosis of these forms is very complicated as the main or even the only evidence of hereditary pathology is family anamnesis; the index of autoimmune genesis is the presence of antithrombotic antibodies.

Thrombocytopathies can be hereditary and acquired.

Acquired thrombocytopathies develop in various pathogenic influences. They are common in many syndromes and diseases.

Acquired thrombocytopathies mostly develop due to:

– disturbance of platelet adhesive-aggregation function (in uremia, liver cirrhosis, tumors and parasitic diseases);

– consumption and structural disorders of platelets in the diseases associated with DIC;

– blockade of platelets by proteins (paraproteinemic hemoblastosis), ADP aggregation disorders (scurvy, B_{12}-deficiency anemia).

Leukocytes

Leukocytes (WBC, white blood cell) are blood cells produced in the bone marrow and lymph nodes. The main function of white blood cells is to defend the body against foreign agents.

Leukocyte count is performed visually or with the help of hematologic analyzers. **Reference ranges are** 4,0-9,0x10^9/L.

Leukocytosis is the increase of white blood cell count to more than 9,0x10^9/L. Leukocytosis can be neutrophilic, eosinophilic and monocytic. Rarer it occurs due to increase of other cells count.

Leukocytosis can occur in healthy people, for example:

– after meals reach in proteins;

– after significant physical activity;

– in case of evident psychoemotional tension; stress; after overheating or cooling.

In most cases leukocytosis usually reflects satisfactory reactivity of bone-marrow blood formation in response to internal and external leucopoiesis stimulants, though vascular reactions should be considered as well as blood flow redistribution, endothelium permeability changes, haematopoietic lineages proliferation in leucosis.

Most evident leukocytosis occurs in chronic and acute leucosis, purulent diseases of internal organs (abscess, gangrene, etc.). Leukocytosis is not characteristic of typhoid fever, paratyphoid fever, some stages of epidemic typhus and many viral infections (influenza, measles, parotitis, viral hepatitis and so on) in which the increase of leukocyte count in the peripheral blood indicates the development of bacterial and other complications. Respiratory tract diseases, smallpox and some other diseases are exceptions.

Leukopenia is the reduction of leukocyte count below $4{,}0x10^9$/l. Leukopenia occurs due to inhibition of leukopoiesis in the blood-forming organs and is observed in many pathological conditions:

1. Viral infections (influenza, measles, rubella, viral hepatitis, AIDS, etc.).
2. Some bacterial (typhoid fever, paratyphoid fever, brucellosis, etc.), rickettsial (epidemic typhus, rickettsiosis, etc.) and protozoan infections (malaria, etc.).
3. All kinds of generalized infection (septicemia, miliary tuberculosis etc.).
4. Hypoplasia and aplasia of the bone marrow (for example, in aplastic and hypoplastic anemias, due to action of ionizing radiation, etc.).
5. Side effect of cytoplastic drugs, antibiotics, sulfanilamides, nonsteroidal anti-inflammatory drugs, thyreostatics and some other drugs.
6. Agranulocytosis, accompanied by evident reduction or disappearance of granulocytes (neutrophils) from the peripheral blood, and in some other conditions.

Leukogram

Leukogram is the percentage of different types of white blood cells in a blood smear.

Changes in leukogram are characteristic of various diseases and are often non-specific ones. (Table 15).

Table 15. Diseases and conditions, accompanied by leukogram shifts.

Regenerative left shifts (metamyelocytes and myelocytes in the blood)	Regenerative left shifts (metamyelocytes, myelocytes, promyelocytes, myeloblasts, erythroblasts in the blood)	Right shift (reduction of stab neutrophils with hypersegmented neutrophil nuclei)
Acute inflammatory processes; purulent infections; intoxications; acute hemorrhages; acidosis and comatose states; physical overstrain.	Chronic leukoses; erythroleukemia; myelofibrosis; methastases of neoplasms; acute leukoses; comatose states.	megaloblastic anemia; renal and liver diseases; states after blood transfusion.

Leukemoid reactions

Sometimes a leukocyte responce can be acute and is accompanied by appearance of young blood cells up to myeloblasts. In such cases we speak about leukemoid reaction. **Leukemoid reactions** are reactive blood changes resembling leukoses according to the stage of leukocyte count increase or to cellular morphology.

Changes in leukocyte morphology

Pelger-Huet anomaly

Among all the hereditary pathologies of Leukocytic morphology Pelger-Huet nucleous anomaly is the most important. Pelger-Huet anomaly is a genetic blood disorder with an autosomal dominant inheritance pattern. Pequliarity of Pelger-Huet leukocytes development is expressed in morphologic changes of neutrophil

nuclei, i.e. their segmentation disturbance (a nucleus is old, but its form is young). The majority of Pelger-Huet neutrophils has unilobar, unsegmented nucleus.

To specify the Pelger-Huet anomaly diagnosis it is advisable to examine the blood of a patient's parents.

Neutrophils

Normally there are segmented neutrophils and some stab neutrophils (1-4%). The main function of neutrophils is to defend the organism against infections, with the help of fagocytosis. Duration of half-life of neutrophil granulocytes circulation is 6,5 hours, then these granulocytes migrate to the tissues. Life cycle of granulocytes in tissues depends on many reasons and may vary from some minutes to some days.

Neutrocytosis (neutrophilia) is the increase of neutrophil count to more than 8,0 x 10^9/L (Table 16).

Neutropenia is a decrease in circulating neutrophils to the level of 1,5 x 10^9/L.

Table 16. Conditions and diseases, accompanied by changes of neutrophil count

Neutrocytosis	**Neutropenia**
Acute bacterial infections: - localyzed (abscesses, osteomyelitis, acute appendicitis, acute otitis, pneumonia, acute pyelonephritis, salpingitis, purulent and tuberculous meningitis, sore throat, acute cholecystitis, thrombophlebitis, etc.); - generalyzed (sepsis, peritonitis, pleural empyema, scarlet fever, cholera, etc.); *Tissue inflammation or necrosis:* - myocardial infarction, extensive burns, gangrene, rapidly	*Bacterial infections* (typhus, paratyphoid fever, tularemia, brucellosis, subacute bacterial endocarditis, miliary tuberculosis); *Viral infections* (infectious hepatitis, influenza, measles, rubella); *Myelotoxic influences and u granulocytopoiesis suppression*: - ionizing radiation; - chemical agents (benzol, aniline, etc.); - anticancer drug (cytostatic drugs and immunosuppressive agent);

developing malignant tumors with decay, periarteritis nodosa, acute rheumatism. *Exogenous intoxication:* - lead, snake venom, vaccines (foreign protein, bacterial); *Endogenous intoxications:* - uremia, diabetic acidosis, gout, eclampsia, Cushing's syndrome; *Medicinal effects;* *Myeloproliferative diseases* (chronic myeloleukemia, erythremia); Acute haemorrhages.	- Vitamin B12 and folic acid deficiency; - acute leukosis, aplastic anemia *Immune agranulocytosis*: - haptenic (hypersensitivity to drugs); - autoimmune (systemic lupus erythematosus, atrophic arthritis, chronic lymphatic leukemia); - isoimmune (in new-born babies, posttransfusion); *Redistribution and sequestration in the organs:* - anaphylactic shock; - splenomegaly of various origin; *Hereditary forms* (cyclic neutropenia, familial benign chronic neutropenia, chronic neutropenia in children)

Agranulocytosis is an acute reduction of granulocyte count in the peripheral blood (lower than $0,5,0x10^9$/L) up to their disappearance. This state leads to decreased resistance to infections and to the development of bacterial complications. Depending on the mechanism of appearance there are immune and myelotoxic agranulocytosis. Myelotoxic agranulocytosis, which develops as a result of cytotoxic factors, is combined with leukopenia, thrombocytopenia, and often with anemia (i.e., pancytopenia). Immune agranulocytosis can be mainly of two types: haptenic and autoimmune, isoimmune.

Degenerative changes of neutrophils

Degenerative changes of neutrophils occur in various pathological conditions (infections, exposure to chemicals, diseases of hematopoietic system, the action of penetrating radiation, ingestion of

radioactive substances, etc.) and can affect both the nucleus and the cytoplasm.

Eosinophils

Eosinophils are the cells, phagocyting antigen-antibody complexes.

Eosinophilia is a condition in which the eosinophil count in the peripheral blood exceeds 5% (>0,4x10^9/L). Most often eosinophilia is associated with parasitic diseases and atopic allergy. Therefore, to determine the cause of eosinophilia it is particularly important to examine stool for detection of intestinal parasites, especially in endemic areas. The presence of an allergic disease is more reliable in detection of serum IgE than skin tests. In some conditions (fibroplastic parietal endocarditis, periarteritis nodosa, lymphogranulomatosis) hypereosinophilic leukemoid reactions with bone marrow eosinophilic hyperplasia and tissue eosinophilic infiltration can be observed.

Eosinopenia is the reduction of eosinophil count to 0,05 x 10^9/L. Usually it is associated with the increased adrenocortical activity which results in eosinophils retention in the bone marrow. Eosinopenia is particularly characteristic of the initial phase of the infectious toxic process. The absence of eosinophils in the blood occurs at the first stage of the inflammatory process, in severe suppurative infections, shock, stress, eclampsia, chemical poisoning, heavy metal intoxication, and during labor.

The reduction of eosinophil count in post-operative period indicates poor patient's condition.

Basophil

Basophils are blood cells, containing coarse purple-blue granules in the cytoplasm. **Basophilia** is the increase of basophil count in the blood (>0,2 x 10^9/L). The conditions and diseases in which basopilia can develop are:

- allergic reactions to food, drugs, injection of foreign protein; chronic myeloleukemia, myelofibrosis, erythremia;
- lymphogranulomatosis;
- chronic ulcerous colitis;
- hypothyroidism;
- estrogen therapy.

Basopenia is the reduction of basophil count in the blood (< 0,01 x 10^9/L). Basopenia is difficult to demonstrate as their normal concentration is low.

Lymphocytes

Lymphocytes, being the main cellular element of the immune system, are produced in the bone marrow and actively function in the lymphoid tissue. The main factors leading to changes in lymphocyte count are given in Table 17.

Table 17. Conditions and diseases associated with changes in lymphocyte count

Absolute lymphocytosis	Absolute lymphopenia
Viral infection Acute infectious lymphocytosis Pertussis Infectious mononucleosis Acute viral hepatitis Cytomegalovirus infection Lymphatic system diseases: – chronic lymphatic leukemia; – Waldenström's macroglobulinemia	Pancytopenia Corticosteroid intake Severe viral diseases Malignant neoplasms Secondary immunodeficiency Renal failure Circulatory deficiency

Monocytes

Monocytes are produced in the bone marrow from monoblasts and are related to the system of phagocytizing mononuclear cells.

Monocytosis is the increase of monocyte count in the blood (>0,8 x 10^9/L). It is associated with many diseases. In tuberculosis monocytosis is the proof of active spread of the tubercular process. In septic endocarditis, subacute sepsis significant monocytosis is possible, which usually occurs in the absence of leukocytosis.

Relative or absolute monocytosis is detected in 50% of patients with systemic vasculitis.

Monocytopenia is the reduction of monocyte count (< 0,09 x 10^9/l). In blood formation hypoplasia monocyte count in the blood is reduced.

Anemia

Anemia is a condition, characterized by the reduced hemoglobin concentration. It can occur due to pathologically decreased RBC count as well. As mature red blood cells are saturated with hemoglobin, their reduction indicates the reduction of hemoglobin. According to WHO (1998) anemia takes 90% among all the blood diseases.

Anemia classifications:

There are two main classifications of anemias: the first is based on kinetic analysis of red blood cells maturation and their destruction, the second is based on morphologic criteria. Both classifications can be recommended and it is preferable to use both of them in complicated cases.

Table 18. Kinetic approach

RBC production disorders
1. **Hypoproliferative** A)Fe-deficiency erythropoiesis ♦ Iron deficiency ♦ Anemia in chronic diseases B) Erythropoietin deficiency ♦ Kidney diseases ♦ Endocrine deficiency C) Hypoplastic anemias ♦ Aplastic anemia ♦ Partial erythroid aplasia D) Infiltration ♦ Leukosis ♦ Metastatic carcinoma ♦ Myelofibrosis
2. **Ineffetive erythropoiesis** A) Megaloblastic anemias ♦ Vitanin B12-deficiency ♦ Folate insufficiency ♦ Other reasons B) Thalassemia C) Some sideroblastic anemias

Increase of red blood cell production
A) Hemolityc anemia ♦ Congenital ♦ Acquired B) Acute posthemorrhagic anemia

Classification of anemia according to pathogenesis:

I. Anemia due to blood loss

A.) Acute posthemorrhagic anemia;

B). Chronic posthemorrhagic anemia;

II. Anemia due to RBC and hemoglobin formation disorders

A.). Iron deficiency anemia;

B). Anemia due to disorders of DNA and RNA synthesis (megaloblastic anemia):

- B_{12}- deficiency anemia
- Folate-deficiency anemia

C). Anemia due to porphyrin synthesis and utilization disorders (sideroachrestic anemias);

D). Anemia due to suppression of bone marrow cells proliferation;

- Aplastic anemia

E). Anemias in chronic diseases;

III. Anemia due to increased destruction of blood (hemolytic anemias)

1. Hereditary:

A.) Associated with destruction of RBC membrane structure;

- Microspherocytic anemia (Minkowski-Chauffard's disease),
- Congenital elliptocytosis (ovalocytosis);
- Abetalipoproteinemia (Bassen-Kornzweig syndrome);
- Hereditary stomatocytosis

B) Associated with deficiency of enzymes in RBC;

- glucose-6-phosphate dehydrogenase deficiency;

C) Associated with hemoglobin synthesis disorders (hemoglobinopathy);

- Sickle-cell anemia

- Thalassemia

2. Acquired

A) Immune;

1) Isoimmune;

- Hemolytic disease of the newborn

2) Autoimmune;

- Autoimmune hemolytic anemia due to partial warm agglutinin;
- Autoimmune hemolytic anemia due to complete cold agglutinin, (Cold agglutinin disease);
- Autoimmune hemolytic anemia due to warm hemolytic antibody paroxysmal cold hemoglobinuria with ;
- paroxysmal cold hemoglobinuria with biphasic hemolysin (Donath-Landsteiner anemia);

B) Hemolytic anemia, associated with drug intake;

C) Traumatic (microangiopathic) hemolytic anemias;

D) Pathologic interaction with activated complement;

- Paroxysmal nocturnal hemoglobinuria

E) Hemolytic anemia due to toxines;

F) Hemolytic anemia, associated with parasitic invasions;

G) Hypersplenism**;**

IV. Porphyria:

A) Erythropoietic;

B) Hepatic;

Below there is information on some types of anemia.

Acute posthemorrhagic anemia

Acute posthemorrhagic anemia is a state which develops as a result of acute massive blood loss. Regardless of the disease pathogenesis special physiological mechanisms are activated in acute posthemorrhagic anemia. These mechanisms are directed to restoration of circulating blood volume, which is reflected in laboratory blood parameters.

The changes in blood picture occur according to phases (in a certain sequence) due to various compensation mechanisms.

During the 1st phase (1 - 2 days) a spasm of the peripheral vessels occurs, bloodstream volume reduces and blood enters systemic circulation from the depot. Despite absolute reduction of RBC mass it leads to restoration of hemoglobin and RBC count and doesn't reflect a true degree of anemization. Though some increase in platelet and leukocyte count with a shift of leukogram to the left is detected.

During the 2nd phase hemodilution occurs. Tissue fluid gets into the blood-vascular system and as a result circulating plasm volume restores. Red blood parameters – hemoglobin and RBC – progressively and steadily reduce without colour index reduction. Exactly in this phase anemia develops. Initially it has normochromic normocytic character.

Three-five days after the blood loss **reticulocytosis (the 3rd phase)** develops. It is accompanied by acute increase of immature reticulocyte fraction (IRF), which, together with active erythrogenesis, reflects regenerative property of the bone marrow (becomes maximal by the 7th-10th day); leukocytosis can be at the level of 12—20 x 10^9mcL with a left nuclear shift.

The appearance of polychromatophilic macrocytes leads to MCV increase and anemia can become macrocytic and normochromic. In combined reticulocytosis and increased MCV hemolytic anemia can be incorrectly diagnosed.

After bleeding arrest normalization of number of reticulocytes is observed in 2 – 3 weeks. Persistent reticulocytosis may indicate continuous bleeding. Immediately after bleeding transient thrombocytopenia can develop, but a few hours later, thrombocytosis and leukocytosis occur.

Minimal blood loss dangerous for a person is 500 ml, rapid blood loss of ¼ of total blood volume leads to a shock, a loss of half of the blood volume is incompatible with life.

Iron deficiency anemia

Iron deficiency anemia is the most widespread one. Its share in all anemias is 80%, and its frequency in pregnant women ranges from 21% to 80%. Along with iron deficiency anemia hidden iron deficiency occurs, the prevalence of which in Europe is about 30% of the population. The main causes of iron deficiency anemia are insufficient iron in the diet, excessive iron loss, or an increased need

for iron. Iron deficiency anemia is most often diagnosed in women of childbearing age, pregnant women and children of various age groups.

Reasons of iron deficiency

Acute blood loss doesn't usually lead to anemia if bleeding is quickly arrested and iron reserves are sufficient. Anemia developing in acute bleeding is normocytic and normochromic. Chronic bleeding, however, can result in the depletion of iron reserves due to the activation of hemoglobin biosynthesis. In this case hypochromic, microcytic anemia is observed.

Iron deficiency can develop if the diet contains food poor in iron. This condition often occurs in infants and is associated with nutrition. It was detected that 15-20% of infants between 9 and 12 months have a relative iron deficiency and 50% of infants in population with a low living standard.

In adults deficiency has a subclinical course and does not always lead to the development of anemia. However, insufficient iron in the diet can increase the deficiency induced by other reasons. Five per cent of healthy women have chronic iron deficiency anemia. Iron deficiency often occurs in malabsorption, pregnancy, menorrhagia, in 40% of women involved in sports due to combined reasons: alimentary reasons, gastrointestinal bleeding, hematuria and hemolysis.

Depending on the bone marrow erythropoietic activity there are regenerative and hyporegeneratory stages, and in accordance with the laboratory parameters iron deficiency anemia has three degrees of severity:

- mild – hemoglobin count is more than 90 g/L;
- moderate - 70 - 90 g/L;
- severe – less than 70 g/L.

In clinical blood analysis in iron deficiency anemia reduction of hemoglobin and RBC levels are registered. Moderate erythropenia can occur if with Hb is <98 g /L, but the decrease in RBC count <2 x 1012 /L is not characteristic of iron deficiency anemia.

In iron deficiency anemia the changes in morphological characteristics of red blood cells and red blood cell indices are registered, which reflects quantitive morphological characteristics of red blood cells.

In chronic iron deficiency hypochromia is observed, as well as various degrees of microcytosis. Decrease in MCV, MCH and MCHC is characteristic. In severe anemia evident anisocytosis (RDW index is increased) and poikilocytosis can be observed. Microcytes in iron deficiency should be differentiated from spherocytes.

The number of reticulocytes is normal in uncomplicated forms of iron deficiency anemia. There is an increase in concomitant blood loss or nutritional iron deficiency.

The signs of chronic iron deficiency are given in Table 19.

Table 19. Detection of chronic iron deficiency

Early preclinical changes

1. Negative iron balance
2. Decrease of hemosiderin level in the bone marrow
3. Decrease of ferritin level in the blood plasma

Further changes

1. Increase of protoporphyrin level in RBC
2. Increase of total binding capacity
3. Decrease of iron level in the blood plasma

Relatively late changes

1. Anemia
2. Microcytosis
3. Hypochromia

Anemia of chronic diseases is a common pathology and takes the 2 place among all anemias in frequency (after iron deficiency anemia). It develops in acute and chronic infectious inflammatory diseases, sepsis, tuberculosis, rheumatoid arthritis, liver diseases, cancer, coronary heart disease, etc. The mechanism of hypochromic anemia in these conditions is associated with the redistribution of iron in the body (it is located mainly in the depot) and violation of iron reutilization mechanism of from the depot. In the above-mentioned diseases macrophage system is activated, when macrophages in the condition of activation restrain iron destroying the process of re-

utilization. In clinical blood analysis a moderate decrease in hemoglobin (<80 g/L) is observed. The main differences from iron deficiency anemia are:

• the increased level of serum ferritin, which indicates an increased content of iron in the depot;

• the level of serum iron can be preserved at a normal level or it can be moderately reduced.

• total iron binding capacity (TIBC) remains normal or reduces, which indicates the absence of Fe-starvation in the serum.

Megaloblastic anemia

Frequency of this anemia comprises 9-10 % of all anemias. Megaloblastic anemias include a group of acquired and hereditary anemias, characterized by megaloblastic hematopoiesis in the bone marrow. There are megaloblastic anemias due to vitamin B_{12} deficiency or folic acid deficiency. Combined deficiency is not common, it occurs only in intestinal absorption disturbance. More often isolated vitamin B_{12} deficiency is observed.

Reasons of vitamin B_{12} deficiency:

1. Disorders of vitamin B_{12} absorption in the intestine (IF factor deficiency)- pernicious anemia.
2. Food poor in vitamin B_{12}.
3. Competitive consumption due to helminthic invasion, intestinal microflora.
4. Absorption disorders in the diseases of the small intestine (resection, tumor, chronic enteritis, malabsorption syndrome).
5. Disorders of vitamin B_{12} release from protein complex
6. Alimentary vitamin B_{12} deficiency (mostly in vegetarians).
7. Hereditary transcobalamin deficiency, which results in disturbance of vitamin B_{12} delivery to the depot (rarely occurs).
8. Due to autoimmune processes antibodies against «internal factor» can appear in the blood.

Vitamin B_{12} deficiency lasting 1-2 years can induce MCV RBC changes as well as changes in the bone marrow which are considered as erythroid hyperplasia and megaloblastic changes. Anemia develops in the peripheral blood 6-18 months later.

RBC count is dramatically reduced (up to 1,0 - 1,5x10^9/L), RBC level is more reduced than the level of hemoglobin. The increase in mean corpuscular volume (MCV> 100 fL) and mean corpuscular hemoglobin (MCH> 32 pg) is observed in macrocytic, hyperchromic anemia. In normal mean corpuscular hemoglobin concentration (MCHC) polychromatophilia is observed as well as remnants of the nuclear substance (Cabot rings, Jolly bodies) and basophilic stippling. In patients with B_{12} deficiency anemia associated with macrocytic and hyperchromic anemia normal or decreased relative number of reticulocytes is observed, but their absolute count, regardless of the relative content, is always reduced. Vitamin B_{12} deficiency is detected by the low level of vitamin B_{12} in the serum (norm for adults is 148 - 616 pmol / L). In clinical practice, a reliable diagnostic test of vitamin B_{12} deficiency is a positive response in the form of reticulocyte crisis on the introduction of minimal doses of vitamin B_{12} (2 mg / day / m).

In majority of patients leukocyte count is reduced (mostly due to neutrophils). A right shift is observed in the leukogram, giant hypersegmented neutrophils appear. Number of eosiniphils reduces up to their disappearance. Number of monocytes reduces. Relative lymphocytosis is observed. Approximately in the half of the patients the reduced (sometimes dramatically reduced) level of platelets is observed. Detection of methylmalonic acid can be useful in the patients with anemia with reduced vitamin B_{12} concentration.

Pernicious anemia

Biermer's anemia or pernicious anemia is an autoimmune disease characterized by formation of antibodies to parietal gastric cells or Castle’s internal factor.

In the blood macrocytic anemia with oval red blood cells and megaloblastic changes in the bone marrow is observed. Bone marrow changes are followed by anemia, thrombocytopenia, leucopenia (neutrophilia) and pancytopenia development.

In the laboratory diagnosis of these anemias **Schilling's test** is very significant: it presupposes intake of vitamin B_{12}, labeled by radioactive cobalt, per os. The usual dosage is 0,5-1,0 mg. Two hours later 1,0 mg of unlabeled vitamin is subcutaneously or intramuscularly injected to a patient (to saturate binding sites) for labeled vitamin could excrete into the urine. In healthy individuals more than 8% of

labeled vitamin is excreted with the urine, while in patients with impaired vitamin B_{12} absorption less than 5% is excreted (in pernicious anemia this index is usually less than 2%).

The detection of Castle's internal factor and its antibodies is used as well: antibodies to the internal factor are detected in 50-70% of patients with pernicious anemia. These antibodies are highly specific: there are no data on any true-positive result in healthy individuals.

Another test in pernicious anemia diagnosis is **the detection of antibodies to parietal cells**: chronic atrophic gastritis and pernicious anemia are commonly accompanied by the appearance of autoantibodies against parietal gastric cells. Antibodies to parietal cells are present approximately in 90% of patients with pernicious anemia, in 50% of patients with atrophic gastritis without pernicious anemia and in 33% of patients with thyroiditis.

Sideroblastic anemia

Sideroblastic, sideroacrestic anemias (iron saturation anemia) are hypochromic microcytic hyporegenerative anemias due to disturbance of intracellular iron utilization for hemoglobin synthesis, in spite of normal or increased content of iron in the mitochondria of erythroblasts. As a result, the number of sideroblasts (normoblasts with characteristic annular arrangement of iron granules around the nucleus— ringed sideroblasts) increases in the bone marrow. There are inherited and acquired forms of anemia.

Hemoglobin synthesis disorders cause the reduction of mean corpuscular hemoglobin, population of hypochromic microcytes appeares. In patients older than 60 years (and in congenital form in teen-agers) anemia can be relatively severe. In the bone marrow erythroid hyperplasia appeares, in smear staining for iron ringed sideroblasts are visible. In the serum there is an increased ferritin level and a significant increase in serum iron with high transferrin saturation. Reticulocytes content is reduced (hyporegenerative anemia). The combination of hypochromic microcytic cells with normocytes or macrocytes is a diagnostic feature of sideroblastic anemia.

Aplastic anemia

Aplastic anemia is a group of congenital and acquired diseases characterized by a sharp depression of the bone marrow hematopoiesis, inhibition of cellular elements proliferation and differentiation with the development of profound pancytopenia in the peripheral blood. The clinical picture is determined by anemic and hemorrhagic syndromes.

The peripheral blood picture is characterized by pancytopenia: erythro-, leuko- and thrombocytopenia. Severe anemia (hemoglobin concentration can drop to 20 - 30 g / L, erythrocyte concentration to 0,7 - 2,5 x109 / L) has normochromic character, with moderate anisocytosis with a tendency to macrocytosis and poikilocytosis. Serum iron level is usually increased with almost complete saturation of iron-binding capacity. Serum iron concentration and its excretion with the urine in aplastic anemia is much higher than in anemias of other origin with the same concentration of hemoglobin. Erythrocyte sedimentation rate is usually increased to 40 - 60 mm/h.

In most cases bone marrow punctuate is very poor, and contains a small amount of hematopoietic cells, most of which are lymphocytes.

The most important method for aplastic anemia diagnosis and assessment of bone marrow hematopoiesis in these diseases is histologic examination of hematopoiesis with the help of blood trepanobiopsy that detects high prevalence of fatty tissue over the active bone marrow.

Hemolytic anemia

Hemolytic anemia is a large heterogenous group of anemias caused by pathologically premature destruction of RBC in the blood vessels or in the cells of reticulohistiocytic system; and the destruction of red blood cells predominates over their formation. Pathological hemolysis is associated with hereditary or acquired changes in the structure and functions of RBC or with influence of some external factors causing destruction of normal RBC.

In hemolytic anemia hemolysis dramatically increases, therefore the content of free bilirubin increases in the blood with its increased excretion into the bile, disturbing its colloidal stability; the preconditions for the development of cholelithiasis are created.

Valuable indicator of hemolysis is the level of haptoglobin: the more intensive is hemolysis, the more haptoglobin is consumed; but its consumption exceeds the synthetic capacity of the liver (haptoglobin is synthesized in the liver and belongs to a class of a_2-globulins). Therefore the level of haptoglobin drastically reduces, which is observed primarily in intravascular hemolysis. In the urine free hemoglobin, and in a few days, hemosiderin is detected.

Hemolytic disease of the newborns

Hemolytic disease of the newborns is the disease based on the hemolysis of RBC of the fetus and newborn, associated with erithrocyte antigens incompatibility of maternal and fetal blood.

The disease develops mostly as a result of maternal-fetal Rh-factor or group antigens incompatibility, rarer other antigenic systems are incompatible due to their lower immunigenity.

- Rh-conflict occurs if a Rh-negative mother has a fetus with Rh-positive blood.
- ABO-conflict develops if a woman has 0(I) group and a fetus has A(II) group (in 2/3 of cases) or B(III) group (in 1/3 of cases).

If the blood of mother and fetus is AB0- or Rh-Ar incompatible hemolytic disease of the newborns develop in 3-6%. There is an opinion that ABO hemolytic disease of the newborns is more common than Rh-factor hemolytic disease, but often has a more favourable course and it is difficult to diagnose. Recently the number of children with severe ABO hemolytic disease has increased especially if newborns have B(III) blood group.

There are edematous (2%), icteric (88%), anaemic (10%) disease forms.

Antenatal and postnatal diagnosis is distinguished.

First of all **antenatal diagnosis** of possible immune conflict is necessary. Erithrocyte antigens incompatibility of parents' blood is considered as well as obstetric and somatic history of the mother (previous abortion, stillbirths, miscarriages, birth of sick children, hemotransfusions without regard to Rh-factor). During the pregnancy the titre of anti rhesus antibodies is detected at least three times in the blood of Rh-negative woman. Titer value is a relative value, since the detection of antibodies in the blood of a pregnant woman can only

assume the probability of disease of the fetus. Type of the curve of Rh-antibody titer dynamic changes ("jumping", i.e, title with sharp fluctuations) has a greater prognostic value than the degree of its increase. In case of detection of the immune conflict risk amniotic fluid obtained by transabdominal amniocentesis is examined. The optical density of bilirubin, protein, glucose, iron, copper, Ig concentration is detected. The factors indicating hemolytic disease of the newborns development are: placenta thickening, its rapid growth due to possible edema, polyhydramnios, fetal abdominal enlargement due to hepatosplenomegaly.

Postnatal diagnosis of hemolytic disease of the newborns is based on clinical manifestations of the disease soon after the birth (jaundice, anemia, hepatosplenomegaly). Very important are laboratory data (increase in concentration of unconjugated bilirubin, positive Coombs' test in Rh-conflict). All the parameters are considered in complex and in dynamics if possible.

LABORATORY ASSESSMENT OF HEMOSTASIS

Hemostasis is a set of mechanisms which, in physiological conditions, is responsible for maintainance of the circulating blood in liquid state.

Hemostasis system includes:

1. thrombocytic-vascular hemostasis (primary);
2. coagulation hemostasis (secondary);
3. Fibrinolysis

Elements of primary hemostasis:

A. Vessels and tissues:

– capillaries, arterioles, venules constrict in response to vasoconstrictive substances production such as serotonin, epinephrine, norepinephrine.

– large and medium vessels – reflex spasm.

– undamaged endothelium of the vessels has anticoagulation properties (heparinoids on the endothelial surface); if damaged it becomes powerful procoagulant

B. Morphological blood elements:

– platelets;

– red blood cells;

– leukocytes.

C. Role of platelets in hemostasis:

– most reactions of plasma hemostasis occur on the surface of platelets

– platelet adhesion is the ability of activated platelets to adhere to the vascular wall at the place of its damage.

– platelet aggregation is the ability of platelets to adhere to each other and form aggregates.

Coagulation hemostasis

Coagulation hemostasis is provided by proteolytic activation of plasma factors; as a result insoluble fibrin is formed from soluble fibrinogen protein. The key reaction of hemostasis is thrombin generation.

Plasma coagulation factors:

Factor I (fibrinogen factor) is the protein synthesized in the liver. Concentration of fibrinogen in blood is about 2-5 g/L (200-500 mg in 100 ml of blood). Reduced concentration of fibrinogen in blood (less than 1 g/L) can induce bleeding in a patient.

Factor II (prothrombin factor) is glycoproteid, synthesized in the liver. Vitamin K is necessary for its synthesis. Influence of multienzyme prothrombinaze complex on this factor causes formation of the key hemostasis enzyme – thrombin. Thrombin turns fibrinogen into fibrin providing clot formation.

Factor III (tissue factor) is a receptor protein of cellular membrane; it is located in all the organs and tissues as well as in vascular endothelium. This factor is receptor for VII factor and provides hemostasis activation.

Factor IV (calcium factor) participates at all the stages of plasma hemostasis.

Factor V (proaccelerin**)** is synthesized in the liver and participates in prothrombin activation, being a part of multienzyme prothrombinaze complex. In its deficiency parahemophilia develops.

Factor VI/VII (proconvertin/convertin) is vitamin K, synthesized in the liver. About 1% of the factor circulates in blood in active VIIa form. VIIa on the surface of the damaged endothelium forms a complex with tissue factor tf, which in its turn activates fX, providing generation of thrombin microquantity. It plays a major role in coagulation process enhancement.

Factors VIII, IX, XI are antihemophilic factors. Activated factors VIIIa and IXa form tenase complex on the phospholipid membrane surface. This complex forms the main prothrombinaze component – factor Xa.

Factor X (Stuart factor) is a key enzyme of prothrombinaze, which transforms prothrombin into thrombin.

Factor XII (Hageman factor) is a factor of contact. This factor deficiency is not usually clinically manifested.

Factor XIII – (fibrin stabilizing factor). It forms D=D links in an unstable fibrin polymer, which stabilizes the latter.

Tests for thrombocytic-vascular hemostasis evaluation

Detection of platelet count is used as a screening of platelet hemostasis component. This test is included in the panel of diagnosis and treatment of disseminated intravascular coagulation (DIC) syndrome and has a principle value in diagnosing of thrombocytopenia, induced by heparin therapy. The reference values of the platelet count depend on the method of calculation. So if manual method in Goryaev chamber is used, normal platelet count in the blood is 180 000-320 000/mcL. If hematologic calculators are used the upper normal range can increase to 450000/mcL or for some analyzers to 550000/mcL.

The platelet count should be carefully evaluated before heparin therapy. Platelet count less than 20000/mkl (for medical patients) and less than 50000/mkl (for surgical patients) is an indication for immediate platelet transfusion.

Standardized bleeding time by Ivy is a test evaluating primary hemostasis function. The increase of bleeding time in normal platelet count indicates their function disturbance (bleeding time is increased by aspirin and nonsteroidal anti-inflammatory drugs - NSAIDs), which requires further evaluation of platelet aggregation properties. The method doesn't detect coagulation hemostasis disorders and doesn't reflect hemostasis system state in the whole. Normal value of this test is 7 min.

Tests for coagulation hemostasis evaluation

Most laboratory methods of hemostasis plasma component are based on a clotting method.

Principle of all clotting tests is based on detection of fibrin clot formation time after addition of calcium ions and activator of the necessary stage of coagulation hemostasis into the examined plasma.

The main tests of coagulation hemostasis evaluation are:

1. Prothrombin time
2. Activated partial thromboplastin time
3. Thrombin time
4. Detection of fibrinogen concentration
5. Detection of concentration of fibrin degradation products.

Prothrombin time (PT)

Prothrombin time concerns to clotting chronometric tests and evaluates the external way of X factor activation. This test is highly sensitive to VII and X factors activity. To a lesser extent prothrombin time reacts to fibrinogen, factor V and prothrombin deficiency. PT depends on coagulation inhibitors, antithrombin, in particular. Though the test is extended only in significant heparin concentration, that is why PT is not recommended for heparin treatment control. Deficiency of factors of X factor internal activation (VIII, IX, XI, XII) are not detected by this test.

Indications for PT detection:

1. INR use is recommended by WHO for control (according to therapeutic INR ranges) in the treatment by indirect anticoagulants. The effective warfarin dose for the treatment of venous thromboembolism is the dose increasing INR to 2-3. If prosthetic cardiac valves use INR values should be 3 – 4.
2. PT is recommended by International Thrombosis and Hemostasis Society to diagnose DIC syndrome .
3. Prothrombin index (time) is used to evaluate synthetic liver function

The ways of PT expression:

1. Prothrombin index (PTI) = $PT_{\text{of standard plasma}} / PT_{\text{of a patient}}$, norm is 0,8-1,2. Increase indicates hypercoagulation, decrease indicates hypocoagulation.
2. Prothrombin ratio (PR) = $PT_{\text{of a patient}} / PT_{\text{of standard plasma,}}$ norm is 0,94 – 1,1.
3. International normalized ratio (INR) is calculated in the following way: $INR = PR^{ISI}$, where ISI is international sensitivity index, comparing activity of tissue factor of animal origin with this factor standard (human origin). Therapeutic range of ISI equals 2 – 3.

Activated partial thromboplastin time (APTT)

APTT concerns to the group of clotting tests of coagulation hemostasis evaluation, it allows to evaluate contact (internal) pathway of factor X activation. The principle of the test: the coagulation time of citrated platelet-poor plasma is detected in the presence of optimal amounts of calcium, as well as contact activator of factor XII and

simulator of phospholipid surfaces - partial thromboplastin. Therefore, this test is called Activated (activator is cephalin, ellagic acid) partial thromboplastin (partial thromboplastin time is a simulator of the phospholipid surface) time (time of clot formation).

This test is especially sensitive to VIII, IX and V factors deficiency. Test sensitivity: APTT is prolonged if factor level is less than 30% of norm. Each laboratory performing examination set reference ranges for APTT.

Indications for application:

1. Control over unfractionated heparin therapy should be performed. Therapeutic level of UFH in blood corresponds to 1,5 – 2,5 time increase of APTT in comparison with mean normal values.
2. Screening of congenital factor deficiency (especially factors VIII, IX).
3. Evaluation of hemophilia replacement therapy effectiveness.
4. Screening of antiphospholipid syndrome.

Prolonged APTT:

1. Treatment by unfractionated heparin or hirudin.
2. Factors VIII, IX and V deficiency. APTT is prolonged if factor level is less than 30% of norm.
3. Consumption coagulopathy.
4. Presence of immune anticoagulants and FDP in patient's blood.
5. Von Willebrand disease.
6. Hypo- and disfibrinogenemia.
7. APTT is physiologically prolonged in newborns.

Thrombin time

Thrombin time is a clotting test, it evaluates activity of the last stage of coagulation hemostasis – fibrinolysis. The method is based on detection of time of fibrin clot formation. Standard solution of thrombin with low activity is added to citrated plasma. Clot formation is registered by coagulometer detecting changes in optical density.

Indications for thrombin time detection:

- Diagnosis of congenital and acquired a/hypofibrinogenia;
- Diagnosis of dysfibrinogenimia (disorders of fibrinogen structure);

– Control over fibrinolytic therapy effectiveness (effective fibrinolysis is observed if thrombine time is 1.5 more prolonged than mean normal values).

In DIC syndrome diagnosis, especially acute and subacute forms, thrombin time directly depends on fibrinogen level and fibrin degradation products.

Problems of interpretation:

1. Influence of drugs:
2. Thrombine time is prolonged in presence of penicillin and protamine sulfate.
3. Is physiologically prolonged in newborns.
4. Is prolonged in hypoalbuminemia (for example in patients with nephrotic syndrome). Normalization occurs if human albumin is added to plasma.

Fibrinogen level detection

Method of choice to detect fibrinogen level is Clauss clotting test, which is standardized (as opposed to the gravimetric method, which can not be subjected to quality control) and has a clinically sufficient sensitivity and specificity. The reference range is 2.0-4.0 g / L (for pregnant women up to 6 g / L).

Indications for application:

– Evaluation of factor consumption level in DIC syndrome.

– Evaluation of liver synthetic function.

– Hereditary disorders of fibrinogen synthesis

– Thrombolytic therapy (in 20% of patients fibrinogen decrease is not observed, which indicates the lack of reperfusion).

– Evaluation of the risk of thrombotic complications in patients with atherosclerosis. Increased fibrinogen level is an independent thrombotic risk factor.

Hemostatic minimum of fibrinogen concentration is 1 g/L. Fibrinogen level lower than this value can induce bleeding.

It should be mentioned that fibrinogen is an acute-phase protein and increase in its concentration is observed in:

1. Acute inflammation.
2. Postoperative period.
3. Malignant neoplasms.

Fibrinogen/fibrin degradation products (FDP)

There are following methods of FDP detection:

Procoagulation tests: ethanol and protaminsulfate tests detect complexes of fibrin monomers with FDP because complexes of fibrin monomers with FDP are able to polymerize and form gel in presence of ethanol or protaminsulfate. These tests are characterized by high sensitivity and low specificity. It means that their positive result indicates activation of thrombin generation and/or fibrinolysis. At the same time negative results have no diagnostic significance.

1. **Immunological semi-quantitative tests of FDP detection** are based on the use of latex particles (latex agglutination test) or standard erythrocytes (hemagglutination reaction) with the adsorbed on them antibodies to fibrinogen-fibrin related antigen - FRA. This semi-quantitative test is highly sensitive to the pathology associated with the activation of clotting processes and especially fibrinolysis. Specificity of the test is significantly higher than paracoagulation tests. In healthy individuals, the concentration range of the FDP is 1 - 5 ug / ml. Diagnostic threshold for DIC syndrome diagnosis is the level of 500 mg / ml. In deep vein thrombosis and pulmonary embolism FDP level is usually in the range of 5 - 500 mg / ml. FDP increase is also observed in treatment by thrombolytic drugs, in myocardial infarction and liver diseases.
2. **D-dimers detection**. Today, D-dimers are the most appropriate diagnostic marker of DIC syndrome, as well as an effective diagnostic test in the management of patients with suspected deep vein thrombosis (DVT) and pulmonary thromboembolism (PE).

Disseminated intravascular coagulation (DIC) and pulmonary thromboembolism (PE) are extremely dangerous complications. This fact, on the one hand, is conditioned by the difficulty of early diagnosis of pathological conditions, and on the other hand by problematic character of the effective treatment at those DIC and PE stages when the diagnosis is not doubtful. Detection of objective diagnostic criteria of these complications allows to diagnose and initiate early appropriate therapy on time. One of these criteria can be D-dimer level in plasma.

Clinical use of D-dimer test:

- Diagnosis, control over the disease course, evaluation of DIC syndrome treatment.

– D-dimer can be used as an effective pre-DIC marker
– D-dimer is an effective marker of DIC development and is included in the laboratory DIC screening together with prothrombin time, fibrinogen level and platelet count.
– D-dimer, as well as antitrombin activity level and platelet count is an effective criteria for DIC course and treatment effectiveness evaluation.

- It is used to exclude deep vein thrombosis and pulmonary thromboembolism.
- Monitoring of heparin therapy and heparin prophylaxis.

– D-dimers are prognostic markers in monitoring of anticoagulation heparin therapy of all clinical conditions, accompanied by thrombosis. Normalization of D-dimers concentration in plasma of patients treated by heparin indicates thrombosis regression; preserving increase of D-dimers during the therapy indicates the unfavourable prognosis.

CLINICAL METHODS OF EXAMINATION

Cerebrospinal fluid examination

Cerebrospinal **f**luid (CSF), Liquor cerebrospinal is clear, colorless fluid filling the cavities of encephalocoel, subarachnoid space and medullary canal, perivascular and pericellular space of brain tissue. Volume of circulating CSF is normally 90–150 ml in adults, in newborns – 10-60 ml. Per day nearly 500 ml (0,35ml/min) is produced, time of complete replacement is 6-8 hours.

Indications for CSF examination:

- Neuroinfections (meningism, meningitis, encephalitis, etc.).
- Disturbed cerebral circulation.
- Craniocerebral and cerebrospinal injuries.
- CNS neoplasia.
- Autoimmune CNS lesions.

The most common method of CSF sampling is a lumbar puncture. This procedure is performed in the operating room, dressing room or in the ward with strict aseptic conditions.

Volume of examinations performed in cerebrospinal fluid:

A. Obligatory tests:

– CSF pressure;

– physical properties detection (quantity, color, clarity);

– cytosis and its character, “true” cytosis;

– protein sedimentation reaction (Pandy and Nonne-Apelt reaction);

– total protein concentration;

– glucose concentration;

– bacterioscopic examination (according to indications);

B. Additional tests:

– microbiological examinations.

– biochemical examinations (lactate concentration, lactate dehydrogenase and creatine kinase activity, C-reactive protein level, electrophoretic separation of CSF proteins, detection of speciphic proteins (immunoglobulins, myelin basic protein, etc.), tumor markers (alfa-fetoprotein, beta-glucuronidase, carcinoembryonic antigen (CEA), human choriogonadotropin (HCG).

CSF pressure detection

To measure CSF pressure water manometer should be attached to the puncture needle as soon as cerebrospinal fluid appears. Normally CSF pressure in the lumbar area ranges from 100 to 180 mm of H2O in horisontal position. In sitting position the pressure increases to 250-300 mm of H2O due to hydrostatic pressure.

CSF pressure increase is observed in:

a) intracranial expansive processes (tumors, abcsesses, hematomas);

b) infectious CNS lesions;

c) chronic cardiac failure;

d) subarachnoid hemorrhages;

e) brain edema.

Decreased CSF pressure is detected in:

a) circulatory collapse;

b) severe dehydration;

c) hyperosmolar syndrome;

d) liquorrhea;

e) subarachnoid area block by a tumor, for example

Detection of physical properties (color, clarity)

Evaluation of CSF physical properties (color, clarity) is performed before and after centrifugation in all the CSF samples. Normally CSF is clear and colorless.

Color

Pink or bloody color can be observed due to an admixture of blood in case of recent subarachnoid hemorrhages or bleeding into the brain matter (if the hemorrhage nidus is associated with CSF pathways), as well as brain damages. Xanthochromia is a light-pink, orange, yellow, or brown CSF coloring, resulting from metabolism of hemoglobin leaving red blood cells. Pink or orange color after centrifugation is associated with the presence of oxyhemoglobin in CSF. It is observed in recent subarachnoid hemorrhages, as well as when red blood cells leave vessels due to congestion in venous or CSF circulation. Yellow CSF color is observed due to biliverdin and bilirubin. Xanthochromia due to the presence of these pigments in CSF is observed in old subarachnoid hemorrhage, tuberculous meningitis, and intracranial hematomas. It should be remembered that CSF xanthochromia is almost always diagnosed in preterm infants.

Clarity

Normally CSF is clear. Opacity is associated with the development of pathologic process and can occur in 2 cases: increased number of the cells (leukocytes more than 400 per mcl (1200/3) or red blood cells more than 500 per mcL.) or increased bacteria content. Appearance of fibrin membrane in CSF is accociated with tuberculous meningitis.

Microscopic CSF examination

Cytological examination of the cerebrospinal fluid is made up of: 1) the calculation of the total number of cells in a Fuchs-Rosenthal counting chamber (cytosis), 2) the differentiation of cellular elements (CSF cytogram) in the preparations made after sedimentation or centrifugation and staining with azure-eosin by Nocht, Pappenheim-Kryuchkov and Romanovsky-Giemsa.

General principles of evaluation of cytosis and its character in CSF include: (1) to detect cytosis and its character only CSF from the last tube is used, (2) the time from CSF sampling and cytosis analysis should not exceed 30-60 minutes, (3) calculation of true cytosis is indicated in the presence of blood in the cerebrospinal fluid, (4) the preparation for CSF stained smear is prepared with the help of cytocentrifugation or Van Slyke sedimentation chamber, (5) In evaluation of cytosis and its character in CSF it is necessary to consider a way of cerebrospinal fluid collection and age of a patient.

RBC count in CSF is performed in Goryaev or Fuchs-Rosenthal chamber and is expressed by the number of RBC per 1 mcL. Simultaneously RBC morphological characteristics are detected for differential diagnosis of subarachnoid hemorrhage and accidental blood in the cerebrospinal fluid. Thus, the presence of the "leached" erythrocytes indicates that there is blood in the cerebrospinal fluid before the lumbar puncture (i.e subarachnoid hemorrhage).

Any pathology affecting the meninges can lead to an increased number of leukocytes in the cerebrospinal fluid (pleocytosis), the degree of pleocytosis will depend on the type of the pathological process, its duration and intensity. Most evident pleocytosis is observed in severe acute infectious meningitis. Pleocytosis severity depends not only on the etiology (bacteria, viruses) of the process, but also on the stage of the disease, its nature and period of therapy,

including the etiotropic treatment. Pleocytosis is evident in acute bacterial meningitis as well. This pathology is characterized by the increase of leukocyte number in CSF to more than 1000 per mcl.

At the same time pleocytosis absence can not absolutely exclude organic lesion of the brain and meninges. Sometimes, especially at the initial stages of the disease, cytosis in SCF can be normal or not evident. It should be noted that in 4% of patients with acute bacterial meningitis pleocytosis can be absent in CSF. In acute bacterial meningitis neutrophilic leukocytes prevail in cerebrospinal fluid, while in viral infections, chronic diseases of the central nervous system, neurosyphilis and tuberculous meningitis mononuclear cells are prevalent.

However, in 20-75% of cases (according to some reports) neutrophils are predominant in CSF obtained from the first puncture in viral meningitis. In addition about 10% of cases of bacterial meningitis are accompanied by lymphocytic pleocytosis.

Another exception is tuberculous meningitis, in which the picture in CSF combines features of both acute bacterial and chronic infections of the CNS. In this case, the predominant cells in the cerebrospinal fluid are lymphocytes, but increase in neutrophil level is observed too.

Uremia can also lead to lymphocytosis in the cerebrospinal fluid in 25% of cases.

Cytosis character detection (CSF cytogram)

In adults normally cytosis in CSF occurs due to lymphocytes (60%±20%) and monocytes (30%±15%), neutrophil number can be 2%±2%. In newborns lymphocyte count is 20%±15%, monocytes are 70%±20%, neutrophils are 4%±4%.

In various pathologies quantitative and qualitative cellular composition can change. The origin of the cellular elements in the cerebrospinal fluid has a dual nature. Lymphocytes, neutrophils, eosinophils, plasma and blasts cells have hematogenous origin. Histogenic origin is characteristic of histoid elements: monocytes, macrophages, tissue basophils, arachnoid cells, ependymal cells, cells of malignant CNS neoplasms and metastatic cells from other organs and tissues.

Evident lymphoid pleocytosis is observed in viral meningitis, neurosyphilis, multiple sclerosis chronic tuberculous meningitis, reactive aseptic meningitis.

Neutrophilia is characteristic of CSF in local and diffuse leptomeningitis, acute tuberculous meningitis. In brain abscesses, cerebral and spinal syphilis a neutrophilic response can vary. In viral meningitis it is short and hardly evident.

CSF eosinophilia is observed in cysticercosis, echinococcosis of the brain, and eosinophilic meningitis. In a small number (2-3%) appear in the cerebrospinal fluid in tuberculous meningitis, cerebral hemorrhages, can accumulate in brain tumors, hydrocephalus, drug intoxication. Two or three percent of eosinophils are detected in SCF in tuberculous meningitis, cerebral hemorrhages, in brain tumors, hydrocephalus, drug intoxication. Basophils are detected in CSF in severe neuroinfections, especially in children.

In normal CSF plasma cells are not detected. In pathology plasma cells are detected at different stages of maturation. Especially characteristic is the appearance of plasma cells in the cerebrospinal fluid of patients with multiple sclerosis, hyperkinetic progressive panencephalitis.

Monocytosis is observed in neurosyphilis, multiple sclerosis, chronic subacute inflammatory processes of the CNS.

Increase in the number of macrophages in CSF is observed in CNS hemorrhages. The number of macrophages in the cerebrospinal fluid increases with any inflammatory processes in the CNS.

Chemical CSF analysis

Protein detection

Preliminary analyses of the protein composition of CSF are Pandey and Nonne-Apelt tests. These tests give approximate information about the level of protein and the ratio of albumin and globulin concentration in CSF. More precise information can be obtained by detection of total protein concentration and electrophoretic separation of cerebrospinal fluid proteins.

Table 20. Reference ranges of protein concentration in lumbar liquor depending on age

Patient's age	**Protein concentration (g/l)**
Adults	0,15–0,45
Adults over 60	0,15–0,60
Children: 0–30 days 30–90 days 90 days –6 monthes	 0,75–1,5 0,2–1 0,15–0,5

Increase in concentration of protein in the cerebrospinal fluid is associated with blood-brain barrier permeability violation (trauma, infection), or with violation of protein reabsorption from CSF by arachnoid cells (infectious lesions, mechanical block of CSF outflow by a tumor, abscess, adhesions), or with the increased synthesis of immunoglobulins by lymphocytes or plasma cells in the central nervous system (multiple sclerosis).

Classical reason for the increase of total protein in CSF is acute bacterial meningitis (74% -99% according to some reports). In bacterial meningitis increase in protein concentration in CSF is usually more than 1 g / L. Detection of total protein in CSF is used as a criterion for differential diagnosis of septic and aseptic meningitis. When concentration of protein in CSF is more than 2 g/L the sensitivity of this test for differential diagnosis of bacterial and viral meningitis is 86%, specificity is 100%. Combination of cytosis with more than 1000 cells/mcL and the level of protein in the CSF more than 5 g / L is associated with a poor prognosis of bacterial meningitis.

A significant increase of protein level in CSF without corresponding increase in the number of cells is known as "protein-cell dissociation." Such a pattern in CSF is observed in Guillain-Barre syndrome, giant cell temporal arteritis, as well as in blockade of SCF outwlow, for example, by spinal cord tumor.

Reduction of total protein concentration in the cerebrospinal fluid is observed in violation of the integrity of the dura mater as a result of an injury or surgery.

Detection of glucose concentration in CSF

Reference ranges: 2,75 – 4,4 mmol/L (60% of blood plasma for adults and 80% for children).

Glucose concentration in cerebrospinal fluid depends on its concentration in blood, that is why its detection only in SCF makes it difficult or impossible to interpret the results. In examination of glucose concentration in CSC it is necessary to detect its concentration in blood and to focus on the ratio of glucose concentration in cerebrospinal fluid and blood. Normally, this ratio is approximately 0.6.

The most clinically significant is the reduction of glucose concentration in cerebrospinal fluid and the ratio of CSF glucose / blood glucose. There are three mechanisms leading to it are: 1) violation of glucose transport in the central nervous system, 2) an increase of glycolytic activity in the CNS, 3) an increase in the intensity of glucose utilization by leukocytes and microorganisms in CSF. With the help of the latter mechanism, the decrease of glucose concentration in CSF in bacterial meningitis (especially tuberculous meningitis) occurs. Besides bacterial meningitis, subarachnoid hemorrhage and metastatic lesion of the meninges can cause the reduction of glucose concentration in CSF.

It is believed that in such CNS diseases as viral meningitis, encephalitis, brain abscess, syphilis, tumors of the brain glucose levels in CSF usually remains in the normal range.

Patients with hyperglycemia to 40 mmol / L (limit of saturation of glucose transport systems through the blood-brain barrier) have an increase in glucose level in CSF though the ratio of CSF glucose and blood glucose is normal. If glycemia is more than 40 mmol / L, this ratio will decrease.

Bacterioscopic CSF examination

An integral element of meningitis diagnosis is microbiological study, including bacterioscopy of stained preparations, detection of pathogen antigens in CSF, the allocation of the pathogen culture on nutrient media and detection of antibiotic sensitivity.

In case of viral meningitis probability the extent of examinations should take into account the patient's age, seasonal prevalence and distribution of the pathogen. The diagnosis of viral meningitis is not always based on allocation of etiologic agent from CSF cerebrospinal

fluid or brain biopsy. For example, it is better to get the cultures of enteroviruses (poliovirus, Coxsackie virus and echovirus) from pharyngeal secretions or feces. Therefore, in patients with a presumptive diagnosis of viral CNS disease not only CSF should be collected but serum (to detect specific IgM in acute phase of a disease), throat and rectal swabbing as well. The epidemiological importance of a pathogen of bacterial meningitis vary depending on the age of the patient.

Microscopy of stained preparations obtained from the sediment of cerebrospinal fluid, is relatively simple and quite effective method of verification of meningitis diagnosis. Properly prepared and stained by Gram's method CSF preparation (if microscopy is carefully performed) helps to identify a pathogen in 60-90% of cases.

In case of detection of Gram-positive cocci, similar to Streptococcus pneumoniae, small Gram-negative coccobacilli similar to Haemophilus influenzae and gram-negative diplococci similar to Neisseria meningitidis, their immediate serotyping using rapid tests is indicated.

Table 21. Basic physiological indices of CSF

VOLUME OF CEREBROSPINAL FLUID (CSF):	
ADULTS	90-150 ml
NEWBORNS	10-60 ml
SPEED OF SCF FORMATION	0,35 ml/min
TIME OF TOTAL SCF REPLACEMENT	6-8 h
TIME OF CSF TRANSFER FROM THE PLACE OF FORMATION TO THE LUMBAR REGION	1 h
CSF PRESSURE (INTRACRANIAL PRESSURE)	90-180 mm CSF
RANGE OF CSF PRESSURE FLUCTUATION IN STRESS TESTING	± 5-10 mm CSF

Table 22. Reference ranges of basic laboratory indices of CSF

Index	Value
RED BLOOD CELL COUNT	0
LEUKOCYTE COUNT	
ADULTS	0-5 in mcL
CHILDREN BEFORE PUBERTY	0-10 in mcL
1-YEAR-OLD CHILDREN	0-20 in mcL
NEWBORNS	0-30 in mcL
CYTOSIS CHARACTER	
ADULTS	
LYMPHOCYTES	60% ±20%
MONOCYTES	30% ±15%
NEUTROPHILS	2% ±2%
NEWBORNS	
LYMPHOCYTES	20%±15%
MONOCYTES	70%± 20%
NEUTROPHILS	4% ±4%
TOTAL PROTEIN	
ADULTS under 60	0.15 - 0.45 g/L
Over 60	0,15 - 0,6 g/L
GLUCOSE	2,75 - 4,4 mmol/L
LACTATE	1,13-3,23 mmol/L

Laboratory analysis of synovial fluid

Laboratory analysis of synovial fluid is essential in the diagnosis of various diseases of the joints, and helps to determine the activity of the process as well as therapeutic approach and its effectiveness monitoring.

Normal synovial fluid usually has a chemical composition similar to the blood plasma one. It is formed as a result of plasma filtration through the vascular endothelium and synovial membrane into the joint cavity. Some components of the synovial fluid are synthesized directly into the joint cavity. They include hyaluronic acid produced by type B synoviocytes and lyubritsin. The presence of hyaluronate distinguishes synovial fluid from other body fluids and provides its

viscosity. Viscosity provides lubricating properties as well as free movement of the joint surfaces. The increased viscosity can cause some difficulties in carrying out certain lab tests, particularly in cytometry.

In norm synovial fluid has a low leukocyte count (<0.2 x 109 / L) with a predominance of mononuclear cells (lymphocytes). Red blood cells and crystals are normally not present, and the liquid is sterile.

The permeability of the synovial membranes and capillaries changes under the action of immunological, mechanical, chemical or bacteriological factors causing various inflammations. Other diseases cause changes in the chemical composition of synovial fluid, cellular composition and crystals too. The study of synovial fluid and crystals morphology, Gram staining and microbiological tests are important for differential diagnosis of joints diseases. Microscopic studies of crystals are also very significant. Using special tests it is possible to diagnose the type of arthritis, which will provide an effective treatment.

The aim of synovial fluid laboratory analysis is to detect the nature and character of the changes occurring in the joint. Rather often this analysis is the basis for diagnosis. Furthermore, it makes possible to evaluate the activity of inflammatory process and has a great importance in approach and monitoring of drug therapy.

Extent of laboratory examinations includes:

1. Detection of physical parameters: color, clarity, viscosity;
2. Microbiological analysis: Gram staining and culture.
3. Leukocytes and percentage of various forms.
4. Analysis of crystal morphology.
5. Other analyses (protein and lipid concentration, detection of rheumatoid factor and antinuclear antibodies).

The first portion of the fluid collected into a sterile syringe is used for bacteriological research. A drop of fluid is placed on a glass slide to prepare native preparation. In a test tube without anticoagulant the fluid for examination of organoleptic properties (color, clarity, viscosity) and pH is collected; Ropes test is performed. In the test tube with anticoagulant the fluid is used to detect the number of cells, as well as viscosity by Ostwald’s method. After that, the synovial fluid is centrifuged in the tube. The sediment is used to prepare native

preparation and smear; the supernatant is used to detect concentration of protein, lipids, rheumatoid factor, antinuclear antibodies.

In the case of high viscosity of the fluid it is usually difficult to perform microscopic analysis. In this case, to dilute the fluid buffer system with hyaluronidase can be used. It should be added to the synovial fluid in a corresponding amount with following incubation at room temperature.

Tests performed without anticoagulant

Evaluation of physical and chemical properties of synovial fluid

The volume of synovial fluid depends on the type of a joint. In the knee joint synovial fluid is usually less than 3.5 ml. If a small amount of the fluid is delivered into laboratory, one drop is used for native preparation, one drop is used to determine the number of cells and one drop - for Gram staining.

Normally the fluid is crystally clear and viscous. In color, it is straw-yellow, light yellow (is similar to white of an egg) or colorless. In various diseases color can vary from bright yellow to dark brown. In joint injuries, hemophilia or arthritis caused by crystals, synovial fluid has a bloody character (the primary cause). It should be remembered that bloody fluid can be the result of a vessel injury during the puncture (secondary cause). To distinguish accidental blood in the fluid it is necessary to evaluate supernatant after centrifugation of a sample. If its color after centrifugation is light yellow or clear the blood is accidental. Dark red or dark brown supernatant indicates bleeding into the joint. Xanthochromic color (yellow) indicates bleeding into the joint, or that the blood has already been some time in the joint.

Turbidity of the sample increases in the presence of protein, cellular elements, and crystal precipitates. Synovial fluid turbidity is usually connected with:

- presence of lipids and crystals;
- increase in number of cells;
- bacterias;
- increased protein concentration;
- presence of amyloid.

Viscosity can be detected during the joint puncture as well as in a laboratory.

Tests performed with anticoagulant

Usually synovial fluid is collected with the addition of heparin (5000 U / ml) - 1 drop per 5 ml. However, in practice, EDTA, sodium citrate or calcium oxalate can be used as an anticoagulant.

Detection of number of cells

Normal synovial fluid contains up to 200 cells per ml. The number of cells significantly increases in inflammation and can reach 200000/ml in a joint affected by bacteria.

Tests performed in the supernatant

Usually to detect protein concentration in the synovial fluid Biuret method is used. Normal values are lower than 3 g / dL. The increase in protein concentration is characteristic of inflammatory processes in the joint cavity.

To detect glucose level in the synovial fluid glucose oxidase methodis usually used. Normal glucose level is about 4.44 mmol/L. Reduced glucose level is characteristic of inflammatory processes.

Rheumatoid factor is represented by antibodies capable of reacting with human and rabbit IgE. These antibodies can belong to different classes of immunoglobulins (IgG, M, A) which can be detected by latex method.

Tests performed in the sediment

From the sediment, obtained by centrifugation and 200g (preferably centrifugated) of the fluid with anticoagulant the native preparation (to detect crystals and amyloid) and smear is made.

Cytogram of the synovial fluid

Normal synovial fluid contains up to 200 cells/mcL. Their qualitative composition is normally represented by:

- monocytes 30 - 47%;
- lymphocytes 25 – 30%;
- neutrophils 6-25%;
- synoviocyte up to 4%.

In various pathological conditions other cells can appear: plasma cells, macrophages, lipophages, Reiter cells, LE cells, ragocytes, cells with fragmented nuclei, eosinophils, basophils, fibroblasts, chondrocytes, adipocytes, cells at different stages of mitosis, cancer cells, etc.

Synovial fluid cells, appearing in different pathologies

Plasma cells are cells of 12-20 microns in diameter. These cells are found in large amounts in chronic viral lesions of the joints and multiple myeloma. **Macrophages** are large-sized cells (20-50 microns in diameter). **Lipophages** are macrophages containing a lot of vacuoles filled with fat drops in the cytoplasm. Lipophages are often found in post-traumatic inflammation of the joints, or hyperlipidemia.

Reiter cells are monocytes or macrophages, which fully phagocytosed a whole neutrophilic leukocyte. The appearance of these cells in large quantities is often observed in reactive inflammation of the joints.

LE-cells – are monocytes or granulocytes which phagocytized altered nucleus of another cell, called hematoxylin body or LE-cell. LE-cells appear in systemic lupus erythematosus complicated by rheumatoid arthritis.

Ragocytes are neutrophils, macrophages, monocytes, synoviocytes, and sometimes lymphocytes which phagocytized immunological complexes containing IgG or IgM. Ragocytes are detected in the synovial fluid in bacterial arthritis, acute inflammatory lesions of the joints caused by crystals, and rheumatoid arthritis.

Cells with nuclear pyknosis are necrotic neutrophils or lymphocytes in which the nucleus is fragmented into 2-6 rounded unstructured homogenous formations of dark-violet color. Vacuoles and granules can be detected in cytoplasm.

Eosinophils can appear during arthrography in patients with eosinophilia in the blood or in hemorrhage into the joint gap.

Atypical cells appear in the synovial fluid in sinovioma or metastatic tumors of other localizations into the joint. Differentiation of these cells is based on cytological, histological and immunological studies.

Crystal analysis in the synovial fluid

Identification of specific crystals in the synovial fluid is particularly important from the point of view of arthritis differential diagnosis.

Crystals can precipitate in the joint cavity and around the joints and cause symptoms of gouty arthritis. Type of arthritic lesions depends on the type of crystals. Identification of specific crystals confirms the diagnosis of joint lesion and requires microscopic examination and accurate interpretation of the results.

Diagnostic value of synovial fluid examination in clinical practice.

Laboratory tests allow to distinguish three basic types of pathological synovial fluid.

Type I – noninflammatory.

It is clear, viscous, straw yellow or yellow fluid, with pH about 7.2-7.4. In Ropes test compact dense clot is formed. Glucose concentration in the fluid is a bit lower than in the serum (the difference does not exceed 0.5 mmol /L). Protein concentration is normal, cytosis is less than 2000 cells per ml, granulocytes are less than 25%.

Type II – inflammatory.

Color of the fluid is yellow or milky-yellow. The fluid is of various turbidity, pH is 7,1-6,8, viscosity is slow. Ropes test (mucin clot test) shows flaky sediment. Glucose concentration is lower than in the serum (the difference exceeds 2.2 mmol /L). Protein concentration increases proportional to the activity of the inflammatory process. Cytosis level depends on the etiological factors causing joint inflammation and can reach 2,000 - 75,000 ml with a predominance of granulocytes. In inflammation caused by crystals, they can be detected within the phagocytic cells or be located extracellularly.

Type III – septic.

Fluid is yellow-gray, turbid; pH is usually about 6.6, viscosity is significantly reduced. Glucose concentration is significantly reduced too (the difference in comparison with the serum can exceed 3.33 mmol / L). Protein concentration is 56 g / L. Cell number increases to 200,000 in mcl with a predominance of granulocytes.

Table 23. The results of synovial fluid laboratory examination in infectious arthritis of various etiology

Infection	**Cytosis (в мкл)**	**Cytosis character**	**Glucose concentration (mmol/l)**	**Character of a clot in Ropes test**
No	200-600	Mononuclear leukocytes	4,44	Compact
Acute bacterial arthritis	10,000 – 100,000	> 90% Peripheral mononuclear leukocytes	<2	Flaky Sediment
Fungal arthritis	3000-30,000	> 70% Peripheral mononuclear leukocytes	Norm or<4,44	Friable
Tuberculous arthritis	10,000-20,000	50-70% Peripheral mononuclear leukocytes	about 3	Friable

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КЛИНИЧЕСКАЯ ЛАБОРАТОРНАЯ ДИАГНОСТИКА

Пособие
для студентов факультета иностранных учащихся
с английским языком обучения
Издается на английском языке

CLINICAL LABORATORY DIAGNOSTICS

Study guide
for the Medical Faculty for International Students

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